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Anglo-Saxon Problems in Science and Technology

RECEIVED doctrine has it that science and technology are the foundations of prosperity in industrialized societies, which is why it is strange to say the least of it that so little has been heard of this principle in the past few weeks, when what the French call the Anglo-Saxon countries have been trimming their sails to the harsh winds of 1968 What has gone wrong ? How was President Johnson able, on January 1, to outline a programme of financial retrenchment overseas without breathing a word about science and technology ? And where now, in Britain, is all the brave talk about the new opportunities which technology provides ? Only three years ago, Britain, thanks to its new government, was buzzing with talk of how technology would magic away the threat of national bankruptcy More recently, technological strength has been proffered as a means by which Europe as a whole could be welded into a gigantic technological community So, has the British Government lost the faith it once possessed in science and technology ? What else can explain Mr Wedgwood Benn's conspicuous silence in the past few weeks ? And, in any case, what policies towards science and technology should the American and British Governments pursue at a time when both of them are being forced to reduce public expenditure quite drastically ?

The first thing to recognize is the difference between the time scales of change in technical development and in high finance Projects in research and development take years to fructify, but financial crises can blow up in a few months, which is why it is that governments have no choice but to fall back on short-term fiscal measures to deal with situations like those which have arisen in Britain and the United States (which is not to imply that the two crises have more than a few elements in common) But the danger, of course, is that in making plans to deal with immediate problems, governments may jeopardize, perhaps inadvertently, long-term prospects And even if their stop-gap policies do no positive harm, too great a preoccupation with immediate problems may obscure the need for long-term solutions

Ironically enough, this danger may be more immediate in the United States than in Britain The annual round of congressional enquiries into the budgets of the federal agencies has already demonstrated plainly enough that Congress fully shares the Administration's zeal for economies in public spending Indeed, in many ways, Congress is the pacemaker, for it now seems to be accepted that a substantial reduction in the scale of government expenditure in the United States will be a precondition for congressional approval of the tax supplement that the Administration would like to have In practice, the economies which have been imposed in recent weeks in the spending of the federal agencies are real enough, and their effects are certain to be palpable Yet it is sensible enough, for example, that the National Aeronautics and Space Administration should be forced to wait for approval of its plans for the use of the Saturn rocket system in the 70s until there is at least some tangible evidence of how the programme of getting to the Moon will work out

Probably there is also enough slack in the budget of the National Institutes of Health for that buoyant institution to be more or less unaffected in the year immediately ahead To be sure, the National Institutes of Health may find itself having to cut back or to postpone some of its imaginative but ambitious plans for development in higher education, particularly in the training of postgraduates, but that will be just as well at a time when the relationship between American universities and government institutions has not been clarified as much as the circumstances require Ironically, there is much more cause for alarm in the ways in which agencies such as the Department of Defense and the Atomic Energy Commission have been forced to economize in the past year, chiefly because these agencies have been converted almost overnight from fairy godmothers to cheeseparers in their support of basic science and technology in the universities and elsewhere The trouble, of course, is that in spite of the way in which organizations such as the US Office for Naval Research have acted as beneficent and visionary supporters of scientific developments in the United States and elsewhere since the war, and even though the armed services and other mission-oriented agencies profess that basic research is somehow inseparable from their more practical endeavours, the amounts being spent on the support of basic science and technology are necessarily marginal At times of scarcity, it is inevitable that they should also seem expendable The trouble is that a great many institutions elsewhere have become dependent on the services and similar agencies for continuing support These will now inevitably feel the draught All this is understandable enough, but the moral should now be plain A greater proportion of the Federal Government's support for basic research in the universities and elsewhere should in future be undertaken by independent agencies such as the National Science Foundation, even if this should entail a radical revision of the structure of that organization Such a shift of emphasis is, indeed, long overdue, and it will be entirely welcome if the present crisis, with its shortage of funds for research and for junior appointments in the larger universities, provides an incentive towards a policy like this

In Britain, by contrast, the danger that enforced economies will cramp the immediate prosecution of science and technology is less real than the danger that the urgency of the present crisis will compel people to postpone yet again decisions on structural problems which affect the support of science and technology in Britain To be sure, it is too soon to know how seriously the finances of the universities will be affected by the economies yet to come, but it is hard to see that even the strongest pressure from the Treasury will enable the Department of Education and Science to reduce still further the amounts of money available to the University Grants Committee in the five years from this September Most probably the chief result will be to cast down still further those who have been hoping against hope that it might be possible to bring about some revision of the straitened terms of grant for the coming quinquennium But these are temporary measures The real question about the character of research in British universities which needs now to be answered is whether they are to be endowed so generously with funds for research that they can make a continuing and important contribution to the development of science in Britain, or whether that role is gradually to be taken over by other institutions—probably the government establishments There is an uneasy sense that the cumulative effect of the decisions which have been taken about research and development in Britain in the past five years have tended to emphasize the educational function of the universities and to diminish their importance as centres of innovation The difficulty which universities now find of increasing the scale of postgraduate teaching is one symptom of this state of affairs It is hard to believe that it can be in the long-term interests of the British economy

The place of the government research establishments is also central to the problem of how to manage the scientific and technical resources of the British economy for long-term prosperity The present government is temperamentally inclined to take the view that even though many of the establishments have outlived the purposes for which they were first established, they still have a valuable part to play in the advancement of industrial expertise and even, sometimes, in the development of pure science And, of course, there is bound to be some truth in this But the question which must really be tackled directly as a public issue is whether there is not now too great an emphasis on the importance of the government establishments at the expense of institutions elsewhere—the universities and industry

It is improbable that the present balance can be right, for after all it has grown by a succession of historical accidents Most probably the government establishments are at present attempting to do more than is good for them or for the rest of the British economy But it would obviously do more damage than good if the establishments were crudely now to be reduced in scale—what is needed, instead, is a mechanism by means of which their resources can be put more fully at the disposal of the British economy The devices in the Science and Technology Act which permit diversification at the establishments are not sufficient Perhaps the new White Paper which the Ministry of Technology has been advertising will have a better solution to propose

Whistling in the Wind

As good trade unionists know, wage claims at times of economic belt-tightening are no more successful than whistling in a blizzard However sweet the music sounds, it never carries far The Association of University Teachers is far from being a trade union, if it were, it would probably not have persisted with its claim that teachers in universities are underpaid The British Government has rewarded the association for its pains by asking the Prices and Incomes Board, a notoriously unsentimental body, to undertake a survey of university salaries When the association's claims were being rebuffed by the Department of Education and Science and the University Grants Committee, it was possible to feel a fatalistic sort of sympathy—but if the Prices and Incomes Board should conclude that there are no grounds for an increase, that is likely to be an end to the matter And once the board, and its chairman, Mr Aubrey Jones, have the bit between their teeth, no government is going to feel moved to set up a review body more sympathetic to the teachers

The burden of the association's case is that academic salaries have been allowed to fall behind those paid to comparably skilled people in other jobs But who is to know whether or not this has happened because academic jobs are so easy to fill ? The very large numbers of young men and women who want to stay in universities may have so exceeded the number of jobs available that the market price has fallen The teachers' hard-luck story might be more convincing if fewer of their students behaved as if all they want to do is follow them into academic life The point is sharpened if one remembers that universities in Britain have been expanding at a great pace for the past few years, and that the supply of manpower seems to have been no limitation to the process It would be good to be able to say the same for other activities employing highly qualified manpower—the application of computers in industry, for example, is likely to be controlled for some years to come by the supply of systems analysts

It is also quite legitimate to ask why university staff should be paid exactly the same as their opposite numbers in industry If the principle of comparability were applied rigidly, it would lead to absurd situations, and to a society forever fixed in a pecking order established years before This is not the same as arguing that industrialists are in some way more valuable than university teachers—that would be an impossible position to sustain—or that long university holidays and the freedoms of academic life should be paid for by smaller salaries, but that academic qualifications should not be regarded as the passport to a guaranteed income. University teachers, above all, should be aware of this. A degree is not a professional qualification. It does not entitle its holder to membership of a select association whose members are paid the same salary whatever job they do. It might, indeed, be better if there were much greater disparities between the salaries of graduates, particularly newly qualified ones. Not every intending academic can be turned into a systems analyst, but a more significant differential could help the process along.

It is also depressing that the association seems determined to establish standard rates of pay at all British universities. One of the striking developments in education in the United States has been the ability of lesser universities to pull themselves up by their own bootstraps by offering generous rates of pay. The University of Texas, supported by local business, is a good example of this. Are universities in Britain to be denied similar opportunities for self-improvement?

The danger implicit in the association's argument is that, if university teachers are to be paid more, industrial salaries will also increase. And the hard-pressed schools, which can really make out a convincing case for a salary review, would be even less able to attract talented graduates. The next few years, which coincide with the graduation from universities of the children born in the "bulge" years, should offer the schools a real chance to recruit graduates. The universities, with the first major period of expansion after Robbins behind them, are likely to be absorbing fewer of their graduates than they have in the past few years. When all this is taken into account, can the association be said to have presented a convincing case? On past form, Mr Aubrey Jones will be only too eager to decide.

Engineering Compared

THE case for the Common Market has seldom been put more cogently than in the recent OECD report on the engineering industries of member countries (*The Engineering Industries in North America—Europe—Japan*, OECD, 1967, 30*s*.). The United States makes a smaller investment in its engineering industries than does Europe, but produces more than twice as much with almost half the number of employees. In spite of higher wage levels, the United States invested only $4,400 million (at 1958 prices) in 1965 to achieve $162,000 million worth of production. Europe, including the United Kingdom, invested $4,500 million in the same year to achieve an output of $83,000 million. Employment figures in 1965 were 6·7 million in the US and 12 million in Europe. During 1966 productivity in the US engineering industry was $25,000 a head, three times the European average. By contrast, productivity was $10,000 a head in France and Japan, $8,000 in West Germany and $6,100 in Britain.

The OECD report observes that these remarkable differences in efficiency are too important to be explained by a few causes. But it points out that disparities in the size and organization of the market, and hence in the scale and structure of production, are likely to be a dominant factor. The necessary reorganization of production in Europe, the report says, "can be fruitful only if accompanied . . . by effective measures to create a single European market, removing not only the traditional barriers (customs) but also all the other obstacles to trade (i.e. technical and administrative regulations, different legal and fiscal systems, currencies, etc.). In a word, the biggest and most acute problem facing the largest industrial sectors in Europe is the difficulty of surmounting those obstacles which prevent really economic production on a large scale."

The growth of British engineering industry compares unfavourably not only with that of the United States but also with its European competitors. As shown in the table below, its growth during the decade 1955–65 has been less than that of France or Germany and its relative share in the total production of OECD member countries has declined.

DEVELOPMENT OF ENGINEERING INDUSTRIES 1955–65

Country	Per cent increase 1965/1955 (1958 prices)	Per cent share of OECD engineering production		
		1955	1960	1965
United Kingdom	37	11·6	11·2	9·4
Germany	91	8·4	10·3	9·6
France	100	5·1	6·0	5·9
Italy	91	2·1	2·7	2·3
United States	57	61·6	54·7	54·7
Japan	611	2·2	6·1	8·6
Europe OECD	72	34·0	37·4	34·5
Total OECD	73	100	100	100

Diagnoses of what is wrong with British industry are two a penny and about as valuable to a patient who has heard it all before in one form or another. More to the point is what is right with American industry. The OECD report emphasizes the widely known fact that the structure of the United States domestic market favours very large-scale production with consequent maximum returns on capital. Greater profits lead to a higher degree of self-financing than in European companies; in absolute terms, investment is high and equipment is constantly renewed. A further difference is that, whereas in Europe new investment must usually be devoted to replacing capital stock or increasing production on the same technical base, the United States engineering industry can afford to divert the major part of new investment to the less immediately profitable ends of innovation and rationalization. Because plant is being used to its technical maximum, manpower is highly productive. The relatively small number of firms reduces administrative and technical personnel needs, which is a further factor in increasing productivity. Wages, and the standard of living, can be allowed to rise accordingly. All this has long been known in Europe but not, it seems, known well enough.

Academic Issues

THE National Board for Prices and Incomes is becoming a maid of all work for the British Government. The latest commission to land at the board's door is a survey of the pay of academic staff in universities. The government asked the board to take over this task, on a continuing basis, in November, and last week the board issued an invitation for written evidence from interested organizations. The Association of University Teachers, quick off the mark, had submitted evidence before it was asked, and other more slowly moving organizations are certain to follow. The Department of Education and Science, the University Grants Committee and the Committee of Vice-Chancellors and Principals are all likely to respond to the call, in their different ways. Some individuals may also feel impelled to write to the board, and their views, too, will be taken into account, although it would be a pity if the board were slowed down in its work by a mass of memoranda from individuals.

Mr Aubrey Jones; new look at university pay.

The last attempt to adjudicate on university salaries was made by the ill-fated National Incomes Commission in 1963. The commission, cosily called "Nicky" by its friends—and much worse things by the TUC, which refused to co-operate—folded up after the Conservative Government was defeated in the 1964 election. Since then, university pay has been settled by the Government, in collaboration with the University Grants Committee. But academics are always inclined to believe that the UGC is in cahoots with the Government, and the Association of University Teachers appealed for an independent review board. Offered the Prices and Incomes Board, it accepted, without, as it says, "prejudice to the future". The AUT argues that salaries of university teachers are much lower than those of people with equivalent qualifications in other walks of life; since the last review, it says, salaries of university teachers have increased by 5 per cent, while those outside academic life have had rises of up to 20 per cent. It rejects the selfless gestures of some professors who have said that in present economic circumstances university teachers should do without a rise. Professors, it implies, are the last people who should make these gestures, as they are well paid—the great mass of the 15,000 membership of the AUT is less well off. Professors would be the first to complain if the standard of academic work fell because universities could not recruit the best people, it suggests.

The board is not saying when it hopes to finish the survey. But it has a reputation for getting things done, and usually takes about 4–5 months, so there could be concrete results by the middle of this year. Mr Aubrey Jones, chairman of the board, has appointed himself chairman of the team which will investigate university pay. He and another two members of the board team will be assisted by a staff working party consisting of at least another three people, who will be able to call on all the available expertise—economists, statisticians, and the like—employed by the board. In addition, the committee can call on an enquiry team to go out and "ferret out the facts", so there seems no lack of professional zeal in the board's approach to the problem.

Bigger Machine at Weston

THE plan to build a new proton accelerator at Weston, Illinois, seems to be moving vigorously ahead. Among the several innovations which have been made since the decision was taken six months ago to go ahead with the machine is that the University of Toronto has joined the Universities Research Association Inc. This consortium of universities is formally responsible to the US Atomic Energy Commission for the construction and operation of the new accelerator. Forty-eight universities now belong to the consortium. The membership of the University of Toronto will no doubt excite the interest of other universities outside the United States.

The flavour of the Weston Project has changed significantly in the past few months, and particularly since the appointment last summer of Professor Robert Wilson as director of the project. For one thing, the accelerator is now known as the 200–400 GeV machine, which amounts to an explicit recognition that at some stage in the development of the project the energy of the protons which the machine produces will be doubled to 400 GeV.

The possibility that accelerators might be graded is not of course new, but Professor Wilson seems to have hit on a particularly simple and economical way of doing this. To begin with, the machine at Weston will be fitted out with magnets designed to operate at a much higher field intensity than is necessary for the production of protons at 200 GeV. This means that when more money is available, it will be possible to increase the operating power of the magnets, chiefly by adding to the power supply. This is a simpler means of increasing the rating of a proton accelerator than some of the other devices which have been suggested—the addition of extra magnets, for example. All this amounts to a neat exploitation of the fact that in the larger accelerators, the costs of buying land and building a tunnel are a larger proportion of the total, which in turn implies that the costs of magnets are comparatively less important. Professor Wilson seems to have been able to push this balance even further by paying close attention to the design of cheap magnets—this is why it is possible for him to think of building into the new machine magnet capacity that will not be used for some time to come.

IBM Displaced

THE United States Air Force has now chosen to buy computers from Burroughs for the second phase of its programme of automation at its bases around the world. This is a plan to install linked computers in USAF bases to deal with problems of logistics. The contract was originally put out to tender last April, and was won by IBM with a bid of $114 million. Honeywell, which had submitted a bid of only $54 million, complained to the General Accounting Office. As a result of this, the Comptroller-General ordered the cancellation of the IBM contract, despite the Air Force's assertion that price was only one of five factors to be considered, and that only IBM, to use its own words, "satisfied these areas of response". All four manufacturers tendered again, and Burroughs' winning bid was to provide 135 of its "third-generation" B 3500 machines for $60 million.

IBM has confessed itself disappointed by the final decision, claiming the superiority of its products. The USAF, which announced details only of the winning bid on both occasions, seems unrepentant. It has confused a none too lucid issue by saying that the tenders for the second selection "were markedly different from the original submission in terms of capacities, speeds and, in some cases, processing techniques". It also claims that the delay caused by the intervention of the Comptroller-General cost $18 million, so that the American taxpayer need only congratulate himself on a saving of $36 million. Honeywell has pronounced itself satisfied that the original complaint has been shown to have been justified; the exercise seems to have lost Honeywell no favour with the US Government, for the company has just received an order to lease twenty-two computers, worth $8·5 million, to the Department of Defense.

Burroughs had won the contract for the first phase of the same programme, a $24 million order to provide computers for organizing supplies at air force bases, so that the USAF will doubtless find advantages in having standard equipment for base logistics. The first two systems of the second phase will be delivered in March, and two further systems will be installed at Sheppard Air Force Base, Texas, in April and May. These will be used for training. A test programme will begin at Langley Air Force Base, Virginia, in September and, if the results of this are satisfactory, the computers will be installed at bases around the world during 1969 and the first half of 1970.

Supersonic Plans

THE introduction of supersonic transport aircraft is bound to increase problems of air transport control and flight planning. As well as flying three times as fast as aircraft now in service, supersonic aircraft will fly much higher, at between 16 and 21 kilometres. Meteorologists, familiar with the problems of long-range forecasting, are already beginning to wonder how the supersonics are going to affect them. Some results of this process of looking ahead have now appeared in the form of a report from the World Meteorological Organization called *Meteorological Problems in the Design and Operation of Supersonic Aircraft*. The report is written by R. F. Jones, R. M. McInturff and S. Teweles.

The difficulties of the sonic boom are already familiar enough. As well as the regular boom which the aircraft makes, the report discusses the phenomenon of "superboom", which is thought to be caused by the simultaneous arrival at the observation point of shock waves from two distinct parts of the flight path. Overpressures as great as 3·6 mbar have been recorded in low level tests, nearly five times as great as the nominal overpressures which the SSTs are expected to produce. It may be possible to eliminate superbooms by avoiding turning manoeuvres or supersonic acceleration in level flight, but atmospheric turbulence may affect the issue by producing quite rapid accelerations and changes in aircraft attitude. Actual experience with the first supersonic airliners will be needed before the position is clear.

The actual incidence of the boom may also be affected by unscheduled changes of flight plan. The thrust of a jet engine decreases with increasing temperature—BOAC, for example, has estimated that a five-degree increase in temperature produces a 25 per cent deterioration in acceleration between Mach 1·1 and 2·0. And because the SSTs will fly much higher than existing aircraft, information about temperatures is relatively more scarce. Most of the handicap of higher temperatures is experienced in the trans-sonic phase of the flight, between Mach 0·8 and 1·2.

The problems of turbulence, and its effects on aircraft, are not well understood. One problem, as the report explains, is that aircraft in flight filter from the whole spectrum of turbulence those frequencies to which they are most susceptible. Thus results with one aircraft may not be duplicated with another. Short wavelength disturbances may affect the fatigue life of the aircraft without actually making passengers feel uncomfortable, while long wavelengths may move the aircraft bodily but smoothly, without imposing great strains on its structure. Between these two extremes there are wavelengths which cause the aircraft to roll or pitch. Some turbulence is associated with cloud structures, and may be possible to avoid, because heavy cloud formations can be detected by radar. Clear air turbulence is a more severe problem, and there is evidence that it will be experienced at SST cruising heights. In flight, warning devices may help, but only if they can detect CAT 100 miles ahead of the aircraft.

There will also be a danger of large doses of radiation, because at supersonic aircraft heights the atmosphere will be too rarefied to provide a protective screen. The report confirms the suggestion that galactic cosmic rays, a continuous background through which the SSTs will have to fly, will not be a hazard. But there does seem to be a danger if exceptional solar events such as solar flares are ignored. The report contains a calculation by M. Haurwitz, previously unpublished, which shows that for a hypothetical event of the magnitude of the event on November 12, 1960, passengers on a three hour flight at 21 km would receive a dose of radiation twice as large as that recommended for a whole year. Warning devices in the aircraft, and careful preflight planning, will be necessary to minimize the dangers. Another hazard is the presence of ozone in the atmosphere; even small concentrations are poisonous, and it is thought that concentrations of more than 0·1 ppm by volume could not long be tolerated in the aircraft Concentrations of this order are common at 15 to 18 km, and may occasionally be ten to fifty times as great; at 21 km a value 100 times the danger level may be encountered. Fortunately, ozone is easily removed by filters, which will be fitted to the aircraft.

Rain and hail are less easy to deal with Hailstones, the report says, constitute one of the greatest dangers to supersonic flight For this reason, it will be vital to avoid penetrating cumulo-nimbus cloud at cruising speed, and detection by airborne radar will be provided But a supersonic aircraft takes 100 miles to change course by 90°, so the equipment will have to be reliable, and carefully watched

Industrial Oceanography

A NUMBER of French industrial companies have formed a scientific and technical association for the exploration of the oceans, ASTEO The association had its origin in a project of the development council of Aquitaine to establish a regional bureau of marine engineering It was soon realized, however, that the task of co-ordinating the large number of industries and scientific techniques would require a national organization The members of the new association, therefore, are companies from all over France, but the association's headquarters will be in Bordeaux Naturally enough, several shipyards belong to the association, and two of the biggest French shipping companies, Messageries Maritimes and the Compagnie Générale Transatlantique, are also among the founder members But there are a number of firms on this list with relatively few nautical associations Hispano and SCNEMA, Nord-Aviation, Dassault and Sud-Aviation, the Compagne Générale d'Electricité, and SEREB (Société pour l'Etude et la Réalisation des Engins Ballistics), whose director-general, M Charles Cristofini, will be the first president of the association

Among the first aims of the association will be to review the possibilities for French industry in oceanography. It is also hoped to establish an information centre which would use the computers of the SEREB at Bordeaux or the Compagnie Générale d'Electricité at Marcoussis M Cristofini has taken care to emphasize that the association is in no way a competitor of the National Centre for the Exploration of the Oceans Rather, the new association, some of whose members are also members of CNEXO, will complement the older organization

In Britain the participation of diverse industries in oceanography is rather less formally ordered This may be partly because certain large British companies active in oceanographical research, such as Hawker Siddeley and Vickers, have many subsidiaries or associates specializing in the techniques involved in this kind of work Co-operation is within the company rather than between companies But it is interesting to note that the British Aircraft Corporation has been working with Imperial College, and with the assistance of the Bristol shipyard of Charles Hill, and Normalair Ltd, on the development of an underwater house (Project Kraken) The Kraken house would be situated a hundred feet below the surface, and would allow divers to work at depth without having to undergo decompression The Guided Weapons Division of BAC will be investigating the type of structure needed for the Kraken house, and working on the features necessary to support life This is the first submarine excursion of this kind by BAC, though the Guided Weapons Division played a small part in the British Polaris programme

The general objectives for Kraken—named after Tennyson's sea-monster—have been laid down by the Imperial College team led by Brian Ray These are for a house capable of providing life support to a 4-man research team living at depths of 80–100 ft for 2–3 weeks or more There would also be an ample margin for visitors on shorter missions This range lies just within the working range of the atmospheric gases Most of the work on saturation dives (in France and the United States) has concentrated on greater depths and on oxyhelium saturation Remarkably little is known about the effects of prolonged saturation with air under pressure Thus a prime objective of the experiment is to gather physiological data on air saturation diving The chosen depth range is practical in a number of ways The cheap gas and modest depth will keep down running costs Much of Britain's continental shelf is within range for 100 foot diving platforms

By living at depth much more work can be done than from surface dives, each one of which must be followed by lengthy decompression Kraken's promoters believe that the practical value of the house will lie in the commercial fields of prospecting, of surveying and for research in support of fish farming

The quarters that BAC has designed round these objectives are a structure rather like a railway coach divided in two internally. The all-purpose living area will be 14 foot long and permanently heated by a system of hot water circulating in pipes The 10 foot work area will not be heated There is no need for a pressure vessel hull since pressure inside and outside Kraken will be at equilibrium (varying a little with the rise and fall of the tide) The house will be permanently open to the sea through a hole in the bottom Gas and power will be piped from the surface The only thing still lacking is the money to build the operational house for next summer

More Social Science

TOTAL research and development expenditure in the United States during 1968 will reach $26,500m—an increase of over $700m or of 3 3 per cent compared with the estimated expenditure for 1967 This is the New Year message from the Battelle Memorial Institute, whose last year's prediction for the 1967 expenditure was in fact 8 per cent below the current estimate

The Federal Government is the largest contributor to research and development expenditure, and during 1968 is expected to provide $17,200m, an increase of 2 2 per cent on its support last year Industry will spend $8,300m, an increase of 4 8 per cent, and the colleges and universities about $865 million, which at 8 8 per cent is the largest proportionate increase on last year's figures The remainder of 1968 spending is made up by the $265 million which will be provided by other non-profitmaking institutions The Battelle Institute points out that although the Federal Government is the major source of research funds, accounting for 65 per cent of the total, 69 per cent of all research is in fact carried out in industry. These figures take account of the reduction in Federal research funds voted by Congress last month (*Nature*, **216**, 1155, 1967).

Analysis of 1968 expenditure shows a relative shift to the social sciences in the distribution of Federal research funds. For the first time since figures became available, the increase in Federal spending in this sector will be greater than the increase in the physical sciences. Research into social problems is a dominant factor in the programmes administered by the Department of Health, Education and Welfare (HEW), the Department of Housing and Urban Development (HUD), the Department of Labor and the Office of Economic Opportunity (OEO). The 1968 fiscal year budgets for these departments are 220 per cent higher than those for comparable programmes in 1961. In contrast the spending on research and development by the Department of Defense, NASA and AEC has increased by only 73 per cent during this period, most of which is a result of the substantial rise in space expenditure. Nevertheless, these three agencies will receive 86 per cent of Federal research funds in 1968, as distinct from their 91 per cent share in 1961.

In the decade 1955–65, research and development expenditure grew at an annual rate of 14 per cent, against an annual growth rate of 5·5 per cent in gross national product. The Battelle Institute feels that this runaway rate of growth has now been contained and will remain so for the foreseeable future. Henceforth research and development is likely to grow at about the same rate as the GNP. Budget administrators in other countries who have watched with alarm the importunate demands of science research will take cheer from the *modus vivendi* now apparently reached in the United States. Scientists may feel differently.

Teaching Aids in Sussex

A GRANT of £10,000 has been awarded by the Department of Education and Science to the Centre for Academic Services at the University of Sussex to assist a co-operative project for the development and application of educational technology at all levels of education in the region. The centre at Sussex is one of the ten which were selected by the University Grants Committee to develop audio-visual aids in education. It was founded in February 1966 with the help of a grant from the Rank Organization.

This particular study will be carried out in association with the Nuffield Resources for Learning Project and the five local education authorities of East Sussex, West Sussex, Brighton, Eastbourne and Hastings. The objective is to analyse the existing audio-visual resources in schools and to estimate the potential for educational technology in the area. Regional co-operation in developing new methods and equipment will be considered and the costs and possible effects of such developments will be analysed. The proposals for joint activity include programmed learning, radio, closed circuit and broadcast television, language laboratories and film and projection systems.

A policy committee has been set up by the university to advise on the project and includes the five chief education officers of Sussex, Mr R. H. Adams (divisional inspector of schools for the south-east of England), Mr Tim McMullen (co-ordinating director of the Nuffield Resources for Learning Project), Professor G. Allen (sub-dean of the University School of Educational Studies), and Mr Norman Mackenzie and Mr Hywel C. Jones (director and deputy director of the Centre for Academic Services), the university will act as co-ordinator for the scheme. The project was started on November 1, 1967, and is expected to last for two or three years.

What is interesting about the scheme is that it involves the getting together of five local education authorities with the university, to their mutual advantage. According to Mr Norman Mackenzie, although the operation is a modest one it will at least show how advantageous such co-operation can be.

Training Computer Users

THE Government and industry are well aware of the need for trained computer manpower and of the shortage of suitable training courses for such personnel. To help satisfy this demand, the British Computer Society is introducing professional qualifications this year. And training courses for computer users, particularly those on the professional and managerial side, are also important. It is therefore welcome that new and extended computer courses are being made available, from the end of January, to people in just this group by Rolls-Royce Ltd.

Rolls-Royce has for many years organized a wide range of courses of various levels in computing and data processing for its own staff. The Ministry of Technology seems to have suggested that the firm's courses should be extended to people from other firms. The suggestion was accepted and the courses now offered have been designed to make them acceptable to people from a broad range of other organizations.

Five-day residential courses in computer systems in management design, research and development will be held in Nottingham at a cost of 150 guineas each (one guinea = £1·05). One-day appreciation courses for executives for 25 guineas will be held in Manchester and London. Four-week courses in systems analysis and design will be held in Largs, Stratford upon Avon, Derby and Droitwich. In addition there will be three short courses for directors of firms with a turnover of £50 million and above. The value of these new courses may lie in the wide experience of Rolls-Royce in specific applications of computers.

New Look Laboratories

THE 39th Building Bulletin of the Department of Education and Science (HMSO, 13*s* 6*d*) deals with the design of a science block for a large boys' grammar school. The block was designed for the Oxford School—a new school formed by the merger of the old-established City of Oxford High School and the prewar Southfields School. Four architects and a quantity surveyor of the Development Group of the Architects and Building Branch at the ministry worked on the project in collaboration with the Oxford local authority. Two consulting firms were called in to advise on heating and electrical work, and the school was built in just over a year and a half.

The importance of science in the curriculum seems to have been fully appreciated from the beginning, partly on the basis of the 1961 policy statements of the Science Masters' Association (SMA) and the Association of Women Science Teachers (AWST) (now merged into the Association for Science Education) Accommodation in the Oxford School was therefore provided for each of the three stages of science education in secondary schools described by the SMA and the AWST statements—two years of general science teaching in the introductory phase, three years of intermediate work leading to O-level and two or three years of advanced work The Oxford School has a four form entry, and a total of about 800 boys Allowance was made for six teaching periods a week of science in the introductory phase, three to four periods for each of chemistry, physics and biology in the middle school and seven periods for each of the three subjects in the sixth form The proportion of time allotted to science under this scheme is higher than in most other schools, with the result that the science block is larger than is usual Twenty-four boys per class was thought to be the best number for middle school science, so that flexibility can be maintained in the timetable, while fifteen was considered the optimum number for experimental work in the advanced stage Mathematics classrooms have added to the size of the block

Self-contained subject departments were provided for the school, following the wishes of the staff, but the bulletin suggests that better use might have been made of space if closer integration had been considered, particularly as the boundaries between science subjects are becoming more blurred Stores and preparation rooms for different subjects which often provide similar facilities and apparatus could well be shared by laboratories of different disciplines The design of the separate departments is described in detail in the bulletin

As well as educational questions the group had definite technical objectives in view The first was to test a dimensional control system proposed by Dr J W Harding of the Building Research Station in his paper "Co-ordination by Design Modules" The second was to develop a rapidly erected "dry" form of construction that can be used for a variety of ten-storey buildings Further use of the lighting system PSALI (permanent supplementary artificial lighting of interiors), first applied by the group to rooms at Harris College, Preston, was the third objective This system is designed to provide strong light during the day to compensate for lack of daylight, but at night provides a lower level of light throughout the room The fourth intention of the group was to gain experience with a computer for processing the bill of quantities, and apply modern management techniques, such as network analysis, to the scheme

The project was under way before even the preliminary conclusions of the Nuffield Science Teaching Project were published, and the bulletin admits that the Oxford block may not be the best answer for the Nuffield requirements, but the detail and ideas put forward in the bulletin will be of considerable value to those designing science buildings in schools.

Advice from Harwell

DESPITE the admonitions of the Select Committee on Science and Technology, Harwell continues to diversify This time it will be lending its experience to engineering companies involved in the problems of heat transfer and fluid flow In return for an annual fee of about £1,000, firms will receive reports on the design of equipment, and there will also be a consultation service available to individual firms The Chemical Engineering and Process Division has for the last ten years been working on heat transfer and fluid flow in the design of nuclear plants, and has therefore come into repeated contact with many of the firms that have shown interest in the new service As early as 1954, for example, APV collaborated with Harwell on the flow of thorium bismuthide through pipes This history of co-operation doubtless explains the enthusiasm felt in industry for the new service The Atomic Energy Authority has announced that there will be long-term support programmes of research into problems chosen in consultation with industry

Perhaps about 10 per cent of Harwell's scientists are now engaged on projects which could be described, in the words of the select committee, as "not inextricably linked to the primary task" of the Atomic Energy Authority Finding ways of filling in the spare time of vast nuclear establishments is not merely a British pastime At Oak Ridge Laboratory, in the United States, for example, there are teams working on desalination and centrifuges for medical use, both areas already tackled at Harwell

Britain Sick

AMONG the winter "epidemics" which have recently been spreading through Britain, influenza at least seems to be on the decline But bronchial complications associated with the infection are still increasing and the red warning for extra available hospital beds is still on

The virus which has been causing this latest outbreak is influenza virus *A*2, which is very similar to that responsible for the pandemic in 1957 and for several outbreaks since then Of the three types of influenza virus, *A*, *B* and *C*, type *A* is the most common cause of influenza in humans and is associated with the pandemics and serious epidemics, while type *B* is usually associated with small outbreaks which are rarely extensive, type *C* usually causes inapparent infections Because the virus is not a new sub-type, it is thought that most people will have acquired immunity to infection, and that the existing vaccines which consist of recent strains of *A*2 are likely to afford protection The principal danger arises when one sub-type mutates to another as in the pandemics of 1891, 1918 and 1957 and the serious epidemics of 1946 The periodicity of outbreaks on a less extensive scale is usually related to the accumulation of susceptible individuals and the removal of immune ones by death

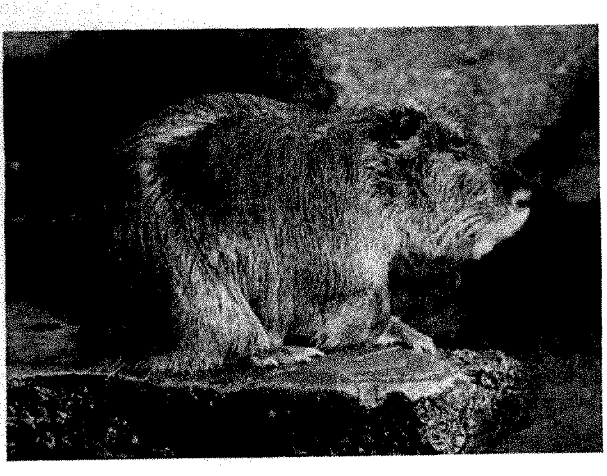
Another RNA virus—respiratory syncytial (chimpanzee coryza agent)—has been identified as the cause of death of five infants in Walsall and two more infants have recently been admitted to hospital with the same infection This virus, which usually causes minor respiratory infections in adults, may, however, be associated with bronchitis and broncho-pneumonia in children and is very widespread—infecting children within the first few months after birth Furthermore, antibodies present in the serum do not always prevent re-infection. According to Dr H. G. Pereira of the National Institute for Medical Research, the prospects of an efficient killed vaccine are not very good and a lot of work remains to be done in developing an attenuated virus vaccine.

Thirteen babies have so far died from a bacterial infection, gastro-enteritis, caused by *E. coli* strain 0–128. Happily, there have been no new cases within the last few days. It now seems that the infection was not the sole cause of death in every case. The involvement of multiple drug resistance transfer is the most likely explanation of how the bacteria became resistant to eight of the eleven antibiotics which have been tested at the Middlesbrough laboratory. The extrachromosomal element, *R* factor, which is responsible for infectious drug resistance is transmitted by conjugation from a resistant bacterium, through a pilus to a sensitive strain which also becomes resistant. The transfer process is repeated from cell to cell and the normal process of cell division also contributes to the rate of spread of multiple resistance in a susceptible population. There has been a suggestion that a combination of drugs might be effective in combating the infection, but this has only been shown experimentally, and *in vivo* applications would not necessarily be successful because the pattern of resistance in different individuals varies. Adults do not seem to be similarly infected; they have probably developed resistance.

Although the strain of *Salmonella* causing typhoid in the borough of Ealing has not yet been named, the same strain has been isolated from three cases. So far, typhoid has been confirmed in two patients, and four people have given positive specimens but have no symptoms. A spokesman at the Ealing Health Department said it was difficult to identify the original carrier of the infection.

Mink in the Wild

WILD populations of mink and coypu, descended from animals which have escaped from fur farms, have been established in England for some years. In spite of vigorous eradication campaigns, the animals are still so numerous that the Ministry of Agriculture has now had extended for five years the legislation whereby occupiers of agricultural land are obliged to report sightings to the ministry. The regulations which govern the keeping and transporting of the animals also remain in force.



Coypu: 136,000 killed in three years.

Coypus closely resemble beavers in appearance. Originally native to South America, they have been established in Suffolk and Norfolk since 1939. The damage they caused was largely compensated by their activity in keeping dykes and waterways free of weeds, and as long as they were worth trapping for their pelts their numbers remained within reasonable limits. But when coypu fur dropped out of fashion the population burgeoned and the damage done to crops such as sugar beet and by the burrows made in river banks reached significant proportions.

A thousand mink are trapped each year.

In 1962 a campaign was mounted to restrict coypus to a few sanctuaries in the Norfolk Broads, and by 1965 136,000 animals had been killed.

Dr R. M. Newson of the Ministry of Agriculture's Infestation Control Laboratory estimates that the survivors of the campaign now number about 5,000. Although a population of this order may be relatively harmless, rigorous control measures are still necessary because of the potential damage which could be caused by another population explosion. Not only have coypus developed a propensity for sugar beet over their usual diet of roots and weeds, but the undermining of dykes by their burrows, combined with high floods, could cause inestimable havoc. The population of wild mink has increased more slowly than that of coypu. Mink are members of the stoat and weasel family and are native to North America. Wild mink have been established for some time and about 1,000 are trapped alive each year.

There appears to be room for disagreement on the precise amount of damage that mink do. The Ministry of Agriculture believes that wild mink are a potentially serious pest to fish, wild birds and poultry. Fur-breeding experts, however, consider that frogs, mice and other economically worthless animals form the major part of their diet, and that mink may often be blamed for the depredations of other animals such as ferrets. But the Ministry is sufficiently convinced to have set up some 2,000 traps throughout the country. Mink fur, of course, still commands a considerable price on the fashion market. There are now 330 mink farms in England which export about £1 million worth of pelts a year. An average pelt sells for £10, and about 70 pelts may be needed to make a coat. The young are born in March at an average of five to a litter; raised on a diet of lights and offal, they are ready for pelting in November. For those with a feel for mink, the farming can be a profitable business.

Help for the Tropics

An appeal for £2 million has been launched by the United Kingdom Foundation for the International College of Tropical Medicine. This foundation, an entirely independent and academic organization—the conception of Mr Geoffrey Myres, a New Zealand surgeon with wide experience in the tropics—has been set up to serve the cause of medicine in tropical and subtropical countries.

The international college aims to help countries in which the shortage of doctors, nurses and other medical staff is acute, by establishing postgraduate tropical medical centres. At the moment, postgraduate tropical medicine training is only available outside the tropics as, for example, at the schools of tropical medicine in Britain. The centres envisaged by the foundation will comprise a hospital for training in clinical tropical medicine at postgraduate level, a school of tropical, social and preventive medicine and a research institute. Under the constitution of the college, it will be able to organize study and correspondence courses, conduct examinations and disseminate information through journals, textbooks, visual aids and libraries.

A positive step has already been made by the donation of land by the government of Uganda for the establishment of the first centre. Two other tropical countries have also offered land. It is hoped to establish other centres in Asia and Latin America. Specialists in tropical medicine from 15 countries form the advisory council, under the presidency of Professor T. A. Lambo, dean of the Medical Faculty, University of Ibadan. The council is backed by a separate board of trustees to manage financial affairs, and by an executive office with a provost to administer the college on behalf of the board and council. Charitable foundations are being established in several countries. The United Kingdom appeal, the first to be made, aims to raise enough money to enable the college to realize its objectives, and in particular to meet the capital expenses of the establishment and running costs of the first centre. Prominent people from the medical, scientific and other professions and industrial and business life are included in the membership of the UK council and executive committee.

Bringing Gas Ashore

Nobody yet knows what natural gas in Britain will cost. Despite this, the Gas Council aims to supply natural gas to all area boards by 1970, and the total expenditure on transmission and distribution over the next five years will be about £800 million. The first of the new pipelines, from Easington in Yorkshire, where gas comes ashore from the West Sole field of the British Petroleum Company, is already transmitting gas. Since July 1967, it has been feeding an average of 100 million cubic feet of natural gas a day into the national transmission system. Details have now been released of a new gas terminal now under construction at Bacton on the Norfolk coast. Initial supplies will go into the Gas Council's No. 2 feeder main, now almost complete.

The council aims for a huge capacity for this terminal, the first part of which is expected to be in operation by the middle of 1968. It is intended to have a maximum capacity of 4,000 million cubic feet of natural gas a day, nearly four times as much gas as is currently used throughout the whole country. The terminal will eventually handle natural gas from the 3 major fields discovered off the Norfolk coast—the Leman and Indefatigable fields which the Shell–Esso Group and Gas Council–Amoco Group share, and the Hewett Bank field, shared by the Arpet and Phillips Groups. Facilities being constructed for the Gas Council are for the reception of the gas and its distribution. Among the principal processes will be filtering of the gas, check-metering if required, heating, blending, odorizing and methanol injection.

A group of architects has been appointed for the design of the terminal, and most of the contracts awarded. The clients are taking due notice of the need for proper landscaping; the basic design for the Gas Council and Shell–Esso, which includes tree planting and grassed earth, has been agreed with the Royal Fine Art Commission. Clearly the hope is that in the rush to get natural gas to the public within the framework of the present economic restrictions, the amenities of the Norfolk coastline will not be sacrificed in aid of progress.

Hydraulics

The Ministry of Technology's Hydraulics Research Station, at Wallingford, Berkshire, has two main functions—to investigate specific problems in the field of civil engineering hydraulics for organizations both in the United Kingdom and overseas, and to conduct research in support of this work. The station's activities in 1966 are recorded in its annual report, just published (HMSO, 11*s*.). Services of the station are provided on a non-profit repayment basis and they appear to be much in demand; 140 investigations were in progress during the year.

One interesting project involves the possible use of synthetic seaweed as an aid in building up beaches. The station is trying to find out whether this material will promote onshore transport of sand and, if so, by what mechanism. Using a special polypropylene of normal density but modified elasticity, a model seaweed bed was laid offshore from a beach in a wave flume. The scale of the model was 1 : 10, and coal particles were used as the bed material. In such a flume, beach changes could only result from changes in onshore–offshore transport. Accumulation on the beach necessarily implied that sediment had come in from offshore, in contrast to the situation on a real beach where accumulation can be caused by changes in littoral drift. With the seaweed in position, the sea-bed eroded offshore and build-up of the beach occurred. Measurements were carried out to find a possible explanation for this. Full-scale tests were made at an offshore synthetic seaweed installation at Bourne-mouth, and confirmed the laboratory tests, although some improvements in the anchoring of the synthetic fibre "pony-tails" were made

Several overseas projects are being investigated One of the most important must be the construction of two major dams on the Orange River, South Africa, as part of the large scheme to provide hydro-electric power and water for irrigation The station has continued and extended its tests on models of the Van der Kloof and the Hendrik Verwoerd dams Work on the Van der Kloof dam now includes investigations into erosion of the river bed some 500 ft downstream of the dam face The aim of tests is to find the most satisfactory method of armouring the alluvium in order to prevent exposure of the shale, and to determine the minimum size of armouring material required and, if possible, to demonstrate a practical method of placing the armouring The report also discusses an analysis being made of the benefits the station brings to the economy and quotes examples based on investigations completed during recent years Initial capital savings of approximately £1 25 million have been reported in connexion with construction of three new bridges for motorway and trunk-road crossings of riverflood plains, and a saving of £2 million resulted from a decision not to build a proposed new training wall for the entrance to a port after it was found that it would not produce the improvements required The saving of £3 25 million on these four projects, the report says, has more than paid the whole cost to public funds of setting up and running the laboratory

Receipts for 1966 totalled over £300,000 The cost of running the laboratory was £482,000 inclusive of interest on capital employed and depreciation of buildings and plant.

More Reliable Pesticides

A RECENT report by the World Health Organization (*Specifications for Pesticides*, WHO, £2) continues the work, begun in 1953, of specifying the quality control procedures for assessing the condition of pesticides The present volume includes specifications for all pesticides currently in widespread use in public health programmes

Pesticides are liable to deteriorate during transit and storage, especially in the harsh conditions of the areas where they are most used Not only the active ingredient itself but the physical properties of the pesticide, such as its suspensibility in water, may also be impaired The specifications drawn up by the WHO Expert Committee enable users and manufacturers to test the quality and durability of commercial pesticides

As the report points out, the standards to which a pesticide is produced are a compromise between what the manufacturer can make, what the user wants, and what he is prepared to pay for it It is therefore necessary that both manufacturer and user know for what purpose a pesticide is required, how it is to be used and what abnormal conditions of storage may have to be met

Estimates that up to a third of world food production is destroyed by pests, and mounting evidence of the damage that can be caused by the unselective application of pesticides, are compelling motives for a more precisely controlled use of toxic chemicals The WHO report is a valuable step in this direction

European Biologists

BIOLOGY is a very diverse and all-embracing subject and the task of establishing "equivalences" for university teaching is by no means easy Indeed, the question arises as to whether it is really necessary for higher education to be systematically organized A new report, *How to Qualify as a "Biologist" in the Universities of Europe*, by Jean Leclercq, professor of zoology at the Faculty of Agronomic Sciences, Gembloux, examines this problem The report, which deals with the teaching of zoology and general biology at university level, is one of a series of publications called "Education in Europe" and published by the Council for Cultural Co-operation of the Council of Europe

Of the eight European countries which Professor Leclercq has examined, Britain, he says, has the greatest academic freedom The system in Belgium, on the other hand, is one of the most rigid operating in Europe and a great deal of importance is attached to the exact sciences—physics, chemistry, calculus and so on—in the basic training of biologists Belgian teaching staff in universities and institutions possessing university status do, however, have complete control over the content of the courses The situation in France is quite the reverse—the system seems to be less rigid but French teachers have less real freedom than their Belgian colleagues There exists a Franco-Belgian cultural agreement, the object of which is to encourage future Belgian students with the qualifying diploma in science to proceed to the second-stage course in a French university or, alternatively, to permit French students with a certificate attesting that they have passed the qualifying examination for higher studies (SPCN) to proceed to take their *licence* in a Belgian university In general, however, this equivalence agreement has produced disappointing results

University entrance from the senior forms of secondary schools into universities in the Federal Republic of Germany is a fairly easy process and both the *Diplom* and *Staatsexamen* courses take five to seven years On paper the German student is entirely free to choose the lecture-series which he wants to attend and, subject to certain restrictions, to draw up his own programme of work considerable academic freedom also exists for the teachers But, according to Professor Leclercq, the German system is ill-designed for the establishing of equivalences and needs to be thoroughly remodelled.

In the Scandinavian countries the university curriculum resembles the studies systems in the universities of Germany and Britain and there is considerable academic freedom The selection process for would-be biologists is very stiff and the number of young Finns hoping to go to university is very large The syllabus for the first two years of the bachelor (FK) can be compared with that of the ordinary *licence* course in France, Belgium and other countries

As Professor Leclercq points out in his general conclusions, "biology studies as carried out in the universities of eight European countries have precious few common characteristics" But he feels that the difficulty of defining equivalences should not prevent students from transferring from one system to another

NEWS AND VIEWS

What are X-Ray Stars?

It is only a few years since rocket flights from North America unexpectedly revealed the presence of discrete sources of X-ray emission in the sky, but the consequences have already been quite considerable. The mere handful of recognizable sources has grown to more than thirty, and there is every prospect that the programmes of rocket exploration planned by several groups in the years ahead will identify many more of them. Already, however, it seems very much as if the known X-ray sources differ from each other in important ways. The source referred to by Dr P. J. Edwards on page 43 of this issue has been observed independently by groups from Australia, Britain and the United States, and is distinguished by the rapidity with which its intensity has changed. Between April 1967, when the first observations were made, and the early autumn last year, the intensity of this source had decreased by a factor of sixty. It is good to see that Dr Edwards has been able to put some flesh on the familiar skeleton of the notion that Centaurus *XR*2 is a nova, but there is probably still some chance that events will bear out one or more of the other theories in the field—for example, that X-ray stars are binary systems able to accrete gas or plasma and which may also be self eclipsing. And clearly, as the work of the Berkeley group reported on page 32 makes plain, there is likely to be continuing interest in the years ahead in the general background of X-ray radiation.

Whether the recent work on the X-ray source called Cygnus *X*2 will simplify these issues it is too soon to tell. Some months ago a group at the California Institute of Technology was able tentatively to identify the X-ray source with a visible star (*Astrophysical Journal Letters*, **148**, 129, 1967). Now it has turned out that the visible star is in fact a double system. Writing in the *Astrophysical Journal Letters* (**150**, 95, 1967) Dr E. M. Burbidge and her colleagues have compared the variations of the Doppler displacement of spectral lines at intervals of a few days, and have found that the observed variations are most easily explained by supposing that the stars concerned are moving in closed orbits at about 250 km a second, which implies an orbital period of a day or so for stars which are as massive as the Sun. Dr Allan Sandage and his colleagues at the California Institute of Technology cap this tale in the same journal (page 99) by describing fluctuations of the optical intensity of the same source which are measured on a time scale of only minutes, and they conclude that the double system responsible for their observations is likely to consist of two roughly comparable stars separated by a distance which is likely to be a fraction of an astronomical unit—a mere million miles or so for stars which are comparable in mass with the Sun. Those who know about the stability of stellar structures will probably have plenty to say about this model. It is, of course, too soon to know how widely applicable it can be.

The Truth about Stacking

from our Molecular Biology Correspondent

Perhaps the most interesting turn taken in recent years by polynucleotide chemistry has arisen out of the discovery that single-stranded polymers such as poly A are not simply random coils in neutral solution. The observation of spectroscopic effects, implicitly associated with defined stereochemistry—hypochromicity, large and characteristic Cotton effects in optical rotatory dispersion or circular dichroism—led to the view that the bases were to a large extent stacked on one another, and it was widely speculated that in the limit of full stacking, the chain would possess the conformation of one strand of the double-helix of a fully base-paired nucleic acid. However, a physical basis for stacking interactions was lacking, for no clear hydrodynamic evidence was available to demonstrate the physical existence of fixed steric relationships between bases. In the absence of such data, the interpretation of the optical phenomena amounted only to a rational guess. The deficiency has now been made good by an elegant piece of physical chemistry by Eisenberg and Felsenfeld (*J. Mol. Biol.*, **30**, 17; 1967).

Neutral poly A was used, and a series of fractions of narrow molecular weight distribution were prepared. The work rests on the discovery that conditions of salt concentration and temperature could be found under which the polymer behaved ideally; this is at the Θ, or Flory, temperature, where interactions between polymer molecules, between polymer and solvent and between different segments within one chain vanish, and the physical properties become independent of the concentration of the solution. Only under these circumstances can it be determined whether the chain indeed forms a rigid structure at low temperatures, as the optical effects suggest that it should, and whether the state of the molecule at any given temperature can be seen in terms of a mixture only of stacked and unstacked bases, with no other, or intermediate, geometries.

All criteria for ideality are fulfilled: the conformation, as observed primarily in terms of the radius of gyration from light scattering, but also by viscosity and sedimentation, showed high rigidity at low temperature, as demanded by short-range interactions between the bases. As the temperature is raised, the stacking is progressively disrupted, and the rigid structure collapses at an early stage of this process, again in a predictable manner. The course of the transition is altogether consistent with a stacked form resembling one strand of a double-helix, though no actual geometry can of course be deduced. It is worth noting that these results are inimical to the concept, which has recently been aired, whereby thermal breakdown of stacking entails oscillations of increasing amplitude of adjacent bases relative to each other

Instead they strongly support a simple two-state stacked–unstacked equilibrium.

As to optical methods, and the assessment of stacking from such measurements, Cantor and Tinoco (*Biopolymers*, **5**, 821, 1967) have compared calculated and observed optical rotatory dispersion curves for nine trimers, seven of them trinucleoside diphosphates (no terminal phosphate). It is confirmed that there is good agreement on the basis of sums of pairwise interactions along the chain, and this work supports the validity of analyses of single-stranded polynucleotides in these simple terms. At the same time, Inoue *et al.* (*J. Amer. Chem. Soc.*, **89**, 5701, 1967) have looked at seven trinucleotides—all with the terminal phosphate group—and obtain less satisfactory agreement with calculated curves; it can be deduced that there is a considerable effect from the terminal charge. It is also found that stacking persists in several cases at alkaline *p*H, so long as not more than one of the bases in the trimer becomes ionized.

Making Safe Vaccines

from a Correspondent

At the fourth meeting of the Cell Culture Committee of the Permanent Section of Microbiological Standardization, held at the National Institute for Medical Research (Hampstead Laboratories) in September last year, much attention was given to the measures that can be taken to eliminate hazards encountered in virus vaccine production, in particular the elimination of oncogenic factors in the final vaccine. It was emphasized that more standardization both of media and of cell substrates would do much to achieve this goal.

As far as cell substrates are concerned, the tissue used on the widest scale is grown from monkey kidney. The primary tissue is not tested for the presence of extraneous agents although attempts are made to isolate viruses from control cultures throughout the period of vaccine production. The recent episode in which a number of laboratory personnel were infected by contact with the blood or tissue of a group of African green monkeys tragically underlines the dangers of using primary cultures of uncharacterized tissues. The further possibility cannot be overlooked that an undetected infectious agent may survive in the final vaccine product and become apparent only after injection into man.

Other primary tissues used for vaccine production are derived from chick and duck embryos and canine kidney. These animals are obviously bred in a much cleaner environment than obtains for monkeys in the wild, but the possible presence of extraneous agents nevertheless enjoins caution. Thus although chickens have been bred under controlled conditions which are free from fowl leukosis viruses, a new infectious agent—that of Marek's disease—has recently been isolated and only a few of the commercial flocks are free from both agents. Rabbit kidney and guinea-pig kidney are also now being used on a small scale; the absence of reported isolations of extraneous agents from primary cultures of these tissues may be due to lack of experience with these cultures rather than freedom from infection.

Any new cell substrate for virus vaccine production must be derived from normal tissue and must remain normal throughout its propagation. The cells must be frozen until they have been cleared for use by the necessary exhaustive tests. It is also necessary that such tissues should be suitable for the growth of human viruses in high titre.

Objections have been raised to the use of human tissue on the grounds that an undetected agent (oncogenic virus or "slow" virus) will be more dangerous in a vaccine to be used in man than similar viruses that may occur in animal tissue. It must, however, be pointed out that none of the primate oncogenic viruses (SV_{40} and adenoviruses) gives rise to tumours in the species in which it is indigenous, although such viruses do produce tumours in other animal species (hamsters and the like). Furthermore, "slow" viruses are known to cross species barriers (scrapie in sheep and mice), and animal tissue would therefore not be safer than human tissue in this respect.

The committee was particularly anxious that greater attention should be paid to the use of standardized cell culture media. The use of a powdered medium made in a large batch and tested for suitability before release has done much to minimize changes that can arise as a result of nutritionally deficient or drastically altered medium. Most antibiotics, although preventing the growth of bacteria, have no effect on the growth of some strains of mycoplasma and their use provides no assurance against mycoplasma contamination. Some batches of serum presumed sterile have been found to be contaminated; they may vary widely in growth promoting activity, and changing batches may occasionally cause chromosomal changes in a cell with an unstable chromosomal complement.

For obvious reasons, much attention is being given to the potential oncogenicity in man of new cell substrates. It has been suggested, for example, that a latent viral genome in the tissue may hybridize with the virus used for vaccine production and thereby be activated. In general, animal inoculation tests are insensitive for the detection of oncogenic viruses, and *in vitro* tests are urgently needed. The fusion of candidate cells for virus production with a variety of other cell types may provide a useful tool for the demonstration, or exclusion, of the presence of incomplete virus in such tissues.

The hazards of making virus vaccines stem primarily from the use of ill defined and potentially contaminated substrates. It is encouraging to learn of the increasing activity in the use of a well characterized human cell (*WI*-38) in the preparation of vaccines for clinical trial and to learn also of their use, without incident, in a total of well over a million human subjects.

Muscle Meeting

from a Correspondent

Passers-by in Gower Street just before Christmas, seeing the large notices proclaiming the "Muscle Meeting", might have been forgiven for supposing that this was a congress of weightlifters. It was in fact merely the winter meeting of the British Biophysical Society: this bore the title "Muscular Contraction" and was organized by Dr H. E. Huxley and Professor D. R. Wilkie. There was an unusually full and international programme and the meeting occupied three full days the first structural, the second biochemical and the third mechanical

The first day was cumulatively a trifle dispiriting The 380 Å and 400 Å repeats flashed across the screen for several hours until the mind reeled, and one was left with an overall impression of many speculations chasing few periodicities A member of the audience was heard to remark that it was like trying to discover how a watch worked by listening to the ticking from the outside Nevertheless there were some interesting papers the meeting began with a comprehensive and highly informative survey of the present state of structural knowledge by H E Huxley F A Pepe (University of Pennsylvania) described new work using antibody-staining to clarify the geometry of the M-line region Observations on staining with fluorescent anti-HMM antibodies in the presence and absence of ATP suggested that on contraction a distortion occurs in the trypsin-sensitive region uniting the light and heavy meromyosin entities This is an important result, the notion of such a distortion as a basis for the movement of the cross-bridges in contraction had also been hazarded by Huxley, and is evidently a concept now generally in the air

Interest was also aroused by a provocative paper by G. F Elliott and E Rome (King's College, London), who have observed the separation of the filaments of glycerinated fibres as a function of *p*H and ionic environment The observed variation has been at least qualitatively explained in terms primarily of coulombic interactions between the filaments, treated as charged rods, and the suggestion was made that the process of contraction should be viewed in terms of disturbance of these charge relationships

The second day provided the most consistently interesting part of the meeting, with an admirable paper by S Ebashi and Y Nomura (University of Tokyo) as its centrepiece These workers have made remarkable advances in analysing the relaxing system, and have isolated and characterized the protein troponin, which is found in association with the rod-like tropomyosin, and promotes its aggregation Calcium ions are involved and two categories of calcium-binding sites have been demonstrated Fixation of purified troponin antibodies gives strong evidence of its location in the *i*-filament region About one troponin molecule is present for every turn of the actin helix Ebashi also discussed α-actinin this has three components, a 6*S* protein, an aggregate of the same protein, and a complex with another protein The 6*S* protein has been shown by antibody methods to reside in the Z-band It has a large promoting effect on superprecipitation of actomyosin and gel-formation of F-actin Other components were also considered, and their location, proportions and activity were surveyed

There were interesting contributions also from A and P Dreizen (State University of New York) on the minor protein components of myosin and on active thiol groups in the myosin heads, from A G Szent-Gyorgyi (Brandeis University) on the relation of nucleotide-binding to the actin-myosin interaction, from J Gergely (Retina Foundation, Boston) on stabilization of actin conformation by divalent cations and nucleotides, and from S V Perry and his associates (University of Birmingham), particularly on the molecular weight and structure of actin, which essentially resolves a current controversy in favour of a low value (about 45,000) for the monomer molecular weight

The final day—on energetics and mechanics—yielded, among other things, some interesting observations by A F Huxley (University College, London) on the transient response of muscle to quick stretch or release, which will have to be considered in the formulation of any general theory of the contractile mechanisms, an informative review by R E Davies (University of Pennsylvania) of ATP conversion and a description of some techniques for its study, and a consideration by D R Wilkie (University College, London) of interpretation of heat and energy measurements on living muscle

The meeting as a whole drew a large audience, and the discussions were more than usually lively and informed One was left at the same time with an impression of specialized interests, and a fairly general failure to make a bridge between the three rather disparate parts of the meeting A number of new ideas were, however, aired and the return in stimulus and hard information was well above average

Earthquake in Turkey

THE UNESCO reconnaissance mission to the earthquake zone of Northern Turkey has concluded the first part of its investigations Dr N N Ambraseys of Imperial College, Dr A Zatopek of Charles University, Praha, and Drs M Tasdemiroglu and A Aytun of Imar ve Iskan Bakanlıgı, Ankara, describe their findings

The earthquake of July 22, 1967, in Turkey was felt over an area of at least 400,000 square kilometres It originated on the extension of the Bolu–Abant fault in the Mudurnu River Valley, an active part of the North Anatolian fault zone

Fig 1 shows the general trend of the North Anatolian fault zone and the extent of faulting since 1939 It is of interest to note from the figure the remarkable trend of the successive faulting along a length of more than 1,000 kilometres, consistently right lateral with the downthrown side to the north Table 1 gives a list of the most important earthquakes in the fault zone With the exception of the earthquakes of 1909 which were not documented, and of the shock of October 1944 which had its epicentre at sea, every other earthquake of magnitude equal to or greater than 6·75 has been associated with surface faulting progressing from east to west It can be shown (Ambraseys and Zatopek, *Bull Seismol Soc Amer*, **58**, 47, 1968) that the seismic energy, in ergs, released by these shocks can be expressed in terms of the length of faulting L, in centimetres, by

$$E(\text{ergs}) = 2\times10^{20} L^{1{\cdot}26} \qquad (1)$$

This suggests that the linear dimensions of the focal volume do not increase in the same proportion with increasing energy content, but that the volume of strained upper crust tends to elongate in the direction of faulting with increasing length of the rupture This is more plausible than a suggestion due to Tocher (Tocher, *Bull Seismol Soc Amer*, **48**, 147, 1958) that the volume of strained rock is not thicker for the largest shocks than for the smallest shocks that produce a fault trace

Table 1

No.	Date	Time	Epicentre	M		L	D	T	h
1	1909 Feb. 9	1124	40°·0 N–38°·0	6·75	—	—	—	—	—
2	1912 Aug. 9	0129	40°·7 –27°·3	7·75	—	(10)	—	—	—
3	1935 Jan. 4	1441	40°·2 –27°·5	6·25	—	—	—	—	—
4	1935 Jan. 4	1441	40°·0 –27°·5	6	—	—	—	—	—
5	1937 Jul. 6	1631	39°·5 –26°·0	6	—	—	—	—	—
6	1939 Sep. 22	0036	39°·0 –27°·0	6·5	—	—	—	—	—
7	1939 Nov. 21	0848	39°·9 –39°·7	6	—	—	—	—	—
8	1939 Dec. 26	2357	39°·7 –39°·7	8	RH	350	370	150	20
9	1941 Nov. 12	1005	39°·8 –39°·3	6	—	—	—	—	—
10	1942 Dec. 20	1403	40°·7 –36°·6	7·25	RH	70	100	50	10
11	1943 Jun. 20	1532	40°·8 –30°·4	6·25	—	—	—	—	—
12	1943 Nov. 26	2220	41°·0 –34°·0	7·6	RH	280	110	100	14
13	1944 Feb. 1	0322	41°·0 –33°·0	7·6	RH	180	350	100	27
14	1944 Oct. 6	0234	39°·4 –26°·7	7·25	—	—	—	—	—
15	1946 May 31	0312	39°·3 –41°·2	5·75	—	—	—	—	—
16	1949 Aug. 17	1844	39°·4 –40°·9	6·5	—	—	—	—	—
17	1951 Aug. 13	1833	40°·8 –33°·2	6·8	—	40	—	—	—
18	1952 Mar. 19	0127	40°·0 –28°·8	6·25	—	—	—	—	—
19	1953 Mar. 18	1906	40°·0 –27°·5	7·4	RH	50	430	0	18
20	1953 Sep. 7	0358	41°·2 –32°·8	6·25	—	—	—	—	—
21	1954 Aug. 19	2103	41°·1 –36°·3	6·1	—	—	—	—	—
22	1957 May 26	0633	40°·6 –31°·2	7·1	RH	50	160	50	8
23	1957 May 26	0936	40°·7 –31°·2	6·0	—	—	—	—	—
24	1957 May 27	1101	40°·7 –31°·2	6·25	—	—	—	—	—
25	1959 Oct. 25	1557	39°·1 –41°·6	6·1	—	—	—	—	—
26	1963 Sep. 18	1658	40°·9 –29°·2	6·2	—	—	—	—	—
27	1964 Oct. 6	1431	40°·3 –28°·2	6·5	—	—	—	—	—
28	1966 Aug. 19	1222	39°·2 –41°·6	6·8	RH	30	—	—	—
29	1967 Jul. 22	1656	40°·6 –31°·0	7·1	RH	90	190	180	10
30	1967 Jul. 26	1852	39°·5 –40°·3	6·0	—	—	—	—	—

Number of events shown in Fig. 1. L, Length of faulting (km); M, magnitude; D, relative horizontal displacement (cm); T, throw (cm); h, focal depth; RH, right lateral movement.

The detailed mapping of the fault zone and the study of the damage associated with the earthquake of July 22, 1967, is now being carried out by the UNESCO Mission to Adapazari. Preliminary results show that at least 80 kilometres of new faulting occurred between Güney and Sapanca. Magnitude of displacements was variable, from a few inches to 7 ft., but the sense of lateral movement was always right-handed, and apart from local irregularities, down to the north. About 25 kilometres of surface rupture, between Güney and Dokurcum, lie within the zone of ruptures associated with the earthquake of May 26, 1957; however, the trace follows neither faults with conspicuous geological evidence of recent activity nor fault-breaks associated with the 1957 earthquake. The next 30 kilometres of faulting lie within a zone of recent tectonic movements; they do not follow faults regarded by geologists as active but produce a new trace. The rest of the fault-break is discontinuous and it is not connected with geological features that show very recent activity.

The ground movements in the fault zone were very severe and lasted about 15 sec. The first movement of the ground was horizontal and strong enough to throw down people and burden animals. This movement was followed by one or two very intense vertical shocks, in places sufficiently strong to lift boulders out of the ground without disturbing the rims of their former seats.

In spite of the intensity of the ground movements in the fault zone, there was very little damage to buildings. This is a consequence of the wood frame construction and comparatively light roofs of the local houses and also of the short duration of severe shaking. Adobe and brick structures suffered varying degrees of damage, particularly heavy at some distance from the fault zone. The mission found that it was nearly always wrong to expect the heaviest damage in the close vicinity of the fault-break. The earthquake killed 87 and wounded 235 people, destroying or damaging beyond repair about 4,000 houses, mainly of timber frame construction. Improperly designed or constructed reinforced concrete structures suffered heavy damage as far as Izmit, 70 kilometres from the fault zone.

The mission found it practically impossible to assess intensities in the epicentral area. On purely vibrational criteria the maximum intensity did not exceed IX on the Modified Mercalli scale. On the basis of the scale, however, the fact that "objects were thrown into the air and lines of sight and level were distorted" implies an intensity XII (MM). This shows how imperfect existing Intensity scales are.

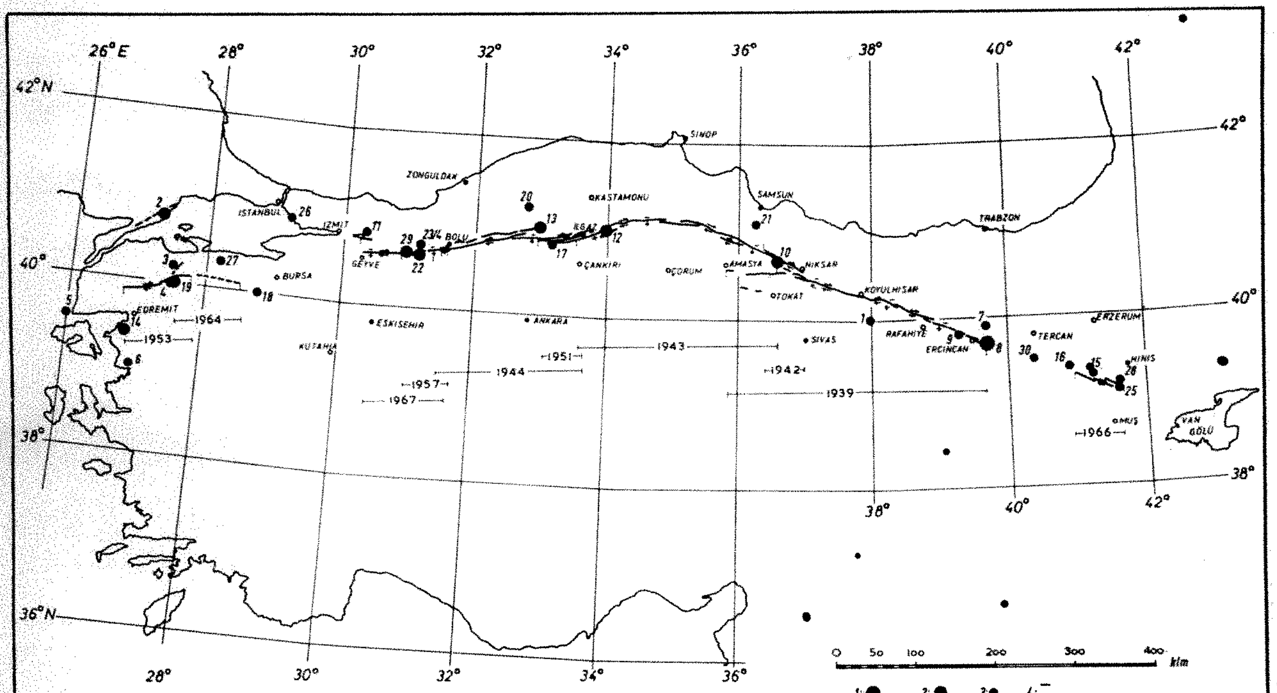


Fig. 1. Anatolian fault system. 1, M≥7; 2, 7>M≥6; 3, 6>M≥5; 4, throw

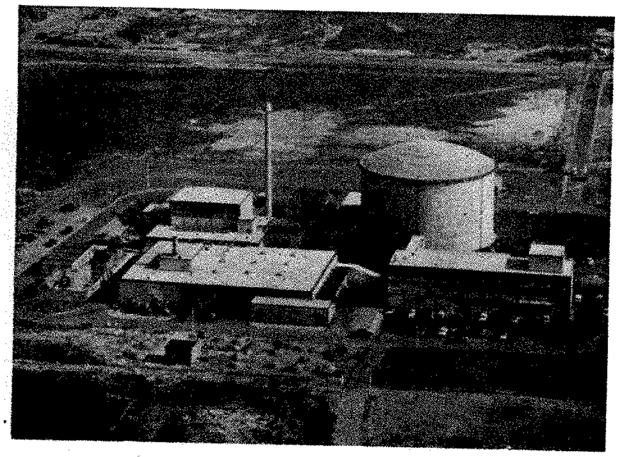
European Nuclear Energy

by
NIGEL HAWKES

Collaboration in Europe is not the rallying-cry it used to be. Organizations set up in the heyday of collaboration are having to adapt to new conditions.

THE European Nuclear Energy Agency is now ten years old, and, like other organizations set up in the fifties, is finding that times have changed. The revival of economic nationalism led by France has meant that organizations which make a virtue of unanimity have found the going hard. Euratom, for example, has been crippled by the reluctance of its members to approve a budget for 1968. The difficulties of ELDO are by no means over. The more flexible organizations have on the whole been more successful in adapting themselves to the new climate, a conclusion which is perhaps no more than a glimpse of the obvious. In these circumstances, the suggestion has been made that Euratom should modify its structure to enable individual countries to opt out of individual contracts without endangering the whole organization. ENEA may well be quietly congratulating itself—its constitution provides exactly this kind of flexibility. This is one of the reasons why ENEA is confident of its future, despite the real doubts about the form it will take.

The most important doubt is about Britain's ability to get into the Common Market. Because this would also mean getting into Euratom—there is no real distinction between the two since the three Common Market commissions were merged into one—it would have a marked effect on the relations between Britain and the ENEA. But until then, there seem unlikely to be any dramatic shifts of policy for the ENEA. There will, however, be a continuing tendency for projects begun by ENEA to be shifted to a world basis under the wing of the International Atomic Energy Agency. In particular, the work on information retrieval and dissemination is already going this way. A plan for a European Distribution Centre for nuclear reports has been shelved for the time being because the IAEA has announced its own plans for a world-wide International Nuclear Information System. In the absence of the European Centre, the ENEA plan is being put into operation through the services of the British National Lending Library for Science and Technology at Boston Spa—if the centre is ever built, it would be intended ultimately to form part of the international system.


The Dragon reactor at Winfrith in Dorset. (UKAEA photograph.)

ENEA EXPENDITURE, 1966–67

	$ million
Headquarters (Paris)	0·5
Ispra and Saclay	0·4
Dragon	6·5
Total so far	71·5
Halden (for the period 1963–69)	9·2
Eurochemic (to meet budget deficits)	5·3

ENEA has been responsible for supporting three important projects; the Dragon high temperature reactor at Winfrith in the United Kingdom, the Halden reactor in Norway, and the Eurochemic fuel re-processing plant at Mol in Belgium. All three are long-standing contracts—Halden came into being in 1958, and Dragon dates from a year later. Both the Halden and Dragon agreements have been extended; Halden comes to an end in 1969, and Dragon is to finish in 1970. In the case of Dragon, however, much will depend on whether Euratom can agree on the budget—at the moment the project is being kept in being by the United Kingdom and the other countries in ENEA who are not members of Euratom. By common agreement, the Dragon project has been a very happy example of collaboration, and has demonstrated the feasibility of the high temperature reactor principle. It has also led to some very optimistic assessments of the economics of fast reactors—in the annual report of the ENEA, just published (OECD, Paris), fuel cycle costs of 0·12–0·13 d per KWh and generating costs of about 0·465 d per KWh are predicted for a 528 MW(e) station. These figures (converted at the new parity of the pound) make high temperature reactors seem an attractive possibility.

The Halden project now finds its principal use as an irradiation facility for testing fuel assemblies. The reactor is of the boiling heavy water type, built originally by the Norwegian Institutt for Atomenergi. Eurochemic, the fuel re-processing plant set up at Mol, came into operation in July 1966. In principle, it is expected to be self-financing, but in the current state of the nuclear fuel business it is unable to make profits. The main difficulty is that there is considerable over-capacity in fuel re-processing, which means that nobody is making much money. As nuclear power expands in Europe, the position could improve, but there seems to be a danger that Eurochemic will be left only the work which member countries are unable to do themselves. The annual report, for example, gives information about a contract lasting ten years to re-process the fuels from the VAK reactor at Kahl and the KRB reactor at Gundremmingen in West Germany. But the contract only covers the amounts of fuel which cannot be handled by a German re-processing plant being built at Karlsruhe. Eurochemic will have to console itself with the thought that if member countries do not use its facilities fully, they will find themselves paying for its deficits. Already the OECD council has agreed that up to $5.3 million should be made available for 1968 and 1969 to cover expenses not covered by receipts.

ENEA also supports a variety of smaller projects. The computer programme library at Ispra in Italy, originally set up for a three year period, is now to continue indefinitely. The library receives computer programmes from member countries, checks them, and then sends them out to interested organizations. Because the checking process normally costs about $1,000 for each programme—and every programme needs correction—the library is a way of avoiding costly duplication in the member countries. Another centre, the Neutron Data Compilation Centre at Saclay, contains a library of neutron data, and the Seibersdorf Project, near Vienna, is a way of gaining experience of the possibilities of food preservation by irradiation. The agency has also been involved in the problems of the disposal of radioactive waste. The latest annual report of the agency gives details of the first dumping of radioactive waste it has organized. Five countries—Britain, France, West Germany, Belgium and the Netherlands—agreed to take part in the experimental programme, and 11,000 tons of packaged waste was collected together, securely packaged and dumped at a depth of 16,400 ft in the eastern Atlantic.

In addition to this, the agency organizes a number of joint committees, covering such subjects as reactor safety, reactor physics, magneto-hydrodynamic power generation and nuclear data. Increasingly, it seems that the agency is moving away from large projects, principally because these are no longer wanted by member countries. Since nuclear power became a commercial activity, collaboration has seemed less and less attractive. But there is now a suggestion in the air for a large scale project in the fast reactor field, and a meeting of ENEA's top scientific committee, with representatives from all member countries, will be discussing the proposal next month. If enough of the countries are interested, the proposal could become a reality, but in the present climate this is unlikely. The United Kingdom, which has already been responsible for shelving one ENEA proposal, for a ultra-high flux research reactor, is unlikely to feel that this is the time for undertaking new expenditure abroad, particularly in a field in which the UKAEA has already made substantial investment at home. The UK would have little to gain and a great deal to give away in a project of this sort. And Euratom is too busy deciding (more accurately, failing to decide) its own research programme to take any interest. It rather looks as if ENEA will have to content itself with more modest projects for the next few years.

Is the Special Theory Right or Wrong?

In *Nature* for October 14, 1967, Professor H. Dingle argued that the special theory of relativity is incorrect, and Professor W. H. McCrea put an opposing view. Some correspondence on the same subject has already appeared. What follows is a further selection from the correspondence on this subject.

Experimental Verifications of the Theory of Relativity

As a postscript to the articles by Professor H. Dingle[1] and by Professor W. H. McCrea[2], we should like to draw attention to two recent experiments which bear directly on items which Dingle has felt obliged to doubt, but which have been mentioned in "News and Views"[3]—the relativity of time as exemplified, in particular, by the "clock paradox" (ref. 4) and the second postulate of the theory of relativity, which asserts that the velocity of light is independent of the motion of the source[5]. The apparent slowing down of moving clocks, long ago verified for straight-line motion[6], has now been established for a circular trajectory as well, a time-dilation factor of 12 being verified to within 2 per cent. In another experiment, bursts of γ-rays emitted from fast moving π^0 mesons have been timed as they cross the laboratory, the velocity obtained is equal to the standard value for the velocity of light c to within 1.3 parts in 10^4 even though the source velocity is itself very close to c.

The result on time-dilation is obtained as a by-product of another investigation. In the muon storage ring[7] at CERN, muons of momentum 1.274 GeV/c are injected into a circular orbit in an almost uniform magnetic field. The design includes vertical and horizontal magnetic focusing to prevent loss of particles. The circulating muons can be detected by means of the decay, $\mu^- \rightarrow e^- + \nu_\mu + \nu_e$ for the electron produced, which has less momentum than the parent μ^-, is bent more sharply in the magnetic field and emerges towards the centre of the ring. Some of these electrons are detected by counters, the number observed at any time being proportional to the number of muons left in the ring at this time. The lifetime of muons at rest is $\tau_0 = (2.200 \pm 0.0015)$ μsec (ref. 8), and special relativity then predicts for the muon in flight, the dilated lifetime $\tau = \tau_0 \ (1 - v^2/c^2)^{-1/2} = 26.72$ μsec. Here it is assumed that no special correction is necessary when the orbit is deflected by a magnetic field, and that the decay rate of the particle in its rest system is not altered by the acceleration experienced. The observations[7] (see Fig. 1) show an exponential decay with lifetime 26.15 ± 0.03 μsec, agreeing with the theory to about 2 per cent. (The marginally shorter lifetime is ascribed at present to a slow loss of muons, perhaps as a result of imperfections in the magnetic field.) This result removes any lingering suspicion that some unsus-

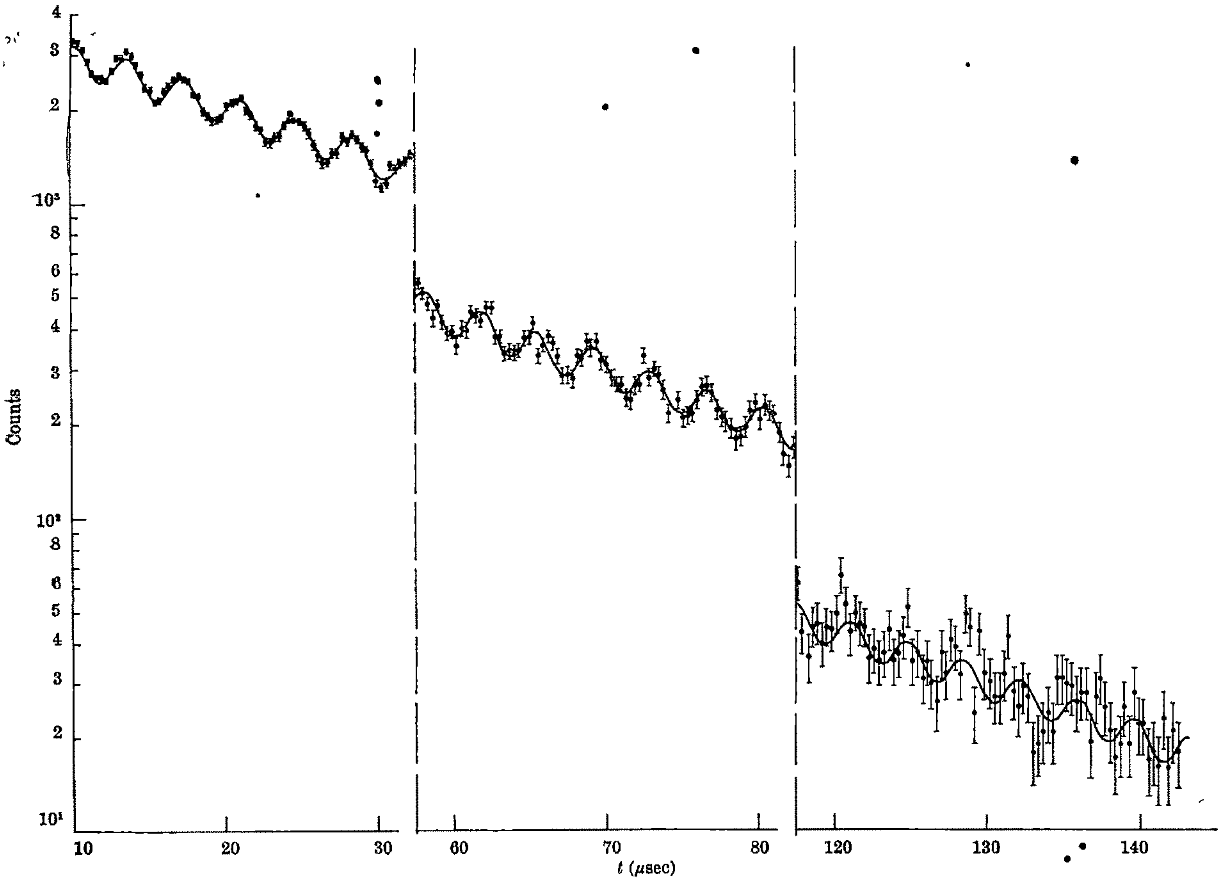


Fig. 1. Decay electron counting rate from stored muons (log-scale) plotted against time. Fitted lifetime $\tau = 26.15 \pm 0.03$ μsec. The small oscillations are due to the precession of the muon spin (see ref. 4).

pected effect associated with acceleration would compensate the time-dilation in a circular or out-and-return journey, the "clock-paradox" is established as an experimental fact associated with the time-keeping of radioactive material in direct comparison with standard laboratory scales of time.

The possible influence of source velocity on the velocity of propagation of γ-rays has also been studied at CERN. When 19 GeV protons hit a beryllium target in the proton synchrotron, the resulting forward-emitted γ-rays have a sharp pulse structure in time as a result of the bunching of the protons in the accelerator by the applied radio frequency. By measuring the time of arrival of the γ-ray bunches ($E_{\gamma} > 6$ GeV) at two points along the beam, and comparing in each case with the radio-frequency phase, the velocity is obtained as a simple product of a laboratory distance (31.4503 metres) and the radio frequency applied to the synchrotron (9.53220 MHz). Including small corrections, the result is $(2.9979 \pm 0.0004) \times 10^{10}$ cm/sec^{-1} (ref. 9), agreeing with the standard velocity of light c to 1.3 parts in 10^4.

In such a high-energy collision, the velocity of the centre of mass is a substantial fraction of c, so it can hardly be doubted that the γ-rays come from a moving source. That the γ-ray spectrum observed in such collisions is dominated by the production of π^0 mesons[10] (followed by $\pi^0 \rightarrow 2\gamma$ giving in the π^0 rest frame two γ-rays of 70 MeV) was established first for cosmic ray events[11] and later in the laboratory[12]. Both the γ-ray intensity and the spectral shape are explained by this hypothesis, admittedly with the use of the relativistic formula for the Doppler shift. In the present experiment, γ-rays with $E_{\gamma} > 6$ GeV are selected, and the corresponding π^0 must have (according to relativity) $V_{\pi^0} > 0.99975\ c$. It is concluded that the second postulate is verified accurately even for very high source velocities.

The Michelson–Morley experiment has shown that the velocity of light is not changed by the motion of source and detector together. Combining with the above result, one may conclude that neither the motion of the source nor the motion of the detector influences the velocity.

The earlier double star observations and other experiments are discussed in ref. 6, including difficulties in interpretation raised by the extinction theorem.

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[1] Dingle, H., *Nature*, **216**, 119 (1967).

[2] McCrea, W. H., *Nature*, **216**, 122 (1967).

[3] *Nature*, **216**, 113 (1967).

[4] See, for example, Dingle, H., *Proc. Phys. Soc.* (London), A, **69**, 925 (1956); *Nature*, **180**, 1275 (1957).

[5] Dingle, H., *Nature*, **183**, 1761 (1959); *Mon. Not. Roy. Astro. Soc.*, **119**, 67 (1959).

[6] Rossi, B., Hilberry, N., and Hoag, J., *Phys. Rev.*, **57**, 461 (1940). Rasetti, F., *Phys. Rev.*, **60**, 198 (1941).

[7] Farley, F. J. M., Bailey, J., Brown, R. C. A., Giesch, M., Jostlein, H., van der Meer, S., Picasso, E., and Tannenbaum, M., *Nuovo Cimento*, **44**, 281 (1966). Bailey, J., Bartl, W., Brown, R. C. A., Farley, F. J. M., Jostlein, H., van der Meer, S., and Picasso, E., *Proc. Intern. Symp. on Electron and Photon Interactions at High Energies, Stanford* (1967).

[8] Feinberg, G., and Lederman, L. M., *Ann. Rev. Nucl. Sci.*, **13**, 466 (1963).

[9] Alväger, T., Farley, F. J. M., Kjellman, J., and Wallin, I., *Phys. Lett.*, **12**, 260 (1964). Alväger, T., Bailey, J. M., Farley, F. J. M., Kjellman, J., and Wallin, I., *Arkiv Fysik*, **31**, 145 (1966).

[10] Bjorklund, R., Crandall, W. E., Moyer, B. J., and York, H. F., *Phys. Rev.*, **77**, 213 (1950). Steinberger, J., Panofsky, W. K. H., and Steller, J., *Phys. Rev.*, **78**, 802 (1950).

[11] Carlson, A. G., Hooper, J. E., and King, D. T., *Phil. Mag.*, **41**, 701 (1950).

[12] Fidecaro, M., Finocchiaro, G., Gatti, G., Giacomelli, G., Middlekoop, W. C., and Yamagata, T., *Nuovo Cimento*, **24**, 73 (1962).

The Error in the Special Theory of Relativity

IN his recent article, McCrea[1] explains the apparent contradiction in the results obtained by Dingle[2] from the special theory of relativity by pointing out that Einstein used two symbols to represent four different quantities. They are defined by Einstein quite carefully and distinguished by qualifying phrases which do not, however, appear in the mathematical treatment. When I discussed this ambiguity some years ago[3,4], I suggested that if Einstein had used four separate symbols he would have avoided the mistake which he made later in his paper. This occurs in the round-trip "thought experiment" in which one of the essential qualifying phrases is omitted even in his descriptive account, thus leading to an incorrect result[5,6]. A brief reference is made to this "experiment" in a comment in *Nature*[7] in which it is stated that the symmetry of the clocks is not preserved. Now this would be true in practice, but Einstein made no mention of any effects caused by acceleration nor did he make any allowance for them. If the "experiment" is carried out correctly it should therefore give the same readings for the clocks as they would have when they are in uniform relative motion. The result given by Einstein does not follow from the "experiment" but from an assumption made implicitly that the clock which does the round trip is actually going slower than the one regarded as stationary and does not simply appear to go slower as viewed by the stationary observer.

This assumption may well be correct and is certainly reasonable. The important point to appreciate is that an additional assumption has been made. The confusion has arisen because of the belief held by Einstein and most writers on the subject that the result of the round-trip "experiment" follows from the initial postulates.

Einstein stressed the tentative nature of his theory and the need for experimental checks. Contrary to popular belief, there is no evidence concerning the special theory as propounded, because no experiment has been made in a force-free space. In practice, gravitational forces or accelerations are always involved[8]. There is now a certain amount of evidence that frequency shifts occur when atomic clocks are at different gravitational potentials or are subjected to rotational acceleration[9,10].

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[1] McCrea, W. H., *Nature*, 216, 122 (1967).

[2] Dingle, H., *Nature*, 216, 119 (1967).

[3] Essen, L., *Proc. Roy. Soc.*, A, 270, 312 (1962).

[4] Essen, L., *Nature*, 180, 1061 (1957).

[5] Essen, L., in *Air, Space and Instruments* (edit. by Lees, S.) (McGraw-Hill, 1963).

[6] Essen, L., *J. Inst. Elec. Eng.*, 9, 166, 389 (1963).

[7] *Nature*, 216, 113 (1967).

[8] Essen, L., *Nature*, 199, 684 (1963).

[9] Essen, L., *Nature*, 202, 787 (1964).

[10] Essen, L., *Proc. Phys. Soc.*, 86, 671 (1965).

The Case against the Special Theory of Relativity

PROFESSOR MCCREA's reply[1] to my disproof of special relativity[2] is both gratifying and disappointing. It is good that, at long last, some comment has appeared, regrettable that this one contains nothing to the point.

One simple thing only is needed to refute the disproof, and it is essential—to show an error in the derivation of my equation (4) that does not invalidate equation (3). This I showed with unmistakable clearness. McCrea's only contribution to it is the following: "Dingle's (3) is meaningless. Correspondingly his (4) is meaningless." This, if true (it is not), would merely kill the theory in another way, for (3) is Einstein's deduction and that of all his followers until now.

Because this conclusively nullifies McCrea's rejoinder, I should leave the matter here, with a final appeal to him now to agree frankly that the theory is untenable, but for the fact that the overlooking of the irrelevant bulk of his statement would, in the prevailing state of thought, be misinterpreted. *Nature's* prediction[3] that "The chances are that most people will be persuaded by what [McCrea] has to say" would only too probably be verified. It is the general view that relativity is beyond the understanding of most, but must be accepted because some mathematicians, who alone understand it, have endorsed it: criticism of it, being on this view merely a sign of incomprehension, can therefore be ignored if a sufficiently imposing mathematical dismissal, intelligible or not, is forthcoming. Euler faced the non-mathematical sceptic, Diderot, with the challenge "Sir, $a+b^n/n=x$, hence God exists, reply!" Diderot did not reply and Euler's case prevailed. McCrea's statement has the same relevance and cogency as Euler's, and if met with silence would produce the same general conviction. Reluctantly, therefore, I dissect it.

First, all the didactic, as distinct from the polemical, part which expounds the mathematics of the theory is superfluous; I do not question it. I distinguished clearly between (*a*) the mathematics and (*b*) the identification of the mathematical symbols with observable quantities. I have enough mathematical insight to see that it is a waste of time to look for mathematical flaws in the theory. Hence McCrea's argument (i), which he says "alone is sufficient to refute Dingle's contention", does not touch that contention. Of course, "equations (III) and (IV) [my (1) and (2)] concern two distinct sets of events, and so they cannot contradict each other". But what McCrea has to show, and has not shown, is why the physical result (3), deduced from one set, can be held true, while the physical result (4), similarly deduced from the other (non-contradictory) set, must be held false.

Not only do I agree that my equations (1) and (2) are mathematically free from contradiction, I agree also that it is perfectly possible (though, of course, not necessary) that if the experiment were made the clocks described would give readings conforming to (1) and (2) (in which case, as far as can be seen at present, we should have to accept Lorentz's theory). But what is impossible is that, in that case, the settings of *H* and *N* in relation to *A* and *B*, respectively, which, according to Einstein's definition, synchronize the pairs, *A*,*H* and *B*,*N*, would be such as to enable us to infer both his reading of *A* for an event occurring on *H* (thus yielding (3)) and a similarly determined reading of *B* for an event occurring on *N* (thus yielding (4)). This I clearly stated.

McCrea's comments on this essential point of synchronization, which alone enables us to compare the readings of separated clocks, are revealing. If Einstein's comparison were, as he says, merely one of "two different ways of putting the readings of A,B into 1–1 correspondence with each other", the whole theory would be an idle mathematical fancy, and a space traveller would return either older or younger than his twin brother according to our capricious choice of correspondence This, which I also clearly pointed out, McCrea ignores We have here another equivoque of the kind which I exemplified by the oscillating interpretation of the "FitzGerald contraction" Either special relativity says nothing physical, or its (physical) statements are contradictory To establish his choice regarding asymmetrical ageing, McCrea supposes that it does speak[4], and selects arbitrarily only one of its contradictory utterances When asked to face the other he shifts his ground and denies that the theory has said anything at all The recognition and abjuration of this specious ambivalence are long overdue

But the passage which shows the deepest misconception is this "About the first thing that relativity theory does is to deny any operational meaning to the notion of simultaneity at two different places Naturally, this fundamental feature in the theory is not affected in the slightest by any arbitrary conventions we may adopt for the synchronization of clocks" What Einstein pointed out (and this shows his great insight into the matter) was that there was no natural meaning of such simultaneity, one could freely be given "by definition" This released him from the previously assumed necessity to assign distant times in conformity with Galilean kinematics and set him free to base a theory on whatever operational definition he chose—the theory then, of course, being riveted to that definition and subject to test by observation But when the observation which it requires is seen to be that one clock goes both faster and slower than another, you cannot plead that that is only because of the arbitrary definition, which does not affect the theory in the slightest The theory is based on the definition, and if you want to regain your freedom to choose another, you must first repudiate the theory and then start again from scratch McCrea's assertion is equivalent to saying that one's freedom to marry any woman who will consent is not affected in the slightest by the fact that one has already married

Here is Einstein's own account of the matter[5] "In order to give physical significance to the concept of time, processes of some kind are required which enable relations to be established between different places It is immaterial what kind of processes one chooses for such a definition of time It is advantageous, however, for the theory to choose only those processes concerning which we know something certain" How it could be advantageous for the theory to be so particular about a process that does not affect it in the slighest is incomprehensible

McCrea's remaining arguments are based on an indefensible misreading of my phrase, "A must be held to read t_1 at E_1" He takes this to imply that I imagined A to be at the place of the event E_1, which no sane person could do "A is not 'at' E_1 in any sense admitted by the theory and it simply has no meaning whatever within the theory to speak of what A must be held to do at E_1", he writes, and he proceeds to draw a space-time diagram that "makes it perfectly clear that α [the world-line of A] does not go through E_1, and so there cannot possibly be a reading of A 'at' E_1" I myself had defined A as a clock permanently distant from H (on which E_1 occurs) and had so shown it in each of four drawings I cannot believe that anyone, reading the phrase in its context, could fail to see that "A must be held to read t_1 at E_1" meant that the reading of A, which, according to the theory, must be associated with the event E_1, is t_1 My definition of E_1 as the coincidence of H and B was accompanied by one of the drawings showing A unmistakably at a distance from that event, and, similarly, another drawing showed B at a distance from E_2 That McCrea should have found it necessary to draw a complex of world-lines which obscures for the non-specialist what my simple figures had already made clear to all can be explained only in Eulerian terms That is not the way to get at the truth, and I am not Diderot

The letters of Dr Barrett[6] and Mr Fullerton[7] reveal elementary misconceptions, and Barrett writes "for A, these two clocks are not synchronized" Synchronized clocks are not synchronized for A or for B, they are simply synchronized The process is experimental if the readings satisfy the condition, $t_3 = t_2 - t_1$, given in my article, the clocks are synchronized absolutely Barrett confuses clock-readings with the times at which the clocks have those readings the same reading is collected, fast or slow, according to the co-ordinates system chosen, but the reading is absolute Clock-readings alone are involved in my disproof of special relativity That is why Einstein rightly concluded that his theory required one clock to work absolutely more slowly than the other Fullerton refers to an observer who "synchronizes A, B, H to zero but not N" Clocks are not synchronized to one reading, synchronized clocks give a common reading in every event B cannot be synchronized with A and H, for it moves with respect to them If Fullerton means only that these clocks read zero at the event E_0, then so does N, but the fact is trivial

I now sum up the situation by stating again what must be done to avoid my conclusion Either my equations (3) and (4) are contradictory or they are not If they are, at least one must be wrong, and if Einstein's (3) is right, then a false step must exist in the deduction of (4) from the commonly agreed (1) and (2) which has no repercussions on the deduction of (3) this false step must be pinpointed If, on the other hand, (3) and (4) are not contradictory, then it must be explained why Einstein's deductions from (3)—for example, that an equatorial clock goes slower than a polar one—are true, while the similar but opposite deductions from (4)—for example, that an equatorial clock goes faster than a polar one—are not equally true In each case, therefore, either the necessary physical implications of (3) must be vindicated and those of (4) discredited, or the theory fails No solution which makes the equations equivalent, whether meaningful or meaningless, has any bearing on the matter

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Received October 18, 1967

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[1] McCrea, W H, *Nature*, **216**, 122 (1967)
[2] Dingle, H, *Nature*, **216**, 119 (1967)
[3] *Nature*, **216**, 113 (1967)
[4] McCrea, W H, *Nature*, **179**, 909 (1957)
[5] Einstein, A, *The Meaning of Relativity*, fourth ed, 27 (Methuen, 1950)
[6] Barrett, W, *Nature*, **216**, 524 (1967)
[7] Fullerton, J H, *Nature*, **216**, 524 (1967)

Special Theory of Relativity

I HAD supposed that the misunderstanding about the "preferred clock", mentioned by Professor Dingle on page 121 of your issue of October 14, arose from attempts to apply the equations of special relativity in a frame which was not "inertial" This seems consistent with what is said in your editorial comment on page 113 about the application of external forces, but it does not seem to explain the statement attributed to Einstein on page 121 if that statement is to be considered in the context of the special theory

The velocity of a point on the equator relative to the pole can presumably be taken as zero in the frame of an observer on Earth What preference is to be given or denied to that frame ?

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The Jodrell Bank Mark III Radio Telescope

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A radio telescope which can be dismantled and reassembled at a new site within 6 months is now being used for interferometer measurements in conjunction with fixed instruments at Jodrell Bank.

SURVEYS have been published[1-4] of the angular sizes of approximately 400 discrete radio sources. Many of these measurements have been made at Jodrell Bank by using the Mark I (ref. 5) or Mark II (ref. 6) telescope linked with smaller telescopes at distant sites to form instruments which are the radio analogues of Michelson's stellar interferometer. The early surveys made it clear that this programme should be extended to fainter sources. This could be achieved with a larger telescope at a remote site. It was also clear that if that telescope were fully steerable, a tracking interferometer[7] would be available to obtain information down to a few seconds of arc about the angular structures of more than a thousand radio sources.

In 1963, a formal request was made to the Department of Scientific and Industrial Research for a grant towards the design and construction of a Mark III radio telescope, of the same size as the Mark II (125 feet by 83 feet 4 inches) and fully steerable. The telescope is intended primarily to be used with the Mark I instrument for interferometric observations at wavelengths down to 21 cm. Because it is not known which separation will be most significant for these measurements of weak sources, the telescope was also designed to be transportable. This implies that the time taken to dismantle it and re-erect it elsewhere should be only about 6 months.

The Mark III telescope was designed by Husband and Co., consulting engineers, of Sheffield and London, to be suitable for automatic operation by remote control by means of radio links and to have a good radio performance at wavelengths of 21 cm and longer. The telescope has been constructed by Fairey Engineering as main contractors with a number of sub-contractors. After some delays in completion and commissioning, it finally came into full service as an interferometer in July 1967 and has been working almost continuously since that time. Fig. 1 is a photograph of the instrument seen from the south-west at its first site, near Nantwich. The focal laboratory is supported on a quadrupod of steel girders. It consists of a temperature controlled enclosure approximately 8 foot cube which is generally similar to that on the Mark II telescope. Access is by a stairway up one of the girders which can be reached from the ground when the bowl is directed to the horizon. The reflecting surface consists of a square mesh of galvanized wires 1 inch apart, which has the correct parabolic shape within a tolerance of 0·5 inch. The radio performance of the reflector has been tested at wavelengths of 21, 18 and 11 cm. It gives approximately 81 per cent of its theoretical performance at 21 cm when directed to the zenith, but the performance at 11 cm is 45 per cent at the zenith. As the telescope is directed to lower elevations, the structure deforms under gravity by not more than 1 inch r.m.s., and at a wavelength of 11 cm the reflector efficiency falls to 14 per cent, when the telescope is directed at the horizon. Thus, the telescope performs satisfactorily at the lower specified wavelength of 21 cm and has a useful performance, particularly at high elevations, down to 11 cm.

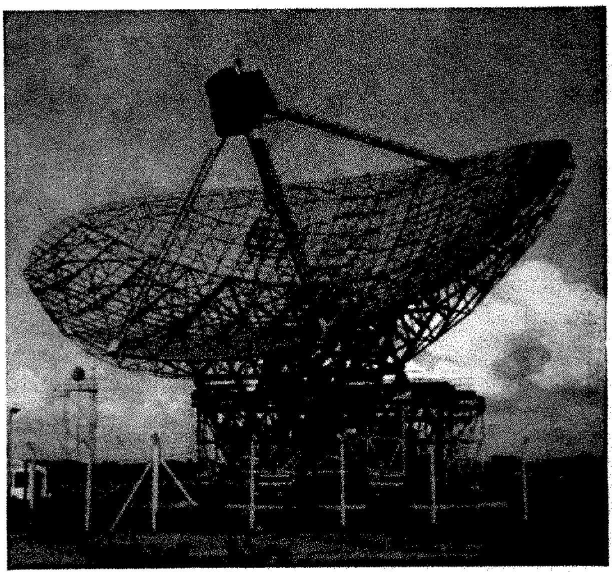

Fig. 1. The Mark III radio telescope at its first site 24 kilometres south-west of Jodrell Bank. The hut containing the receiving and control equipment, and the tower carrying the radio link aerials, can be seen to the left of the telescope.

At an early stage it was decided to use hydraulic drive systems for both motions. The drive in azimuth on the 60 foot diameter rail track is provided at two of the six bogies, while the motion in elevation is controlled by two hydraulic pistons each approximately 16 feet long. A 5 horse-power electric motor drives separate hydraulic pumps for the elevation and the azimuth systems. The telescope can be driven in each motion at speeds of 5° per minute, or at one of three pre-selected lower speeds. These motions can be controlled locally or, as is now more usual, from Jodrell Bank through the UHF and microwave radio links. (Details will be described elsewhere by B. Rowson.) The attitude of the radio telescope is indicated by magslips, and their dials can be viewed by a television system on the microwave link. In addition, this attitude is continuously communicated to Jodrell Bank by signals sent over the microwave link, which drive magslip repeater dials on the remote control console. During remote operation of the telescope, the site can be viewed by television camera and the

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control dials can also be inspected at will. When the Mark III telescope is used in conjunction with the Mark I, it can be arranged that the magslip signals from the two telescopes are compared and differences can be used to actuate the hydraulic motors of the Mark III. The two telescopes then move in step to an accuracy of 2 or 3 minutes of arc, and the Mark III moves at an appropriate low speed for approximately 10 seconds in every half-minute.

The hydraulic rams and motors are protected by a comprehensive system of over-pressure alarms, cut-outs and relief valves. During operation, two alarm systems are used to detect any dangerous malfunction of the control or driving of the telescope. The first sounds warning tones at the telescope, while the second sounds tones and flashes lights at Jodrell Bank. These alarms operate if the telescope approaches the end of its permitted travel in any direction, if unexpectedly high hydraulic pressures are generated in either the elevation or the azimuth system or in the event of unexpectedly high wind speeds at the remote site. At the present stage of the programme, it is normal practice for the scientist operating the telescope from Jodrell Bank to use a television camera to observe the control panel and the telescope itself periodically. In due course, it is possible that some of these control and monitoring functions will be taken over by the Argus 400 computer at Jodrell Bank.

The first site selected for the Mark III telescope gives a baseline to the Mark I of 24 kilometres length in azimuth 225°. The effective resolving power of this baseline and the position angle in which it is obtained change as the source being observed moves across the sky. These effects are shown in Fig. 2 for sources at declinations 0°, +20°, +40° and +60°. The resolving power at any instant may be represented by a vector drawn from the origin to the point on the appropriate curve at the hour angle of observation. It will be seen that for circumpolar sources all position angles can be obtained, but that for sources south of the celestial equator the range of resolving power and position angle is comparatively small. This diagram is applicable at any wavelength, but the scales on the x and y axes will vary with the wavelength of observation. The range of source sizes which can be investigated in the various frequency bands allocated for radio astronomy is shown in Table 1. Column 1 gives the observing frequency and column 2 the separation of the telescopes in wavelengths at that frequency. In column 3 are listed the angular sizes of radio sources having gaussian distributions of surface brightness which would give fringe visibilities $\gamma = 0{\cdot}8$ and $\gamma = 0{\cdot}2$. It will be seen that at this baseline we shall be able to concentrate on sources smaller than 20 seconds of arc and at the shorter wavelengths go down to sources smaller than 1 second of arc.

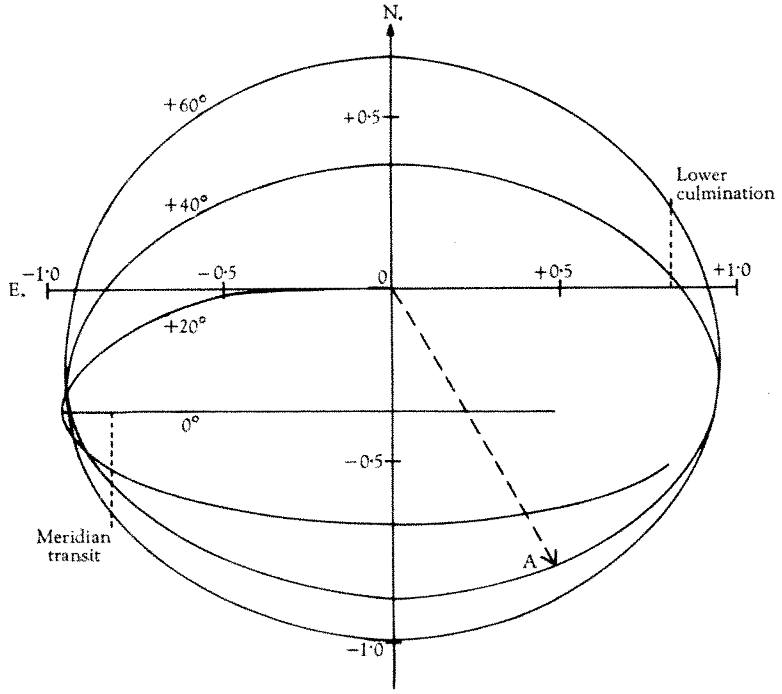

Fig. 2. These curves show the variation with hour angle of the effective resolving power and its position angle for the Mark I–Mark III baseline. Curves are shown for sources at declinations 0°, +20°, +40° and +60° for the hours when they are above the horizon. The resolving power obtainable at any instant may be represented by a vector from the origin to a point on the appropriate curve. An example, OA, is the vector for a source at hour angle 06^h, dec +40°.

Table 1. RANGE OF THE ANGULAR SIZES OF RADIO SOURCES WHICH CAN BE MEASURED WITH THE MARK I–MARK III INTERFEROMETER AT A NUMBER OF FREQUENCIES

Frequency (MHz)	Maximum baseline (No. of wavelengths λ)	Size of source (sec of arc) giving fringe visibility γ	
		$\gamma = 0{\cdot}8$	$\gamma = 0{\cdot}2$
38	3,000	17″	40″
151	12,000	4·25″	9″
408	32,000	1·5″	4″
1420	112,000	0·45″	1·5″
2695	213,000	0·23″	1″

In addition to the radio tests mentioned earlier, interferometric observations have now been completed during observations of flare stars and of a few interesting strong radio sources at 38 MHz. Brief initial programmes at 408 MHz and 151 MHz have also been carried out and some of these first results were described recently[8].

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[1] Allen, L. R., Anderson, B., Conway, R. G., Palmer, H. P., Reddish, V. C., and Rowson, B., *Mon. Not. Roy. Astro. Soc.*, **124**, 477 (1962).
[2] Maltby, P., and Moffet, A. T., *Ap. J. Supplement*, **7**, 141 (1962).
[3] Fomalont, E. B., *Observations of the Owens Valley Radio Observatory*, No. 7 (1967).
[4] Clark, B. G., and Hogg, D. E., *Ap. J.*, **145**, 21 (1966).
[5] Lovell, A. C. B., *Nature*, **180**, 60 (1957).
[6] Lovell, A. C. B., *Nature*, **203**, 11 (1964).
[7] Rowson, B., *Mon. Not. Roy. Astro. Soc.*, **125**, 177 (1963).
[8] Miley, G. K., Rickett, B. J., and Gent, H., *Nature*, **216**, 974 (1967).

Kinetics of Association of Carbon Monoxide with Haemoglobin at Low Temperature

by

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Carbon dioxide binds to haemoglobin at −55° C, in methanol and water, in a manner basically similar to binding in ordinary conditions.

FOR a long time the combination of haemoglobin with gases has been studied chiefly by measuring dissociation curves. Such equilibrium data have led to the discovery of the haem–haem interaction, and until some years ago were the only basis of its quantitative assessment. Since the development of flow methods for the measurement of rapid kinetics and particularly that based on flash photolysis of HbCO, attempts have been made to describe the phenomenon in terms of kinetic parameters.

The kinetic and thermodynamic parameters of this reaction may not always be related in a simple manner, but some well defined kinetic expressions seem to apply to the haem–haem interaction. Particularly instructive is the comparison of the combination velocity constant obtained by rapid mixing with that observed after photolysis. The combination of haemoglobin and carbon monoxide when the two components are mixed follows a time-course mostly described by a second order rate constant (l') of about $1{\cdot}2\times10^{5}$ M^{-1} sec^{-1} at 20° (ref 1). When observed after flash photolysis, particularly after very limited photodissociation (to about 5 per cent), the combination rate is much faster[2]. Gibson originally assimilated these rapid kinetics with l'_4, the fourth combination constant in the Adair model; more recent data[3] in concentrated salt solutions (on $\alpha\beta$ sub-units) might justify a revision of nomenclature, but the basic concept relating this difference (between l'_4 and l') to the haem–haem interaction seems to be well founded.

When the light energy used for photolysis is sufficient to induce total photodissociation, the combination reaction is biphasic, an initial rapid phase (with a rate constant about twenty times as great as l') is followed by a slower phase with a rate essentially equal to that obtained by mixing (l').

The situation on and after flash photolysis of HbCO in the presence of excess carbon monoxide has been described by Gibson[4] as

$$\mathrm{HbCO}\xrightarrow{h\nu}\mathrm{Hb}^{*}+\mathrm{Hb}+\mathrm{CO}$$
$$\mathrm{Hb}^{*}+\mathrm{CO}\xrightarrow{l^{*}}\mathrm{HbCO}$$
$$\mathrm{Hb}+\mathrm{CO}\xrightarrow{l'}\mathrm{HbCO}$$
$$\mathrm{Hb}^{*}\xrightarrow{k_1}\mathrm{Hb}$$

He called the species Hb* a quick reacting form of haemoglobin which, while combining with carbon monoxide at a much faster rate (l^{*}) than does ordinary haemoglobin (l'), is itself unstable, and reverts quite rapidly to the ordinary form. The experimental reaction rates obtained by Gibson were complex, but fitted with this scheme when chosen numerical values were assigned to l^{*}, l' and k_1. He also reported activation energies for each of these reactions.

Whatever the underlying phenomena, the overall rate $\left(\frac{d\,\mathrm{HbCO}}{dt}\right)$ was dictated by a number of concurrent reactions, some of them quite fast at ordinary temperatures. We think that measurements made at widely different temperatures might in in such cases make resolution easier, for the rates of different reactions would vary by amounts determined by their respective activation energies. It is usual to operate down to 0° C, but regions below this have so far hardly been explored except in a few cases[7].

We have tried to follow some such haemoglobin kinetics at a low temperature (−55° C) in mixtures of methanol and water. The choice of the solvent at a given temperature is dictated by the stability of the protein which in this case might depend on the dielectric constant of the medium. A mixture of methanol and water 60v/40v has a reduced polarizability (compared with water) at ordinary temperatures, but the dielectric constant is close to 80 at about −55° C (ref 8). Preliminary tests showed that haemoglobin and its liganded forms (HbO_2, HbCO) are not only quite stable in these solvents but could be made to undergo the usual cycle of transformations.

$$\mathrm{Hb}+\mathrm{O_2}\rightarrow\mathrm{HbO_2},\ \mathrm{HbCO}\xrightarrow{h\nu}\mathrm{Hb}+\mathrm{CO},\ \mathrm{Hb}+\mathrm{CO}\rightarrow\mathrm{HbCO}$$

The visible absorption spectra of the respective species are not significantly different from those in water, the kinetics of these transformations can thus be followed spectrophotometrically in the usual bands.

The dark reactions were found to be much slower (by several orders of magnitude) at a low temperature than at an ambient temperature, as might be expected. The reaction, $HbO_2+CO\rightarrow HbCO+O_2$, proceeds so slowly at −55° C (even in the presence of an excess of carbon monoxide) as to be imperceptible, no doubt because of the rate-limiting step, $HbO_2\rightarrow Hb+O_2$, which, already slow (12 sec^{-1}) at 19° C and pH 9.1 (ref 9), is almost stopped at low temperature.

We report here some observations on the kinetics of the combination of carbon monoxide with haemoglobin after photodissociation of HbCO at −55° C. In one series of experiments, photodissociation was designed to be small

(5–10 per cent of total HbCO). The other series of experiments involved a much greater degree of initial photodissociation.

The optical cuvette, 10 mm long (for measurement), 3 mm thick (for photolysis) and 45 mm high, was sealed to a long cylindrical tube. It was placed in the centre of a quartz cryostat (with two pairs of parallel flat windows) where circulating cold gaseous nitrogen, measured with a thermocouple immersed in the solution itself, ensured (to ±2° C) the desired temperature[10]. The cuvette and cryostat were placed in the upper (measurement) compartment of a recording spectrophotometer, the Cary 15. A photographic shutter screwed over a hole in the external shield let in the photolysing light when desired. The latter consisted of a parallel beam recovered integrally from a mercury source of 200 W and filtered across a rectangular water trough. Except when longer exposures were wanted the shutter was set 0.25–1 sec. The recording was started manually, and so about 1 sec was lost after the end of the period of illumination. This lost time did not prove to be a serious handicap, for all the reactions were found to have slowed down and more than 50 per cent of the overall reaction could be recorded even in cases where there was a rapid phase.

The experimental solution was manipulated under nitrogen and contained at all stages an excess of dithionite. The following example describes the procedure for a typical preparation containing, say, 2.6 µmolar HbCO, 56 µmolar CO, 60 mmolar *tris* buffer, and with a solvent composition of 60 methanol/40 water, at −55° C. For solvent (+CO) 1.90 ml of deoxygenated methanol +0·95 ml of deoxygenated buffer solution (0.2 molar, *p*H 7.8) were mixed at 20° C. with a pinch of dithionite to ensure removal of traces of oxygen. Then 0.142 ml. of water saturated with carbon monoxide at 20° C was added under 1 atm pressure. The mixture (always under nitrogen) is immediately cooled to −55° C.

For HbCO which is 3.43 mmolar in aqueous buffer saturated with carbon monoxide at 20° C in the presence of a few crumbs of dithionite, 0.2 ml of the solution cooled to 0° C was added under nitrogen to 0.2 ml. of methanol at 0° C. A sample (46 µl.) of this mixture at 0° C was added immediately afterwards to the solvent at −55° C. The final solution thus has the required composition. The intermediate dilution in methanol (to 50 per cent) at 0° C is indispensable in order to avoid freezing.

In order to ensure that no denaturation had occurred, the following precautions were taken. Before each kinetic determination, the absorption spectrum in the Soret band of the experimental solution was recorded. The maximum was found to be at 421 mµ, as in aqueous solution. After the stages of photolysis and recombination, the spectrum was again recorded. The experiment was accepted only when the height and the position of the Soret band were identical with those observed initially. In some rare cases the Soret band spectrum recorded after the experiment was reduced in height with the maximum shifted to lower wavelength (to 414 mµ in the extreme—these results were discarded). This shift was no doubt caused by the formation of HbO_2 from traces of oxygen in the solution, for the spectrum thus obtained corresponded to that of HbO_2 (in aqueous solution or in a mixture of methanol and water at −55° C when prepared as described, starting from HbO_2 and omitting dithionite). Spectral anomalies, when found, were limited to those stated above, and never proceeded to stages indicating denaturation.

The kinetics of formation of HbCO after controlled photolysis were followed at 421 mµ, the stages of photolysis and combination could be repeated a number of times with little or no effect on the time course of the combination reaction. The rates obtained (thirty-four tracings from fourteen independent experiments) were consistent and when analysed gave the following results.

After slight photodissociation (~10 per cent), the rate of formation of HbCO can be described by a second order rate constant for the whole reaction. The value of this constant, which we shall call l'_4, is found to be 7×10^{-3} M^{-1} sec^{-1} at −55° C. This is not far from what would be expected from Gibson's value of 6.9×10^{-6} at 19° C and activation energy (10 kcal) determined in aqueous solution. We are aware that factors other than temperature (that is chemical composition, organization and viscosity of the solvent) may play a part in determining the rates, but the agreement seems to show that, at least for this reaction, the secondary effects are not much involved. Bielski and Freed[7] obtained continuous and expected change of rates from water to aqueous methanol in the case of hydrolysis of substrates by chymotrypsin.

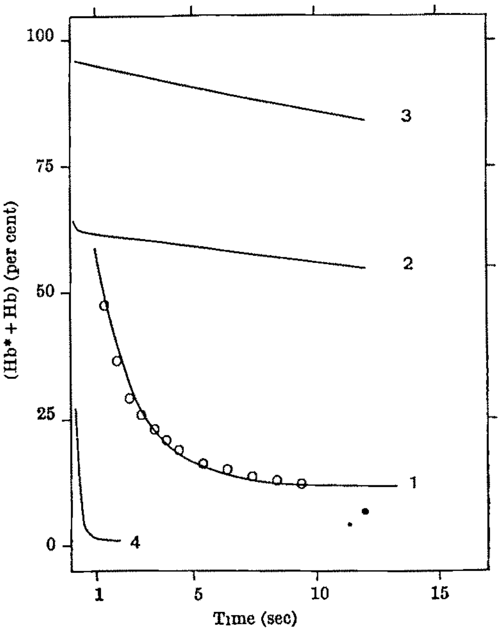

Fig. 1. Time course of formation of HbCO observed in a typical experiment at −55° C after high photodissociation. Solvent was 60 per cent methanol v/v containing *tris* buffer (*p*H 7.8 at 20° C). Initial concentration of HbCO was 2.6 µmolar (in haem), with 56 µmolar carbon monoxide. ○, Points read from the experimental tracing. Curve 1 was calculated following Gibson[4] but taking $l^* = 9.75 \times 10^3$ M^{-1} sec^{-1}, $l' = 1.91 \times 10^2$ M^{-1} sec^{-1}, $k_1 = 0.08$ sec^{-1}, assuming total photodissociation and all resulting haemoglobin initially to be the quick reacting form. Curve 2, expected time course with activation energies of (5.6 and 6 kcal for l^* ($l^* = 1.3 \times 10^5$ M^{-1} sec^{-1}) and k_1 ($k_1 = 11.9$ sec^{-1}) respectively. Curve 3, $l^* = 9.75 \times 10^{-3}$ M^{-1} sec^{-1} ($E = 11.1$ kcal), $k_1 = 11.9$ sec^{-1} ($E = 6$ kcal). Curve 4, $l^* = 1.3 \times 10^5$ M^{-1} sec^{-1} ($E = 5.6$ kcal), $k_1 = 0.08$ sec^{-1} ($E =$ 16.6 kcal).

Many of the uncertainties about the chemical and physical properties of the solvent should disappear when we consider the kinetics of the reaction at a given temperature (say −55° C) and compare the time course of formation of HbCO after slight photodissociation with that after high initial photodissociation. First, as said before, the reaction is strictly monophasic. After high initial photodissociation, the kinetics are heterogeneous, and can be fitted to Gibson's scheme in terms of the constants l^*, l' and k_1; in a typical experiment (Fig. 1), a good fit was obtained following Gibson's procedure[4] with $l^* = 9.75 \times 10^3$ M^{-1} sec^{-1}, $l' = 1.9 \times 10^2$ M^{-1} sec^{-1} and $k_1 = 0.08$ sec^{-1}. It is implied that the photodissociation is total and all the resulting haemoglobin is initially in the quick reacting form. These assumptions are justified from relevant logarithmic plots (not shown in the figure) which extrapolate at zero time to zero concentration of HbCO. While the value of l' agrees very well with that calculated from l' at 20° C and $E = 10.5$ kcal, those of l^* and k_1 are much smaller than what would be expected from activation energies and rate constants (at 1° C) reported by Gibson (Table 1). In the experiment reported in Fig. 1, the rapid phase lasted until about 9 sec, 8 sec after the recording was initiated. Values of l^* and k_1 consistent with the reported activation energies (5.6 and 6 kcal respectively)

Table 1 APPROXIMATE RATE CONSTANTS AND ACTIVATION ENERGIES DURING THE COMBINATION OF CARBON MONOXIDE WITH HUMAN HAEMOGLOBIN AT −55° C

Proportion of initial photo-dissociation	Rate constants	−55° C Solvent 60 vol methanol/40 vol water Found	Activation energy (kcal) Calc †	 Reported
Small	l'_4	$7\ 3 \times 10^3\ M^{-1}\ sec^{-1}$	11 6	10
High	l^*	$9\ 75 \times 10^3\ M^{-1}\ sec^{-1}$	11 1	5 6
High	l'	$1\ 9 \times 10^2\ M^{-1}\ sec^{-1}$	10 9	10 5
High	k_1	$0\ 08\ sec^{-1}$	16 6	6

The temperature was maintained at ±2° C Buffer salts (*tris*-) were present, but it seems impossible to give accurate *p*H values for the reaction media Some of these rate constants have been reported to vary with *p*H, but the difference in rates observed with solutions containing *tris* buffer at *p*H 6 7 or *p*H 7 8 was not larger than the experimental error The overall limit of error may be taken as about 1 kcal in terms of activation energies

† Calculated from rates at 19° C or 1° C reported by Gibson, and those observed during this work at −55° C

would have made the rapid phase disappear in 1 sec (curve 2) before we would have noticed it Likewise, it seems impossible to get a fit by assigning small values to either of l^* and k_1 separately (curves 3 and 4) Both of these rates seem to have a much greater temperature dependence than reported The value (11 kcal) we found for the activation energy of l^* seems to be in the expected range for a reaction of this rapidity involving gaseous ligand binding by haemoglobin[9]

The results show in general that gas binding by haemoglobin at −55° C in methanol and water is basically similar to binding in ordinary conditions It is agreeable to find evidence for haem-haem interaction, although there are no apparent reasons why this should not be so if the protein can be maintained in the native state On the other hand, these data may be considered in the light of recent work[5,6] on the identity of Hb* and the mechanism of its conversion to ordinary haemoglobin The quick reacting form has been suggested to consist of isolated chains in their monomeric form, with the deactivation step ($Hb^4 \rightarrow Hb$) simply the combination of deoxy α and β chains to give α β or $\alpha_2\ \beta_2$. We do not propose at this stage that our data bear on these considerations, but it is significant that the combination of ligand-free α and β chains to form α β or $\alpha_2\ \beta_2$ has been found by Antonini *et al* [11] to be very temperature dependent ($Q_{10} = 3\ 13$)

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[1] Gibson, Q H , and Roughton, F J W , *Proc Roy Soc* , B, 146, 206 (1957)

[2] Gibson, Q H , *J Physiol* , 134, 123 (1956)

[3] Antonini, E , Chiancone, E , and Brunori, M (in the press)

[4] Gibson, Q H , *Biochem J* , 71, 293 (1959)

[5] Gibson, Q H , and Antonini, E *J Biol Chem* (in the press, cited in ref 6).

[6] Antonini, E , Brunori, M , and Anderson, S (in the press)

[7] Bielski, B H J , and Freed S , *Biochim Biophys Acta*, 89, 314 (1964).

[8] Åkerlof, G , *J Amer Chem Soc* , 54, 4125 (1932)

[9] Gibson, Q H , *Prog Biophys Biophysical Chem* , 9, 1 (1959).

[10] Douzou, P , *J Chim Phys* (in the press)

[11] Antonini, E , Bucci, E , Fronticelli, C , Chiancone, E , Wyman, J , and Rossi-Fanelli, A , *J Mol Biol* , 17, 29 (1966)

Radio Emitting Electrons in the Galaxy and the Solar Modulation of Cosmic Rays

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The energy spectrum of electrons in interstellar space needed to explain the Galactic background radio emission has been derived. Information can then be deduced about the solar modulation of cosmic ray electrons.

A WIDE variety of observations, including measurements on heliocentric cosmic ray intensity gradients[1], has now established that even during the time of minimum activity of the Sun in its 11 yr cycle, there remains an appreciable residual modulation of the nucleonic components of the cosmic radiation These observations have prompted many attempts to understand the mode and magnitude of the solar modulation suffered by the nucleonic component existing in the near interstellar space, particularly within the framework of the solar wind model originally suggested by Parker[2] Such studies have revealed the following observational characteristics for the period of solar minimum which are now generally accepted by workers in this field (1) At rigidities, *R*, greater than 5 to 10 BV there seems to be very little or no modulation (2) For rigidities between about 0 5 BV and about 5 BV the modulation function $\exp(-K(t)/R\beta)$, where *K* is a time dependent constant and β is the ratio of the particle velocity to the velocity of light, can best represent the observations with a value of $K \approx 0\ 4$ to 1 0 BV (refs 3 to 5) (3) For lower rigidities the observational data suggest that the modulation is β dependent[1,6,7].

On the other hand, because the first experimental observations for a finite flux of cosmic ray electrons[8,9] were reported only in 1961, adequate electron flux measurements over a long enough period of time are not available, as in the case of the nucleonic component, to enable us to deduce, in a similar manner, information on the solar modulation of electrons As an important consequence of this, our present knowledge on this subject is in a rudimentary and, to some extent, controversial state[10–12] Nevertheless, information about electron modulation is very important because a comparison of the modulation suffered by electrons and nuclei will allow us to distinguish between contributions caused by rigidity and velocity dependent modulation, and thus lead to definitive conclusions regarding the mechanism of solar modulation It is now well recognized that cosmic ray electrons existing in Galactic space are responsible for the background radio emission; we have in the present treatment taken advantage of this special feature to infer directly the electron spectrum in interstellar space needed to account for the observed background radio spectrum We then compare this electron spectrum with that observed near the Earth during the period of minimum solar modulation, to draw valuable information on the nature of the modulation of electrons

In Fig 1 are summarized the data available on the differential flux of cosmic ray electrons[13–16] measured in the neighbourhood of the Earth near the time of minimum solar modulation (1965–66) It is seen that sufficient data exist for this period to construct a reliable energy spectrum from about 100 MeV to about 50 BeV;

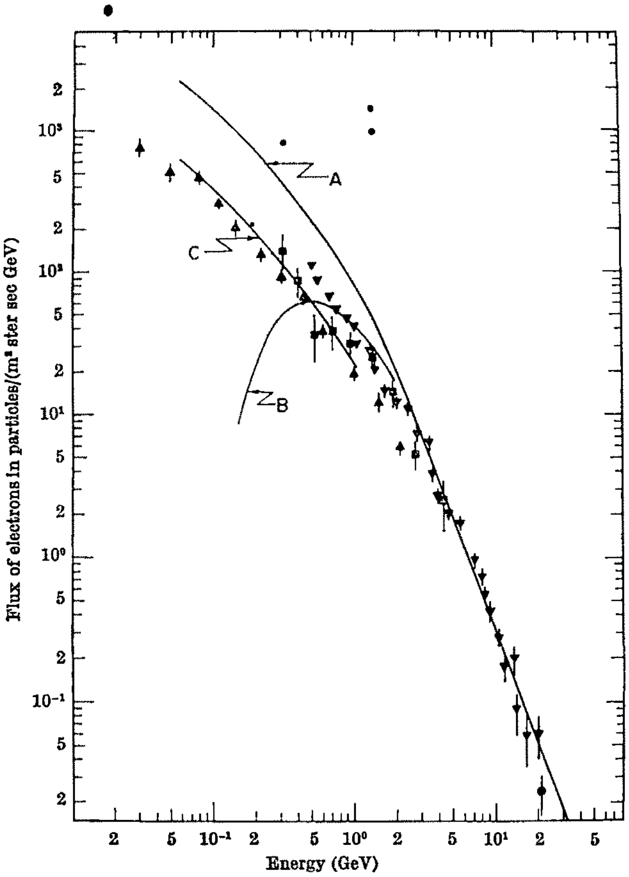

Fig 1 All data points refer to experiments made during 1965–66 except the highest energy point[15] Curve *A* is the electron differential energy spectrum derived for the near-interstellar space Curves *B* and *C* are the modulated spectra expected near the Earth from an *R* or *Rβ* dependence between 0 5 and 5 BV and a constant or a *β*-dependence below 0 5 BV respectively ▲, Beedle and Webber[16], ▼, Bleeker *et al* [18], ■, L'Heureux[14], ●, Daniel and Stephens[15]

it is also seen that the data above 5 BeV can be well fitted by the power law energy spectrum

$$\frac{\mathrm{d}J(E)}{\mathrm{d}E} = 126\, E^{-2.6 \pm 0.1}\ (\mathrm{m}^2\ \mathrm{sec\ ster\ BeV})^{-1} \qquad (1)$$

We would like to point out here that present evidence[17] strongly suggests that for rigidities in the range of 10 to 50 BV, the hydrogen, helium and heavy nuclei of the cosmic rays observed near the Earth all have power spectra with exponents Γ close to 2 6

From the many radio surveys made of the sky using wide angle beams, we have been able to construct the radio brightness distribution as a function of frequency for three general Galactic directions, namely, the Anticentre ($l'' \approx 140°$–$190°$ and $b'' \approx 10°$ N –10° S), the Galactic radio minimum (right ascension ≈ 10 h and $\delta \approx 20°$–$60°$) and the North Halo, but clearly avoiding the north polar radio spur (right ascension ≈ 10–17 h and $\delta \approx 30°$–$60°$) In addition, using narrow angle beam surveys, we have also been able to obtain the brightness distribution in the direction of the Galactic Ridge ($l'' = 0$ and $b'' = 3\ 6°$ N and 3 6° S) Details on the compiling of the radio and electron data and how they have been used to draw a number of meaningful conclusions regarding the electrons, and in general cosmic rays and magnetic fields in the Galaxy, are described in a separate paper[18] For purposes of the present analysis we have employed the radio spectra obtained for the direction of the Anticentre and the North Halo only; these are summarized in Fig 2 While constructing the radio spectrum for the Halo we have included data for only the northern Galactic hemisphere[19–24] because of the fact that the solar system is situated significantly above the Galactic plane, this helps in minimizing, in this direction, the contributions from the Galactic Radio Disk It may be seen from Fig. 2 that the observational data have been plotted only up to 10 MHz, below which Galactic absorption and other effects become progressively important It is also seen that the absolute sky brightness at 10 MHz in these two directions is nearly the same, with power index α in both cases close to 0 3–0 4 at low frequencies, slowly increasing to about 0 8 at the highest frequencies The value of $\alpha \approx 0\ 8$ at high radio frequencies can also be seen to be consistent with the power index of the high energy cosmic ray electrons (equation (1)) as expected from synchrotron mechanism.

Beginning with the first attempt in 1960 by Biermann and Davis[25], many investigators have used the background radio emission in the direction of the Galactic Halo to deduce information on the magnetic fields existing therein Okuda and Tanaka[26] have recently carried out such an analysis for the Galactic Centre and Anticentre In all these attempts, the simplified assumption is made that the observed radio emission and the radiating electrons have power law spectra connected by the relation $\alpha = (\Gamma - 1)/2$ Calculations are then carried out for the radio emission at a convenient frequency with an assumed electron spectrum made to fit the electron intensities observed near the Earth at some suitable electron energy Unlike these attempts, in what follows we derive the complete spectrum of radio emitting electrons (100 MeV to 10 BeV) needed to explain the observed radio emission (which is not a power law spectrum) in the frequency range of 10 to 400 MHz

Because we are primarily interested here in obtaining the electron spectrum as it exists in the near-interstellar space, we have chosen for the present analysis the Galactic Radio Disk in the direction of the Anticentre as resembling closest the near-interstellar space The dimension of the radio disk in this direction is thought to be reasonably well known and has a value of ≈4 kparsec Also, it seems reasonable to expect that the shape and intensity

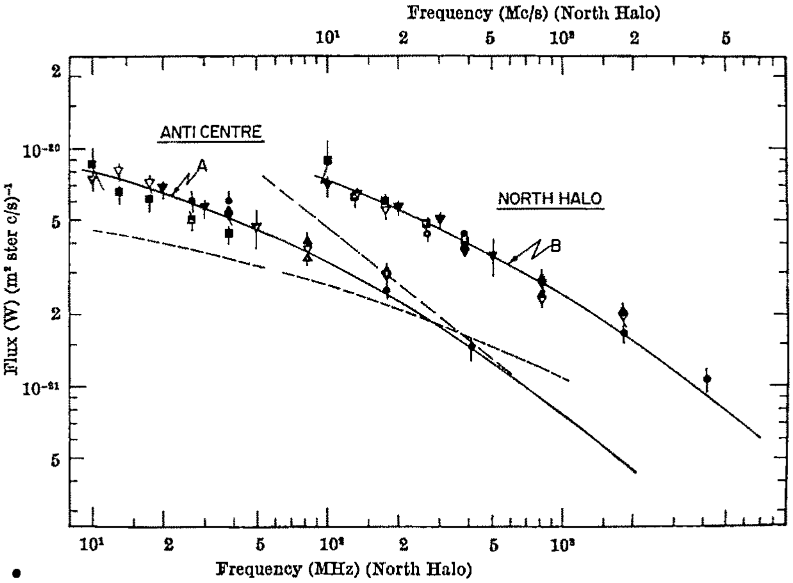

Fig 2 Radio brightness distributions in the directions of the Galactic Anticentre and North Halo Curve *A* for Anticentre and curve *B* for the North Halo were obtained using the electron spectrum *A* of Fig 1 - - -, Expected radio spectrum if experimental electron spectrum of Fig 1 and $\langle H_\perp \rangle = 7 \times 10^{-6}$ gauss are used, — — — —, expected radio spectrum if the power law electron spectrum given by equation (1) extending down to 100 MeV and $\langle H_\perp \rangle = 5 \times 10^{-6}$ gauss are used ◆, Costain[24], ●, Turtle *et al* [19], ▼, Parthasarathy and Lerfald[20], ■, Andrew[21], ▲, Purton[22], △, Bridle[23]

of electrons above 5 GeV are well preserved in the interstellar space as there are known to be in the case of the nucleonic component Thus we first attempt to deduce the electron spectrum in the interstellar space below 5 BeV by making use of the radio spectrum towards the Anticentre and later show that such a spectrum, if it exists in the Halo, would also be able to explain the observed radio spectrum in this direction

It is appropriate to mention briefly here the effect, in the present analysis, of any isotropic metagalactic radio component that may exist Though many attempts have been made by radio astronomers to estimate the magnitude of such a component, the situation is still far from satisfactory It is generally thought, however, that its contribution is likely to be $\lesssim 20$ per cent of the Galactic background emission The influence of this component, even if it exists to the extent of 20 per cent, on the derived electron spectrum depends on the assumed shape of the metagalactic radio spectrum and it can be easily shown[18] that in any case its effects are small and will not alter any of our general conclusions For this reason we have neglected in the present analysis the effects, if any, caused by the metagalactic radio component and hence all observed background radio emission has been attributed as being of Galactic origin

We will now proceed to describe the method of determining the electron energy spectrum responsible for the Galactic background radio emission The intensity of the synchrotron radiation at a frequency ν emitted along the line of sight by radiating electrons having a spectrum of energies can be written as[27]

$$I_\nu = \frac{\sqrt{(3)}e^3 < H_\perp >}{4\pi mc^2} L \int_{E_1}^{E_2} G_\nu (E)\, N(E)\, \mathrm{d}E \tag{2}$$

where $< H_\perp >$ is the mean component of the magnetic field perpendicular to the line of sight in the radiating region, L is the radiating distance along the line of sight

$$G\nu(E) \equiv x \int_x^\infty K_{5/3}(\eta)\mathrm{d}\eta \tag{3}$$

through the relation

$$x = \frac{\nu}{\nu_c} = \frac{4\pi mc}{3e < H_\perp >}\left(\frac{mc^2}{E}\right)^2$$

and $N(E)\,\mathrm{d}E$ is the number of electrons per unit volume with random directions of motion and having energies in the interval E and $E+\mathrm{d}E$ (all notations used here are the same as in ref 27) The limits of integration E_1 and E_2 are chosen such that

$$G_\nu (E_1)\, N(E_1)\, \mathrm{d}E \approx G_\nu (E_2)\, N(E_2)\mathrm{d}E \lll [G_\nu(E)N(E)\mathrm{d}E]_{\max}$$

The value of $[G_\nu(E)N(E)\mathrm{d}E]_{\max}$ would depend on the spectral shape of the electrons and can be estimated suitably For a radio spectrum which is not a simple power law, as is the case in Fig. 2, the electron spectrum required to give rise to it can best be obtained by a method of trial and error

Of the two free parameters L_A and $< H_\perp >_A$ relevant to the Anticentre, the former was assumed to be 1 25 × 10^{22} cm Because it has also been assumed that electrons with $E_e > 5$ BeV in the radiating region have the same spectrum as in the vicinity of the Earth, it is possible to set quite meaningful constraints on $< H_\perp >_A$ because it has to be such that the electron spectrum deduced for lower energies smoothly joins with that at energies > 5 BeV near the Earth It may also be pertinent to stress here that the fitting in the high frequency end of Fig 2A depends primarily on the assumed magnetic field because in this frequency region $I_\nu \propto L - < H_\perp >^y$, where $y > 1$ It is then possible by trial to fix a single value of $< H_\perp >_A$ and a unique, smoothly varying electron spectrum to match the observed radio spectrum The electron spectrum shown by curve A in Fig 1 and a value $< H_\perp >_A = 5 \times 10^{-6}$ gauss are thus obtained corresponding to the solid curve shown in Fig 2A for the radio brightness spectrum It is expected that any uncertainty in the electron spectrum derived for the interstellar space (curve A of Fig 1) will primarily result from uncertainties associated with the radio observations It is now interesting to notice that if we assume the same electron spectrum deduced for the Anticentre (curve A of Fig 1) to exist also in the Halo region, we get an exceedingly good fit (solid curve in Fig 2B) with $L_H = 4 \times 10^{22}$ cm and $< H_\perp >_H = 2 \times 10^{-6}$ gauss We therefore conclude that the electron spectrum given by curve A in Fig 1 represents the spectrum of electrons as they exist in the near-interstellar space, this may also be the case in the Galactic Halo.

We now proceed to compare curve A in Fig 1 representing the electron spectrum deduced for the near-interstellar space with that observed near the Earth in 1965–66 and deduce information on the electron modulation Such a comparison reveals the following important features (a) between about 0 5 BV and 5 BV, the modulation can be described equally well by an $R\beta$ or R dependence with a value for the constant $K \approx 0$ 65 (expected shape near Earth is then given by curve B of Fig 1), and (b) for $R < 0$ 5 BV there is a constant modulation which can also be understood as β dependent with a value for the constant $K \approx 1$ 3 (expected shape near Earth is then given by curve C of Fig 1) It is seen that inferences (a) and (b) are also consistent with our knowledge of the nucleonic modulation summarized early in this article We would further like to mention that the residual solar modulation of electrons in the entire energy region 0 1 BV to 5 BV can be represented well by a single modulation function of the form $\exp[-K/(a+R)\beta]$ where K and a are constants with values of $K \approx 1$ 4 and $a \approx 1$ because $\beta \approx 1$

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[1] O'Gallagher, J J , and Simpson, J A , *Ap J* , **147**, 819 (1967)

[2] Parker, E N , in *Interplanetary Dynamical Processes* (Interscience Publishers Inc , New York, 1963)

[3] Biswas, S , Ramadurai, S , and Sreenivasan, N , *Phys. Rev* , **159**, 1063 (1967)

[4] Gloeckler, G , and Jokipii, J R , *Ap J* , **148**, L41 (1967)

[5] Ramaty, R , and Lingenfelter, R E , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[6] Balasubrahmanyan, V K , Boldt, E , and Palmeira, R A R , *Phys Rev* , **140**, B1157 (1965)

[7] Durgaprasad, N , Fichtel, C E , and Guss, D E , *J Geophys Res* , **72**, 2765 (1967)

[8] Earl, J A , *Phys Rev Lett* , **6**, 125 (1961)

[9] Meyer, P , and Vogt, R , *Phys Rev Lett* , **6**, 193 (1961)

[10] Bleeker, J A M , Burger, J J , Deerenberg, A J M , Scheepmaker, A , Swanenburg, B N , and Tanaka, Y , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[11] L'Heureux, J , Meyer, P , and Verma, S. D , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[12] Webber, W R , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[13] Bleeker, J A M , Burger, J J , Deerenberg, A J M , Scheepmaker, A , Swanenburg, B N , and Tanaka, Y , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[14] L'Heureux, J , *Ap J* , **148**, 399 (1967)

[15] Daniel, R R , and Stephens, S A , *Proc Indian Acad Sci* , **65**, 319 (1967)

[16] Beedle, R E , and Webber, W R , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[17] Anand, K C , Daniel, R R , Stephens, S A , Bhowmik, B , Krishna, C S , Aditya, P K , and Puri, R K , *Proc Indian Acad Sci* (in the press), *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[18] Anand, K C , Daniel, R R , and Stephens, S A , *Proc Indian Acad Sci* (in the press)

[19] Turtle, A J , Pugh, J F , Kenderdine, S , and Pauliny-Toth, I I K , *Mon Not Roy Astro Soc* , **124**, 297 (1962)

[20] Parthasarathy, R , and Lerfald, G M , *Mon Not Roy Astro Soc* , **129**, 395 (1965)

[21] Andrew, B H , *Mon Not Roy Astro Soc* , **132**, 79 (1966)

[22] Purton, C R , *Mon Not Roy Astro Soc* , **133**, 463 (1966)

[23] Bridle, A H , *Mon Not Roy Astro Soc* , **136**, 219 (1967)

[24] Costain, C H , *Mon Not Roy Astro Soc* , **120**, 248 (1960)

[25] Biermann, L , and Davis, L , *Zeit Astrophysik*, **51**, 19 (1960)

[26] Okuda, H , and Tanaka, Y , *Proc Intern Conf Cosmic Rays, Calgary* (1967)

[27] Ginzburg, V L , and Syrovatskii, S I , *Ann Rev Astro Astrophys* , **3**, 297 (1965)

Discrete Potentials in the Dark-adapted Eye of the Crab *Limulus*

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Two types of discrete depolarizing potentials are recorded intracellularly from thoroughly dark-adapted visual cells of small *Limuli* stimulated with dim light. The larger type of discrete potential seems to be associated with the generation of nerve impulses in the eye in these conditions.

One of the striking discoveries made by intracellular recording from visual cells in *Limulus* was of small, discrete, depolarizing potentials which appear on stimulation with dim light or, occasionally, spontaneously in darkness[1]. In the first reports of such potentials, it was suggested that they are responses to single quantum absorptions, and so they were given the name "quantum bumps"[1,2]. Later studies showed that a second, larger type of discrete potential can often be elicited from the visual cells in *Limulus*, particularly when the cells are slightly hyperpolarized[3,4]. This second type of discrete potential is frequently superimposed on a quantum bump or other small depolarization of the cell membrane and is usually several times larger in amplitude. It has been suggested therefore that the large discrete potentials are a type of non-propagated regenerative response that arises in the visual cells in certain conditions[3-6].

The contribution of the two types of discrete potentials, particularly the regenerative responses, to the generator potential of the ommatidium and to the generation of nerve impulses in the eye has not been clarified. This report describes some intracellular recordings obtained from the ommatidia of thoroughly dark-adapted small *Limuli* which show very clear and large discrete potentials. The recordings also suggest a role for the regenerative responses in the dark-adapted eye.

Lateral eyes from small *Limuli* (3–4 in across the carapace) were excised and clamped firmly by an attached piece of shell in a small chamber containing sea water. The eyes were sliced approximately in half, with a razor blade, to expose intact ommatidia. White light from a microscope illuminator was focused through the cornea on to a single exposed ommatidium. The time of illumination was controlled by mechanical shutters, and light intensity by neutral filters inserted in the beam. A micropipette filled with 2 molar KCl and with a resistance varying from 30 to 70 megohms when measured in sea water was inserted into the ommatidium with the aid of a micromanipulator. Ag–AgCl electrodes connected the micropipette and chamber to a Keithley negative-capacitance electrometer. The amplified responses were displayed on an oscilloscope and recorded photographically.

The successful penetration of a cell was signalled by a sudden negative shift of the micropipette and the appearance of large positive responses to light stimuli. In this study, only cells showing large resting potentials (usually 70–90 mV) and large generator potentials (80–120 mV for retinular cells) were used. Numerous cells with lower resting and generator potentials (30–50 mV) were encountered. These cells were usually 1–2 log units less sensitive to light and did not show large discrete potentials, and so they were not carefully studied. The experiments were carried out at room temperature (25°–27° C), and after a satisfactory cell had been penetrated, the preparation was left undisturbed in the dark until it was thoroughly dark adapted. This took at least 30 min.

Two distinct types of response to moderate or bright light stimuli are encountered after penetration of a cell of the *Limulus* ommatidium[7]. The first type of response is a smooth depolarization, consisting of an initial transient wave and a steady potential which is maintained for the duration of the light stimulus. A notch is occasionally observed on the ascending portion of the transient wave, which suggests that there is also an initial spike-like component in the response[6,8]. The second type of response consists of a similar but smaller depolarization on which large nerve impulses are superimposed. The current interpretation of these responses is that the smooth depolarizing potential is a generator potential, which originates in the rhabdom region of the retinular cells and spreads by electrotonus into an eccentric cell, where it generates nerve impulses[9-12]. In support of this view is evidence that retinular and eccentric cells are electrically coupled[13]. The large, smooth depolarizing potentials are therefore believed to derive from the retinular cells, and the responses with superimposed impulses are thought to come from the eccentric cells. There are between ten and fifteen retinular cells in an ommatidium and usually one eccentric cell[14]. Thus in random penetrations, responses of retinular cells are encountered very much more frequently than are responses of eccentric cells.

Typical responses of a fully dark-adapted retinular cell to graded intensities of light are shown in Fig. 1. In the dark, such cells often show little spontaneous activity. Distinct responses to light are first detected when the light stimulus is attenuated about 7 log units. These initial responses consist of two types of discrete potentials. The first type is a regular, wave-like potential, 4–8 mV in size, which occurs repetitively during a prolonged threshold stimulus. The individual waves often appear in groups and summate to give a more sustained depolarization of the cell. The second type of discrete potential occurs less frequently. In Fig. 1, for example, only one such potential is observed with the threshold stimulus. These potentials are very large (up to 60 mV) and their shape, particularly their peak, is much sharper than that of the smaller potentials The small type of potential is believed to be the previously described quantum bump[1,2], while the large type of discrete potential is probably a regenerative response[3,4] This terminology will be adopted here, although the present experiments have little to add concerning the accuracy of these designations

With somewhat brighter stimuli (6 log unit attenuation of the light stimulus) a small but steady depolarization of the cell lasts for the duration of the stimulus (From inspection of the tracings in Figs 1–3, it seems likely that the small sustained depolarization in response to dim light is caused simply by summation of numerous quantum bumps) Superimposed on the sustained depolarization are several regenerative responses These regenerative responses are quite similar in size and shape, and resemble the single regenerative response observed with the dimmer (threshold) stimulus With yet brighter light stimuli, greater sustained depolarization is observed; and it is no longer possible to recognize either individual quantum bumps or regenerative responses. With progressively brighter stimuli, the responses become increasingly smooth until (with 2 log unit attenuation of the stimulus beam) an entirely smooth generator potential is observed

Thus only with dim light stimuli are the quantum bumps and regenerative responses easily distinguished Additional records illustrating the two types of discrete responses are shown in Fig 2 The regenerative responses are usually observed arising from the top of one or a group of quantum bumps Occasionally, a regenerative response seems to arise independently of the quantum bumps (Fig 3) This is difficult to judge, however, because if a quantum bump and a regenerative response superimpose closely, the underlying quantum bump would not be obvious

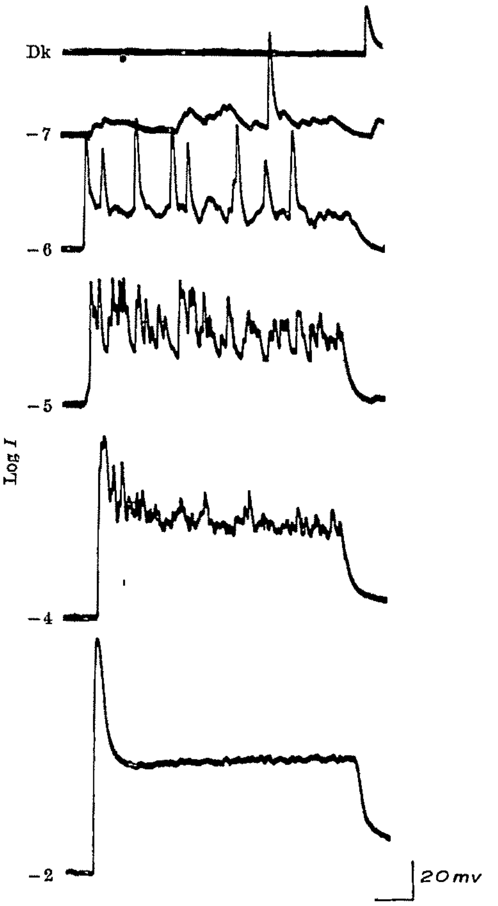

Fig 1 Responses of a thoroughly dark-adapted retinular cell to graded intensities of light. The numbers to the left of each record indicate the stimulus attenuation in log units The bar below the record indicates the approximate duration of the light stimulus See text for details

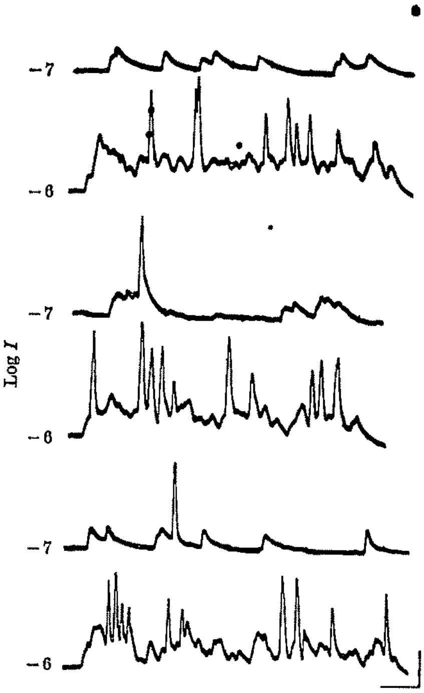

Fig 2 Responses of a retinular cell to dim illumination (7 and 6 log unit attenuation of the stimulating beam) These records show clearly the two types of discrete potentials elicited from the dark-adapted cell Vertical calibration, 20 mV, horizontal calibration, 0 5 sec

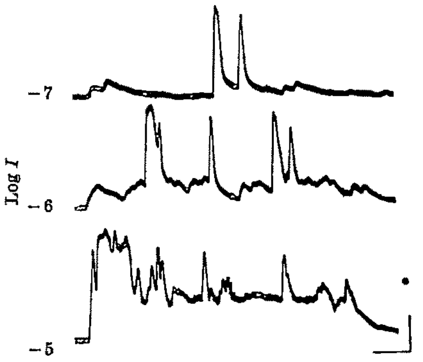

Fig 3 Responses of a dark-adapted retinular cell to graded intensities of light, showing decreasing latency of first regenerative response with increasing intensity Calibration as in Fig 2

One feature of the responses of retinular cells to moderate and bright light which was noted previously is the presence of a notch on the leading edge of the generator potential[6,8] This notch may be caused by a single regenerative response which occurs initially after stimulation of the dark-adapted eye with moderate or bright light A record suggesting this is shown in Fig 3; all three tracings in the record show regenerative responses of similar size and form With brighter stimuli, however, the first regenerative response appears earlier, until it is observed preceding most of the sustained depolarization In the bottom tracing in Fig. 3 the initial regenerative response is also the largest and clearest, suggesting, perhaps, that full-sized regenerative responses are elicited only for a narrow range of membrane polarization This may

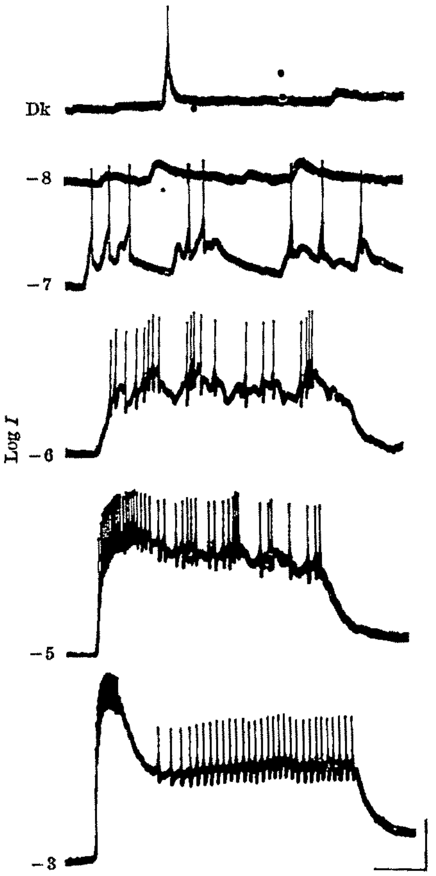

Fig 4 Responses of a thoroughly dark-adapted eccentric cell to graded intensities of light See text for details Calibration, 20 mV and 0 2 sec

explain why only one regenerative response is ordinarily observed after stimulation of the eye with moderate or bright light

It was assumed that an eccentric cell had been penetrated when impulses of 15–60 mV were observed superimposed on the generator potential Occasionally, small impulses are detected in retinular cell recordings, but the amplitude of these impulses never exceeds 1–5 mV. In records from retinular cells that show large discrete potentials, impulses are not usually observed (Figs. 1–3).

As already noted, generator potentials observed in eccentric cell recordings are usually smaller than the generator potentials noted in retinular cell recordings The discrete potentials are also smaller in eccentric cell recordings and thus cannot be distinguished as readily as in retinular cell recordings Nevertheless, in many recordings from eccentric cells, especially when the impulses are not very large (15–30 mV), discrete potentials of two varieties can be observed

A set of responses of one such eccentric cell to stimuli of increasing intensity is shown in Fig 4 In the dark, this cell showed an occasional spontaneous discrete potential and nerve impulse The first responses to dim light (log $I = -8$) consisted of small waves of depolarization These waves vary considerably in size and duration, suggesting that they are not single quantum bumps but probably groups of summating bumps, similar to the quantum bumps of varying size observed in retinular cell records (compare Figs 1 and 4)

With brighter stimuli (log $I = -7$) a more sustained depolarization of the cell is observed Superimposed on the sustained depolarization are sharp, discrete potentials which seem similar to the regenerative responses seen in retinular cell recordings. Nerve impulses are also seen in the record and are associated with each of the regenerative-like responses

With still brighter stimuli a greater sustained depolarization is evoked, and regenerative-like responses are more difficult to detect and to associate with the numerous impulses The brighter the light stimulus, the smoother is the generator potential and the more regular is the discharge of impulses. The responses of eccentric cells shown in Fig. 5 are about 1 log unit more sensitive than the comparable responses of the retinular cell shown in Fig 1 This is the usual finding, and it probably reflects the summated effects of the ten to fifteen retinular cells on the single eccentric cell of an ommatidium

In Fig. 5*a* the close relation between the regenerative-like responses and the impulses in the eccentric cell is shown more clearly. In all these records, impulses are observed only in conjunction with regenerative-like responses. For example, with 7·5 log attenuation of the stimulating beam, a very small sustained depolarization was observed together with five clear and prominent regenerative-like responses. Five impulses are seen in Fig 5*a*, superimposed on the five regenerative-like responses In Fig. 5*b*, recorded at a much faster sweep speed, one such regenerative-like response is shown with superimposed impulse The impulse has a duration of about 4 msec; the regenerative-like response, about 100 msec The regenerative-like response has a smooth wave-form, interrupted only by the impulse.

The regenerative responses, in occurring repetitively in an all or none fashion when elicited with a prolonged dim light, have characteristics similar to those of nerve impulses. It is known that tetrodotoxin blocks the transient influx of sodium ions that occurs when a nerve impulse is generated but does not appreciably affect slow, generator-type potentials[15].

In Fig 6 the effect of tetrodotoxin on the nerve impulses, quantum bumps, and regenerative responses of the photoreceptor of *Limulus* is shown The concentration of tetrodotoxin used in these experiments was 2×10^{-6} g/100 ml of sea water. Within 3 min of infusion of the drug, impulses are blocked in the eye and remain blocked as long as the drug is present. When the drug has been washed away, nerve impulses reappear, but if the con-

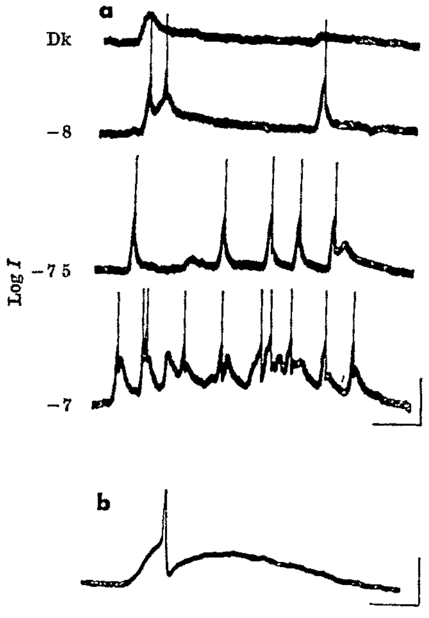

Fig 5 *a*, Records from an eccentric cell showing the close relation of the regenerative-like potentials and nerve impulses in the dark-adapted eye stimulated with dim light Calibration, 20 mV and 0 2 sec *b*, Regenerative-like response with superimposed impulse, recorded at fast sweep speed Vertical calibration is same as Fig 5*a*, horizontal calibration, 20 msec

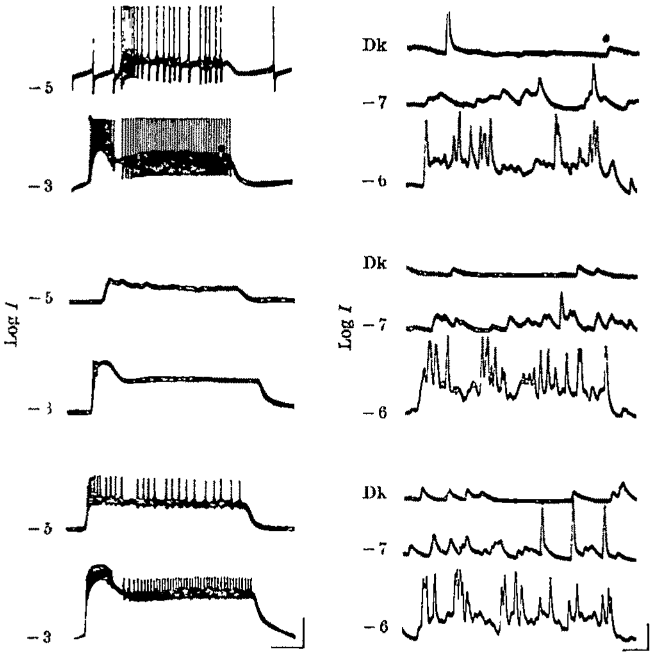

Fig 6 Effect of tetrodotoxin on the nerve impulses of eccentric cell (left) and on quantum bumps and regenerative responses of retinular cell (right) The top tracings are the controls recorded before addition of the drug Middle tracings are responses recorded 10 min after infusion of the drug, and bottom tracings are responses recorded 20 min after start of sea water wash The impulses are abolished by tetrodotoxin, but the quantum bumps and regenerative responses are unaffected Drug concentration was 2×10^{-6} g/100 ml Vertical calibration, 20 mV, time calibration 0 2 sec on left, 0 5 sec on right

centration and duration of the drug exposure were high, as here, only partial recovery of the impulse amplitude is noted.

No effects on either the quantum bumps or regenerative responses are observed after exposure of the eye to the same concentration of tetrodotoxin that rapidly eliminates the nerve impulses. So far, I have not observed tetrodotoxin to affect any component of the generator potential Benolken and Russell have reported some effects of tetrodotoxin on the generator potential in *Limulus*, but the concentration of tetrodotoxin they used was 5 to 1,000 times greater than was used here[8]

Recordings from thoroughly dark-adapted retinular cells show two types of discrete potentials clearly Recordings from eccentric cells are somewhat more equivocal because the discrete potentials are smaller, but in many instances two types of discrete potentials can be recognized which seem to correlate with the discrete potentials of the retinular cells The present experiments indicate that the discrete potentials observed in the eccentric cell originate at some distance from the impulse-generating site, because in those recordings that show large nerve impulses the discrete potentials (and the rest of the generator potential) are small, and vice versa These observations therefore suggest that the discrete potentials (like the rest of the generator potential) originate in the retinular cells and spread electrotonically into the eccentric cells

When the dark-adapted ommatidium is stimulated with dim light, the generation of nerve impulses seems to be correlated with the presence of regenerative responses. With brighter light stimuli, regenerative responses are not clearly recognized, but the sustained depolarization induced by the light is large and apparently sufficiently large to generate impulses in the eccentric cell. It is therefore tempting to suggest that the regenerative responses provide the potential to generate action potentials in the dark-adapted eye, when too few quanta are absorbed to provide sufficient sustained depolarization to induce action potentials It has long been realized that visual systems, including that of *Limulus*, can be excited by very few quanta[7,16] If, however, the small discrete potentials observed in retinular cell recordings are the quantal responses, it seems unlikely that only a few such quantum bumps could provide sufficient depolarization to generate an impulse at the spike generating site in the eccentric cell, which is perhaps several hundred microns away The regenerative responses may be, therefore, the amplification mechanism by which action potentials are elicited in the dark-adapted eye when only a few quanta are caught To confirm these ideas it will be important to make recordings from retinular cells and optic nerve simultaneously, to show unequivocally the correspondence of regenerative responses in the retinular cells and impulses in the optic nerve

The regenerative responses are interesting potentials that warrant further study In the dark-adapted eye these potentials are large (up to 60 mV), quite constant in size and shape, and are evoked repetitively in dim light They are not affected by tetrodotoxin, but are profoundly reduced in amplitude by light adaptation. A single bright flash will eliminate them, and 15 min or more in the dark is often necessary before they recover their initial amplitude These responses seem to be regenerative, but they probably do not propagate, because they are usually smaller when recorded in the eccentric cells Thus they share properties of both slow, graded potentials and nerve impulses

The final point concerns the conditions in which discrete potentials, especially regenerative responses, are best seen. Four factors seem to be important These are (*a*) the use of small (3–4 in) *Limuli*, (*b*) thorough dark-adaptation of the preparation (30 min at 25°–27° C), (*c*) the use of relatively fine micropipettes (30–70 megohm resistance in sea water), and (*d*) the use of cells with large resting potentials and large generator potentials Although on several occasions one or more of these factors have varied and good discrete potentials have still been observed, the best recordings have invariably been made with these conditions fulfilled It also seems likely that the micropipette must be in a particular location in the cell, perhaps close to the rhabdom region, if large discrete potentials are to be observed On one or two occasions, a slight shift of the pipette during an experiment significantly changed the size of the discrete potentials but did not appreciably affect the resting potential This suggests that the pipette did not withdraw from the cell, but simply moved within the cell to a new position, one that was more (or less) advantageous for recording the discrete potentials

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[1] Yeandle, S , *Amer J Ophthalmol* , **46** (pt 2), 82 (1958)
[2] Fuortes, M G F , *Arch Ital Biol* , **97**, 243 (1959)
[3] Adolph, A R , *J Gen Physiol* , **48**, 297 (1964)
[4] Fuortes, M G F , and Poggio, G F , *J Gen Physiol* , **46**, 435 (1963)
[5] Benolken, R M , *Cold Spring Harbor Symp Quant Biol* **30**, 445 (1965)
[6] Yeandle, S , *Kybernetik*, **3**, 250 (1967)
[7] MacNichol, E F , *Exp Cell Res* , Suppl 5, 411 (1958)
[8] Benolken, R M , and Russell, C J , in *Functional Organization of the Compound Eye* (edit by Bernhard, C G), 231 (Pergamon, London, 1966)
[9] Hartline, H K , Wagner, H G , and MacNichol, E F , *Cold Spring Harbor Symp Quant Biol* , **17** 125 (1952)
[10] Tomita, T , Kikuchi, R , and Tanaka, I , *Electrical Activity of Single Cells* (edit by Katsuki, Y), 11 (Igakuyshoin, Tokyo, 1960)
[11] Borsellino, A , Fuortes, M G F , and Smith, T G , *Cold Spring Harbor Symp Quant Biol* , **30**, 429 (1965)
[12] Behrens, M E , and Wulff, V J , *J Gen Physiol* , **48**, 1081 (1965)
[13] Smith, jun , T G , Baumann, F , and Fuortes, M G F , *Science*, **147**, 1446 (1965)
[14] Miller, W H , *J Biophys Biochem Cytol* , **3**, 421 (1957)
[15] Loewenstein, W R , Terzuolo, C A , and Washuizu, Y , *Science*, **142**, 1180 (1963)
[16] Hecht, S , Shlaer, S , and Pierenne, M H , *J Gen Physiol* ,**25**, 819 (1942)

Detection of an Anisotropic Soft X-ray Background Flux

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The cosmic X-ray background has been found to be anisotropic. It is interpreted as being made up of two components: an isotropic background, possibly of extragalactic origin, and an anomalous emission, perhaps located within the Galaxy.

On December 13, 1966, at 06.30 UT, an instrumentation package carrying several X-ray detectors was launched on an Aerobee 150 rocket from Natal, Brazil. The X-ray background observed in the 44–70 Å band on the flight was anisotropic and is interpreted to consist of an extragalactic component and a component which may possibly be associated with the galactic corona.

The instrument which supplied the data reported here was a proportional counter with an effective collecting area of 500 cm². The detector was collimated to 8° full width half maximum (FWHM) by a honeycomb collimator. A constant pressure gas flow system was used to ensure stable operation of the counter throughout the flight. The pressure regulator employed was capable of holding the preset counter pressure to better than 0.1 per cent. Counting gas was continuously flushed through the counter through an umbilical before the launching. After lift-off, flow was maintained from an onboard gas supply bottle.

A calibration detector with an internal iron-55 source was connected in parallel with the primary detector and shared the high voltage and counting gas of the detector. The electronics chain servicing the primary detector was switched to the calibration detector periodically throughout the flight to check the overall system gain. These calibrations indicated that the primary detector was stable to the limit of readability of the data or to better than 0.2 per cent.

The window employed in the detector was nominal 1/8 mil 'Mylar' with a 200 Å 'Nichrome' coating. The counting gas was 90 per cent argon and 10 per cent methane. A laboratory calibration of the transmission of the window was made at 7 Å and 44 Å. The detection efficiency of the counter is shown in Fig. 1. The data from the detector were amplified and fed to an eight-channel pulse height analyser. The output from each channel was then telemetered to ground.

The rocket was despun at 70 km to a roll period of 12 sec by a gas despin mechanism. As the rocket spun and precessed, it scanned much but not all of the overhead sky. The determination of the rocket aspect as a function of time was greatly complicated by the failure of the onboard stellar photometers. An aspect solution was eventually obtained by combining the data from two onboard magnetometers with X-ray data from the proportional counters. The spin rate, precession rate, coning angle and the angle between magnetic north and the angular momentum vector were determined from the magnetometer data. A unique position sighting necessary to specify completely the solution was provided by a roll on which both the Crab Nebula and Sco*XR*-1 were observed by the X-ray detectors. These two sources were unambiguously identified by their intensities and by their angular separation.

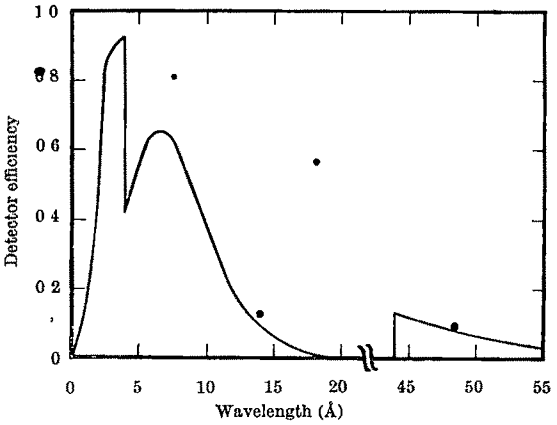

Fig. 1. Detector efficiency of the proportional counter. Photons producing counts in the 44–70 Å band were discriminated electronically and telemetered directly to ground.

Although this aspect solution does not possess the strong internal verification of a solution based on stellar signals, several checks on self consistency were available. The solution obtained was consistent with a rigid body solution based on the moments of inertia of the rocket and the observed roll rate. In addition, a strong source was observed approximately 100 sec before the Sco*XR*-1 sighting. The position of this source is within 10° of the reported location of Cen*XR*-2 (ref. 1).

From this aspect solution, the detector scans were plotted in galactic co-ordinates. Photons detected in the 44–70 Å band were handled in the following way. The celestial sphere was divided into elements of 50 square degrees, corresponding to the approximate resolution area of the collimator. The midpoints for all scans passing through a given resolution element were noted, and the number of photons detected in 0.5 sec about those points were averaged and recorded for that element. The photon count in each element was then reduced by a background count of 4 counts/0.5 sec. This counting rate was observed throughout the flight when the detectors were looking in the downward direction. The statistical validity of the count in each element varied from ±8 per cent to ±50 per cent depending on the number of scans and the number of counts/scan contributing to the average count in that element. Typically elements were valid to ±20 per cent.

A simplified version of this plot was superimposed on a map of the galactic hydrogen distribution taken from ref. 2, and is shown in Fig. 2. In this composite map, the photon counts are grouped into four intensity categories. A general trend is evident which shows that the count rate is higher near the pole than it is near the galactic equator.

The data from the higher energy channels have not as yet been completely analysed. As a preliminary step, however, the rate of counting uncorrected for detector efficiency was determined for the region within 20° of the galactic pole and the region within 10° of the galactic plane. These results, along with the 44–70 Å data, are given in Table 1. It is clear that the correlation rate of counting with galactic latitude is lost at higher photon energies in agreement with earlier work[3].

To interpret the data of Fig. 2, we have tentatively divided it into two components: (*a*) an intrinsically isotropic background, possibly extragalactic in origin, and

Table 1

Wavelength band	Galactic pole	Galactic plane	Ratio of plane to pole
	Counts/half sec		
44–70 Å	10±1	0·3±0·9	0·03±0·09
5–16 Å	26±1·4	22 ±2	0·85±0·09
3– 5 Å	14±1	18 ±2	1·29±0·17
2– 3 Å	9±1	10 ±2	1·11±0·25

(*b*) an anomalous emission in $300° < l < 30°$ and $b < 70°$, perhaps located within the Galaxy. The first component may be identified with the background detected at higher energies where the attenuation by interstellar gas is negligible. The fact that it correlates with latitude in the present experiment is interpreted as interstellar attenuation of an extragalactic source, an effect predicted by Gould and Sciama[4]. We have analysed the data to obtain the isotropic intensity corrected for attenuation and the magnitude of attenuation within the Galaxy.

This analysis is based on the relation

$$R = A\Omega \int_0^{\varepsilon_0} j(\varepsilon)\, E(\varepsilon)\, e^{-\tau(\varepsilon)}\, d\varepsilon \tag{1}$$

where R is the count rate, A is the detector area, Ω is the effective solid angle ($2·05 \times 10^{-2}$ ster), $j(\varepsilon)$ is the unattenuated photon spectrum at energy ε (quanta cm^{-2} sec^{-1} ster^{-1} keV^{-1}), $E(\varepsilon)$ is the detector efficiency and $\tau(\varepsilon)$ is the optical depth of the interstellar gas. The detector cut-off is at $\varepsilon_0 = 0·28$ keV, corresponding to 44 Å.

The optical depth τ equals σN_H, where σ is the effective cross-section per hydrogen atom and N_H is the atomic hydrogen column density based on 21 cm measurements[2]. In the present energy range, σ is caused entirely by hydrogen and helium, which are assumed to be neutral. The most recent calculations of σ yield[5]

$$\sigma = \sigma_0 \left(\frac{\varepsilon}{\varepsilon_0}\right)^{-b}$$

$$\sigma_0 = (0·84 + 21·4\xi) \times 10^{-21}\ \text{cm}^2 \tag{2}$$

$$b = 3·2,$$

where σ_0 is the effective cross-section at $\varepsilon_0 = 0·28$ keV, and $\xi = n(\text{He})/n(\text{H})$. We fitted E by $E_0(\varepsilon/\varepsilon_0)^c$, with $E_0 = 0·158$ and $c = 6·2$. Although $j(\varepsilon)$ is not yet known, we assumed a power law $j_0(\varepsilon/\varepsilon_0)^{-a}$, where $a \simeq 1·6$ from previous results below 100 keV (ref. 6). When these approximations are inserted into equation (1), the result can be written

$$R = R^0\, F(\sigma_0 N_H)$$

where

$$R^0 = \frac{A\Omega j_0 E_0 \varepsilon_0}{1 + c - a} \tag{3}$$

is the count rate outside the galactic disk and $\sigma_0 N_H$ is the optical depth (at 44 Å) for the direction in question. While $F(\sigma_0 N_H)$ can be evaluated exactly in terms of incomplete Γ functions, a sufficiently accurate approximation is

$$F = \exp(-1·47\sigma_0 N_H) \tag{4}$$

The count rates corrected for background were divided into eight groups according to the value of N_H at the observed position in the sky. Mean values of $R_{0·5}$ (counts/0·5 sec interval) and dispersions based on count statistics were computed. The results are presented as log $R_{0·5}$ against N_H in Fig. 3. (The point for $N_H > 1·6 \times 10^{21}$ is $1·2 \pm 0·3$ and is consistent with the data plotted.) The correlation between log $R_{0·5}$ and N_H is good, confirming the qualitative result from Fig. 2. The fit of a straight line (equations (3) and (4)) yields

$$R^0 = 2R^0_{0·5} = 22 \pm 3\ \text{c.p.s.}$$

and

$$\sigma_0 = (0·91^{+0·25}_{-0·14}) \times 10^{-21}\ \text{cm}^2$$

The errors quoted are estimates of the maximum permissible error. From equation (3), the corrected count rate corresponds to

$$j_0 = 270 \pm 40\ \text{quanta cm}^{-2}\ \text{sec}^{-1}\ \text{ster}^{-1}\ \text{keV}^{-1}\ \text{at } 0·28\ \text{keV}$$

or

$$J\nu = (5·1 \pm 0·7) \times 10^{-25}\ \text{erg cm}^{-2}\ \text{sec}^{-1}\ \text{ster}^{-1}\ \text{Hz}^{-1}$$

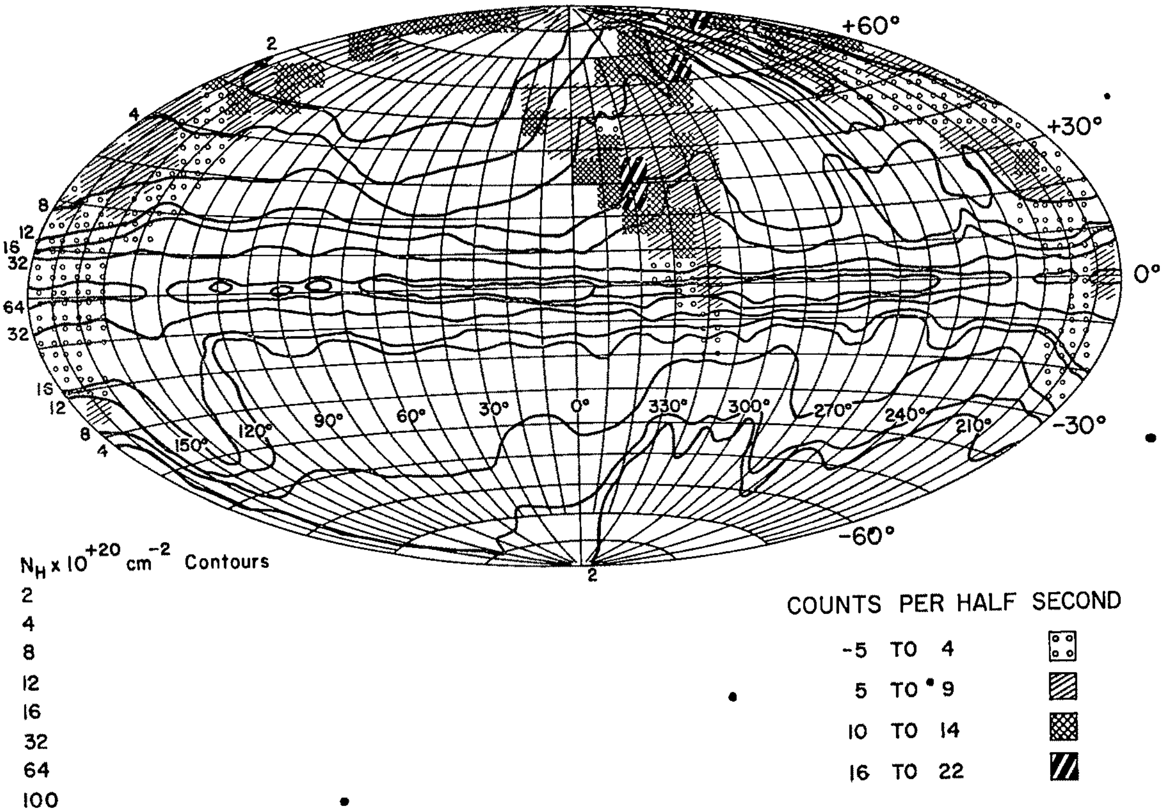

Fig. 2. 44–70 Å photon counting rates superimposed on a plot of the galactic hydrogen distribution. The resolution areas employed correspond to the effective resolution area of the collimator and the count rates refer to 0·5 sec averages.

The cross-section σ_0 inferred from the data is surprisingly small. According to equation (2), it formally implies a helium abundance $\xi = n(\text{He})/n(\text{H}) = (0.3^{+1.3}_{-0.7})$ per cent. This is only a small fraction of the value observed in emission nebulae[7], and in *O* and *B* stars[8] and is not acceptable. One may question the validity of the adopted cross-section for helium in equation (2). It agrees, however, within 10 per cent with an experimental determination at 0.28 keV (ref. 9) and correlates well with other data at lower energies[10]. Furthermore, if additional interstellar hydrogen were present in a form other than atomic (such as H_2 or H^+) the discrepancy would be still worse.

It is interesting that ultraviolet observations of stars also indicate a discrepancy with the 21 cm column densities[11]. Lyman-α measurements indicate that N_H (21 cm) may be as much as a factor 6.5 too high. Our measurements indicate that N_H (21 cm) may be a factor 3.3 too high if ξ is 0.10. If this discrepancy is real, it has profound implications for the interstellar medium.

We find that j_0 may be reasonably interpreted as a continuation of the background spectrum already observed above 1 keV. For example, a power law with $\alpha = 1.8$ fits the points from 0.28 to 30 keV quite well (confirming our use of $\alpha = 1.6$ in the data reduction). Such a spectrum is consistent with either emission by distant discrete sources or inverse Compton scattering of 3° black-body background quanta in intergalactic space. In this case, the observed steepening of the spectral index by about 0.5 somewhere between 10 and 100 keV may have great significance.

The present measurement may be employed to place limits on the temperature of a dense intergalactic medium. It was shown by Field and Henry[12] that an upper limit on the temperature of a postulated intergalactic gas can be obtained from the observations of the diffuse background in the 2–10 Å region. If the gas is assumed to be heated at the epoch $z = 1$, and if its density is sufficient to give $q_0 = 1$, the present gas temperature must be less than 2×10^6 °K. Weymann[13] has computed more realistic models, based on definite heating mechanisms and including free-bound emission and emission by helium in addition to free–free emission by hydrogen. His upper limit from the 2 to 10 Å data is also about 2×10^6 °K because the latter dominates at high temperatures.

The present data at lower photon energies are sensitive to lower temperatures. From Weymann, we find $T < 3 \times 10^5$ °K if the predicted thermal emission is not to exceed the observed flux. Even if helium and free-bound emission are omitted[12], $T < 5 \times 10^5$ °K.

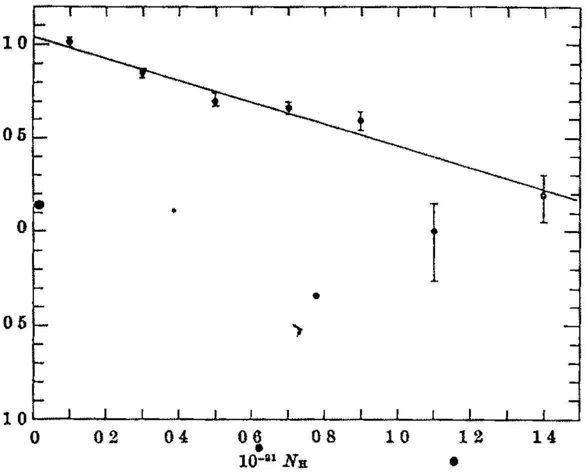

Fig. 3. Correlation between observed count rate and calculated interstellar absorption. The abscissa is the column density of interstellar atomic hydrogen, which is proportional to the expected absorption, and the ordinate is count rate (0.5 sec intervals). The correlation represented by the line confirms the hypothesis of extragalactic origin. The intercept yields the count rate corrected for absorption, and the slope yields the cross-section per hydrogen atom.

If quasars are at cosmological distances, Weymann has shown that $T > 1 \times 10^6$ °K from the absence of intergalactic Lyman-α absorption. We may set a lower limit on T from the present data alone, which is independent of the controversial issue of quasar distances, although it does depend on the assumption that the abundance of primordial helium is about 10 per cent. If T were lower than 10^5 °K, thermal emission at 44 Å would be negligible, requiring that the observed flux be attributed entirely to nonthermal sources. If, as seems very likely, these sources are at a great distance, they will be attenuated by the intergalactic gas[4,14] if any atoms are present which are not completely ionized. For $q_0 = 1$ and 10 per cent helium abundance, this requires that less than a few per cent of the helium can be neutral or singly ionized. From ref. 13, this in turn requires that $T > 10^5$ °K.

We may therefore conclude that the present temperature of a postulated dense intergalactic gas must lie in the narrow range $1 \times 10^5 < T < 3 \times 10^5$ °K. Such values agree very well with a theoretical prediction by Sciama[15]. It is important to note, however, that a valid alternative explanation is simply that the intergalactic gas is not dense and that it contributes a negligible amount to the mass of the Universe. If future experiments eliminate the permitted range of temperature altogether, we shall be forced to the latter conclusion.

Finally, we consider the interpretation of the anomalous emission component. If it is indeed a real effect, its direction is not far from the direction of the galactic nucleus. Its intensity decreases at low galactic latitude as would be expected for a distant source. We have considered the possibility that this source is caused by a galactic corona of the type proposed by Spitzer[16]. We estimate that its maximum intensity at 0.28 keV, corrected for interstellar attenuation, is about 1.8×10^{-24} erg cm^{-2} sec^{-1} ster^{-1} Hz^{-1}. If we include a small contribution by helium and by bound-free emission, the thermal radiation from the hot gas would equal the value suggested by the observations if $T = 10^6$ °K and the emission measure $\int N_e^2 \, dl = 0.7$. Such a corona would not be observable optically or at radio wavelengths. If we suppose it to be a spherical cloud centred on the galactic nucleus, its radius would be about 5 kparsec and N_e would be about 10^{-2} cm^{-3}. The gas pressure would be about 2×10^{-12} dynes cm^{-2}, about twenty times the value suggested by Spitzer. Its mass would be $10^8 M_\odot$. If the anomalous emission is caused by another effect, these figures represent an upper limit on a possible galactic corona.

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[1] Harries, J. R., McCracken, K. G., Francey, R. J., and Fenton, A. G., *Nature*, **215**, 38 (1967).
[2] Garmire, G., and Kraushaar, W. L., *Space Sci. Rev.*, **4**, 123 (1965).
[3] Seward, F., Chodil, G., Mark, H., Swift, C., and Toor, A., *Astrophys. J.* (in the press).
[4] Gould, R. J., and Sciama, D. W., *Astrophys. J.*, **140**, 1634 (1964).
[5] Bell, K. L., and Kingston, A. E., *Proc. Phys. Soc.*, **90**, 31 (1967); *Mon. Not. Roy. Astro. Soc.*, **136**, 241 (1967).
[6] Gould, R. J., *Amer. J. Phys.*, **35**, 376 (1967).
Faulkner, D. J., and Aller, L. H., *Mon. Not. Roy. Astro. Soc.*, **130**, 393 (1965).
[8] Underhill, A. B., in *The Early Type Stars*, 171 (Reidel Publishing Co., Dordrecht, 1966).
[9] Lukirskii, A. P., Brytov, I. A., and Zimkina, T. M., *Optika i Spektroskopiya*, **17**, 438 (1963); transl. in *Opt. and Spectr.*, **17**, 234 (1964).
[10] Baker, D. J., Bedo, D. E., and Tomboulian, D. H., *Phys. Rev.*, **124**, 1471 (1961).
[11] Jenkins, E. B., and Morton, D. C., *Nature*, **215**, 1257 (1967).
[12] Field, G. B., and Henry, R. C., *Astrophys. J.*, **140**, 1002 (1964).
[13] Weymann, R., *Astrophys. J.*, **147**, 887 (1967).
[14] Felten, J. E., and Gould, R. J., *Phys. Rev. Lett.*, **17**, 401 (1966).
[15] Sciama, D. W., *Quart. J. Roy. Astro. Soc.*, **5**, 196 (1964).
[16] Spitzer, jun., L. S., *Astrophys. J.*, **124**, 20 (1956).

Conformational Relationship between the O–C–C–N+ System of Phospholipids and Substrates of Muscarinic and Cholinergic Systems

by

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The O–C–C–N+ system is a common structural feature of phospholipids and nerve amines and it displays the *gauche* conformation about the carbon–carbon bond. The characteristic *gauche* conformation is probably associated with the biological activity of the phospholipids and the nerve amines. The close structural relationship between the phospholipid components and acetylcholine suggests that the lecithins and the sphingomyelins of cell membranes may be competitive inhibitors and sites of concentration of acetylcholinesterase

In a recent communication on the crystal structure of the phospholipid component L-α-glycerophosphorylcholine cadmium chloride trihydrate[1], it was pointed out that the choline residue displayed a *gauche* conformation about the C–C bond (Fig 1) Choline and ethanolamine derivatives are widely distributed as components of membrane phospholipids and nerve amines Examination of several of these compounds, the solid state structures of which are known, revealed that the O–C–C–N+ system always exists in the *gauche* form (my unpublished results, see also ref 2), rather than the *trans* form which would be expected on purely steric grounds I contended that electrostatic attraction between the positively charged nitrogen and the electronegative oxygen (ester, ether or alcohol oxygen) stabilizes the *gauche* conformation

There can be two enantiomeric *gauche* conformations, I and II, in I the OCCN+ angle is positive while in the other this angle is negative Crystal structures, which have a mirror plane (glide plane) of symmetry or a centre of symmetry, would display both these forms The torsion angles φ_{CC} (for definition of φ_{CC} (ref 3) see Fig 2), for the compounds under consideration, are listed in Table 1 Irrespective of whether the compound is a zwitterion or one that possesses only a positively charged nitrogen, it is observed that the conformation about the C–C bond is *gauche* The intramolecular N+ O distances shown in the figures may be regarded as being close to or slightly less than the van der Waals contacts, indicating that there is substantial electrostatic interaction between the quaternary nitrogen, N+, and the electronegative oxygen, thus supporting the contention that N+ O coulombic attraction is primarily responsible for the folded conformation

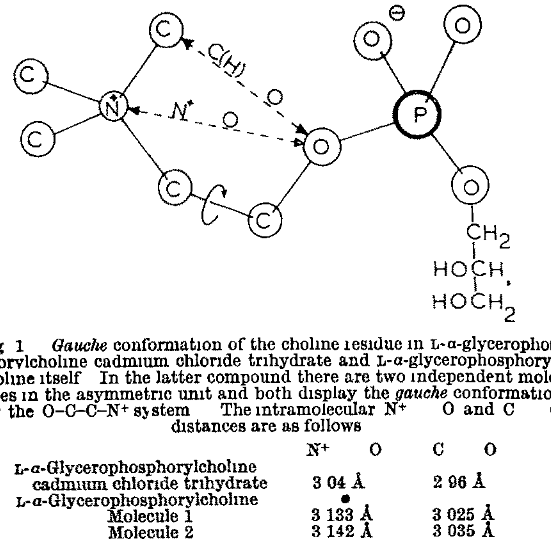

Fig 1 *Gauche* conformation of the choline residue in L-α-glycerophosphorylcholine cadmium chloride trihydrate and L-α-glycerophosphorylcholine itself In the latter compound there are two independent molecules in the asymmetric unit and both display the *gauche* conformation for the O–C–C–N+ system The intramolecular N+ O and C O distances are as follows

	N+ O	C O
L-α-Glycerophosphorylcholine cadmium chloride trihydrate	3 04 Å	2 96 Å
L-α-Glycerophosphorylcholine		
Molecule 1	3 133 Å	3 025 Å
Molecule 2	3 142 Å	3 035 Å

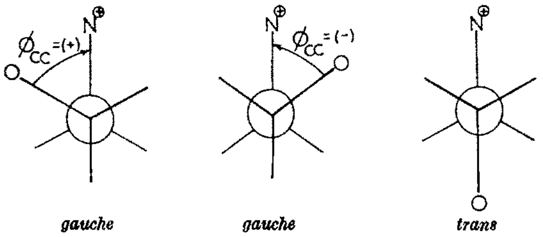

Fig 2 The three possible staggered arrangements for the O–C–C–N+ system as viewed down the C–C bond The torsion angle φ_{CC} (ref 3) is said to describe a *gauche* conformation when the projected angle between the C–O and C–N+ bonds is ±60°, the positive angle is measured clockwise from the C–O bond to the C–N+ bond

Furthermore, the positive charge on the nitrogen would be expected to be at least partially delocalized into the methyl groups Possible C–H O hydrogen bonds[3] involving the C–H bond of the methyl group closest to the oxygen atom can therefore provide additional stabilization of the *gauche* conformation in the choline derivatives where the H–C N–C–C–O chain achieves a quasi six-membered ring, as shown in Fig 3 Even in the zwitterions it seems that the *gauche* conformation is stabilized by the interactions discussed (N+ . . . O) rather than the electrostatic interaction between the positively charged nitrogen and the anionic oxygen.

The cadmium chloride complex of L-α-glycerophosphorylcholine exhibits a torsion angle of about +60° In L-α-glycerophosphorylcholine itself[4], however, while the two independent molecules comprising the asymmetric unit of the crystal structure are *gauche*, their torsion

Table 1. CONFORMATION OF THE N+–C–C–*X* SYSTEM, WHERE *X* = OXYGEN, SULPHUR OR SELENIUM*

Compound	System	Zwitterion	Conformation	φ_{CC}
L-α-Glycerophosphorylcholine CdCl₂ 3H₂O[1]	N+–C–C–O	Yes	*Gauche*	+50 3°
L-α-Glycerophosphorylcholine[4] (2 molecules/asymmetric unit)	,,	,,	,,	+71 5, −75 3
Muscarine iodide[5]	,,	No	,,	−61 9
Acetylcholine bromide[8]	,,	,,	,	~ ±60
Palmitylcholine bromide[9] (and iodide)	,,	,,	,,	Projection work
Stearylcholine bromide[10] (and iodide)	,,	,,	,,	,, ,,
Choline chloride[8]	,,	,,	,,	−84
2-Aminoethanol phosphate[11]	,,	Yes	,,	±52
DL-Serine[13]	,,	,,	,,	±60
L-Serine phosphate[12]	,,	,,	,,	~ +50
Ls-Threonine[14]	,,	,,	,,	−55
d,l-2-Aminobutylthiosulphuric acid[21]	N+–C–C–S	,,	,,	±58 1
2-Aminoethylsulphonic acid[20]	,,	,,	,,	±60 2
Acetylthiocholine bromide (private communication from Shefter and Mautner)	,,	No	*Trans*	~180
Acetylselenocholine iodide[2]	N+–C–C–Se	,,	,,	~180

* After this article was submitted for publication, Carlstrom and Bergin (*Acta Cryst*, **23**, 313, 1967) reported the crystal structure of the brain amine noradrenaline hydrochloride, in which the O–C–C–N+ system also occurs in the *gauche* conformation with φ_{CC} about −60°

angles have opposite signs, namely +71·5° and −75·3° (Table 1) The remaining choline derivatives in the table with the O–C–C–N$^+$ system are not zwitterions and, interestingly, are related to the substrates of muscarinic and cholinergic systems, for example, muscarine iodide[5], choline chloride[6], acetylcholine bromide[7,8], and palmityl- and stearylcholine bromides[9,10] These compounds exist in the *gauche* conformation in their respective crystals (Fig. 4)

The two ethanolamine derivatives—ethanolamine phosphate and L-serine phosphate—are components of the important phospholipids (cephalins), phosphatidylethanolamine and phosphatidylserine, respectively Again, both these components demonstrate the *gauche* form in the solid state, although here the substituents on the N$^+$ are hydrogen and not methyl groups The N$^+$ . . O distance in ethanolamine phosphate[11] is 2·878 Å, which not only is appreciably shorter than the corresponding values found in the choline derivatives, but is also significantly shorter than the van der Waals contact distance (Fig 5) The absence of methyl groups on the nitrogen in the ethanolamine derivatives therefore makes possible a closer approach of the quaternary nitrogen and the phosphate ester oxygen, and the N$^+$ . . O coulombic attraction is probably the only dominant force that twists the molecule into the *gauche* form Hydrogen bonding interactions involving the hydrogens on N$^+$ and the ester oxygen are of little importance here because not only are the H . . O distances greater than 2·90 Å but also the N–H . . O angles, close to 90° or less, are unfavourable for an N–H . . O hydrogen bond The N$^+$ . . O distance in L-serine phosphate[12] is not available because the atomic co-ordinates were not reported, however, the *gauche* arrangement in L-serine phosphate was deduced from the published perspective drawing (redrawn, Fig 6)

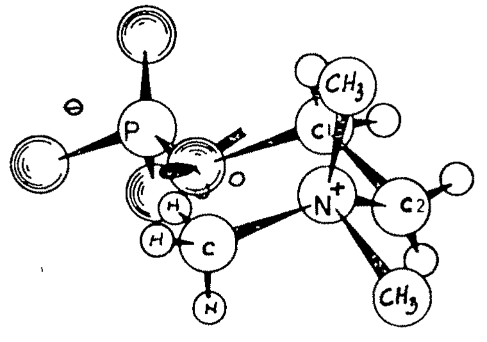

Fig 3 C–H . . O interaction between a methyl C–H bond and the ester oxygen atom in the glycerophosphorylcholines is achieved by the quasi six-membered ring formed by the O–C–C–N$^+$–C–H chain

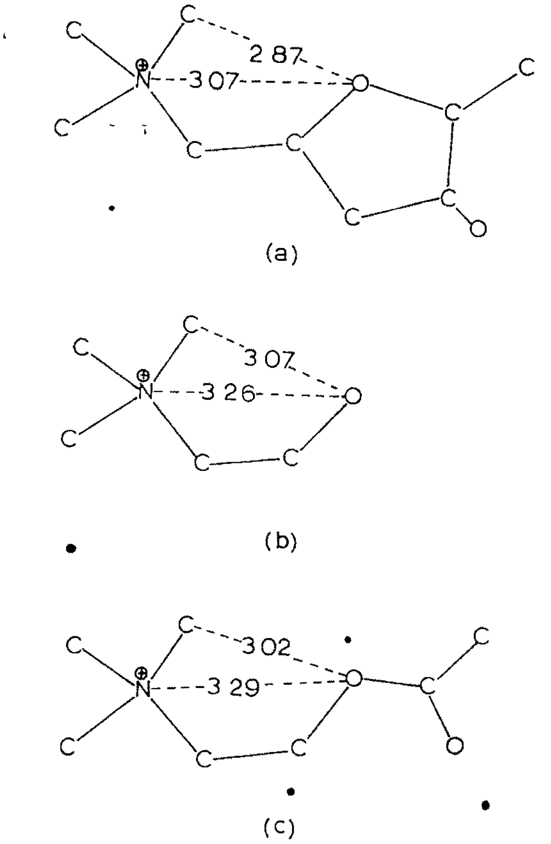

Fig 4 The *gauche* conformation of the O–C–C–N$^+$ system in muscarine and choline derivatives (*a*) muscarine iodide, (*b*) choline chloride and (*c*) acetylcholine bromide In all three compounds the organic residue is a cation rather than a zwitterion as found in the phospholipid components, ethanolamine phosphate, L-serine phosphate and L-α-glycerophosphorylcholine

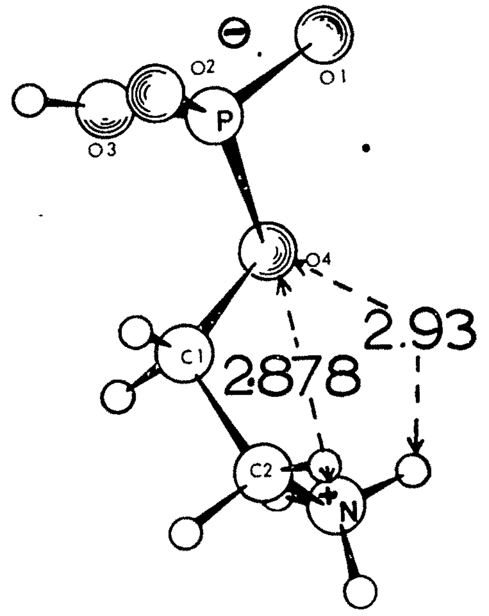

Fig 5 The *gauche* conformation in ethanolamine phosphate

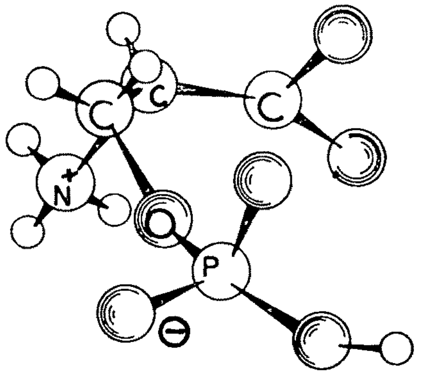

Fig 6 The *gauche* conformation in L-serine phosphate.

DL-Serine[13] also exists in the *gauche* form (Fig 7) Apparently the presence of the alpha carboxyl group in L-serine phosphate and DL-serine does not affect the preferred *gauche* conformation of the O–C–C–N$^+$ system Serine and threonine are the only amino-acids which possess the O–C–C–N$^+$ system While phosphatidylserine occurs abundantly in cell membranes, phosphatidylthreonine is not widespread in biological membranes, a surprising fact in view of the *gauche* arrangement of the O–C–C–N$^+$ system in the crystal structure of L$_S$-threonine[14] While φ_{CC} is negative (about −60°) in L-threonine, it is positive in L-serine phosphate The lack of occurrence of phosphatidylthreonine in cell membranes may be attributable to the presence of the beta methyl substituent in threonine, which might interfere with the conformation of the phospholipid, the packing of the phospholipid in the membrane, and the formation of the phospholipid–cholesterol complex in the membrane.

Although detailed structural information on the phospholipids is not yet available, it is very likely that their choline and ethanolamine components prefer the *gauche* conformation, at least in the solid state. Recently, Culvenor and Ham[15] have also found the *gauche* conformation for acetylcholine from studies of the proton magnetic resonance spectra of aqueous solutions of acetylcholine bromide. In the solid state this compound displays the enantiomeric *gauche* forms[8] by virtue of the presence of the glide plane of symmetry in the crystal.

Acetylcholine, a nerve transmitter, when released at nerve endings is rapidly hydrolysed by the readily available enzyme acetylcholinesterase, which is found associated with the cell membranes. The presence of the choline residue in the lecithins and sphingomyelins of membranes has prompted me to speculate that these membrane phospholipids may function as competitive inhibitors of the enzyme acetylcholinesterase (phosphorylcholine itself is a strong inhibitor of acetylcholinesterase[16]) and may play a part in the storage of this enzyme in cell walls, in which the cationic groups ($(CH_3)_3N^+-$) of the phospholipids may serve as binding sites[17] for acetylcholinesterase.

The release of acetylcholine in nerve endings is accompanied by an increase in the ion permeability of excitable membranes. This increase in ion permeation is probably correlated with the acetylcholinesterases detaching themselves from the cationic sites of the membrane phospholipids in order that they can now employ their cationic sites to carry out the hydrolysis of acetylcholine. The charged groups of the "freed" ($(CH_3)_3N^+-$ group of phospholipids not bound to cholinesterase) phospholipids are then available to take part in the transport of ions through the cell wall. In addition, the free phospholipids may undergo conformational changes in the vicinity of the charged groups that could also promote ion transport. When the acetylcholinesterase has completed the hydrolysis of acetylcholine, however, salt secretion is once again arrested. This may be interpreted as being caused by the "deactivation" of the phospholipids ($(CH_3)_3N^+-$ group of phospholipids bound to cholinesterase) by the re-formation of the cholinesterase–phospholipid complex. It must be remembered that the above is only one of the numerous processes that probably occurs during ion transport.

Apparently, the N^+–C–C–O system is one of the requirements for the membrane phospholipids. It will be of interest to investigate the effect of $N^+(C)_nO$ chain length on these phospholipids. It has not yet been established whether the *gauche* conformation (about $+60°$ or about $-60°$) of N^+–C–C–O is necessary for biological activity of the phospholipids. It is possible, however, that there is a critical distance between the positively charged nitrogen (cationic site) and the anionic phosphate oxygens (esteratic site) for phospholipid activity (see also refs. 18 and 19). If the N^+–C–C–O chain of the lecithins and cephalins occurs in the *gauche* form, this critical distance probably is achieved, and therefore permits the formation of the enzyme–substrate complex.

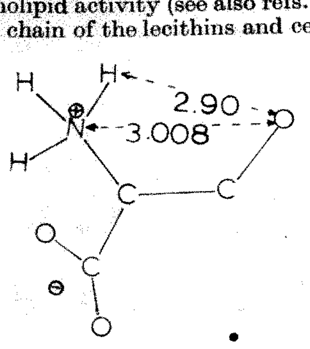

Fig. 7. The *gauche* conformation in DL-serine.

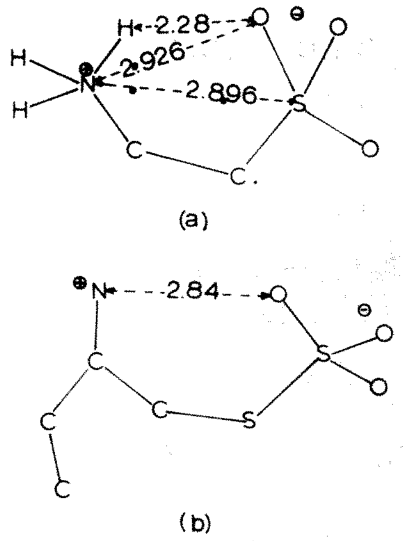

Fig. 8. The *gauche* conformation of the N^+–C–C–S system is stabilized by intramolecular N–H . . . O hydrogen bond in (*a*) 2-aminoethylsulphonic acid, and (*b*) *d,l*-2-aminobutylthiosulphuric acid.

The S–C–C–N^+ system in acetylthiocholine (private communication from E. Shefter and H. G. Mautner) and the Se–C–C–N^+ system in acetylselenocholine[2] display the *trans* conformation about the C–C bond. Increase in the nonbonded interactions, S . . . N^+ and Se . . . N^+, arising from the larger substituents (sulphur, selenium) and a decrease in the coulombic attractions (because sulphur and selenium are less electronegative than oxygen) are possibly responsible for the preferred *trans* conformation in these compounds.

It should be noted that the *gauche* conformation of the S–C–C–N^+ system in 2-aminoethylsulphonic acid[20] and 2-aminobutylthiosulphuric acid[21] (Fig. 8) is stabilized for a different reason, namely the presence of an intramolecular N^+-H . . . O^- hydrogen bond between the quaternary nitrogen and the anionic oxygen of the sulphonic acid and the thiosulphuric acid, thus forming a quasi six-membered ring zwitterion in the first and a quasi seven-membered ring zwitterion in the second.

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[1] Sundaralingam, M., and Jensen, L. H., *Science*, **150**, 1035 (1965).

[2] Shefter, E., and Kennard, O., *Science*, **153**, 1389 (1966).

[3] Sutor, D. J., *J. Chem. Soc.*, 1105 (1963).

[4] Abrahamson, S., and Pascher, I., *Acta Cryst.*, **21**, 79 (1966).

[5] Jellinek, F., *Acta Cryst.*, **10**, 277 (1957).

[6] Senko, M. E., and Templeton, D. H., *Acta Cryst.*, **13**, 281 (1960).

[7] Sörum, H., *Acta Chem. Scand.*, **13**, 345 (1959).

[8] Canepa, F. G., Pauling, P., and Sörum, H., *Nature*, **210**, 907 (1966).

[9] Stora, C., *Bull. Soc. Chim.*, **199**, 867 (1949).

[10] Stora, C., *Bull. Soc. Chim.*, **200**, 874 (1949).

[11] Kraut, J., *Acta Cryst.*, **14**, 1146 (1961).

[12] McCallum, G. H., Robertson, J. M. and Sim, G. A., *Nature*, **184**, 1864 (1959).

[13] Shoemaker, D. P., Barieau, R. E., Donohue, J., and Lu, C. S., *Acta Cryst.*, **6**, 241 (1953).

[14] Shoemaker, D. P., Donohue, J., Shoemaker, V., and Corey, R. D., *J. Amer. Chem. Soc.*, **72**, 2328 (1950).

[15] Culvenor, C. C. J., and Ham, N. S., *Chem. Commun.*, No. 15, 537 (1966).

[16] Tammelin, L. E., *Arkiv Kemi*, **12**, 287 (1958); *Svensk Kem Tidskr.*, **70**, 157 (1958).

[17] Wilson, I. B., in *The Enzymes* (edit. by Boyer, P. D., Lardy, H., and Myrbäck, K.), **4**, 501 (Academic Press, New York, 1960).

[18] Foldes, F. F., van Hees, G., Davis, D. L., and Shannor, S. P., *J. Pharmacol.*, **122**, 457 (1958).

[19] Wilson, I. B., and Quan, C., *Arch. Biochem. Biophys.*, **73**, 131 (1958).

[20] Okaya, Y., *Acta Cryst.*, **21**, 726 (1966).

[21] Okaya, Y., *Acta Cryst.*, **21**, A124 (1966).

Pathogenic Effects of Rubella Virus on Embryos and Newborn Rats

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In rats with congenital rubella, following maternal infection during pregnancy, the pathogenic effects and persistence of the virus after birth are remarkably similar to conditions prevailing in human infants with congenital rubella.

ALTHOUGH the rubella virus has been cultured *in vitro* and the methods for its propagation, identification and quantitation are available, the teratogenic effects of the virus and cellular pathological effects in various animal species *in vivo* have not been well established. Several attempts have been made to induce congenital rubella disease in animals. Sever and co-workers infected pregnant rhesus monkeys with rubella virus but found no evidence of malformation or infection in the foetuses which were delivered by caesarean section[1]. Experiments of Parkman *et al.* indicated a transplacental passage of the virus in monkeys and the possibilities of inducing a congenital rubella syndrome in animals[2]. There are, however, practical reasons for avoiding the monkey as an experimental animal. These are principally the problems of breeding and mating primates, the need for accurate determinations of the beginning of pregnancy, the length of the pregnancy (6 months) and the final surgical delivery of the babies. Rabbits, hamsters and ferrets have been injected with rubella virus during pregnancy and there was a decrease in the size and growth of the offspring[3]. In the series of experiments reported here, we found that the rat presents definite advantages for studies of experimental congenital rubella.

Pregnant albino rats, weighing 200–250 g, were obtained from the Hormone Assay Laboratories (Chicago, Illinois) and housed in individual cages. The day of pregnancy had already been established by vaginal smear at the supplying laboratories and found to be correct in more than 95 per cent of animals. The rats had a 3 week pregnancy period and the ova became implanted 4 days after fertilization. Approximately two hundred mother rats were used (thirty–forty rats in each experiment). The animals were injected intramuscularly in the hind legs with 0·25–0·60 ml. of a rubella virus suspension containing 10^3 $TCID_{50}$ (tissue culture infective dose) of the Bodak strain which was initially obtained from the Fifth Army Infectious Diseases Laboratories (St. Louis, Missouri), and propagated in $LLCMK_2$ cells (African green monkey kidney cells from Eli Lilly Company, Indianapolis, Indiana) which were cultured in roller tubes. Viral titrations and identifications were established before injection by the indirect interference techniques with Echo II virus developed by Parkman *et al.*[4]. Control rats had either been injected with heat inactivated virus or else had not been injected.

Rubella virus was identified in the tissues of embryos and newborn rats by the immunofluorescent technique of Woods *et al.*[5]. The embryos were obtained from 15 day pregnant mothers which had been injected on the fifth or sixth day of pregnancy as described. The embryos were frozen in liquid 'Freon' at −150° C, and serial frozen sections were processed. Alternate sections were exposed to human serum containing rubella antibodies (titre 1 : 32). All sections were incubated at 38° C with a 1 : 8 dilution of fluorescein-labelled purified goat antiserum to human gamma globulin. The tissue sections, as well as simultaneously processed tissue cultures which had been grown on cover slips and which had either been infected with rubella, or else were uninfected, were then examined with the ultraviolet Leitz microscope, and photographed on high speed 'Ektachrome *ASA* 160'. Viral antigens, when present in rubella infected cells, showed up as intense perinuclear and diffuse cytoplasmic fluorescence.

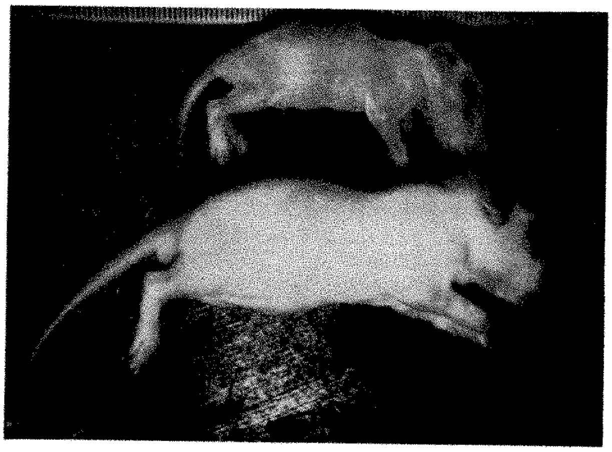

Fig. 1. 15 day old congenital rubella rat (top) and control rat of same age (bottom).

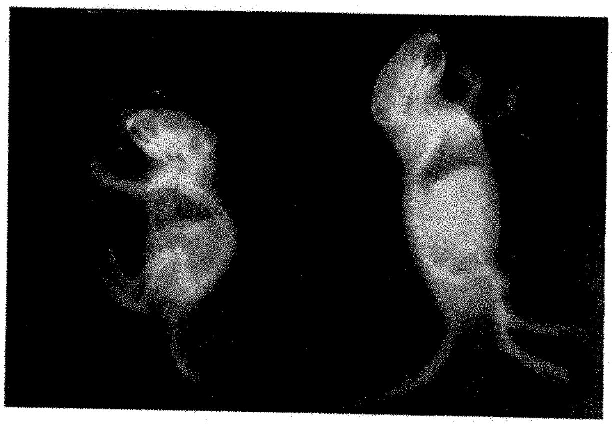

Fig. 2. Skeletal radiograph of rats in Fig. 1. Left: congenital rubella rat. Right: control rat.

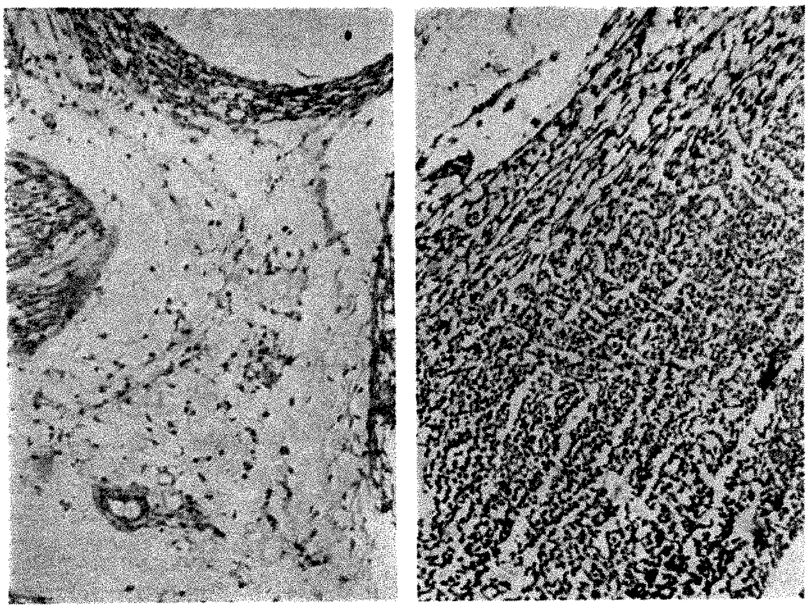

Fig. 3. Myocardial ventricular septum of (*A*) newborn congenital rubella rat and (*B*) newborn control rat (haematoxylin and eosin, × 200).

Controls and rats which had been injected with rubella were allowed to deliver. A most evident finding was the high incidence of neonatal deaths in the offspring of rats which had been injected with rubella, as compared with control rats. The results of four successive series of experiments are shown in Table 1. The intramuscular injection of rubella virus of the Bodak strain during the fifth and sixth day of rat pregnancy resulted in a high incidence of death of the offspring during the first month and a half after birth. Daily examination and measurements of the animals showed a marked decrease in the size and weight of the rubella offspring (weight 20–40 per cent of that of controls) (Fig. 1), and the X-rays showed alterations which were typical of retarded bone growth (Fig. 2). The rubella offspring were cyanotic (bluish abdominal skin) and extremely cachectic in the days preceding death. Rats with congenital rubella had purpura (10 per cent), opacities in the lenses (25 per cent) and obvious malformations such as open fontanelles (2 per cent) and missing hind legs (1 per cent). After either intramuscular injection or intramuscular and oral spraying, the mother rats did not show signs of rubella rash or lymphadenopathy and moved about the cages in the same way as their corresponding controls.

The rats with congenital rubella showed pathological changes in the intraventricular septum of the hearts and in the crystalline lenses and retinae of the eyes: intraventricular septa were markedly thin and atrophic (Fig. 3*A* and *B*), and infected crystalline lenses showed destruction of the lens fibres and epithelial cells, necrotic areas in the centre of the lens and vacuolation of the cortical areas. The pathological condition of the lens was similar to that described in the lenses of human rubella syndromes by Boniuk and Zimmerman[6]. As seen by electron microscopy, some lens cells had lost all electron opaque DNA and contained a nucleus partially filled with debris, while others had marked "clumping" of DNA material (Figs. 5 and 6). Although the magnification which was obtained did not allow definite statements, isolated, round viral-like particles of approximately 500 Å in diameter could be identified among nuclear debris. Immunofluorescent techniques showed that rubella virus was present in crystalline lens, heart and retina of embryos in newborn rats (Fig. 4). Other tissues from newborn animals were negative.

Table 1. MATERNAL INFECTIONS WITH RUBELLA VIRUS (BODAK STRAIN) AND RATE OF NEONATAL DEATH

Pregnancy day	Dose		Newborns surviving at the indicated days after delivery: 3 days	45 days	Percentage death
		Controls:	80	**72**	**10**
5	0·1 ml. IM		46	23	50
	0·2 ml. IM		93	54	42
	0·4 ml. IM		20	5	75
		Total	159	82	48·5
6	0·1 ml. IM		51	49	4
	0·2 ml. IM		68	40	41
	0·3 ml. IM		38	22	42
		Total	157	111	29
8	0·2 ml. IM + 0·2 ml. oral		15	15	0
9	0·2 ml. + 0·2 ml. oral		23	19	18
11	0·2 ml. IM + 0·2 ml. oral		25	25	0

IM, Intramuscular injection.

The surviving litters of rats with congenital rubella were of smaller size and weight. The histology of the kidneys, livers, brains, lungs, bones and eyes of these animals was normal. No additional deaths were recorded among the litters 45 days after birth.

Oxford has described the growth of rubella virus in small laboratory animals, and suggested that it might be

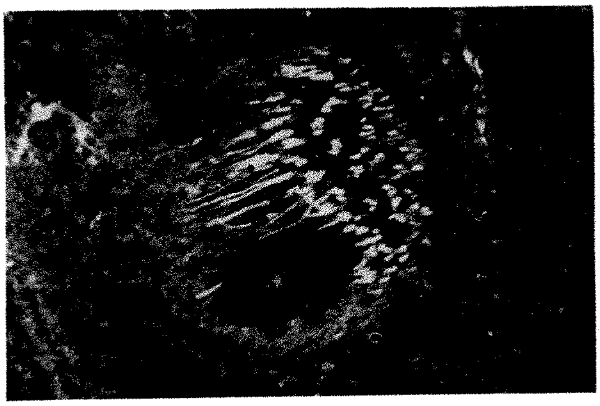

Fig. 4. Section through lens and optic vesicle of 15 day old rat embryo infected with rubella virus at day 6 of gestation. Fluorescence of rubella virus in nuclei and elongating epithelial cells of the lens vesicle (× 200).

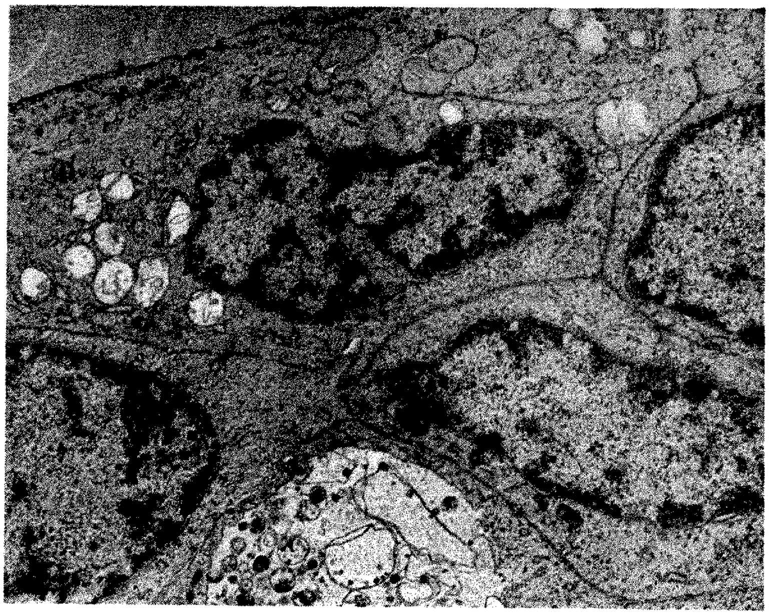

Fig. 5. Lens epithelium of 14 day old rubella rat. In the upper left area: the intact lens capsule. Below and centre: a cell showing destroyed nuclear DNA. Other epithelial cells are not infected (× 15,000).

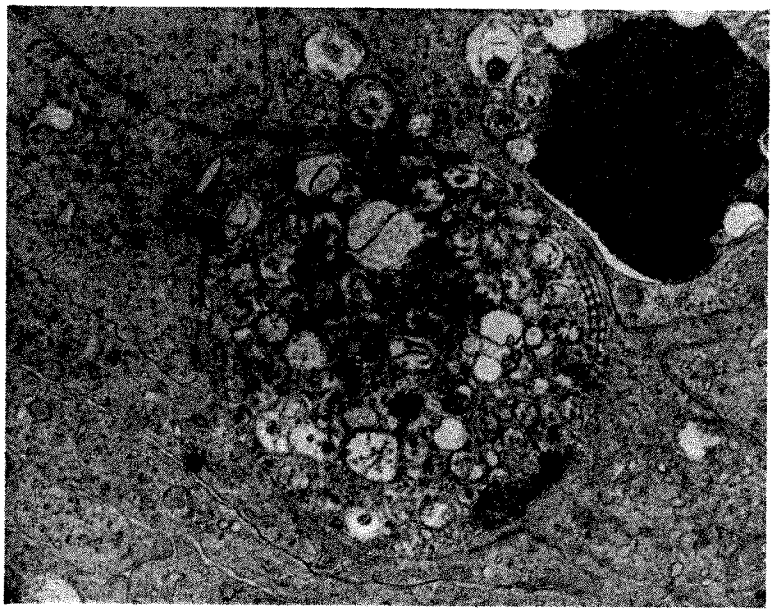

Fig. 6. Destruction and "clumping" of DNA in the nuclei of two equatorial lens fibres (× *c*. 18,670).

used for studies of the teratogenic effects of the virus[7]. In rats with congenital rubella the pathogenic effects and persistence of virus after birth are remarkably similar to those described in human infants with congenital rubella. The prominent clinical signs in the human disease are the intraventricular septal defect, cataracts, retinopathy and deafness[8]. Of interest is the specific pathogenic effect of the rubella virus on the nuclear DNA of isolated lens epithelium cells. It would seem that the virus infection and destruction of the genetic apparatus of these cells are in some way related to the decreased rate of replication of cells and size of organs infected by the virus. Plotkin and Vaheri have reported on the inhibition by rubella virus of growth and cell count of normal human diploid cells which were cultured *in vitro*. In their studies, the antimitotic activities on rubella virus infected cells were exerted on DNA synthesis as shown by a cumulative reduction of cells taking up tritiated thymidine[9].

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[1] Sever, J. L., Meier, G. W., Windle, W. F., Schiff, G. M., Monif, G. R., and Fabiyi, A., *J. Infect. Dis.*, **116**, 21 (1966).

[2] Parkman, P. D., Phillips, P. E., and Meyer, H. M., *Amer. J. Dis. Child*, **110**, 390 (1965).

[3] Belcourt, R. J. P., Wong, F. C., and Walcroft, M. J., *Canad. J. Publ. Hlth.*, **56**, 253 (1966).

[4] Parkman, P. D., Buescher, E. T., Artenstein, M. D., *Proc. Soc. Exp. Biol. and Med.*, **111**, 225 (1962).

[5] Woods, W., Johnson, R., Hostetler, D., Lepow, M., and Robbins, F., *J. Immunol.*, **96**, 253 (1956).

[6] Boniuk, M., and Zimmerman, L. F., *Arch. Ophthalmol.*, **77**, 455 (1967).

[7] Oxford, J. S., *J. Immunol.*, **98**, 697 (1967).

[8] Krugman, S. J., *Pediat.*, **67**, 159 (1965); Korones, S. B., Ainger, L. E., Monif, G. R., Roane, J., Sever, J. L., and Fuste, F. J., *Pediat.*, **67**, 166 (1965). Cotlier, E., and Fox, J., in *Current Concepts in Ophthalmology* (edit. by Becker, B., Drews, R., and Mosby, C. V.), 178 (1967).

[9] Plotkin, S. A., and Vaheri, A., *Science*, **156**, 659 (1967).

LETTERS TO THE EDITOR

ASTRONOMY

Radio Sources in Opposite Directions as a Cosmological Test

FROM the observed distribution of quasi-stellar sources versus red-shift an excess of sources with red-shifts between 1·9 and 2·1 is noticed. Petrosian *et al.*[1] showed that this effect may be explained in a cosmological model where the pole is in this red-shift range.

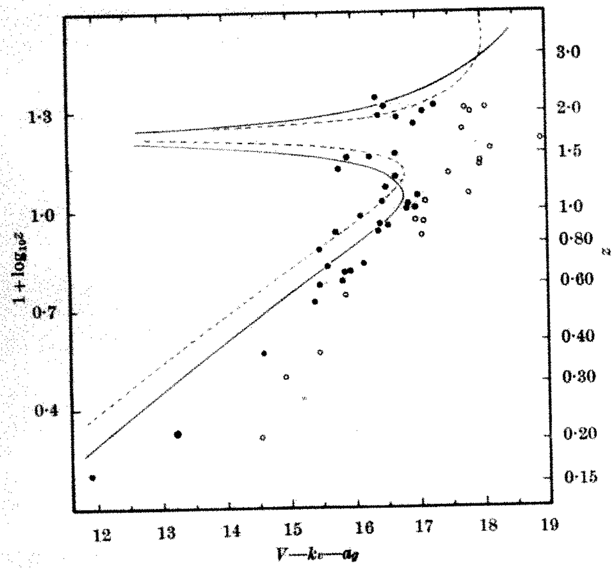

Fig. 1. Magnitude–red-shift relations for brighter quasars. The magnitudes are corrected for K-correction[3], k_v, and galactic absorption (unpublished work of G. de Vaucouleurs and G. Malik), a_g, assuming 0·25 mag. absorption at the galactic pole. The black dots represent the brightest quasars which may represent a class of objects with a standard absolute magnitude. The selection is arbitrary; a few of the fainter quasars are shown as open circles. The two models shown are based on a Hubble parameter $H_0 = 100$ km sec^{-1} Mparsec^{-1}, q_0 is the deceleration parameter and σ_0 a density parameter, $\sigma_0 = 4\pi G\varrho/3H_0^2$ where ϱ_0 is the mean density of the universe. Models: ——, $q_0 = -1·5$, $\sigma_0 = 0·132$, $M_v = -27·3$; – – – –, $q_0 = -1·4$, $\sigma_0 = 0·085$, $M_V = -27·8$.

In Fig. 1 the red-shifts and magnitudes are shown for the brighter quasars. The data are from a list compiled by Barbieri *et al.*[2] and the magnitudes plotted are corrected for K-correction[3] and for absorption in our own galaxy (unpublished work of G. de Vaucouleurs and G. Malik). Two theoretical models with a pole for z about 1·7 are shown which are in good agreement with the observations.

Models where the pole has a red-shift of the order 2–3 or less suggest an interesting cosmological test—that is, the possibility of observing the same object in two opposite directions in the sky. The question discussed here is the possibility that sources in the 4*C* radio catalogue[4] may show this effect.

The 4*C* radio catalogue contains one part between declination −7° and +7° where it is possible to compare positions 180° apart in the sky. In the following the notation R is used for a test of sources 180° apart and C_1 and C_2 for two control tests (areas opposite in declination or opposite in right ascension only).

In the catalogue, we have mean errors in position up to 12′ in declination for the fainter sources. This may give mean separations up to 14′ on sources which are exactly opposite. If we have a cosmological model as shown in Fig. 1, we may see two images of the same object at different epochs. The time difference may be of the order 10^9 years. If the object has a proper motion of 50–100 km/sec, which is found for ordinary galaxies[5], this will easily make a separation of the two images of the order 10′ because of the enlargement of apparent size close to the pole. If double image objects could be observed, we should then expect them to show a characteristic separation of at least 10′ from exact opposite positions.

In the project described here, pairs with separations up to 75′ have been investigated. The results are shown in Fig. 2 for R and the two control areas C_1 and C_2. The quantity plotted is the number density of sources in different rings of separations or N/a, where N is the number of sources and a is the separation. The pair count is performed for all sources in the strip of the catalogue and also for faint and bright sources separately. Pairs for which the total flux is less than 6·35 flux units are considered as faint. This represents about half the pairs.

In Fig. 1 the expected pair densities are shown as broken lines. The slopes of these lines are explained by

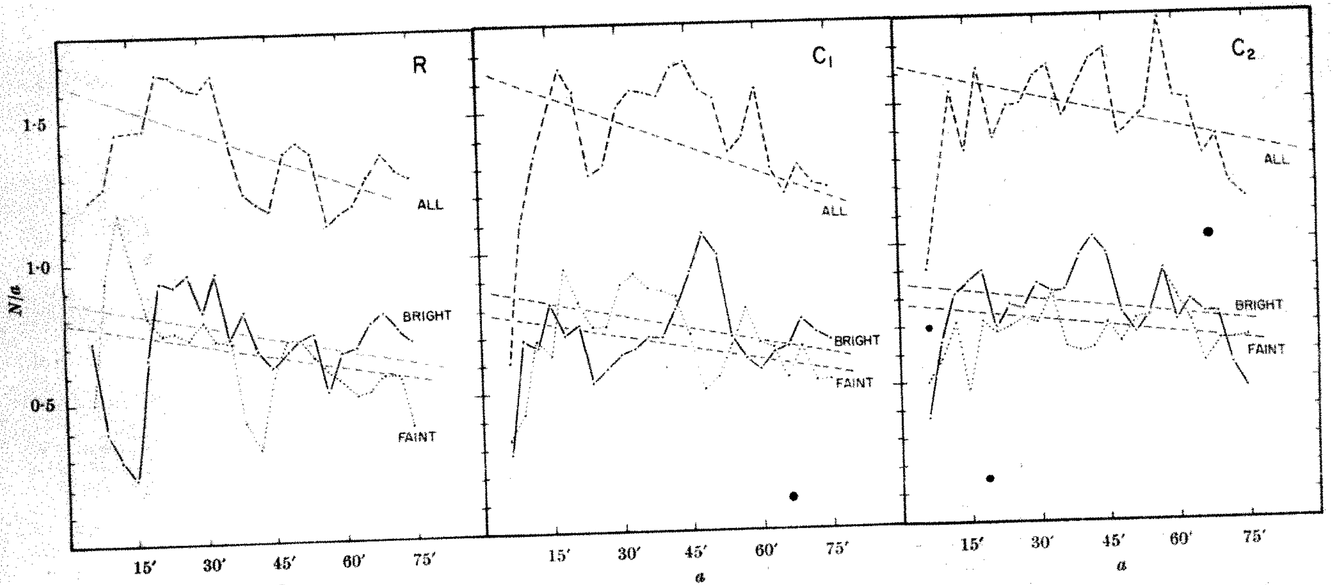

Fig. 2. The surface density (N/a) for the three different test areas shown as a function of the separation a. The distributions of the brighter and fainter pairs are also shown. The broken lines indicate the expected distribution. The separation $a = 3′$ is used as a unit.

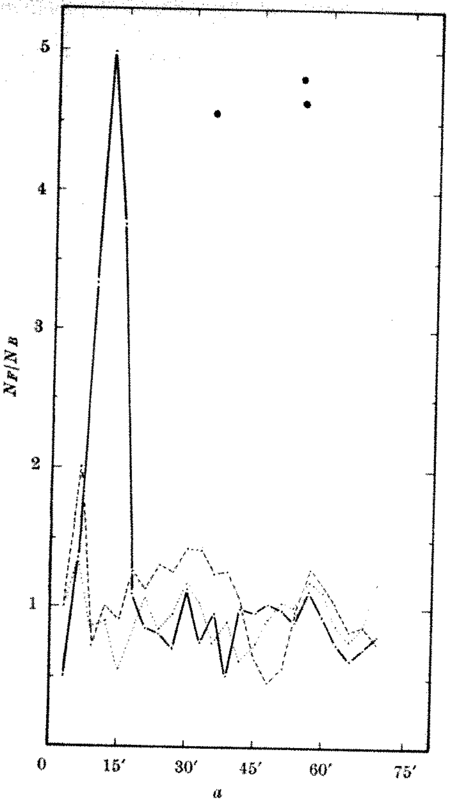

Fig. 3. The ratio of faint to bright pairs in the three different test areas. ----, C_1; ····, C_2; ——, R.

the loss of possible pairs at the boundaries of the areas investigated. Areas of confusion, as listed in the catalogue, are excluded as are sources less than ± 10° from the galactic plane. The figure shows that the distribution of all the pairs together is what might be expected from a random distribution. When we study the distribution of the faint and the bright pairs separately, we note a difference in R for separations less than 15′. We find an excess of faint close pairs and a deficiency of bright close pairs in R. The deficiency of bright sources in R is somewhat more pronounced than in C_1 and C_2, but may not have any special significance. The excess of faint sources in R may be significant, if real.

The difference between the tests R and C_1 or C_2 is evident in Fig. 3, which shows the proportion of faint to bright pairs at different separations. The effect demonstrated is the combined result, in the area R, of the excess of faint sources and the deficiency of bright sources with small separations. The excess of faint sources in R is concentrated in the range $10' < a < 16'$. Comparing the counts with the expected number of pairs in this range for a random distribution, we find an excess of 5·8 ± 2·3 faint pairs. The probability of this excess happening by chance is 0·012. If we instead compare the count in R with the actual observed distribution of faint pairs in C_1 and C_2, we find an excess of 6·5 ± 2·3 faint pairs. The probability of this departure is 0·006. We conclude that, at the 98–99 per cent confidence level, there is evidence for an excess of faint sources in almost exactly opposite positions as given in the 4*C* radio catalogue.

If this effect is real, possible explanations may perhaps be found in some properties of the distribution of faint radio sources such as clustering effects, or perhaps in unrecognized systematic errors of position determinations in the catalogue. I prefer, however, to suggest that the effect is the result of the well known cosmological property that in some closed universe models it should be possible to see the same object in two opposite directions.

In closed universe models, in which the pole is within the particle horizon[6], information can be received from objects beyond the pole; such objects should be observable in two nearly opposite directions with suitable instruments. For this purpose we require objects such as radio galaxies or quasars with a lifetime of the order of the difference in travel time between the two opposite light paths (about 10^9 years). Disturbances of the light rays caused by passage through clusters of galaxies may introduce a small displacement of the two images with respect to each other and may also cause blurring[1] in addition to the displacement from proper motion described here.

The observed excess of faint pairs is in the range of separations from 10′ to 15′, the mean separation being 13′·2 ± 2′·9. The chief component of this separation may be the result of errors in determining positions, but may also contain a component indicating a real position difference of the order 5′ to 10′ linked with the effects mentioned here.

In uniform relativistic world models, the observation of the same object in opposite or nearly opposite positions would indicate rather specific values of the cosmological parameters q_0 and σ_0 (ref. 7, Fig. 8). The red-shift of the pole cannot be much greater than 2 if we are to observe this effect, which restricts the value of q_0 to close to −1·5 and σ_0 of the order 0·1 if we also have the magnitude–red-shift distribution of quasars in mind (Fig. 1). This is not in contradiction with the values obtained by testing red-shift–magnitude relations for galaxies in clusters when evolutionary effects are taken into account[9].

As a check on the reality of the interpretation presented here, we expect that in the 4*C* data a characteristic separation would show up more clearly in right ascension than in declination because the positions are more accurate in right ascension. Fig. 4*a* shows the distribution in total separation of the closest nineteen pairs at the fainter part of the catalogue, and Fig. 4*b* and *c* shows the distribution of their separations in right ascension ($\Delta\alpha$) and in declination ($\Delta\delta$). By comparing the distributions as shown in these figures we find, as expected, more pronounced peaks in the ($\Delta\alpha$) distribution at separations of about 5 and 10 minutes, while the peak in ($\Delta\delta$) distribution is centered around zero separation, which indicates that a significant separation is lost because of the inaccuracy in this co-ordinate.

In an attempt to identify optically the suspected double image pairs, priority should be given to all eleven opposite pairs with a separation 10′ to 15′. If they have a displacement in both right ascension and declination corre-

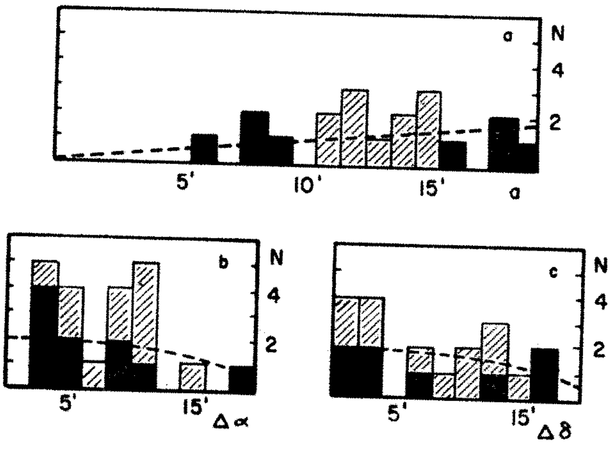

Fig. 4. *a*, The detailed distribution of close pairs in *R*. The excess seems to be concentrated in the separation range 10′–15′ (shaded). *b* and *c*, The same objects distributed due to the separation in right ascension ($\Delta\alpha$) and declination ($\Delta\delta$). The shaded objects are the same as in Fig. 4*a*. Expected distributions are shown as broken curves.

Table 1 MOST PROBABLE REAL OPPOSITE PAIRS

Priority 1	Priority 2	Priority 3
4C–04 02, 4C04 42	4C–05 08, 4C05 58	4C–02 62, 4C02 09
4C–04 07, 4C04 48	4C–04 58, 4C04 14	
4C–04 34, 4C04 77	4C–05 81, 4C05 32	
4C–03 40, 4C03 53	4C–05 87, 4C05 39	
4C–02 57, 4C02 03	4C–01 60, 4C01 29	

sponding to the excess distribution in these co-ordinates, we give priority 1 If they have excess in only one of the co-ordinates we give priority 2, and in none priority 3 The eleven pairs can then be sorted as shown in Table 1 Obtaining optical identifications of some of these pairs for closer study would enable us to decide if the observed effect is cosmological In particular, red-shifts of both members of pairs must satisfy a unique relation in order to satisfy the cosmological explanation

A programme for optical identification has been started at McDonald Observatory for a few of the most promising pairs, but it is likely that better radio positions will be necessary before any positive identifications can be expected

I thank G de Vaucouleurs for valuable suggestions

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[1] Petrosian, V , Salpeter, E , and Szekeres, P , *Astrophys J* , **147**, 1222 (1967)

[2] Barbieri, C , Battistini, P , and Nasi, E , *Pubbl Osserv Astro Padova*, *N* 141 (1967)

[3] Sandage, A , *Astrophys J* , **146**, 13 (1966)

[4] Gower, J F R , Scott, P F , and Wills, D , *Mem Roy Astro Soc* , **71**, 49 (1967)

[5] de Vaucouleurs, G , *Ap J* , **63**, 262 (1958)

[6] Rindler, W , *Mon Not Roy Astro Soc* , **116**, 662 (1956)

[7] Refsdal, S , Stabell, R , and deLange, F G , *Mem Roy Astro Soc* , **71**, 143 (1967)

[8] Tinsley, B , thesis, Univ Texas (1967), *Astrophys J* (in the press)

Nova Model of Centaurus *XR*-2

RECENT observations of the Centaurus region[1–3] have firmly established the existence of a variable X-ray source, Centaurus *XR*-2 This source is unique in that its variability has been established beyond doubt[2–4]

Francey *et al* [4] compared their data from two rocket flights in April 1967 with those of Chodil *et al* [2] obtained in May, 44 days later They found the data to be consistent with monotonic cooling of a thin plasma source from 4 2 ± 0 6 × 10⁷ °K to 1 8 × 10⁷ °K, accompanied by a five-fold decrease in the 2–8 keV energy flux during this period Data presented by Cooke *et al* [3] suggest a somewhat faster decrease

These observations, together with the assumption (supported by the detailed energy spectrum of Chodil *et al* [2]) of an optically thin X-ray source, suggest a model of the cooling X-ray source which is satisfied by the physical conditions expected[5,6] in a circumstellar envelope shortly after a nova outburst

At the observed temperatures, the radiation originates principally in electron proton bremsstrahlung The spectral intensity, j_E, at distance d from an isothermal thin plasma of volume V, containing n_e electrons/cm³, is then given by[7,8]

$$j_E\,\mathrm{d}E \sim (1\times10^{-28})\left(\frac{n_e{}^2V}{d^2}\right)T^{\frac{1}{2}}\left(\frac{\mathrm{e}^{-E/kT}}{kT}\right)\mathrm{d}E \text{ ergs/cm}^2\text{ sec}$$

where we take the gas to have the normal chemical abundances Using this relation we find that $(n_e{}^2V/d^2)$, initially about 5×10^{17} cm⁻⁵, had decreased by a factor of about 2 at the time of the Lawrence Research Laboratory rocket flight[2] 44 days (4×10^6 sec) later Assuming that the mass of radiating gas was conserved during this period, this at once implies a polytropic gas expansion, $TV^{\gamma-1}$ equal to a constant, with exponent γ equal to $2^{+1}_{-0\ 5}$. Although this is consistent with steady adiabatic expansion (γ = 5/3), isothermal motion (γ = 1), as proposed by Manley[9], is clearly at variance with the observations Tucker[6] has pointed out the difficulty of maintaining steady gas expansion through gravitational constraint at these high temperatures It seems highly probable therefore that the expansion was explosive and that the X-radiation originated in a shell of shock heated gas Before showing that the time decay of the source can be explained naturally in terms of an adiabatic blast wave propagating radially out through a circumstellar gas envelope, we note the following orders of magnitude

The gas temperature, (1 5–4) × 10⁷ °K, limits the radial expansion velocity to v about 10⁸ cm/sec Also, the mass conserving (n_eV = constant) volume change sets a radial expansion $\Delta R/R$ of order one over a period of 4×10^6 sec When first seen on April 4, the source was therefore no more than a month or so old, having expanded to a radius R_1 of about 5×10^{14} cm (30 astronomical units) An upper limit to the mean electron density of $n_e \leqslant 5\times10^8$ cm⁻³ then follows from the extreme assumption that the plasma cooled by radiation alone Taking $n_e = 10^8$ cm⁻³, we find a total mass (n_em_HV) of about 10²⁹ g (5×10^{-5} $m_\odot$) and an emission measure, $n_e{}^2V$, of about 4×10^{60} cm⁻³ on April 4 The thermal energy content, $3n_ekTV$, circa 10⁴⁵ ergs, and luminosity, L, ~ $10^{-27}T^{\frac{1}{2}}\,n_e{}^2V$, circa 10³⁷ ergs/sec, give a radiative life time of about a year and the distance to the source (from $n_e{}^2V/d^2$, ~ 5×10^{17}) becomes about 1 kparsec This distance may be regarded as an upper limit because there is no need to invoke significant radiative cooling, and the electron density may well be considerably lower than 10⁸ cm⁻³

These parameters are typical of those deduced for recurrent novae by Wallerstein[5] from coronal line measurements We now use the blast wave solutions of Rogers[10] in order to find a more detailed model of the expansion The cooling data for Centaurus *XR*-2 show that any such model must conserve the quantity $(n_e{}^2V/T)$ during the expansion This condition is satisfied for an adiabatic strong shock which propagated radially outward through a non-uniform gas envelope in which the density

$$n \propto r^{-\alpha} \text{ with } \alpha = 2$$

The shock wave solutions give the radius, R, of the shock front at time t as

$$R = kt^{1/\lambda}$$

and the velocity of the shock as

$$v = R/\lambda t$$

where λ is an expansion parameter which for an adiabatic shock is related[10] to α by

$$2\lambda = 5 - \alpha$$

Because $n_e{}^2 \propto R^{-2\alpha}$, $V \propto R^3$ and $T \propto v^2 \propto R^{2(1-\lambda)}$, we have $n_e{}^2V \propto T$ as required from the data, providing that

$$2\lambda = 2\alpha - 1$$

Thus

$$\alpha = 2$$

and

$$\lambda = 3/2$$

With this solution, the density behind the shock is a linear function of the radial distance from the origin of the explosion, increasing to $(\gamma+1/\gamma-1) = 4$ times the pre-shock envelope density at the shock front. The gas velocity similarly increases linearly with radial distance up to the shock boundary The emission measure, $n_e^2 dV$, falls off rapidly behind the shock front (as $(r/R)^4$), and we may take the effective X-radiator to be a nearly isothermal shell of thickness ΔR, about $R/4$, and volume $2 5R^3$, having the density, temperature and radius of the front

Because the 2–8 keV energy flux has suffered a six-fold decrease and the temperature, T, a 2 3-fold decrease in 4×10^6 sec, we find from $n^2 V \propto T \propto R^{-1}(t^{-2/3})$ that the shock wave would have been about 2 weeks old when first seen by the Universities of Adelaide and Tasmania group The shock wave temperature at that time determines an expansion velocity v of about 2×10^8 cm/sec and a corresponding shock radius of $R = \lambda tv$, about 3×10^{14} cm This would have increased to about 8×10^{14} cm when the source was seen by Chodil *et al* [2] The distance to the source becomes d about 300 parsec for $n_e = 10^8$ cm^{-3} and the energy of the blast, E about 10^{44} ergs

If the source is indeed an average nova at this distance, we might expect it to have reached a brightness greater than 4^m in March or April 1967 Some additional support for the nova hypothesis comes from the optically observed deceleration of nova envelopes[11] in which $v^2 \propto 1/R$ as in the present model of the X-ray source

In conclusion we note that the total X-ray luminosity decreases faster than t^{-1} even without radiative cooling, and that, 10^7 sec after the initial observation, the energy flux above 2 keV would have decreased by nearly two orders of magnitude

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[1] Harries, J , McCracken, K G , Francey, R J , and Fenton, A G , *Nature*, 215, 38 (1967)

[2] Chodil, G , Mark, H , Rodrigues, T , Seward, F , Swift, C D , Hiltner, W A , Wallerstein, G , and Mannery, E J , *Phys Rev Lett* , 19, 681 (1967)

[3] Cooke, B A Pound, K A , Stewardson, E A , and Adams, D J , *Ap J* (in the press)

[4] Francey, R J , Fenton, A G , Harries, J R , and McCracken, K G , *Nature*, 216, 773 (1967)

[5] Wallerstein, G , *Pub Astro Soc Pacific*, 73, 153 (1961)

[6] Tucker, W H , *Ap J* , 148, 3 (1967)

[7] Karzas, W J , and Latter, R , *Ap J* , 6, suppl , 55, 167 (1961)

[8] Tucker, W H , and Gould, R J , *Ap J* , 144, 1, 244 (1966)

[9] Manley, O P , *Phys Rev Lett* , 19, 19 (1967)

[10] Rogers, M H , *Ap J* , 125, 478 (1957)

[11] Friedjung, M , *Mon Not Roy Astro Soc* , 132, 317 (1966)

Extinction by Interstellar Silica Grains

INTEREST has recently been expressed in the possibility that the interstellar dust contains a silica component (ref 1, and R C Gilman, private communication) The purpose of this communication is to point out that silicon dioxide has strong absorptions in the 8–14μ atmospheric "window" which should be observable with ground-based infrared telescopes We have computed normalized extinction spectra (extinction cross-section divided by geometrical cross-section) for crystalline quartz and vitreous silica

The refractive index of quartz varies between 1 52 and 1 57 in the visible region of the spectrum To determine approximately the size of interstellar silica grains, we have compared the Mie calculation of Lowen[2] for $m = 1 55$ with the extinction curve of Boggess and Borgman[3] between 0 3 and 1 5μ A particle of radius 0 2μ gives agreement to 15 per cent over this range Although a single size model is probably too simple to describe the interstellar dust completely, this size should at least be indicative of the real situation

The extinction in the infrared by quartz spheres of radius a can be calculated by a generalization of the expressions given by van de Hulst[4]

$$Q_{\text{sca}} = \frac{8x^4}{9}\left[2\left|\frac{m_o^2-1}{m^2+2}\right|^2 + \left|\frac{m_e^2-1}{m_e^2+2}\right|^2\right] \qquad (1)$$

$$Q_{\text{abs}} = -\frac{4x}{3}\, Im\left[2\left(\frac{m_o^2-1}{m_o^2+2}\right) + \left(\frac{m_e^2-1}{m_e^2+2}\right)\right] \qquad (2)$$

where m_o and m_e are the ordinary and extraordinary complex refractive indices and $x = 2\pi a/\lambda$ For vitreous silica we have included terms in x^3 and x^4 in Q_{abs}, as given by van de Hulst (ref 4, p 270) Optical constants were obtained from refs 5–7 for quartz and from refs 5, 6 and 8 for vitreous silica The imaginary part of the refractive index between 2 7 and 7μ for both cases was calculated from the absorption data of Drummond[6] The results give a mismatch between these optical constants and those above 7μ

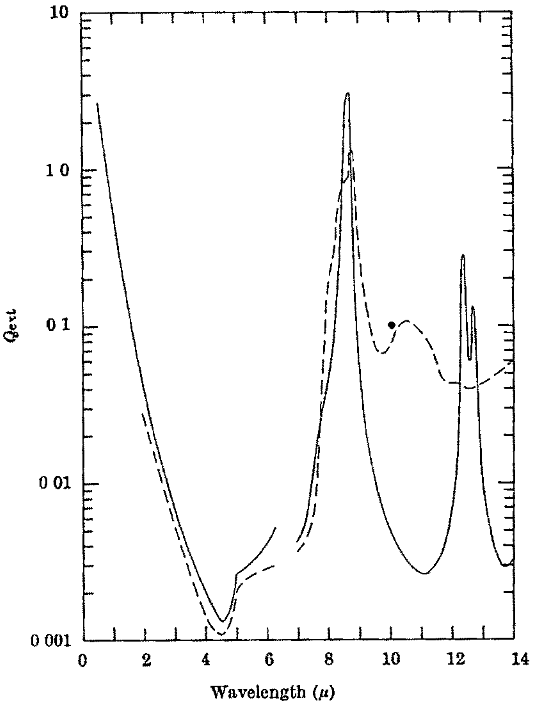

Fig 1 Normalized extinction cross-section for particle radius of 0 2μ ——, Quartz, - - -, vitreous silica

Results are shown in Fig 1, where Q_{ext} ($Q_{\text{sca}} + Q_{\text{abs}}$) is plotted against wavelength for particle radius $a = 0 2\mu$ Below 2μ where x becomes large, Q_{ext} is taken from the Mie extinction calculation of Lowen for $m = 1 55$ For quartz, the extinction at 8 7μ is 60 per cent of that at 0 6μ

Quartz is strongly dichroic in the double absorption at 12 6μ The ordinary ray is absorbed at 12·7μ and the extraordinary at 12 4μ This feature, apparently a property of the long range order of the crystal, is not evident in the vitreous phase The position of the absorption at 8 7μ is noteworthy The imaginary part of the refractive index has maxima at 9 3 and 9 2μ for the ordinary and extraordinary rays of quartz, but the extinction by small spheres is greatest at 8 7μ At this wavelength the real part of the dielectric constant m^2 approaches -2, so that there is a resonance in equation (2) A similar effect in metallic spheres has been described by Unsold[9]

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[1] Dorschner, J, *Astro Nach* (in the press)
[2] Lowen, A N, *Nat Bur Stand Appl Math Ser*, 4 (1949)
[3] Boggess, A, and Borgman, J, *Ap J*, 140, 1636 (1964)
[4] van de Hulst, H C, in *Light Scattering by Small Particles* (J Wiley, 1957)
[5] *Amer Inst Phys Handbook* (McGraw-Hill, New York, 1963)
[6] Drummond, D G, *Proc Roy Soc*, 153, 328 (1935)
[7] Spitzer, W G, and Kleinman, D A, *Phys Rev*, 121, 1324 (1961)
[8] Simon I, and McMahon, H O, *J Chem Phys*, 21, 23 (1953)
[9] Unsold, A, *Pub Roy Obs Edinburgh*, 4, 35 (1964)

PLANETARY SCIENCE

Precipitated Electrons as a Cause of the Irregular Behaviour of the F_2 Region

It has been established that electrons precipitated from the outer radiation belt produce ionospheric disturbances[1-3] Most of these were found to involve decreases in f_0F_2, suggesting that the fluctuations in precipitated electron flux will produce a spread in f_0F_2 values about the hourly means We therefore compared latitudinal and longitudinal variations in precipitated electron flux with similar variations in the root-mean-square deviation of f_0F_2

For the longitudinal analysis, a number of ionosphere stations lying along $L=4$ in the southern hemisphere were selected For the latitudinal analysis, five southern hemisphere stations lying over a fairly narrow range in longitude (about 140° E to 170° E) and extending from $L=1\ 5$ to $4\ 0$ were used The latter stations lie well outside the South Atlantic geomagnetic anomaly and so are not affected by this

Using the data for the three months November, December 1962 and January 1963, the root-mean-square deviation of f_0F_2 was calculated for each hour for the selected stations It sometimes happens that a value for f_0F_2 cannot be obtained In the event of a record being lost because of a total blackout, f_0F_2 was regarded as being at least as small as $f_{\min}$, which was estimated by interpolation In cases where the measurement of f_0F_2 was influenced by spread—F, absorption, atmospherics, etc — the observations were omitted from the count in calculating the root-mean-square deviation.

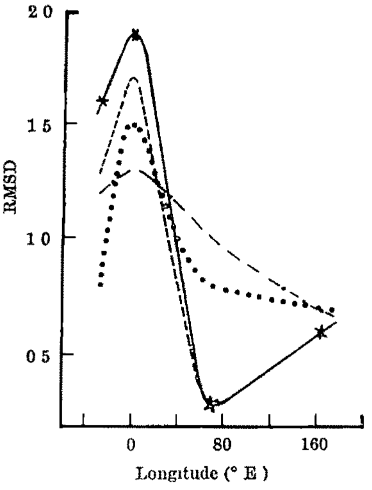

Fig 1 Longitudinal variations of the root-mean-square deviation (RMSD) of f_0F_2 (Mc/s) at different times of day ———, 00 LT, - - - , - - 06 LT, , 12 LT, — —, 18 LT

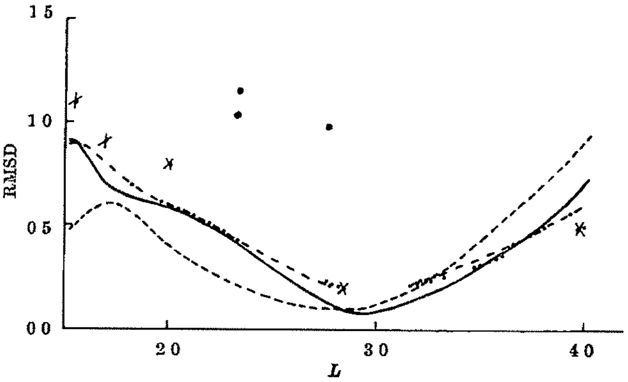

Fig 2 Variation of the root-mean-square deviation (RMSD) of f_0F_2 (Mc/s) with L at different times of day ———, 00 LT, - - - , 06 LT, , 12 LT, — —, 18 LT

Fig 1 shows a plot of the root-mean-square deviation against longitude for four southern hemisphere stations on $L=4$. The stations are Halley Bay (26 6° W.), Sanae (2 3° W), Kerguelen (70 2° E) and Campbell Island (169 1° E) Sanae and Halley Bay lie in the region where high intensities of charged particle fluxes have been observed[4,5] It is known that the intensity of precipitation of electrons is inversely dependent on B, the total geomagnetic intensity On $L=4$, B reaches its lowest surface value in the vicinity of Sanae We therefore expect precipitation of electrons to be greatest in this region and Fig 1 shows that the root-mean-square deviation is also greatest in this region.

Fig 2 shows a plot of root-mean-square deviation against L The stations in this case are Brisbane ($L=1\ 55$), Woomera ($L=1\ 70$), Canberra ($L=2\ 0$), Hobart ($L=2\ 85$) and Campbell Island ($L=4{\cdot}0$) There is a decrease in root-mean-square deviation at $L=3$ corresponding to the decrease in precipitated electron flux which has been observed at the same shell[6-8]

A further point may be briefly speculated on here. It can be seen in Fig. 1 that the curve for midnight shows a far more pronounced longitudinal variation than that for mid-day It has recently been suggested[9-12] that atmospheric winds result in ionosphere drifts having a vertical component which is usually downward during the day and upward during the night It is therefore possible that at night the ionization produced by the incoming electrons be blown upward into a low loss rate region, magnifying the effect

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[1] Gledhill, J A, and Torr, D G, *Space Res*, 6, 222 (1965).
[2] Gledhill, J A, Torr, D G, and Torr, M R, *J Geophys Res*, 72, 208 (1967)
[3] Sharp, G W, Imhof, W L, and Johnson, R G, *Space Res*, 6, 203 (1965)
[4] Ginsburg, V L, Kurnosova, L V, Logachev, V I, Rozarenov, L A, Sirotkin, I A, and Fradkin, M I, *Plan Space Sci*, 9, 845 (1962)
[5] Seward, F D, and Kornblum, jun, H N, *Univ California Res Lab*, 6693 (1963)
[6] Armstrong, T, *J Geophys Res*, 70, 2077 (1965)
[7] Williams, D J, and Kohl, J W, *J Geophys Res*, 70, 4139 (1965)
[8] Rose, D C, in *Radiation Trapped in the Earth's Magnetic Field* (edit by McCormac, B M), 191 (Gordon and Breach, New York, 1966)
[9] King, G A M, *J Atmos Sci*, 21, 231 (1964)
[10] Hines, C O, *Plan Space Sci*, 13, 169 (1965)
[11] Geisler, J E, *J Atmos Terr Phys*, 28, 703 (1966)
[12] Kohl, H, and King, J W, *J Atmos Terr Phys*, 29, 1045 (1967)

Evolutionary Processes and Reversals of the Earth's Magnetic Field

It has been suggested that there is a connexion between reversals of the Earth's magnetic field and evolutionary processes[1-3]. This topic has excited much interest since the discovery of an apparent correlation between levels of field reversal and microfaunal discontinuities in deep sea sediment cores[4]. The Earth's magnetic field is known to have reversed its polarity at least nine times during the past 4×10^6 yr[5,6] and at frequent intervals before that except during the Permian[7]. Briefly, the ideas of Uffen[1,2] and Simpson[3] are that during the process of a reversal there is a period of reduced or zero intensity of the dipole field during which there should be a great increase of cosmic radiation at the Earth, which is no longer shielded by its magnetic field. This enhanced radiation will produce a higher mutation rate, which will result in some kind of evolutionary discontinuity. There is good evidence that during the process of reversal, the Earth's dipole field is reduced in strength, probably passing through zero[4,8]. The other assumptions are less acceptable.

Organisms receive radiations from many different sources[9] (Table 1). Of those listed in Table 1, a change in the intensity of the Earth's magnetic field will change only the radiation from cosmic rays and carbon-14.

Table 1. DOSE RATE IN MILLIREMS/YR ON HUMAN GONADS*

	Source of radiation	Dose rate
External	Cosmic rays	50
	Terrestrial (from rocks, etc.)	50
Internal	Elements of radium and thorium series	5
	Potassium-40	20
	Carbon-14	1

* Because we are interested in mutations, the gonadal dose rate is appropriate.

Primary cosmic rays consist of protons with energies between 10^7 and 10^{20} eV (ref. 10). The Earth's magnetic field deflects cosmic rays and produces a shielding at the Earth which is dependent on the horizontal component of the field. Above the atmosphere it is found that the cosmic ray intensity is reduced by a factor of about ten on going from (magnetic) pole to equator. At the bottom of the atmosphere, the latitudinal change is only 14 per cent[11]. This is because the atmosphere absorbs the low energy cosmic rays which are those chiefly responsible for the large latitudinal effect above the atmosphere.

Lingenfelter[12] has estimated that the present polar carbon-14 production rate (attributed to cosmic rays) is not more than 2.1 times the average global production. So, on the assumption that during a reversal the whole Earth receives the present polar cosmic ray intensity, we can estimate that the equatorial dose rate caused by cosmic rays should increase from 50 to 57 millirems/yr and that the dose caused by carbon-14 should increase from 1 to 2.1 millirems/yr. This is an increase of 16 per cent.

If the Earth's dipole moment were larger than at present, the latitude effect would be greater. There have undoubtedly been variations in the Earth's magnetic field strength. Studies of the palaeofield intensity show that the field has probably not been more than 1.5 times its present intensity for significant lengths of time and that it was less intense during the Tertiary[13,14].

To calculate the shielding effect of a larger field, Table 3 from Compton[11] has been used. The geomagnetic latitude (for latitudes less than 45°) was converted to horizontal field strength and this was plotted against cosmic ray intensity (Fig. 1). Assuming that the shielding is directly proportional to horizontal field strength, the regression line shows that if the field strength were 1.5 times the present strength, the equator would receive 65 per cent of the polar dose. During a reversal the equatorial dose rate would therefore change from 37 to 57 millirems/yr because of cosmic rays, and from 0 to 2.1 millirems/yr (at the most) because of carbon-14. This gives a total change from 37 to 59.1 millirems/yr or an increase of 60 per cent.

Mutations are produced by radiation, chemical effects and errors in replication. Cosmic rays produce only a small percentage of all observed mutations. For example, it has been estimated that the total mutation rate in humans is equivalent to that which would be produced by 100 rems/generation[15,16] or 3.3 rems/yr. This is known as the doubling dose. Even in the case of a field 1.5 times the present field intensity, the change in mutation rate when the field goes to zero will be only 0.6 per cent at sea level on the equator. This seems much too small to cause an evolutionary discontinuity.

The doubling doses for mice[9] and *Drosophila*[17] are somewhat greater than for humans and, because of the much shorter generation length for these organisms, the effect of a reversal of the Earth's field will be minute. In general, it is extremely unlikely that a reversal of the Earth's magnetic field could have anything but a small effect on the mutation rate of organisms living at sea level unless these organisms have a much lower spontaneous mutation rate than humans, mice or *Drosophila*.

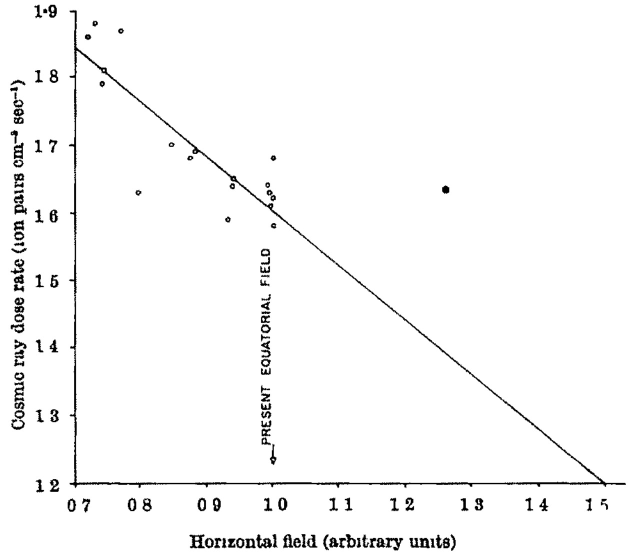

Fig. 1. Variation of cosmic ray dose rate with horizontal field intensity. The points are taken from Compton[11]. The least squares regression line is shown. Correlation coefficient = −0.8668.

It has been suggested that the present atmosphere is the result of a slow degassing of the rocks forming the Earth which has been occurring throughout geological history[18]. Although this could lead to an increase of the latitude effect at sea level further back in time, it is undoubtedly counteracted by the decrease in the strength of the Earth's magnetic field[13].

Despite these arguments, it does seem that there may be a correlation between evolutionary discontinuities and reversals of the magnetic polarity in deep sea cores[4]. These cores all came from latitudes greater than 40°, where the change in the cosmic ray intensity during a reversal would be much less than at the equator. One possible cause for such a correlation might be a climatic change at the time of a reversal. I can suggest two possible mechanisms whereby a reversal could cause a climatic change.

First, there seem to be several meteorological effects which are to some extent controlled by the Earth's magnetic field. King and Kohl[19] and King-Hele and

Allan[20] have suggested the importance of the Earth's magnetic field in controlling the wind velocity near the top of the F layer What the effect of removing the dipole field would be, and whether this effect could change the climate of the Earth, have not been studied Jacchia and Slowey[21] state, "It looks as though the role of the magnetic field of the Earth in controlling the temperature of the upper atmosphere might be considerably more important than was supposed heretofore"

Second, although the latitude effect at sea level is only about 14 per cent, above the atmosphere it is much greater[12] The result of removing the Earth's magnetic field would be to increase several times the amount of ionization produced in the atmosphere by cosmic rays, and also by the solar wind It seems that between altitudes of about 50 and 80 km the main source of ionization today is cosmic radiation[22] It is possible that a large increase in the ionization at this level could have an effect on the climate

During a reversal of the Earth's magnetic field, organisms at the equator will receive about 14 per cent more cosmic radiation than they do at present. Because of the comparatively high spontaneous mutation rate of organisms studied so far it is extremely unlikely that this increase in radiation could have a significant effect on mutation rates It is suggested that a possible cause of the correlation between microfaunal discontinuities and reversals should be looked for in a climate change Climatic changes could be brought about because of the control that the Earth's magnetic field may have on meteorological phenomena, or because of an increase of ionization at certain levels in the atmospheres These are only suggestions and it would require investigation by meteorologists to ascertain if the climate could be affected in such a way.

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[1] Uffen, R J , *Nature*, **198**, 143 (1963)
[2] Uffen, R J , *Proc 22nd Intern Geol Cong , New Delhi* (1964)
[3] Simpson, J F , *Bull Geol Soc Amer* , **77**, 197 (1966)
[4] Opdyke, N D , Glass, B , Hays, J D , and Foster, J H , *Science*, **154**, 349 (1966)
[5] Doell, R R , Dalrymple, G B , and Cox, A , *J Geophys Res* , **71**, 531 (1966)
[6] Doell, R R , and Dalrymple, G B , *Science*, **152**, 1060 (1966)
[7] Irving, E , in *Paleomagnetism* (Wiley, 1965)
[8] Harrison, C G A , and Somayajulu, B L K , *Nature*, **212**, 1193 (1966)
[9] *Rep UN Sci Com Effects of Atomic Radiation, Gen Ass* , official records seventeenth session, suppl 16 (A/5216) (1962)
[10] Ginzburg, V L , and Syrovatskii, S I in *The Origin of Cosmic Rays* (trans by Massey, H S H , edit by ter Haar, D) (Macmillan Co , 1964)
[11] Compton, A H , *Phys Rev* , **43**, 387 (1933)
[12] Lingenfelter, R E , *Rev Geophys* , **1**, 35 (1963)
[13] Smith, P J , *Geophys J Roy Astro Soc* , **12**, 321 (1967)
[14] Bucha, V , *Nature*, **213**, 1005 (1967)
[15] Kato, H , Schull, W J , and Neel, J V , *J Human Genet* , **18**, 339 (1966)
[16] *The Effects of Nuclear Weapons*, rev ed (edit by Glasstone, S) (US AEC, 1964)
[17] Muller, H J , *Proc Second UN Inter Conf Peaceful Uses of Atomic Energy*, **22**, 313 (1958)
[18] Rubey, W W , *The Crust of the Earth, Geol Soc Amer Spec Paper*, **62**, 631 (1955)
[19] King, J W , and Kohl, H , *Nature*, **206**, 699 (1965)
[20] King-Hele, D G , and Allan, R R , *Roy Air Estab Tech Rep* 66212 (1966)
[21] Jacchia, J G , and Slowey, J , in *The Shape and Location of the Diurnal Bulge in the Upper Atmosphere* (Smithsonian Institution, 1966)
[22] Mitra, A P , in *Advances in Upper Atmosphere Research* (edit by Landmark, B), 57 (Pergamon, 1963)

Group Analysis of Variance for Earthquake Location and Magnitude

Two central problems in seismology are the location of earthquake epicentres and the allocation of magnitudes to earthquakes We show in this communication that these problems can be reduced to the classic statistical model of two-way analysis of variance Applying this theory to the reduction of pooled data from many stations and earthquakes leads to estimation of source and station terms and the interaction between them In particular, it provides the theoretical framework for the method of group (or regional) adjustment of epicentres used by Freedman[1] and Douglas[2] The embedding of the problems in this theory opens the way for strict statistical tests of various hypotheses of geological interest

Consider I seismic regions and J seismological stations Arrange the measurements under study into a two-way layout in which the "i, j cell" contains the measurements from region i (i = 1, 2, I) at station j (j = 1, 2, J) For simplicity, suppose that no cell is empty (Approximate methods of dealing with a layout with empty cells are discussed by Freedman[3]) Denote the number of measurements in the "i, j cell" by K_{ij} (K_{ij} = 1, 2, K)

First, we examine the analysis of the layout for the earthquake location problem Let r_{ijk} denote the travel time residual (for the P wave, say) from the kth earthquake (k = 1, 2, . . K_{ij}) in the ith seismic region, to the jth station, let η_{ij} denote the cell mean Now the main effect, α_i, of the ith region is the excess of the ith row mean, A_i, over the general mean, μ, of the residuals, that is, $\alpha_i = A_i - \mu$ In seismological terms, α_i is the average source adjustment Similarly, the main effect β_j of the jth station ($\beta_j = B_j - \mu$) is the average adjustment ("station correction") for the jth station

There is now strong evidence that P residuals at many stations vary markedly with azimuth[4] One functional relation used is

$$h_j(\theta) = \beta_j + D_j \sin (\theta + E_j)$$

where θ is the (station to source) azimuth and D and E are constants (Recent work with data from Californian stations indicates that, for some stations, this representation is too simple, the function may be discontinuous)

Azimuthal variations, at both source and station, give rise to interactions, that is, the observed variation in travel times contains a term $\gamma_{ij}(\theta)$ which measures the effect of the ith seismic region at station j over and above the main effect α_i of the region, that is

$$\gamma_{ij}(\theta) = \eta_{ij} - \alpha_i - \beta_j - \mu$$

The term $\gamma_{ij}(\theta)$ may be interpreted as the combined source and station azimuth anomaly function, where for simplicity the dependence on epicentral distance (angle of emergence) has been ignored (The interpretation of the argument θ needs care, it might be azimuth of station to source or vice versa)

Because hypocentral locations are computed so that average P residual is close to zero, the overall mean μ, for a group of earthquakes, will be close to zero Then, if the errors involved are both independent and normally distributed about zero mean with common variance (that is $N(0, \sigma^2)$), the travel time problem is reduced to the classic model of two-way analysis of variance Devices must be applied to minimize any non-normality in the distribution of errors For example, contaminations such as extreme residuals can often be dealt with by Jeffreys' method of uniform reduction[5]

The least squares estimates (assuming equally weighted observations) for the source and station adjustments can then be simply calculated The azimuth anomaly function is estimated by

$$\gamma_{ij} - r_{ij} \quad - r_{i} \quad - r_{j} \quad + r_{\bullet}$$

where a dot indicates that the arithmetic mean has been taken over that subscript. As an illustration, the analysis of variance model may be used to test whether the β_j for stations in one continental region are significantly different from those in another The mathematical formula and methods are given, for example, by Scheffé[6]

This general theory shows at once why it is advantageous in epicentral determinations to combine earthquakes into regional groups during the calculations The underlying idea is that the error distribution arises from errors in reading the time of arrival, t_{jk}, of the P phase Thus separation of the source and station terms by appropriate averaging allows the variations in the reading errors to be reduced to nearly zero.

Consider a particular seismic region with K earthquakes (we now suppress the suffix i) selected so that the true station residual against the global tables $r_{jk} = r_j = r(\Delta_j)$ Apart from an additive constant, the M residuals $r(\Delta_j)$, for M ranges of distance, can be estimated if necessary along with the epicentral adjustments For brevity, we simply combine the source and station terms so that $g_j(\theta) = \alpha_i + \beta_j + \gamma_{ij}(\theta)$ Then the standard linearized equation[1] for the location of the kth earthquake is

$$\hat{r}_{jk} = r(\Delta_j) - \delta\tau_k - a_{jk}\,\delta x_k - b_{jk}\,\delta y_k - \delta g_j(\theta) + \delta t_{jk} \qquad (1)$$

where τ is the origin time, x is longitude, y is latitude, $a = \dfrac{\partial t}{\partial \Delta} \sin \hat{\theta}$, $b = \dfrac{\partial t}{\partial \Delta} \cos \hat{\theta}$, and Δ is the epicentral distance In this equation, the focal depth term has been ignored and δ is used to denote adjustment to provisional values

Assuming that the errors δt_{jk} are independently $N(0, \sigma^2)$, an analysis of variance yields the adjustments $\delta\tau_k$ δx_k δy_k for the K earthquakes as a group as well as δg_j and $r(\Delta_j)$ (Formulae are given by Freedman[1] in a more restricted case than given here) There are $J+3K+M$ unknowns and JK equations of condition

In a numerical test of the method, P travel times to five stations of the Berkeley network from the two Bikini atomic explosions Bravo and Union with known positions and shot-times were used The co-ordinates were interchanged but the shot-times kept fixed The longitude change was 0 114° and the latitude change 0 036° A relocation, using the foregoing method, shifted, with the correct signs, the latitudes by 0 04° and the longitudes by 0 09°

In the published example by Douglas[2], times at thirty stations from six earthquakes and the explosion Longshot in the Aleutian Islands are used to make a group (or joint) location The stations were well distributed with azimuth The estimated position of Longshot was much closer to the known shot-point than when a location was made for Longshot alone The previous theory provides the explanation without the separate estimates of the $\delta g_j(\theta)$ terms, the error terms are non-normal

It should be remarked that Douglas does not make clear the full complexity of his "station correction" term A comparison between his equation (2) and our equation (1) shows that the numbers which he computes are estimates of the sum of the station and source adjustments (relative to the Jeffreys–Bullen tables) as well as an interaction term which may be azimuthally dependent As a consequence, the numbers assigned as "station corrections" apply only for the selected group of earthquakes

The way now appears open, using analysis of variance techniques, for more reliable location of earthquakes Regional and global seismological centres doing routine hypocentral determinations can easily adapt the group method to their special requirements It would seem wise if a calibration event be included when possible in each group calculation Underground nuclear explosion, well located earthquakes or, for local networks, a timed quarry blast would all serve Any regional bias in location, perhaps caused by poor azimuthal distribution of stations, could in this way be detected On this point, solutions are not optimized by using readings from all available stations, on the contrary, some uniform azimuthal distribution should be used

Finally, the group method outlined here seems promising also for the routine estimation of the magnitudes of both teleseisms and local earthquakes In place of time residuals, consider measurements from seismograms of $\log_{10}(A/T)$, denoted by $\hat{a}_{jk}$, where A is the amplitude of a wave of period T The computed magnitude of the kth earthquake at station j is $\hat{M}_{jk} = \hat{a}_{jk} + q(\hat{\Delta}_{jk})$, where q is some standard function[7] For a particular region, equation (1) is replaced[8] by

$$\hat{M}_{jk} = M_k - \delta q(\Delta_j) - \delta g_j + \delta a_{jk} \qquad (2)$$

in which M_k denotes the true magnitude of the kth earthquake, δa_{jk} is the reading error, δq is the correction needed to the standard distance term and δg_j, as before, denotes the combined station and regional source adjustments (including azimuthal interactions)

Simultaneous computation of the magnitudes of K earthquakes from measurements at J stations will provide maximum likelihood estimates of M_k, $\delta q(\Delta_j)$ and δg_j if the errors are independently $N(0,\sigma^2)$ Because the formulation (2) allows the calculation of the systematic station-source terms δg_j separately from the random variation, the assumption is likely to be a reasonable one Suppose $q(\Delta_{jk})$ be considered in N piecewise constant segments, then the analysis of variance would require the solution of JK equation with $J+K+N$ unknowns

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[1] Freedman, H W , *Bull Seism Soc Amer* , 57, 545 (1967)
[2] Douglas, A , *Nature*, 215, 47 (1967)
[3] Freedman, H W , *Bull Seism Soc Amer* 56, 677 (1966)
[4] Bolt, B A , and Nuttli, O , *J Geophys Res* , 71, 24 (1966)
[5] Jeffreys, H , *Bur Cen Seism , Inter A* , Trav Sci , No 14, 3 (1936)
[6] Scheffé, Henry, in *The Analysis of Variance* (Wiley, New York, 1959)
[7] Gutenberg, B , and Richter, C F , *Ann Geofisica*, 9, 1 (1956)
[8] Carpenter, E W , Marshall, P D , and Douglas, A , *Geophys J Roy Astro Soc* , 13, 61 (1967)

Late Tertiary Slumping on the Continental Slope of Southern Portugal

THE geological relationship of the Atlantic Ocean to Europe is recorded west of the British Isles and France in a sequence of continental shelf and slope deposits, although the latter seem to be missing on the northern and western sides of Iberia[1] Accordingly, the southern continental slope of Iberia was examined (Fig 1) for the missing parts of the record, particularly south of Portugal where a detailed bathymetric survey had revealed a series of long, sub-parallel ridges with a westerly to west–south–westerly trend (Hydrographer of the Navy, personal communication)

The examination was made from RRS Discovery by a continuous reflexion profiler, using a modified airgun of Lamont type as the sound source The peak pressure at its face was 1×10^6 dyne cm^{-2} at a distance of 1 m The amplifier and hydrophone were built to a National Institute of Oceanography design The preferred hydrophone was 50 ft long with ten elements spaced to taper the beam, so reducing the side lobes and presenting maximum sensitivity to the sea floor at the frequencies used The two profiles illustrated have been traced directly from the originals, which were recorded on a modified Mufax recorder

The records showed that the continental slope, south of Portugal, is composed of folded, well bedded sediments at least 0 6 sec thick, equivalent to more than 2,000 ft at a compressional velocity of 6,560 ft sec^{-1} (ref 2) The folds observed bear an intimate relationship to the relief, such that the anticlinal and synclinal axes correspond exactly with the axes of the ridges and troughs (Fig 2 and profile *a*–*b* of Fig 4) Further east, a profile (Fig 3 and profile *d*–*e* of Fig 4) extending almost along the strike of the folds shows that the folds have been buried by the Quaternary continental shelf and upper slope sediments South of Portugal (Fig 2) the absence of sediment fill in the synclines can be ascribed to the large canyons, which transect the folds, acting as paths for the transport of sediment to deeper water

The distribution of folds, shown by the profiles and extrapolated from the relief, is illustrated in Fig 4 There is a general southerly decrease in wavelength and increase in amplitude to a maximum of 1,200 ft as well as a change in trend from westerly to west–south–westerly The belt of folded sediments is terminated to the south by an area of gentle gradient and irregular topography which exhibited a very high reflectivity and beneath which no bedding was seen This area is believed to be the submerged extension of the highly contorted and metamorphosed rocks of the Betic Alps, the development of which culminated in the Burdigalien

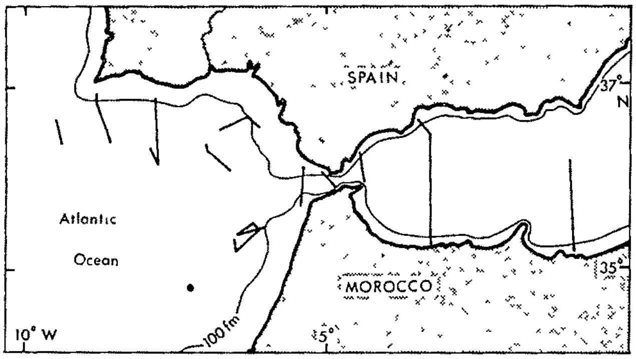

Fig 1 Location of continuous reflexion profiles obtained by RRS Discovery south of Iberia

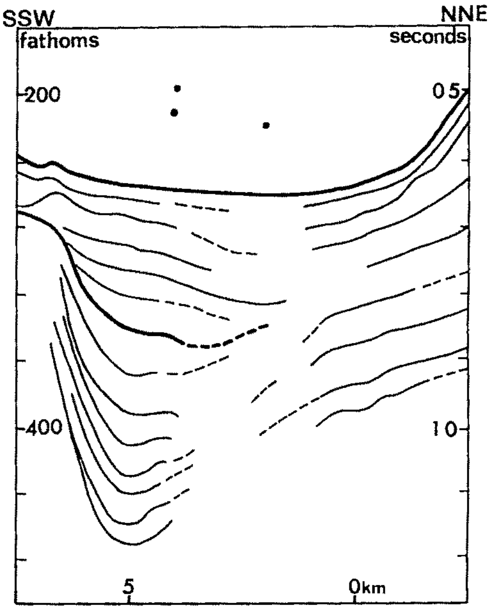

Fig 3 Continuous reflexion profile (*de* of Fig 4) showing the burial of folded sediments (upper surface shown by a heavy line) at the base of the Quaternary continental slope

The gentle folding of the late Tertiary sediments might have been produced by a north–south compression between the principal tectonic units of southern Spain

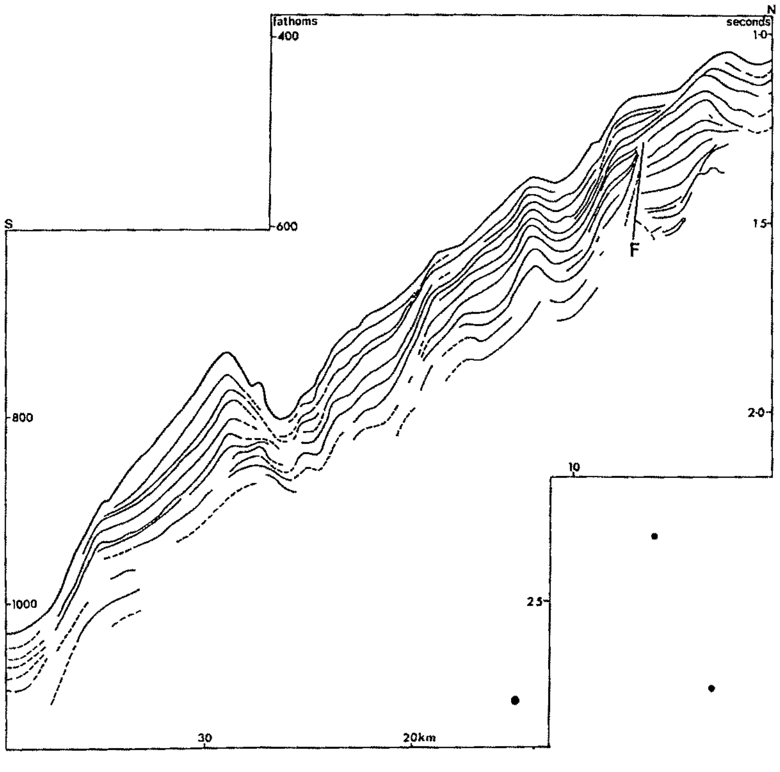

Fig 2 Continuous reflexion profile south of Portugal (*ab* of Fig 4) showing the close relationship of folds to relief *F* Fault zone Note the absence of sediment fill in the troughs

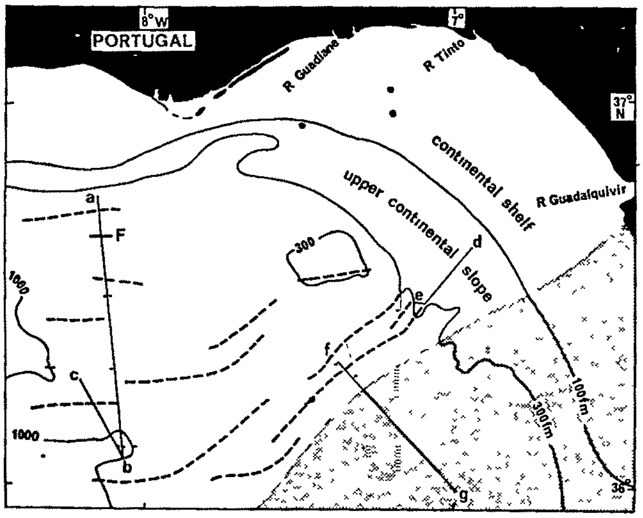

Fig 4 Distribution of slump folds (anticlines ----, synclines) from reflexion profiles *abc*, *de* and *fg* and inferred from the relief *F* Fault zone Shaded area is the supposed submarine extension of the contorted and metamorphosed rocks of the Betic Alps

and Morocco (Fig 1) The change in fold style downslope and the absence of complementary folds in the western Mediterranean, however, are not in agreement with such a mechanism The preferred explanation is that the sediments, which had accumulated on the continental slope south of Portugal, were given an enhanced oceanward dip during the Pliocene uplift of Iberia and consequently moved downslope by creep and crumpled against the submerged northern edge of the Betic block (shaded in Fig 4)

The same episode of slumping may be partly responsible for the apparent absence of continental slope deposits on the northern and western sides of Iberia It is possible that slumping on existing continental slopes and those of the geological past is more generally associated with regional uplift of continents than has previously been supposed

We wish to thank the master and officers of RRS Discovery and our colleagues for their willing co-operation at sea, D G Bishop and A R Stubbs for their work on the continuous reflexion profiling equipment and N Kenyon for his valuable discussion.

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[1] Curray, J , *et al* , *Science*, **154**, 265 (1966)

[2] Drake, C L *et al* , *Coll Repr Lamont Geol Obs* , No 328 (1959)

Radio Emission from Geomagnetically Trapped Particles

Many experiments to measure the radio noise intensity in the topside ionosphere have found an intense "band" of noise in the range of frequency between the plasma frequency and the upper hybrid resonance frequency[1-4] Various explanations have been suggested, including plasma instability, radiation by energetic particles[5] and anomalous behaviour of the antenna impedance[4] Data received by the *UK*-2 (*Ariel* 2) satellite suggest that the energy for this noise comes from the particles trapped in the inner Van Allen radiation belt

The *UK*-2 radio astronomy experiment has been described elsewhere[3,6] A 40 m dipole antenna was connected to a receiver with an input impedance of 10 kΩ and a bandwidth of 20 kHz, and the frequency of reception was varied linearly between 0 65 and 3 50 MHz once every 28 sec The satellite orbit had an inclination of 51° and an apogee of 1,385 km

In the experiment reported here, the noise bands were of three principal types (1) noise between the plasma frequency and the upper hybrid resonance frequency, as already mentioned, (2) anomalously intense noise below

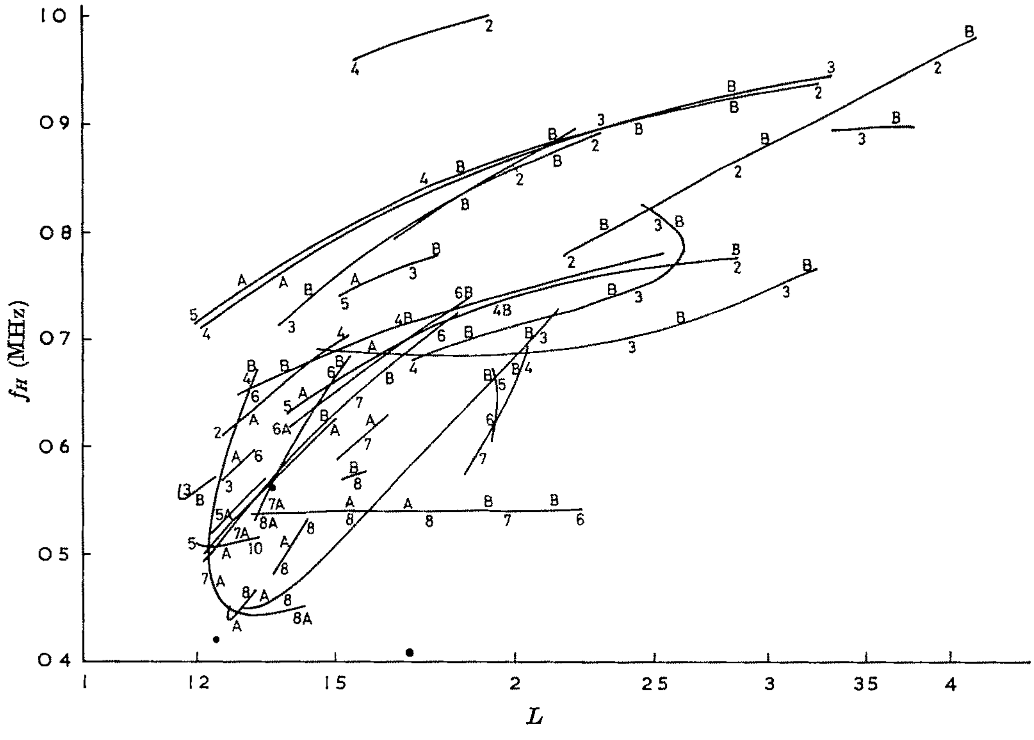

Fig 1 The trajectories of passes near to apogee plotted in *B*/*L* space, *B* being measured in terms of the gyro frequency for electrons

the local plasma frequency and above the extraordinary wave cut-off frequency, (3) low frequency noise with no definite cut-off frequency

I have examined in detail the interference-free passes near to apogee The trajectories of these passes in B/L space[7] are shown in Fig 1, B is measured in terms of the gyro-frequency for electrons Beside the curves are numbers representing the intensity of the noise band of type (1) It will be seen that the intensity is a maximum near the value $L = 1\ 35$ This is the position of the maximum energetic particle intensity in the inner Van Allen radiation belt[8]

A ray tracing programme has been used to show that electromagnetic waves cannot propagate over many degrees of latitude in the frequency range of the noise of type (2); this noise must be generated close to the point where it is observed The occurrence of this type of noise is qualitatively marked in Fig 1 A is written where it is observed, and B where it is not observed It is always observed near to the equator at $L = 1\ 35$, and never at points far from these co-ordinates

The noise of type (1) is used to determine the plasma and gyro frequencies at the satellite, and the latter frequency usually agrees well with the value calculated from a 48 coefficient spherical harmonic expansion of the geomagnetic field[9] Sometimes the agreement is rather bad this disagreement happens only at points well removed from $L = 1\ 35$

This evidence strongly suggests that the source of energy for the noise of types (1) and (2) is the particle radiation of the inner Van Allen radiation belt

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Received December 11, 1967

[1] Walsh, D , Haddock, F T , and Schulte, H F , *COSPAR Fourth Inter Symp , Warsaw*, 1963

[2] Huguenin, G R , Lilley, A E , McDonough, W H , and Papagiannis, M D , *Plan Space Sci* , 12, 1157 (1964)

[3] Harvey, C C , *Ann d'Astrophys* , 28, 248 (1965)

[4] Alexander, J K , and Stone, R G , *Ap J* , 142, 1327 (1965)

[5] Lacombe, C , thesis, Univ Paris (1967)

[6] Hugill, J , and Smith, F G , *Mon Not Roy Astro Soc* , 131, 137 (1965)
McIlwain, C E , *J Geophys Res* , 66, 3681 (1961)

[8] Paulikas, G A , Blake, J B , and Freden, S C , *J Geophys Res* , 72, 2011 (1967)

[9] Jenson, D C , and Cain, J C , *J Geophys Res* , 67, 3568 (1962)

PHYSICS

Duration of Sensitive Period for Track Recording in Mica

THERE is evidence which suggests that the tracks of muons and of electrons in electron showers may leave detectable marks in muscovite mica during a sensitive period following crystallization of the mica[1,2] The observation that the tracks of electrons may in special conditions leave detectable records suggests that mica is responsive to particles of low specific ionization during the sensitive period Consequently, charged particles of higher specific ionization such as alpha particles and fission fragments would also be expected to leave detectable records

It has been suggested that observable tracks of charged particles may be produced in mica as a result of the exsolution of an excess iron content on nucleation centres, formed by ionization of the crystal structure by the charged particles[1] Following the initial precipitation of the iron impurity at a nucleation centre, decoration of such centres will occur as a result of accretion of the impurity as exsolution continues The original nature of the initial precipitation will be masked to a considerable extent by the continued decoration For example, the individual dots delineating the track of an electron are commonly of the order of 1,000μ in diameter, while the track length of an alpha particle from natural nuclear decay is typically 25μ Indeed, the appearance of a fully decorated alpha track is unlikely to retain any of the initial characteristics such as direction of track or recordable length, only the fact of occurrence and general location being important As decoration of such a track continues, the crystal is liable to local fracture resulting in a characteristically star-shaped figure resembling a "percussion" figure, as would be expected

Fortunately, most crystals contain trace amounts of the naturally radioactive elements, and so isolated regions showing decoration by the iron impurity would be expected in mica specimens showing staining If the occurrence of such decorated regions or spots can be related to the radioactive impurity content, then the duration of the sensitive period of the mica for the recording of the passage of charged particles can be found for each specimen of mica

The occurrence of isolated regions of decoration or "spotting" in muscovite mica is well known A rough estimate of the duration of the sensitive period is made next Typically, muscovite mica may contain uranium and thorium in concentrations of a few parts in 10^9, or about 10^{13} atoms of uranium-238/cm^3 In every thousand decays, about one will lie sufficiently close to the (001) plane of the mica to be recorded, so in each year there should be about ten recordable alpha decays The density of these spots is roughly 100/cm^3, suggesting that the sensitive period lasts for something like 10 years

Measurements on these spots have been restricted to those regions also showing line staining, that is, decorated crystal defects and tracks In Fig 1 a plot is shown of the size distribution of these spots The observed distribution can be explained by assuming a two-dimensional growth of decoration on nucleation centres formed by tracks of alpha particles from natural nuclear decay together with a constant rate of exsolution of iron impurity Although fission fragments should also produce nucleation centres, the low probability for spontaneous fission relative to alpha decay makes this a negligible contribution It is well known that the spots are essentially flat disks with

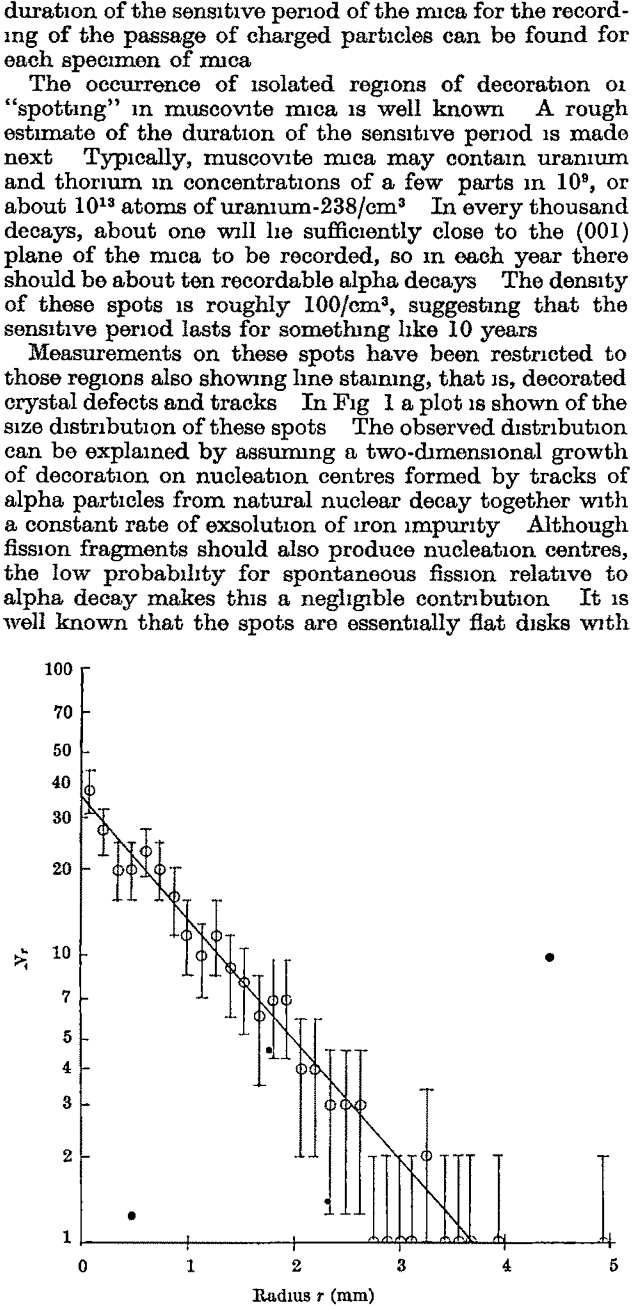

Fig 1 Plot of the mean radius of decorated spots in muscovite mica N_r is the number of spots occurring within a 100μ interval of radius

nearly constant thickness, so that growth must occur principally at the periphery, giving a two-dimensional growth by diffusion The details of the exsolution process are not known, but it is not unreasonable to assume that once exsolution has started it continues at an approximately constant rate until terminated by depletion of the impurity Because the change in temperature associated with the exsolution stage is probably small, it is also probable that the rate of diffusion changes only slightly during the sensitive period.

At the high temperatures involved, although mica is still essentially an insulator, the ionization produced by the passage of a charged particle will not persist for long, so that only those decays which occur after exsolution has started can form recordable nucleation centres On the short time scale involved, the number of nucleation centres will thus increase approximately linearly with time so that the amount of impurity available to each centre will decrease as more centres are formed Let r be the mean radius of a spot and q the quantity of impurity contained within the spot It follows that for a given rate of exsolution,

$$\frac{\mathrm{d}q}{\mathrm{d}t} = \text{constant} \quad \frac{r}{N}$$

where N is the number of centres present Because $2\pi r^2 \alpha \int \mathrm{d}q$ and N is proportional to t for small t, then

$$\frac{\mathrm{d}r}{\mathrm{d}t} = K\frac{1}{t}$$

giving the radius of a spot, formed at t_1 with decoration terminating at t_2, as

$$r = K \log (t_2/t_1)$$

The size distribution is then $\mathrm{d}N/\mathrm{d}r = Ce^{-r}$ where C is a constant and is shown in Fig 1 by the solid curve

A third reason for relating these spots to alpha decays is the observed correlation of spot density with concentration of uranium Because the duration of the sensitive phase of the mica is relatively short, it is probable that most of the alpha decays result from the thorium series The chemistry of thorium and uranium, however, has enough in common to permit a reasonable estimate of the thorium content from the measured uranium content The concentration of uranium in the specimens was determined by counting the number of fission tracks intersecting a given surface area of crystal after irradiation of the specimens in a known flux of thermal neutrons, the fission tracks being rendered observable by etching[3]. A definite correlation between spot and fission track densities has been observed (Fig 2)

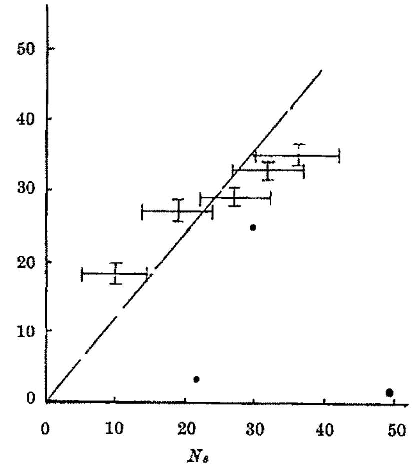

Fig 2 Correlation plot between the number of spontaneous fissions, N_f, and the number of spots, N_s, per unit of area, for a sample of mica showing considerable variation in the spot density

To determine the duration of the sensitive phase from the observed density of spots/unit volume requires a knowledge of the radioactive impurity concentration and of the mean detection efficiency of the mica to alpha particles The relatively long half-life of the uranium-234 alpha decay mediates the uranium-238 series so that only one alpha particle is associated with each uranium-238 decay In the thorium series, however, the relatively short half-life of radium-228 is the mediating element and more than one alpha particle is to be expected from each thorium-232 decay site during the sensitive phase The maximum number of alpha particles from each thorium-232 decay site is six, all with energies of 4 MeV or more Although the mean probability that any one will produce a nucleation centre is approximately the same, once a nucleation centre has been formed at a given decay site further alpha decays would be ineffectual On the average, the number of alpha particles from each thorium-232 decay site effective in producing new nucleation centres will be half the maximum From the known terrestrial thorium to uranium abundance ratio, the average number of alpha particles effective in nucleation centre formation, after allowing for the longer half-life of thorium-232 compared with uranium-238, per uranium-238 decay, is approximately three Finally to determine the mean detection efficiency it is necessary to specify the magnitude of the effective cut-off angle out of the (001) plane within which tracks of alpha particles may be recorded A lower limit for this angle is provided by the value pertaining to low energy muons namely about 2 milliradian Even though the specific ionization of fission fragments greatly exceeds that of alpha particles and so there may not be any restriction in angle for spot formation the half-life for spontaneous fission of uranium-238 is such that only a small fraction of the nucleation centres could arise from this cause

From measurements made on some mica samples from the Madras region of India, the duration of the sensitive phase was found to be about 16±8 yr The principal uncertainty is in the magnitude of the cut-off angle for alpha particles, and has been set by assuming the angle is proportional to the specific ionization The effect of this uncertainty is mitigated by the compensating influence of the radium-228 half-life In the other sense it is unlikely that the sensitive time for the recording of muons exceeds that for alpha particles

Because mica of the type considered here is usually associated with zoned pegmatites, the pegmatites being intrusive into the country rocks, a time scale of the order of a few tens of years is not unreasonable because this is commensurate with the probable growing time of the mica crystals It is not known yet whether the entire crystal becomes sensitive or only a small portion, perhaps close to the growing surface, the integrated sensitive time in either case being the same.

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[1] Russell, F M, *Phys Lett*, 25B, 4, 298 (1967)
[2] Russell, F M, *Nature*, 216, 907 (1967)
[3] Fleischer, R L, Price, P B, and Walker, R M, *Science*, 149, 383 (1965)

Acceleration Produced Electric Fields in Charcoal Bodies

An analysis of the electrodynamics of accelerated charged bodies reveals a new method by which Einstein's principle of equivalence may be tested If a uniformly charged spherical shell of radius r is given an acceleration a, there will be within it a uniform electric field E along the direction of acceleration the magnitude of which is given by

$$E = -\frac{q}{r\,c^2}\,a$$

This may be seen as follows[1] The scalar potential φ within the shell is constant The vector potential A which exists within a shell moving with velocity V is also constant This may be derived by Lorentz transformation of the static case with the result,

$$A = \varphi\frac{v}{c} = \frac{q}{rc}\,v$$

to first order in v

The retardation of the vector potential may be neglected for an accelerated shell provided

$$|a| \ll \frac{c^2}{r}$$

The acceleration produced electric field is obtained from the relationship

$$E = -\nabla\varphi - \frac{1}{c}\frac{\partial A}{\partial t}$$

Therefore $$E = -\frac{q}{r\,c^2}\,\dot{V} = -\frac{q}{r\,c^2}\,a$$

If, instead of an insulating shell, a charged conducting body of arbitrary shape were accelerated, the same phenomenon would occur, although the field might then be annulled by a redistribution of charge on the surface of the object

Schiff and Barnhill[2] have also derived an electric field which may be produced in metals in an acceleration field, but their field is independent of the excess charge in the body Provided that the scalar potential is constant within the accelerated object, the electric field produced by acceleration will be uniform and therefore conservative That is, the integral of electric field strength around any closed path is zero

$$\oint E\,\mathrm{d}l = 0$$

Permanent potential differences can be produced by placing two dissimilar metals in contact and utilizing the contact potential phenomenon[3] In this case, non-conservative electric fields can arise from which energy can be extracted if a current flows around a suitable path A case of interest is shown in Fig 1

The internal electric fields produced by an acceleration along the line joining the junctions, J_1, J_2, are in opposite directions in the two limbs of the device which carry opposite charges and which differ in potential by φ_c, the contact potential In this case,

$$\oint E\,.\,\mathrm{d}l \doteqdot \varphi_c\,\frac{h}{c^2}\,|a|$$

Because the field is non-conservative, it cannot be cancelled by a simple rearrangement of the surface charges If the principle of equivalence is valid, the same results as are obtained for an accelerated apparatus should apply to a similar system supported vertically in a gravitational field

If φ_c is about 1 V then $\varphi_c\frac{gh}{c^2} \sim 10^{-18}\,h$ V where h is in cm

The measurement of such a small voltage is technically feasible by using superconducting limbs of low inductance and the SQUID, a measuring device based on the Josephson effect[4].

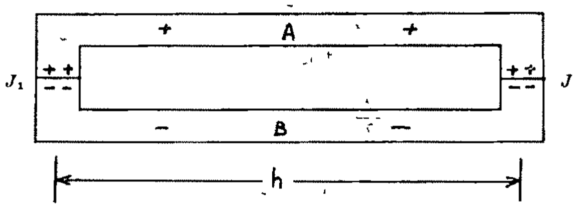

Fig 1 A shell made of two dissimilar metals There will be a contact potential across junctions J_1 and J_2

Witteborn and Pallesen[5] have used different experimental techniques which yielded an upper limit to any gravitational shift in contact potential of $\pm 10^{-9}$ V/m

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[1] Sciama, D W, *Mon Not Roy Astro Soc*, **113**, 953 (1953)
[2] Schiff, L I, and Barnhill, M V, *Phys Rev*, **151**, 1067 (1966)
[3] Kittel, C, in *Elementary Solid State Physics*, 232 (John Wiley, 1962)
[4] Zimmerman, J E, and Silver, A H, *Phys Rev*, **141**, 367 (1967)
[5] Witteborn, F C, and Pallesen, M R, *Phys Rev Lett*, **19**, 1123 (1967)

Effect of Pipe Diameter, Vibration Amplitude, and Fluid Viscosity on Pipe Flow

IN view of the comments made by Silberman[1] on the communication by James and myself[2], I should like to draw attention to other experimental work that I have carried out to determine the effect of fluid viscosity, pipe diameter and amplitude of vibration on the flow of a fluid in a pipe Although at this stage the work does not cover as wide a range of significant variables as would be required for a complete investigation of the effect of vibrations in pipe flow, it does give an indication of the magnitude of the effects which are likely to be obtained for the conditions which were described by Silberman

This communication describes results obtained from two types of experiment, one of which was basically similar to that described by James and myself[2] This consists of determining the friction factor λ for a pipe where the static pressure tappings are situated 24 in apart and there is an entry length of 12 in The whole of the pipe was connected to an aluminium channel and vibrated in a plane perpendicular to the flow of fluid in the pipe by means of a slotted bar mechanism which was driven by a constant speed motor The pipe was free at its ends and tended therefore to bend and twist slightly when vibrating It was not flexibly mounted as in the previous experiments because it is believed that the mountings subject the pipe to conditions of shock The results of tests using this experimental arrangement are referred to as test *L*

In the other experiment, to be referred to as test *S*, a 12 in length of pipe, midway between two static pressure tappings which were 24 in apart, was attached to a 12 in length of aluminium channel and vibrated as described here Each static pressure tapping and a 2 5 in length adjacent to it on the vibrated side was rigidly clamped to a frame so that it was kept stationary The inlet length of 12 in and the pipe downstream of the test section was also clamped to the frame

The results of experiments, which were carried out on a pipe of diameter 0 164 in which was vibrated according to test *L*, are shown in Fig 1, where the effect of the amplitude of vibration and viscosity on the friction factor can be seen as a function of Reynolds number, Re The test fluids were water and an oil A which had a kinematic viscosity approximately four times that of water

The effect of amplitude of vibration and viscosity is also shown in Fig 2 where a pipe, 0 0714 in in diameter, was vibrated according to test *S* The test fluids in this case were water and an oil B which had a kinematic viscosity approximately 1 4 times that of water The results are essentially the same as those obtained for a pipe of 0 0714 in diameter which was vibrated according to test *L*.

Figs 1 and 2 both show that for similar amplitudes of vibration, a reduction in kinematic viscosity increases the magnitude of the friction factor at a particular

Reynolds number. Furthermore, for a particular fluid at a fixed Reynolds number an increase in the amplitude of vibration increases the friction factor. The shape of the λ–Re curve is interesting because it shows that at certain vibration amplitudes the effect of increasing the friction factor extends down to a Reynolds number of 800 and lower, whereas in other tests and those shown for oil B in Fig. 2 the curves in the turbulent region for vibrating and non-vibrating conditions are similar.

It is noticeable from Figs. 1 and 2 where all the tests were carried out at a frequency of 25 c/s that there is a diameter effect present because the curves of λ–Re for amplitudes of 0.175 in. and 0.37 in. for water are much closer together in the 0.164 in. diameter pipe than they would have been in the 0.0714 in. diameter pipe.

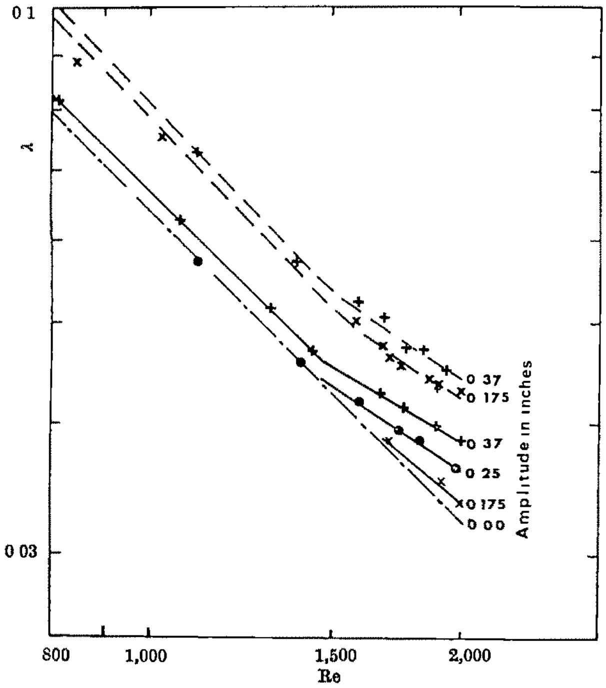


Fig. 1. Test *L* ———, Oil A; - - -, water

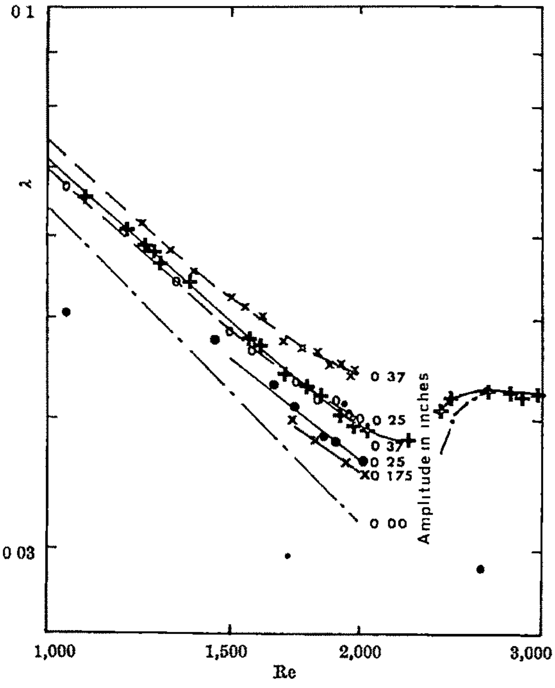
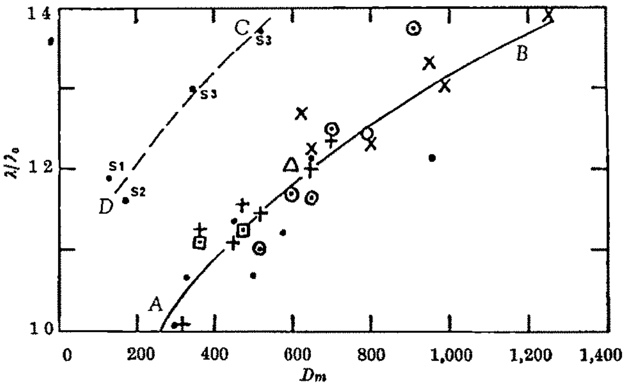


Fig. 2. Test *S* ———, Oil B; - - -, water



Test or test length	Pipe diam. (in.)	Frequency (c/s)	Fluid	Mounting	Symbol
24 in.	0.0714	10	Water	Shock	•*S1*
24 in.	0.127	10	Water	Shock	•*S2*
4 in.	0.0714	20	Water	Shock	•*S3*
L	0.164	25	Oil A	Free	•
L	0.0714	25	Oil B	Free	□
L	0.0714	25	Water	Free	Δ
L	0.164	25	Water	Free	×
S	0.0714	25	Water	Rigid	○
S	0.0714	25	Oil B	Rigid	+

Fig. 3

It is interesting to note that the curves for λ–Re are similar to those obtained by White[3] for flow through curved pipes. The streamline motion of a fluid in a curved pipe was analysed theoretically by Dean[4,5], who showed the significance of the function $\frac{1}{2}\mathrm{Re}\left(\frac{r}{R}\right)^{\frac{1}{2}}$ (now known as the Dean number) to this type of flow. In the function r is the radius of the pipe and R is the radius of the bend.

It would seem that when a fluid flows along a pipe which is being vibrated in a direction perpendicular to the flow, as a particle of fluid moves along the pipe, it seems to flow through a series of bends. Suppose that the pipe is being vibrated with an amplitude A and a frequency n. If the mean velocity of flow in the pipe is u, then a particle of fluid will have moved a distance $u/4n$ along the pipe while the pipe moves a distance A perpendicular to the flow direction. To a first approximation the radius of curvature of the bend (assumed to be of a circular form) is given by R where

$$R=(u^2+16A^2n^2)/32An^2$$

The flow through such a pipe is more likely to depend on A/R than on r/R, except when A/r is very small. Thus the Dean number can be modified to

$$D_m=\frac{1}{2}\ \mathrm{Re}\ (A/R)^{\frac{1}{2}}$$

Substituting for R and putting $\mathrm{Re}=2ur/v$ where v is the kinematic viscosity

$$D_m=\mathrm{Re}\sqrt{x^2/2(1+x^2)}$$

for $x=\frac{4}{\mathrm{Re}}\left(\frac{And}{v}\right)$ and $d=2r$.

In order to determine the effect of vibration on friction factor, experimental values of λ/λ_0, where λ_0 is the friction factor for the unvibrated pipe, are plotted in Fig. 3 against the modified Dean number as curve *AB*. This curve is represented by the relationship

$$\frac{\lambda}{\lambda_0}=0.33\ D_m^{0.20}$$

For values of $x>1.0$ the flow path has a minimum radius of curvature which is smaller than that obtained by using the formula which was mentioned earlier. The magnitude of the effect depends on whether x is being increased by varying the term n/u or the amplitude A. At Reynolds numbers of 2,000, 1,500 and 1,000 the respective values of Dean number for $x = 1{\cdot}0$ are 1,000, 750 and 500. Where $x < 1{\cdot}0$, the friction factor equation given here fits the experimental points quite well, but the points showing most scatter are in general those where $x > 1{\cdot}0$.

The effect of the shape of the displacement time curve is another factor among others which could well influence such an expression because results which were obtained where the pipe was subject to shock conditions lay above the general shape of the curve AB at the points S. These results (curve DC) were obtained using the experimental arrangement described by James and myself[2] where the tests were carried out on pipes having test lengths of 4 in. and 24 in.

The oscillations which were set up by the flow round a bend were analysed by Hawthorne[6] in the case of a non-viscid fluid; this could also be an important factor which influences the flow of a fluid through a vibrating pipe which seems to be sinuous in form to a particle.

With a pipe rigidly fixed at each end and vibrated at the centre as described by Silberman it is not likely that the results would be much different (subject to the comment expressed later) to those expected from the above relationship.

Values of Adn/v and a modified Dean number are tabulated in Table 1 for a range of the most significant vibrating conditions referred to by Silberman.

Table 1

Frequency (c/s)	Amplitude (in.)	Loops	$\frac{Adn}{v}$	D_m Re = 2,000	D_m Re = 1,000
Magnetic vibration					
23·3	0·188	1	47	132	131
46·7	0·125	3	62	175	171
93·3	0·093	3	92	256	244
187	0·063	5	125	343	316
Hydraulic vibration					
31·7	0·250	1	84	233	232

It can be seen from Fig. 3 that in the tests which were carried out by Silberman using an oil of kinematic viscosity $2{\cdot}4 \times 10^{-4}$ ft.^{2}/sec, the effect is likely to be small. The amplitude referred to in Table 1, however, presumably refers to that of the vibrator at the centre of the pipe. For one loop, only the centre of the pipe will have this amplitude, the rest of the pipe being subject to vibrations where the amplitude reduces to zero at the clamps. This would suggest that the amplitude of vibration which is equivalent to the tests on which the formula is based should be reduced. Furthermore, with such a small change in the friction factor, this effect coupled with an additional 28 in. of non-vibrated pipe outside the clamps, yet between the static pressure tappings, could reduce the effect to an unmeasurable quantity. Similar reasoning could be applied to the cases of three and five loops where the amplitude in the loops not connected to the vibrator could also be less.

It is interesting to note that Silberman refers to a series of tests on water where at Reynolds numbers above 2,200 the flow could be turned from laminar to turbulent with certain types of vibration. Calculations based on the formula here indicate that, for water, very definite changes would be expected in friction factor for the vibrations mentioned in Table 1 if the pipe had been vibrated as a whole.

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[1] Silberman, E., *Nature*, **216**, 259 (1967).
[2] James, D. D., and Morton, A. S., *Nature*, **214**, 692 (1967).
[3] White, C. M., *Proc. Roy. Soc.*, A, **123**, 645 (1929).
[4] Dean, W. R., *Phil. Mag.*, S.7, **5**, 673 (1928).
[5] Dean, W. R., *Proc. Roy. Soc.*, A, **121**, 402 (1928).
[6] Hawthorne, W. R., *Proc. Roy. Soc.*, A, **206**, 374 (1951).

Possible Systematic Error in the Measurement of Normal Stress Differences in Polymer Solutions in Steady Shear Flow

It is well known[1] that, in suitable flow conditions, the normal stress differences $p_{11} - p_{22}$ and $p_{22} - p_{33}$ are determined by the values of pressure, $-p_{22}$, on the stationary and rotating walls of cone and plate (cp), plate and plate (pp) and concentric cylinder (cc) apparatuses. The common use of small holes (filled with static liquid) for measuring these pressures must give rise to a systematic error, p_{H} say, where

$$p = p_{\mathrm{H}} - p_{22} \tag{1}$$

is the pressure recorded using a hole and $-p_{22}$ is the pressure which would act on the wall if the hole were absent.

In spite of a warning by Schultz-Grünow[2], p_{H} has hitherto been neglected in the analysis of polymer solution data, partly because Greensmith and Rivlin[3] found that holes of diameters 1·0, 1·7 and 2·6 mm gave indistinguishable results, and partly because estimates for Newtonian liquids suggest that p_{H} will be small[4] (p_{H} about $0{\cdot}05\, p_{21}$, where p_{21} denotes the value of shear stress near to but unperturbed by the hole).

Some recent measurements, however, lead us to suggest that polymer solutions generate much higher values of p_{H} (p_{H} about $2\, p_{21}$) and that, when the $p_{\mathrm{H}}-$ contribution is taken into account, it will be possible to resolve certain known disagreements[5] between the results of measuring normal stress differences in different ways.

If we assume (*a*) that p_{H} is determined by p_{21}, and (*b*) that p_{H} is independent of the gap between the walls, then the equation[1]

$$\Delta_{cp} \equiv -\left(r\frac{\mathrm{d}p}{\mathrm{d}r}\right)_{cp} = p_{11} + p_{22} - 2\, p_{33} \tag{2}$$

for the pressure gradient in the cone and plate apparatus is unaltered, but the equation for the rim pressure $p(R)$ becomes

$$p(R) = p_{33} - p_{22} + p_{\mathrm{H}} + p\mathrm{a} \tag{3}$$

where pa denotes atmospheric pressure, R denotes the radius of the rotating member, and r denotes distance from the axis of rotation. The equations[1] for the plate and plate and concentric cylinder systems become

$$\Delta_{pp} \equiv -\left(r\frac{\mathrm{d}p}{\mathrm{d}r}\right)_{pp} = p_{11} - p_{33} + G\frac{\mathrm{d}}{\mathrm{d}G}(p_{22} - p_{33}) - \left(G\frac{\mathrm{d}p_{\mathrm{H}}}{\mathrm{d}G}\right)_{pp} \tag{4}$$

$$\Delta_{cc} \equiv -\left(r\frac{\mathrm{d}p}{\mathrm{d}r}\right)_{cc} = p_{11} - p_{22} + 2\left(p_{21}\frac{\mathrm{d}p_{\mathrm{H}}}{\mathrm{d}p_{21}}\right)_{cc} \tag{5}$$

G denotes the value of shear rate.

Using the apparatus of Adams and Lodge[5], with the hole diameters increased to 1·3 mm, we find that, for a 2 per cent solution of a high molecular weight polyisobutene ('Oppanol *B*200') in a low molecular weight liquid polyisobutene ('Oppanol *B*1'), Δ_{cp} is independent of r, consistent with equation (2). This is our reason for assumption (*b*). We also find that $p_{11} - p_{22}$, determined from values of the total thrust, F, in a second cone and plate apparatus[6] (which uses no holes for pressure measurement) according to the equation[1]

$$p_{11} - p_{22} = \frac{2F}{\pi R^2} \tag{6}$$

is equal to $2\, p_{21} \cot 2\chi$, where χ denotes the flow birefringence extinction angle measured in the concentric cylinder apparatus of Kaye and Saunders[7]. Values of p_{21} measured in the two latter apparatuses agree with one another.

It is therefore tempting to suppose that our data for Δ_{cp} and F do give correct values for $p_{11}-p_{22}$ and $p_{22}-p_{33}$ by means of equations (2) and (6); we find that, in the range $5<G<25$ (units, sec^{-1}),

$$p_{11} - p_{22} = 240\,G + 500 \qquad (7)$$

$$0 < p_{33} - p_{22} < 0{\cdot}07\,(p_{11} - p_{22}) \qquad (8)$$

With these values for $p_{11}-p_{22}$ and $p_{22}-p_{33}$ and measured values of $p(R)$, we may now calculate the value of p_{H}, using equation (3); we find that, in the range $200<p_{21}<500$,

$$p_{\mathrm{H}} = -3{\cdot}07\,p_{21} + 414 \qquad (9)$$

(units, dynes cm^{-2}).

Thus the holes generate a negative pressure greater in magnitude than the shear stress caused by the primary shear flow.

We can now test the validity of this procedure by using the above values of p_{H}, $p_{11}-p_{22}$ and $p_{22}-p_{33}$ to evaluate the right hand side of equation (4) and comparing the result with values of Δ_{pp} measured in the apparatus of Adams and Lodge. We find that equation (4) is satisfied within the accuracy of measurement, and that the contribution from the term involving p_{H} is substantial. In this way, a disagreement of the type noted by Adams and Lodge[5] (between equations (2), (3) and (4), with $p_{\mathrm{H}}=0$) is resolved.

A further test is afforded by equation (5) which, with equations (6) and (9), implies that

$$\Delta_{cc} < 2F/(\pi R^2) \qquad (10)$$

Our values for Δ_{cc}, obtained from a new concentric cylinder apparatus (J. M. Broadbent and A. S. Lodge, in preparation), with pressure-measuring holes of diameters 3 mm and 6 mm, are consistent with this inequality. Moreover, we find that in certain circumstances negative values are obtained for the pressure on the inner cylinder (even when the appropriate inertial force corrections have been made for the rotation of liquid in the gap and in the inner cylinder); similar results have recently been reported for an aqueous detergent solution[8]. These could be explained by a dominant negative value for p_{H}.

It is possible that, for certain tests of the validity of methods of determining normal stress differences, the contributions from p_{H} in different apparatus might partially cancel one another. For example, for the test obtained by elimination of $p_{11}-p_{22}$ and $p_{22}-p_{33}$ between equations (2), (4) and (5), we see that

$$\Delta_{pp} - \Delta_{cp} + \tfrac{1}{2}\left(G\frac{\mathrm{d}}{\mathrm{d}G} - 1\right)(\Delta_{cc} - \Delta_{cp})$$
$$= \left(G\frac{\mathrm{d}}{\mathrm{d}G} - 1\right)\left(p_{21}\frac{\mathrm{d}p_{\mathrm{H}}}{\mathrm{d}p_{21}}\right)_{cc} - \left(G\frac{\mathrm{d}p_{\mathrm{H}}}{\mathrm{d}G}\right)_{pp} \qquad (11)$$

In this way, it might be possible to explain Markovitz's result[9] that the left hand side of this equation is small for a 5·4 per cent solution of polyisobutene ('*B*100') in cetane, if the liquid properties and hole sizes were such that the right hand side was small.

If assumption (*b*) is valid, and if the constitutive equations for the liquid involve no material constants of the dimension of length, then dimensional considerations[5] suggest that p_{H} will not depend on the hole diameter, because there is no other quantity to form a dimensionless group with this. This rather surprising suggestion might explain the result of Greensmith and Rivlin[3], and suggests that changing the hole diameter might not be a sufficient test for the absence of a pressure contribution from the hole.

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[1] For example, Lodge, A. S., *Elastic Liquids*, chap. 9 (Academic Press, London and New York, 1964).
[2] Schultz-Grünow, F., *Ver. Deut. Ing.-Z.*, **97**, 409 (1955).
[3] Greensmith, H. W., and Rivlin, R. S., *Phil. Trans. Roy. Soc.*, *A* **245**, 399 (1953).
[4] Thom, A., and Apelt, C. J., *Rep. Mem. Aero. Res. Coun.*, No. 3090 (HMSO, London).
[5] Adams, N., and Lodge, A. S., *Phil. Trans. Roy. Soc.*, *A* **256**, 149 (1964).
[6] Adams, N., and Jackson, R., *J. Sci. Instrum.*, **44**, 461 (1967).
[7] Kaye, A., and Saunders, D. W., *J. Sci. Instrum.*, **41**, 139 (1964).
[8] Papenhuijzen, J. M. P., and Van der Tempel, M., *Rheol. Acta*, **6**, 311 (1967).
[9] Markovitz, H., *Phys. Fluids*, **8**, 200 (1965).

THE SOLID STATE

Epitaxy as a Growth Factor in Urinary Calculi and Gallstones

Epitaxy[1] consists of the growth of one crystal, in one or more particular orientations, on a substrate of another, with a near geometrical fit between the respective networks which are in contact. Thus anthraquinone can grow on rocksalt[2] and *d*- or *l*-camphor on *d*- or *l*-quartz[3]. That there is some influence of the structures themselves (apart from the geometry) has been proved, but it is of surprisingly minor importance[3]. There is also a dependence of the degree of misfit which can be tolerated on the degree of supersaturation of the solution from which the epitaxial crystals are deposited[4].

One curious feature of many kidney and bladder stones is the relatively abrupt change of composition which can occur from time to time during growth. Often there are alternating layers of differing composition around a nucleus, combined with a radial arrangement of the crystals[5] in the successive layers (Fig. 1).

Examination of the usual habits of crystals of the common stone-forming compounds (Table 1), together with the dimensions of the networks in the occurring faces, indicates that epitaxy may be of considerable importance in causing the stone to grow larger, once a suitable seed has been deposited. Without any such seed, the urine

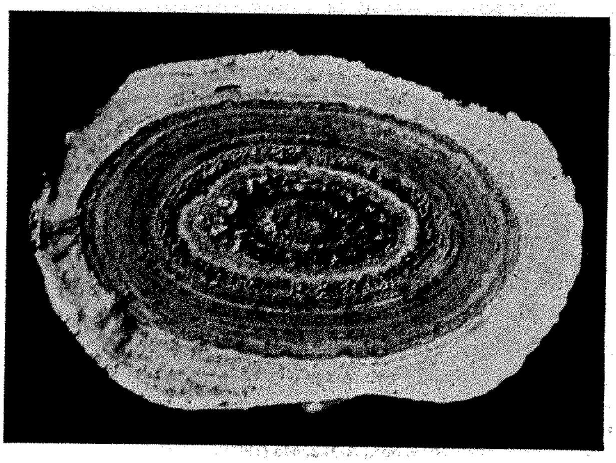

Fig. 1. A bladder stone showing the alternating layers of different composition around a nucleus. The relatively abrupt changes of composition during growth can be seen clearly.

Table 1. COMMON STONE-FORMING COMPOUNDS AND THEIR USUAL HABITS

Compound	
Anhydrous uric acid $C_5H_4N_4O_3$	$\{100\}\ \{210\}\ \{\bar{1}01\}\ \{021\}\ \{010\}\ \{111\}$ $a \doteq 13{\cdot}12\ b = 7{\cdot}40\ c = 6{\cdot}21\ \beta = 90{\cdot}5°$
Uric acid dihydrate $C_5H_4N_4O_3.2H_2O$	$\{100\}\ \{101\}\ \{210\}\ \{021\}$ $a = 17{\cdot}52\ b = 7{\cdot}40\ c = 6{\cdot}35$
Whewellite $CaC_2O_4.H_2O$	$\{100\}\ \{010\}\ \{001\}\ \{011\}\ \{11\bar{1}\}\ \{12\bar{1}\}\ \{131\}$ $a = 6{\cdot}28\ b = 14{\cdot}57\ c = 10{\cdot}11\ \beta = 109{\cdot}4°$
Weddellite $CaC_2O_4.2H_2O$ (to 2·5 H_2O)	$\{101\}$ only $a = b = 12{\cdot}30\ c = 14{\cdot}32$ (tetragonal)
Apatite $Ca_{10}(PO_4)_6(OH)_2$ (or carbonate-apatite)	$\{0001\}\ \{10\bar{1}0\}\ \{11\bar{2}0\}\ \{41\bar{5}0\}\ \{10\bar{1}1\}\ \{10\bar{1}2\}$ $\{20\bar{2}1\}\ \{11\bar{2}1\}\ \{11\bar{2}2\}\ \{21\bar{3}1\}\ \{31\bar{4}1\}\ \{31\bar{4}2\}$ $a = b = 9{\cdot}42\ c = 6{\cdot}88$ (hexagonal)
Brushite $CaHPO_4.2H_2O$	$\{010\}\ \{001\}\ \{\bar{1}01\}\ \{120\}\ \{\bar{1}11\}\ \{1\bar{1}1\}$ $a = 5{\cdot}81\ b = 15{\cdot}18\ c = 6{\cdot}24\ \beta = 116{\cdot}4°$
Whitlockite $\beta = Ca_3(PO_4)_2$	$\{0001\}\ \{11\bar{2}0\}\ \{01\bar{1}2\}\ \{01\bar{1}3\}\ \{10\bar{1}4\}$ etc. $a = b = 10{\cdot}32\ c = 37{\cdot}0$ (rhomb.)
Octacalcium phosphate $Ca_8H_2(PO_4)_6.5H_2O$	Habit not known: $a = 19{\cdot}87$, $b = 9{\cdot}63$ $c = 6{\cdot}88$; $\alpha = 89{\cdot}3°\ \beta = 92{\cdot}2°\ \gamma = 108{\cdot}9°$
Struvite $MgNH_4PO_4.6H_2O$	$\{001\}\ \{010\}\ \{110\}\ \{101\}\ \{011\}\ \{012\}\ \{021\}$ $\{103\}\ \{111\}$ $a = 6{\cdot}94\ b = 6{\cdot}14\ c = 11{\cdot}20$
l-Cystine $[-SCH_2CH(NH_2)COOH]_2$	$\{0001\}$ Probably $\{10\bar{1}0\}$ or $\{11\bar{2}0\}$ $a = b = 5{\cdot}42\ c = 56{\cdot}28$ (hexagonal)
Gallstones only[7-9]	
Cholesterol $C_{27}H_{45}OH$	$\{010\}$ flake face; possibly $\{100\}\ \{001\}$ $a = 14{\cdot}00\ b = 33{\cdot}71\ c = 10{\cdot}46$; $\alpha = 94{\cdot}5°\ \beta = 90°\ \gamma = 95{\cdot}9°$
Calcite $CaCO_3$	$\{0001\}\ \{10\bar{1}0\}\ \{11\bar{2}0\}\ \{10\bar{1}1\}\ \{01\bar{1}2\}$ etc. $a = b = 4{\cdot}99\ c = 17{\cdot}06$ (rhomb.)
Aragonite $CaCO_3$	$\{001\}\ \{010\}\ \{110\}\ \{011\}\ \{012\}\ \{021\}$ etc. $a = 4{\cdot}96\ b = 7{\cdot}97\ c = 5{\cdot}74$
Vaterite $CaCO_3$	Spherulitic or hexagonal plates or lenses $a = b = 7{\cdot}16\ c = 16{\cdot}98$ (hexagonal)

Note: Ammonium acid urate, sodium acid urate and xanthine are not included because neither their habits nor their unit-cell dimensions are known. Ammonium acid urate does, however, sometimes give a slightly orientated powder pattern, with its 3·43 Å spacing either equatorial or meridional when the radial direction of a stone sample is set vertical in a Unicam X-ray photogoniometer. Xanthine also sometimes shows a tendency towards orientated overgrowth.

Newberyte, $MgHPO_4.3H_2O$, although of common occurrence on the surface or exposed sections of old stones, is not included because it appears to be a decomposition product of struvite, and therefore is not a primary component.

can attain a very high degree of supersaturation of certain relatively insoluble components without any deposition[6]. Deposition from a saturated or supersaturated solution of a stone-forming compound will occur at once on a suitable seed or substrate provided that there is an epitaxial relationship. Deposition might be prevented, however, either by habit-modification (provided that a suitable non-toxic additive could be found) or by a sufficient modification of network size, for example, by the substitution for part of the calcium of another divalent ion of different effective radius. Magnesium is the obvious choice (average distance Mg—O is 2·10 Å as compared with 2·40 Å for Ca—O) as long as it does not stimulate the production of struvite. If growth can be prevented, the chance of natural elimination of seed crystals is greatly increased.

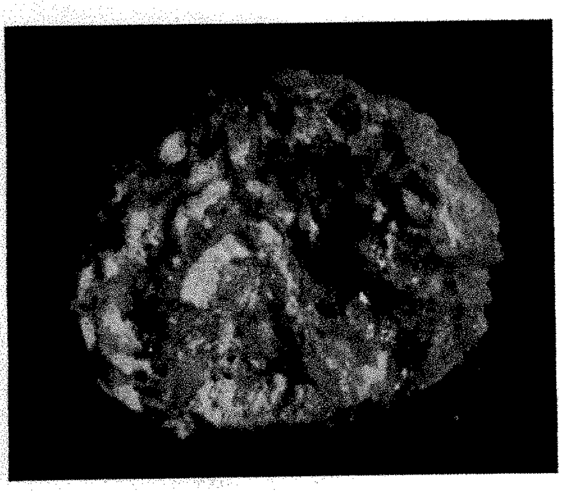

Fig. 2. The interior of a kidney stone, consisting of an intimate mixture of weddellite and apatite.

It is clear from Table 2 that a variety of excellent fits (too many to be purely coincidental) are possible for epitaxial growth on a nucleus of uric acid, uric acid dihydrate, whewellite or weddellite (in any order). These are the substances most frequently found in nuclei of adult urinary calculi. The phosphates more often occur in the outer layers, but frequently stones are found without any apparent nucleus; they seem to consist simply of agglomerations of crystals, some in very finely divided form. Thus Fig. 2 shows the interior of a kidney stone from a 4 year old boy; this consists of an intimate mixture of weddellite and apatite. The (101) network of weddellite and the (0001) network of apatite differ in linear dimensions by only 2·0 and 3·9 per cent. Each presumably can act as substrate for the other.

The role of any complex organic components in the growth of stones is not easy to define. It may be that they take part in the epitaxy, or they may act as a cementing material where epitaxy has not occurred. Nor is it easy to test which kind of epitaxy does actually occur, although it can be shown by X-ray diffraction that there

Table 2. POSSIBLE EPITAXY IN URINARY CALCULI AND GALLSTONES

Substance	Face	Dimensions of net	Percentage misfit	
		Bladder or kidney stones		
Uric acid	(100)	6·21 × 7·40 (90°)	—	—
	(021)	13·12 × 14·46 (90°)	5·6	2·3
Uric acid $2H_2O$	(100)	6·35 × 7·40 (90°)	2·2	0
	(101)	18·64 × 7·40 (90°)	0	0
	(210)	6·35 × 22·93 (90°)	2·2	3·2
Whewellite	(001)	6·28 × 14·57 (90°)	1·1	1·6
Weddellite	(101)	12·30 × 14·32 (90°)	1·0	3·3
Apatite	(0001)	32·62 × 28·26 (90°)	5·0	4·5
Brushite	(010)	6·36 × 30·72 (85·5°)	2·4	3·8
	(101)	6·36 × 15·18 (90°)	2·4	2·5
	(001)	5·81 × 15·18 (90°)	6·4	2·5
Struvite	(001)	6·14 × 6·94 (90°)	1·2	6·2
	(011)	12·77 × 6·94 (90°)	2·8	6·2
	(010)	11·20 × 6·94 (90°)	9·8	6·2
Uric acid	(210)	6·21 × 19·78 (90°)	—	—
Whewellite	(010)	6·28 × 19·07 (90°)	1·1	3·6
Apatite	$(10\bar{1}0)$	18·84 × 20·64 (90°)	1·1	4·3
	$(11\bar{2}0)$	32·62 × 20·64 (90°)	5·0	4·3
	$(41\bar{5}0)$	39·94 × 20·64 (90°)	7·3	4·3
Brushite	(010)	6·36 × 20·48 (85·5°)	2·4	3·5
Whitlockite	(0001)	17·88 × 20·64 (90°)	4·0	4·3
	$(11\bar{2}0)$	17·88 × 37·00 (90°)	4·0	6·4
Octacalcium phosphate	(100)	19·26 × 20·64 (89·3°)	3·4	4·3
	(010)	19·87 × 20·64 (92·2°)	6·7	4·3
Struvite	(101)	6·14 × 39·34 (90°)	1·2	0·5
	(001)	6·14 × 20·82 (90°)	1·2	5·2
		Cystine stones		
Cystine	(0001)	5·42 × 9·39 (90°)	—	—
Apatite	(0001)	16·31 × 9·42 (90°)	0·4	0·3
Struvite	(110)	11·20 × 9·27 (90°)	3·4	1·3
Whewellite	(100)	10·11 × 29·14 (90°)	7·5	3·4
	$(10\bar{3})$	21·22 × 29·14 (90°)	2·1	3·4
		Gallstones		
Cholesterol	(010)	14·00 × 31·38 (90°)	—	—
	(001)	14·00 × 33·71 (96°)	0	6·6
Calcite	$(10\bar{1}0)$	14·97 × 34·12 (90°)	7·0	8·8
Aragonite	(001)	14·88 × 31·88 (90°)	6·3	1·6
Vaterite	$(10\bar{1}0)$	14·32 × 33·96 (90°)	2·3	7·3
Apatite	$(11\bar{2}0)$	13·76 × 32·62 (90°)	1·6	3·1
Cholesterol	(010)	[201] [103] 29·89 × 34·37 (93·5°)	—	—
Calcite	$(10\bar{1}0)$	29·94 × 34·12 (90°)	0·2	0·7
Aragonite	(001)	29·76 × 31·88 (90°)	0·4	7·2
Vaterite	$(10\bar{1}0)$	28·64 × 33·96 (90°)	4·2	1·2

Normally occurring crystal faces with (near-) orthogonal networks which could grow on one another (including alternatives) in some common components. Note that epitaxy can occur between $a \times b$ and $na' \times mb'$ if a, a' (and b, b') only differ by a few per cent.

is orientated crystal growth (ref. 5, and unpublished work in this laboratory) in striated or layered calculi. X-ray diffraction, however, cannot determine the nature of the interfaces between neighbouring crystallites. Experiments to determine relative orientations of calculus components are, however, being made, and we propose also to attempt the growth of artificial stones on suitable crystal substrates and then to find out whether growth can be prevented by substrate modification. Similar experiments in other laboratories would be welcomed.

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[1] Royer, L., *Bull. Soc. Franç. Minéral.*, **51**, 7 (1928).

[2] Willems, J., *Naturwissenschaften*, 44/52, 324 (1944).

[3] Kleber, W., Ickert, L., and Vollstädt, N., *Zeit. Phys. Chemie.*, **222**, 285 (1963).

[4] Newkirk, J. B., and Turnbull, D., *J. Appl. Phys.*, **26**, 579 (1955).

[5] Carr, J. A., *Brit. J. Urol.*, **25**, 26 (1953).

[6] Vermeulen, C. W., Lyon, E. S., and Gill, W. B., *Invest. Urol.*, **1**, 370 (1964); Fried, F. A., and Vermeulen, C. W., *ibid.*, **2**, 131 (1964).

[7] Sutor, J., and Wooley, S. E., *Science* (in the press).

[8] Rains, A. J. Harding, in *Gallstones, Causes and Treatment* (W. Heinemann Medical Books Ltd., London, 1964).

[9] Juniper, jun., K., and Woolf, W. E., *Amer. J. Med.*, **20**, 383 (1956).

Refractive Indices of Mixed Crystals of Phenazine and *N*-Oxyphenazine

REFRACTIVE indices have been measured for the complete range of mixed crystals of phenazine and *N*-oxyphenazine. These measurements, which were repeated several times for each batch of crystals, indicate the presence of a discontinuity in the series, extending from 8 to 30 mole per cent of *N*-oxyphenazine.

Crystals of phenazine ($C_{12}H_8N_2$) and *N*-oxyphenazine ($C_{12}H_8N_2O$) crystallize out of methyl ethyl ketone. The crystallographic data are as follows

$C_{12}H_8N_2$: $P2_1/a$, $Z=2$,
$a=13{\cdot}20(\pm 0{\cdot}005)$, $b=5{\cdot}05(\pm 0{\cdot}02)$, $c=7{\cdot}08(\pm 0{\cdot}005)$ Å,
$\beta=109{\cdot}2(\pm 0{\cdot}05)°$,
$D_x=1{\cdot}341$ g/cm^3 (using $N=6{\cdot}023\times 10^{23}$ mole^{-1}),

$C_{12}H_8N_2O$: $P2_1/a$, $Z=2$,
$a=14{\cdot}29(\pm 0{\cdot}01)$, $b=4{\cdot}60(\pm 0{\cdot}02)$, $c=7{\cdot}44(\pm 0{\cdot}005)$ Å,
$\beta=108{\cdot}8(\pm 0{\cdot}05)°$,
$D_x=1{\cdot}404$ g/cm^3

The cell dimensions were determined at room temperature (21° C) from Straumanis-loaded films with cobalt radiation, the calculations being done by least squares refinement on five to seven reflexions (λ K$\bar{\alpha}=1{\cdot}7902$ Å). The structures of these substances are known[1,2].

In the crystal structure of *N*-oxyphenazine, the molecules are arranged in a disordered manner, thus producing a statistical centre of symmetry. A similar situation occurs in anthrone[3].

Phenazine and *N*-oxyphenazine are reported[2] to form a continuous solid solution series. According to Kitaigorodskii[4], this would only be possible if overall symmetry were retained on substitution of foreign molecules in a "host" lattice. Some evidence for this has been provided by studies of several organic mixed crystal series, which were grown from the melt, for example, acridine–anthracene[5], anthracene-phenanthrene[6], dibenzyl-stilbene[7] and tolane–mercury diphenyl[8].

Phenazine and *N*-oxyphenazine easily produce flat, needle-like crystals with well developed (20Ī) faces from solution[2]. Because there is some inherent structural disorder in one end member (*N*-oxyphenazine) we felt that this would be an interesting system to study.

The crystals were grown from methyl ethyl ketone in sealed glass containers and the compositions were determined to ~1 per cent by ultraviolet spectroscopy (phenazine peak at 250 mμ, *N*-oxyphenazine peak at 266 mμ; in ethanol). This was necessary because the difference in solubility between the two components meant that the composition of the mixed crystals was not the same as that of the original mixed solute.

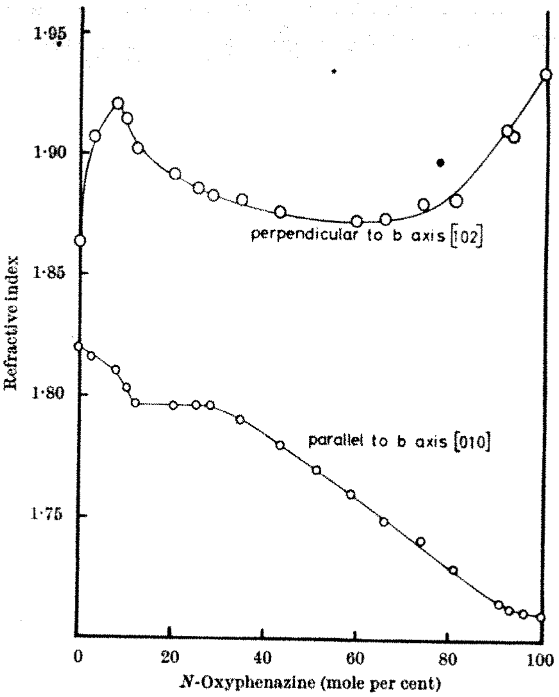

Fig. 1.

It was immediately noticed that crystals having a composition between 10 and 40 mole per cent *N*-oxyphenazine were fibrous and badly formed, the worst crop of crystals having a composition of 25 mole per cent. It therefore seemed possible that there was a region of immiscibility; that is that the system was one of partial miscibility and not a continuous solid solution series.

Attempts to construct a phase diagram were hampered by the thermal decomposition of *N*-oxyphenazine. A graph of cell dimensions against composition seemed to give a set of smooth curves, with a break only where the crystals were too poor for X-ray study. It was thought that an investigation of refractive index against composition might give evidence for or against a partially miscible system.

At first, a great deal of difficulty was encountered in measuring the high refractive indices (above 1·86) and immersion media in this region tended to attack and dissolve the crystals. Eventually it was found that suitable results could be obtained by using a mixture of arsenic tribromide, arsenic sulphide and arsenic triiodide[9,10]. This was a very stable mixture, giving refractive indices higher than 1·94. Using a Leitz–Jelley refractometer, with sodium light, measurements of up to 1·92 could be made, to an accuracy of ± 0·001. Methylene iodide was used to dilute the medium in order to vary the refractive index. Because solution of the crystals still occurred, observations of the Becke line were made as rapidly as possible. The effect of dissolved material on the refractive index liquid was a change of the order of 0·001. Measurements were made parallel and perpendicular to the *b* axis. In the case of phenazine, the refractive index along *b* was the β index and agreed with the literature value[11]. It was not possible to ascertain whether this was also true for *N*-oxyphenazine because a suitable interference figure could not be obtained. A graph of refractive index parallel and perpendicular to the b axis is plotted against composition in Fig 1 This shows that there is a remarkably large peak at a composition of 8 mole per cent of N-oxyphenazine for refractive indices which were measured perpendicular to the b axis [102], while measurements parallel to the b axis [010] show a depression starting at 8 mole per cent and ending at 30 mole per cent X-ray photographs showed that the crystals, at all compositions, had the same developed faces Thus the refractive index measurements perpendicular to the b axes were always obtained with the same crystal face perpendicular to the microscope axis

The exact reason for the two effects, however, is not yet apparent and an X-ray investigation of the series, at present in progress, may throw more light on the shapes of these curves

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[1] Herbstein, F H , and Schmidt, G M J , *Acta Cryst* , 8, 399 (1955)
[2] Curti, R , Riganti, V , and Locchi, S , *Acta Cryst* ,14, 133 (1961)
[3] Srivastava, S N , *Acta Cryst* ,17, 851 (1964)
[4] Kitaigorodskii, A I , *Dokl Akad Nauk SSSR*, 113, 604 (1957)
[5] Kitaigorodskii, A I , and Myasnikova, R M , *Kristallografiya*, 3, 160 (1958)
[6] Kitaigorodskii, A I , and Kozhin, V M , *Kristallografiya*, 4, 209 (1959)
[7] Kolosov, N Ia, *Kristlalografiya*, 3, 700 (1958)
[8] Kitaigorodskii, A I , Myasnikova, R M , and Samarskaya, V D , *Kristallografiya*, 8, 393 (1963)
[9] Borgström, L H , *Comm Géol Finlande* , Bull No 87, 58 (1929)
[10] Meyrowitz, R , *Science*, 122, 1272 (1955)
[11] Wood, R G , and William, G , *Phil Mag* , 31, 115 (1941)

CHEMISTRY

Initiation of Free Radical Polymerization by Manganese Carbonyl and Carbon Tetrachloride

Initiation of free radical polymerization by metal carbonyls in the presence of organic halides has been shown, in all cases studied, to involve a rate determining primary activation of the carbonyl, in which the halide does not participate[1,2] In the system $Mn_2(CO)_{10}$ + CCl_4 + monomer, the monomer does not enter into this process[2], which would therefore seem likely to consist either of scission of carbon monoxide from the carbonyl molecule or rupture of the metal–metal bond After considering both possibilities, we concluded that experimental data from polymerization studies favour the former[2] Wawersik and Basolo reached a similar conclusion for substitution reactions of manganese carbonyl[3] On the other hand, Hopgood and Poe reported that substitution, oxidation and decomposition reactions of $Mn_2(CO)_{10}$ involve primary fission of the manganese–manganese bond[4], although more recently Haines and Poe have stated that carbon monoxide-scission contributes an additional rate determining step[5] Haines and Poe therefore propose[5] that in the initiation of polymerization both primary steps occur, giving rise to two independent mechanisms, A and B

$$(A) \qquad \mathrm{Mn_2(CO)_{10}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \{\mathrm{2Mn(CO)_5}\} \tag{1}$$

$$\{\mathrm{2Mn(CO)_5}\} + \mathrm{CCl_4} \xrightarrow{k_2} \mathrm{Mn(CO)_5Cl} + \mathrm{Mn(CO)_5} + \dot{\mathrm{C}}\mathrm{Cl_3} \tag{2}$$

$$\mathrm{Mn(CO)_5} + \mathrm{CCl_4} \xrightarrow{k_3} \mathrm{Mn(CO)_5Cl} + \dot{\mathrm{C}}\mathrm{Cl_3} \tag{3}$$

$$\mathrm{Mn(CO)_5} \xrightarrow{k_4} \text{inactive products} \tag{4}$$

$$(B) \qquad \mathrm{Mn_2(CO)_{10}} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \mathrm{Mn_2(CO)_9} + \mathrm{CO} \tag{5}$$

$$\mathrm{Mn_2(CO)_9} + \mathrm{CCl_4} \xrightarrow{k_6} \dot{\mathrm{C}}\mathrm{Cl_3} + \text{inactive products} \tag{6}$$

$$\mathrm{Mn(CO)_9} \xrightarrow{k_7} \text{inactive products} \tag{7}$$

Unfortunately, these authors make an elementary error in their kinetic treatment by assuming that the rates of polymerization, corresponding to the separate mechanisms, are additive, and so arrive[5] at the incorrect rate expression (8) In this equation a, b are given by equations (9) and (10), ω is the rate of polymerization, M represents monomer and k_p, k_t are the velocity coefficients for chain propagation and termination, respectively

$$\omega = \omega_A + \omega_B = k_p k_t^{-\frac{1}{2}}[M]\,[\mathrm{Mn_2(CO)_{10}}]^{\frac{1}{2}}(a+b) \tag{8}$$

$$a = \left(1 + \frac{k_3[\mathrm{CCl_4}]}{k_4 + k_3[\mathrm{CCl_4}]}\right)^{\frac{1}{2}} \left(\frac{k_1 k_2[\mathrm{CCl_4}]}{k_{-1} + k_2[\mathrm{CCl_4}]}\right)^{\frac{1}{2}} \tag{9}$$

$$b = \left(\frac{k_5 k_6[\mathrm{CCl_4}]}{k_{-5}[\mathrm{CO}] + k_7 + k_6[\mathrm{CCl_4}]}\right)^{\frac{1}{2}} \tag{10}$$

The correct rate expression, based on the additivity of rates of initiation, is given in equation (11)

$$\omega = k_p k_t^{-\frac{1}{2}}[M][\mathrm{Mn_2(CO)_{10}}]^{\frac{1}{2}}(a^2+b^2)^{\frac{1}{2}} \tag{11}$$

The validity of Haines and Poe's claim that their mechanism is in excellent quantitative agreement with our experimental data is called in question by these considerations In specified conditions[5], the rate of initiation predicted by equations (1) to (7) with their parameters is only 57 per cent of that measured by us[2], while equation (8) suggests rather precise agreement Perhaps more significant is the fact that the mechanism does not account satisfactorily for the behaviour of the system in the presence of added carbon monoxide It is easy to show from equation (11) that, at high $[\mathrm{CCl_4}]$,

$$\frac{\omega([\mathrm{CO}]=0)}{\omega([\mathrm{CO}]=\infty)} = \left(\frac{2k_1+k_5}{2k_1}\right)^{\frac{1}{2}} \tag{12}$$

so that, with the values of Haines and Poe for k_1, k_5, the ratio has the value 1 08, compared with the experimental figure[6] of 1 21 for a carbon monoxide pressure of 775 mm of mercury Fig 1 of Haines and Poe, in which they purport to demonstrate a sensitivity to carbon monoxide similar to that observed experimentally, is incorrect, because the calculated rate for $[\mathrm{CO}]=0$ is derived from equation (8) Furthermore, it seems to be assumed, with no obvious experimental justification, that a pressure of carbon monoxide of 760 mm of mercury is kinetically equivalent to $[\mathrm{CO}]=\infty$

Whether or not the mechanism of equations (1) to (7) may be modified to overcome these shortcomings is debatable, it is important to note that our measured rates of polymerization are initial values, and thus are not affected by reactions of intermediates with half-lives comparable with that of $Mn_2(CO)_{10}$ It is relevant to the postulation of $Mn(CO)_5$ radicals that in the polymerization of methyl methacrylate at 25° C photosensitized by $Mn_2(CO)_{10}+CCl_4$ the quantum yield for initiation is unity and unsymmetrical fission of the manganese–manganese bond has been postulated as the primary act[7]

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[1] Bamford, C H, and Finch, C A, *Proc Roy Soc*, A 268, 553 (1962)

[2] Bamford, C H, and Denyer, R, *Trans Farad Soc*, 62, 1567 (1966), this paper gives earlier references

[3] Wawersik, H, and Basolo, F, *Chem Commun*, 366 (1966)

[4] Hopgood, D, and Poe, A J, *Chem Commun*, 831 (1966)

[5] Haines, L I B, and Poe, A J, *Nature*, 215, 699 (1967)

[6] Bamford, C H, and Finch, C A, *Trans Farad Soc*, 59, 540 (1963)

[7] Bamford, C H, Crowe, P A, Hobbs J, and Wayne, R P, *Proc Roy Soc*, A 292, 153 (1966)

Adsorption Efficiency in the Collision of Molecular Oxygen with Clean Tungsten Surfaces

Morrison and Roberts[1] obtained consistent calculations for the variation of the accommodation coefficient of neon on a tungsten filament during the adsorption of oxygen using the assumption that the sticking probability for oxygen on tungsten is initially unity and independent of coverage Later work on filaments and ribbons, using the sophisticated techniques of ultra-high vacuum technology and the more direct flash filament method for measuring sticking probabilities[2] has, however, produced values between 0 1 and 0 2 (refs 3 to 6), although Schlier[7] has reported a value of 0 65

The sticking probability can be obtained directly using a reflexion detector technique in which a detector (ionization gauge or mass spectrometer) is so placed relative to adsorbent and gas supply that gas molecules can only enter it after collision with the adsorbent The cell used in the present work is diagrammatically illustrated in Fig 1, and is a modification of a previous design[8,9] Using modern ultra-high vacuum techniques[10], vacua of about 2×10^{-11} torr (nitrogen equivalent, measured by a Redhead modulated Bayard-Alpert ionization gauge[11] with a thoria-coated filament) were achieved in the cell The adsorbent was obtained by depositing metal vapour from a thoroughly outgassed tungsten wire (0 3 mm diameter, Murex Ltd, grade 1) on to the walls of the 1 litre spherical reaction cell Oxygen gas was directed at the film from a centrally positioned retractable diffuser, and the gauge side-arm designed so that gas from the diffuser could not enter the gauge tubulation without first colliding with the metal film Because even low concentrations of non-adsorbable impurities from the gas supply accumulate in the cell, giving an anomalous pressure reading and thus a low apparent sticking probability[8], great care was taken in the preparation of impurity-free oxygen by heating very thoroughly outgassed potassium permanganate British Oxygen Company bottled oxygen was found to be unsuitable It is necessary to use a modulated ionization gauge to measure oxygen pressure because, as shown by Redhead[11] and Singleton[12] and confirmed in our work (results to be published), the residual pressure varies with the amount of exposure of the gauge to oxygen To reduce the effect of pumping by the gauge, it was "saturated" with oxygen, at a pressure of about 10^{-7} torr, after outgassing and before film deposition The sticking probability s can be evaluated from the simple relationship

$$s=\frac{\text{rate of adsorption}}{\text{rate of bombardment}}=\frac{r_i}{r_i+\text{reflexion rate}}$$
$$=1/(1+ZP/r_i) \qquad (1)$$

where Z is the Knudsen collision factor, P the gauge pressure reading and r_i the rate of gas inflow into the cell

With the cell maintained at 78°, 195° or 300° K, when the gas supply was first opened ($r_i=6\times10^{11}$ molecules sec^{-1} cm^{-2} geometric area of film) to a clean film the pressure rise was $<1\times10^{-11}$ torr, from equation (1), this indicates an initial sticking probability s_0 within the limits

$$1\geq s_0>0\ 997$$

Furthermore, after adsorption for about 20 min the gauge pressure had only risen by 8×10^{-11} torr at 195 or 300° K, while at 78° K no rise was observed, the initial sticking probability is therefore very close to unity and is virtually independent of coverage up to at least 10×10^{14} molecules/cm^2 geometric area of film These results therefore confirm the original self-consistent assumptions made by Morrison and Roberts[1] but apparently contradict the more recent flash filament results

It is not possible to attribute the discrepancy to differences in either gas or surface temperatures The possibility that differences may be attributable to differences in the nature of the adsorbent surfaces should be considered (1) Metal films are porous and present a rough surface to the impinging gas molecules, resulting in multiple collisions (as discussed in detail elsewhere[13]) while filaments possess relatively low roughness factors But $s=1$ for a rough surface requires that s is also unity for a perfectly smooth surface (2) The exposed crystal planes in tungsten film and filament surfaces may occupy different fractional areas The value of unity for s, however, implies that all planes exposed in the film are capable of adsorbing oxygen with $s=1$, it is most unlikely that planes exposed in filaments are not present in the

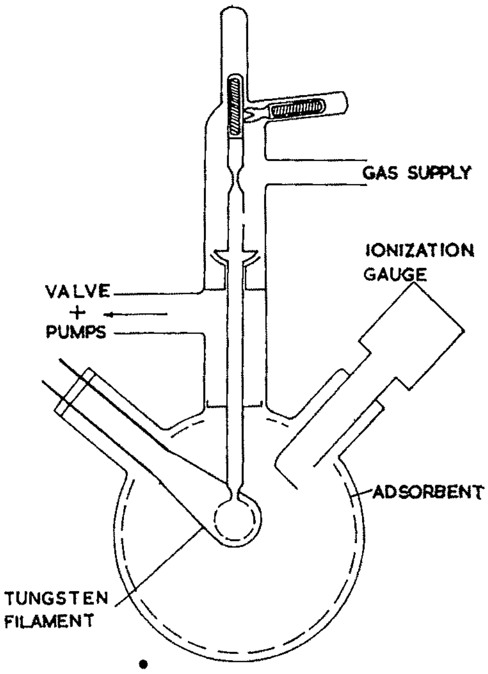

Fig 1 The adsorption cell ('Pyrex' glass)

polycrystalline films Thus the discrepancy seems to be caused by an experimental artefact in the flash filament technique for the oxygen-tungsten system

Reflexion detector techniques (rdt) possess a number of advantages over flash filament techniques (fft) As $s \to 1$, $P \to 0$ in equation (1) and the calculated value of s becomes virtually independent of the gauge calibration factor with rdt, while it is a critical factor with fft With rdt, by operating at low adsorption rates the experiment can be made to last hours, while with fft experiments are usually over in seconds, with a concomitant loss of detail and accuracy. With rdt the gauge can be operated intermittently, thereby reducing the possibility of gauge phenomena affecting the results, while with fft gauge effects are magnified because the area of the adsorbent is approximately equal to that of the gauge grid (usually molybdenum), and the gauge must be operated continuously Finally, with fft adsorption rates are measured from the pressure drop occurring when the filament is cooled, and reversible desorption from the gauge grid would result in an anomalously small pressure drop, and thus an anomalously low calculated value for the sticking probability

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---

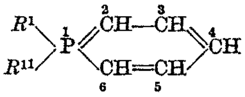
[1] Morrison, J L, and Roberts, J K, *Proc Roy Soc*, A, **173**, 1 (1939)
[2] Hayward, D O, and Trapnell, B M W, in *Chemisorption*, second ed (Butterworths, London, 1964)
[3] Becker, J A, *Solid State Phys*, **7**, 379 (1958) Becker, J A, Becker, E J, and Brandes, R G, *J Appl Phys*, **32**, 411 (1961)
[4] Eisinger, J, *J Chem Phys*, **30**, 412 (1959)
[5] Ageev, V N, and Ionov, N I, *Zhur Tekhn Fiz*, **35**, 2109 (1965) (English trans *Soviet Phys—Techn Phys*, **10**, 1614 (1966))
[6] Singleton, J H, *J Chem Phys*, **47**, 73 (1967).
[7] Schlier, R E, *J Appl Phys*, **29**, 1162 (1958).
[8] Hayward, D O, King, D A, and Tompkins, F C, *Chem Comm*, 178 (1965)
[9] Hayward, D O, King, D A, and Tompkins, F C, *Proc. Roy Soc*, A, **297**, 305 (1967)
[10] Ehrlich, G, *Adv Catalysis*, **14**, 255 (1963)
[11] Redhead, P A, *Vacuum*, **13**, 253 (1964)
[12] Singleton, J H, *J Chem Phys*, **45**, 2819 (1966)
[13] King, D A, and Tompkins, F C, *Trans Farad Soc* (in the press)

Huckel Molecular Orbital Models for Heterocycles with Tetra-coordinated Phosphorus Atoms

MANY heterocyclic compounds with tetra-coordinated phosphorus atoms have been synthesized[1,2]. Several models for Huckel molecular orbital calculations[3–6] have been proposed for heterocycles containing atoms with *d*-orbitals, but the most suitable model should first be found before such calculations are made for these phosphorus heterocycles The most suitable model can be found by comparing the experimental data for phosphabenzene (more exactly 1,1-diphenylphosphabenzene)[1,7,8] with the Huckel parameters calculated for all these models

Three models could be used for Huckel molecular orbital calculations in tetra-coordinated pentavalent phosphorus heterocycles. Fukui *et al.*[3] considered one normal $d\pi$ orbital (a d^3s hybrid) and one π electron for the phosphorus atom. According to Mason[4] and Craig and Paddock[5], the $d\pi$ orbital of the phosphorus atom should be a $3d_{xy}$ orbital, which yields β exchange energy integrals differing in sign from the two adjacent $2p\pi$ carbon orbitals. According to Dewar[6], the phosphorus atom should have two orthogonal $3d\pi$ orbitals, conjugation being interrupted at the phosphorus atom We used four models in our Huckel molecular orbital calculations of formally unsubstituted phosphabenzene (1) the normal Fukui model with $\alpha_P = \alpha - 0{\cdot}6\beta$, $\beta_{CP} = 0{\cdot}9\beta$, (2) the "anti-Huckel" model of Mason and Craig with $\alpha_P = \alpha - 0{\cdot}6\beta$, $\beta_{CP} = 0{\cdot}9\beta$, $\beta_{C'P} = -0{\cdot}9\beta$, (3) the Dewar model, with 7 atomic orbitals $\alpha_P = \alpha_{P'} = \alpha - 0{\cdot}6\beta$, $\beta_{CP} = \beta_{C'P'} = 0{\cdot}9\beta$, $\beta_{PP'} = 0$, and (4) the weak phosphorus–carbon interaction model (normal Fukui model) with $\alpha_P = \alpha - 3\beta$, $\beta_{PC} = 0{\cdot}3\beta$ The α_P parameter was chosen so that the $3d\pi$ phosphorus orbital was slightly more electronegative than the $3p\pi$ phosphorus orbital, for which Pauling electronegativity considerations (work to be published) indicated that $\alpha_P = \alpha - 0{\cdot}4\beta$ The phosphorus parameters for weak phosphorus–carbon interaction are those of Brown and McCormack[9] The results are listed in Table 1

Table 1. HÜCKEL MOLECULAR ORBITAL CALCULATIONS FOR PHOSPHABENZENE



	Model 1	Model 2	Model 3	Model 4
Σ_{LE}	$\alpha - 1{\cdot}000\beta$	$\alpha - 0{\cdot}222\beta$	$\alpha - 0{\cdot}317\beta$	$\alpha - 1{\cdot}000\beta$
Σ_{HO}	$\alpha + 0{\cdot}727\beta$	α	$\alpha + 0{\cdot}483\beta$	$\alpha + 0{\cdot}040\beta$
R	$1{\cdot}916\beta$	$1{\cdot}098\beta$	$1{\cdot}752\beta$	$1{\cdot}496\beta$
q_1	0·687	0·410	0·892	0·029
$q_2 = q_6$	1·074	1·384	1·168	1·324
$q_3 = q_5$	1·016	0·784	0·834	1·000
q_4	1·142	1·333	1·070	1·324

According to model (1), phosphabenzene should be a stable aromatic heterocycle The first ultraviolet band should be somewhat displaced towards the red in comparison with benzene, for 1,1-diphenylphosphabenzene $\Sigma_{LE} - \Sigma_{HO} = -1{\cdot}376\beta$ and with the usual spectroscopic $\beta = -2{\cdot}5 \times 10^4$ cm^{-1} the first absorption maxima should be at 2900 Å. According to model (2), phosphabenzene should be unstable towards both electron accepting and electron donating reactions, towards radical reactions (dimerizations) or should be, in fact, biradical (small $\Sigma_{LE} - \Sigma_{HO}$ differences) The electronic spectrum should be at very long wavelengths According to model (3), phosphabenzene should be stable The first absorption maxima (calculated with $\beta = -2{\cdot}5 \times 10^4$ cm^{-1}) should be at about 5000 Å According to model (4), phosphabenzene should behave similarly to the $R(\mathrm{CH})_5R^-$ anion It should be an amphiionic substance with a basicity similar to the

$$(\mathrm{CH_3})_3\mathrm{N^+}\text{—}\mathrm{CH^-}\text{—}\mathrm{CH{=}CH_2} \overset{\pm\mathrm{H}^+}{\rightleftharpoons} (\mathrm{CH_3})_3\mathrm{N^+}\text{—}\mathrm{CH_2}\text{—}\mathrm{CH{=}CH_2}$$

equilibrium As shown in the *Beilstein's Encyclopaedia*[10], the quaternary ammonium hydroxide with allyl substituents is a strong base and does not show a tendency to deprotonate in aqueous media

1,1-Diphenylphosphabenzene is obtained as a yellow precipitate from aqueous solutions with an electronic maxima at 4090 Å in aetheric solution The substance is stable in water but is protonated in position 2 in acidic solutions It is extremely prone to auto-oxidation[1,7,8]

The electronic spectra and the general stability eliminate the "anti-Huckel" model (2) Auto-oxidability is a general tendency of organic phosphorus compounds and does not seem to be caused by peculiarities of the π electronic system. The moderate basicity eliminates model (4) based on the weak phosphorus–carbon interaction There remain the normal Fukui model (1) and the Dewar model (3); to decide between them, more comparisons between the Huckel calculations and the experimental data for tetracovalent phosphorus-heterocycles are necessary

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---

[1] Markl, G , *Angew Chem* , **77**, 1109 (1965)

[2] Purdela, D , and Vîlceanu, R , in *Chimia compusilor organici ai fosforului si ai acizilor lui*, chap 8 (Academia R S Române, Bucuresti, 1965)

[3] Fukui, K , Morokuma, K , and Nagata, Ch, *Bull Chem Soc Japan* **33**, 1214 (1961)

[4] Mason, S F , *Nature*, **205**, 495 (1965)

[5] Craig, D P , and Paddock, N L , *J Chem Soc* , 418 (1962)

[6] Dewar, M J S , Lucken, E A , and Whitehead, M A , *J Chem Soc* , 2423 (1960)

[7] Märkl, G , *Angew Chem* , **75**, 168 (1963)

[8] Markl, G , *Angew Chem* , **75**, 1121 (1963)

[9] Brown, D A , and McCormack, C G , *J Chem Soc* , 5385 (1964)

[10] *Beilstein's Encyclopaedia*, **4**, 206, Hauptband

BIOCHEMISTRY

Comparison of the Kinetics of Photosynthetic Carbon Dioxide Fixation in Maize, Sugar Cane and Tobacco, and its Relation to Photorespiration

Most plants exhibit the phenomenon of photorespiration that is to say they give out a certain amount of carbon dioxide in the light This effect is usually masked by the occurrence of photosynthesis which takes up carbon dioxide at a greater rate than photorespiration releases it Photorespiration can, however, usually be demonstrated by allowing leaves to photosynthesize in an enclosed space when they are unable to reduce the carbon dioxide concentration of the atmosphere below about 50–100 p p m This is because at this point carbon dioxide starvation reduces the rate of photosynthesis to a level at which it equals photorespiration and therefore no further net uptake of carbon dioxide is observed More recently, a small group of plants, including maize and sugar cane, has been investigated, which can reduce the carbon dioxide concentration of a closed system virtually to zero[1] This could be the result either of their having a poor photorespiratory mechanism or of their possessing an unusually efficient photosynthetic mechanism There is evidence that the mechanism of photosynthetic carboxylation at least is different in maize and sugar cane from that in most other plants, for the initial product of photosynthetic carbon dioxide fixation in maize and sugar cane is oxalo-acetate, rather than the phosphoglycerate which is the normal product of most plants[2] Thus if different carbon dioxide fixing enzymes were operating in maize and sugar cane, this could well account for an increased affinity for carbon dioxide in these species

To test this, the apparent Michaelis constants for photosynthesis of maize and sugar cane were determined and compared with that of tobacco, which exhibits normal photorespiration[3] and normal photosynthesis with phosphoglycerate as initial product[2] The apparent Michaelis constant is the external carbon dioxide concentration which enables the leaf, at saturating light intensity, to fix carbon dioxide at half the maximal velocity While it is not claimed that this represents the true Michaelis constant for the photosynthetic carboxylating enzyme, it does, by analogy, represent the affinity of the overall photosynthetic system for carbon dioxide—the lower the apparent Michaelis constant the greater being that affinity In order to eliminate the effect of photorespiration on measurements of photosynthesis, photorespiration was suppressed by measuring the uptake of carbon dioxide in an atmosphere of nitrogen This suppression can be clearly shown by the fact that in these conditions a photosynthesizing tobacco leaf segment can reduce the concentration of carbon dioxide in the atmosphere almost to zero

The technique for determining the apparent Michaelis constants for the tissues examined was as follows The apparatus consisted of a 'Perspex' leaf chamber submerged 10 cm beneath the surface of a water bath at 20° C One to five leaf segments, cut to a total area of 100 cm², so as to exclude the main veins, were floated, undersurface upwards, on 20 ml of distilled water in the leaf chamber and illuminated from above at 6,000 ft -candles from a tungsten source A gas stream was pumped from the leaf chamber through a magnesium perchlorate drying tower to an infrared carbon dioxide analyser and then returned to the chamber so as to complete the circuit The rate of gas flow was approximately one circuit/min The leaves were illuminated in air for at least 1 h before measurements were commenced The system was then charged with gas by flushing it with 6,000 p p m carbon dioxide in nitrogen, the circuit sealed off and the concentration of carbon dioxide plotted against time The relative rate of fixation at any particular concentration of carbon dioxide was measured as the slope of a tangent drawn to the curve at that concentration

Table 1

	First replicate		Second replicate		Third replicate	
Species	Max rate	*Km*	Max rate	*Km*	Max rate	*Km*
Tobacco	500	330	520	330	450	290
Sugar cane	400	300	440	330	380	270
Maize	250	260	380	270	330	325

Km (apparent Michaelis constant) is expressed as p p m carbon dioxide

Maximum rate is expressed as p p m reduction in the carbon dioxide concentration in the circuit/min In all cases the rate of fixation was constant at all concentrations of carbon dioxide tested above 1,000 p p m

Table 1 shows that the apparent Michaelis constants for the photosynthesis of all three species are similar, the value being approximately equal to the natural carbon dioxide concentration of the Earth's atmosphere This finding indicates that the affinity for carbon dioxide of these three species is not greatly different It may therefore be argued that the apparent lack of photorespiration in maize and sugar cane is the result of a true deficiency in this process rather than the possession of an unusually efficient photosynthetic mechanism

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[1] Forrester, M L , Krotkov, G , and Nelson, C D , *Plant Physiol* , **41**, 428 (1966)

[2] Hatch, M D , Slack, C R , and Johnson, H S , *Biochem J* , **102**, 417 (1967)

[3] Krotkov, G , *Photosynthetic Mechanisms in Green Plants*, Nat Acad Sci — Nat Res Council Publication 1145 (1963)

Effect of Kinetin on the Phosphatase Enzymes of *Acetabularia*

The effect of auxins on higher plants has been extensively studied[1–3] but their action on algae is not clear Thimann and Beth[4] concluded that while various auxins promoted some aspects of algal growth, there was a wide variation in the effect and the specificity of action was uncertain They also measured the effect of indole acetic acid and naphthalene acetic acid on the growth of nucleate and enucleate *Acetabularia mediterranea* They found that these auxins increased the growth rate but did not affect cap formation in mature cells Similar effects occurred to a smaller extent in enucleate cells

Table 1 EFFECT OF KINETIN ON WHOLE CELLS

Initial size (mm)	Weeks	Control			10^{-9} molar			10^{-7} molar			10^{-5} molar		
		%*	*p*H 5 †	*p*H 12 ‡	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12
7	0	0	0 15	0									
	3	0	0 17	0	0	0 23	0	0	0 22	0	0	0 15	0
	6	0	0 19	0	0	0 24	0	0	0 23	0 02	0	0 2	0
	11	0–10	0 29	0 03	10–40	0 28	0 06	20–44	0 29	0 11	5–27	0 35	0 05
15	0	0	0 2	0 07									
	4	2–4	0 22	0 13	0	0 27	0 07	2–8	0 33	0 11	0–2	0 22	0 05
	6	3–5	0 25	0 12	1–4	0 27	0 12	5–23	0 35	0 21	1–3	0 26	0 13
	10	9–30	0 32	0 16	2–6	0 29	0 12	15–35	0 45	0 3	2–6	0 3	0 18
	14	20–30	0 4	0 26	7–25	0 38	0 15	40–45	0 65	0 34	4–10	0 4	0 22
25	0	0	0 22	0 08									
	3	30–40	0 34	0 3	20–20	0 32	0 28	20–16	0 28	0 3	20–42	0 33	0 27
	5	40–46	0 43	0 38	30–44	0 45	0 32	25–33	0 43	0 31	30–36	0 38	0 3
	9	60–28	0 57	0 64	45–33	0 59	0 46	55–35	0 89	0 62	40–44	0 76	0 45

* Large caps more than 3 mm in diameter, small caps less than 3 mm in diameter
† Units of acid phosphatase activity/cell[8] at *p*H 5
‡ Units of alkaline phosphatase activity/cell[8] at *p*H 12

Table 2 EFFECT OF KINETIN ON ENUCLEATE CELLS

Weeks	Control			10^{-9} molar			10^{-7} molar			10^{-5} molar		
	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12
0	0	0 3	0 1									
4	0–6	0 22	0 09	2–8	0 22	0 11	10–14	0 2	0 19	2–6	0 2	0 12
6	2–6	0 25	0 1	6–8	0 34	0 2	14–14	0 35	0 26	4–6	0 35	0 18
8	4–6	0 3	0 15	10–10	0 4	0 3	18–15	0 5	0 3	8–6	0 56	0 25

Brachet[5] has reported that kinetin inhibits the growth of *Acetabularia* at 4 6 × 10^{-9} molar, but later de Vitry[6] found an increased growth rate at the same concentration Zetsche[7] has measured growth rate and cap formation of *A mediterranea* in the presence of kinetin and gibberellin over the range 10^{-9} to 10^{-4} molar He found that the maximum effects on stalk elongation and cap formation occurred at different auxin concentrations

The presence of phosphatase enzymes in *Acetabularia* has been described by Spencer and Harris[8] They found an acid phosphatase (optimum *p*H 5 0) which steadily increased in amount as the cell developed, and an alkaline phosphatase (optimum *p*H 11 7) which only appeared during cap formation This enzyme was also synthesized during cap formation in the enucleate cell

The present communication describes the effect of kinetin on these two enzymes and on cap formation in nucleate and enucleate *A crenulata* The effect of 2,3,5-triiodobenzoic acid (TIB) is also reported At 10^{-4} molar this substance antagonizes plant growth and is toxic at higher concentrations[4] *A crenulata* were grown as previously described[8] The cell density was fifty cells in 100 ml of Erdschreiber medium[9] The necessary amount of kinetin (6-furfurylaminopurine) was added from a stock solution in sterile sea water[7] TIB was dissolved in the minimum volume of sodium hydroxide and was adjusted to *p*H 8 3 with hydrochloric acid, it was then diluted with sterile sea water to give a stock solution The cells were transferred to fresh medium at 14 day intervals Prior to enucleation, the cells were kept in the dark for 10 days The nucleus was removed by amputation of the rhizoid[8] The cells were allowed to recover for 2 days in weak light before addition of the kinetin or TIB This procedure gave a higher recovery rate than when the amputated cells were maintained for 1 day in the dark The cells were collected and the number of caps counted as small (less than 3 mm in diameter) or large (above 3 mm) The phosphatase enzymes were then assayed as previously described[8]

Preliminary experiments showed that kinetin did not affect the cap morphology and a concentration of 10^{-7} molar gave the greatest increase in the rate of cap formation for small nucleate cells The time course for cap formation was therefore followed at 10^{-9}, 10^{-7} and 10^{-5} molar kinetin The results for cells which were initially 7 mm long are shown in Table 1

It can be seen that cap formation is accelerated in the presence of kinetin with the greatest effect at 10^{-7} molar In all cases, cells which were treated with kinetin formed caps on shorter stalks than in the controls The synthesis of acid phosphatase was not greatly affected by the presence of kinetin and again the alkaline phosphatase only appeared when the cell was about to form a cap. Table 1 also shows the effect of kinetin on cells which were 15 and 25 mm long The auxin effect is reduced as the cell matures and is negligible for cells of length 25 mm.

Survival of *Acetabularia* after enucleation necessitates keeping the cells in the dark for 10 days before removal of the rhizoid[10,11] The results obtained for the effect of kinetin on enucleate cells of length 25 mm are shown in Table 2 The growth promotion was greater than for nucleate cells of the same size and cap formation was coupled with the appearance of alkaline phosphatase

The inhibition of growth in the presence of 10^{-6} and 10^{-4} molar TIB is shown for 10 and 30 mm long nucleate cells in Table 3 The results for enucleate cells measuring 25 mm are shown in Table 4 In all cases there was some inhibition of growth at 10^{-6} molar TIB and almost complete inhibition at 10^{-4} molar TIB The inhibition of cap formation also prevented synthesis of the alkaline phosphatase

Table 3 EFFECT OF TIB ON WHOLE CELLS

Initial size (mm)	Wks	Control			10^{-6} molar			10^{-4} molar		
		%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12
10	0	0	0 2	0						
	6	0–50	0 6	0 07	0–40	0 24	0 09	0	0 24	0 04
	11	40–48	0 7	0 3	20–60	0 4	0 16	0–15	0 46	0 1
30	0	0	0 35	0 05						
	3	10–16	0 43	0 25	10–22	0 54	0 34	0	0 31	0 1
	6	30–16	0 5	0 34	40–20	0 9	0 42	0–10	0 7	0 15

Table 4 EFFECT OF TIB ON ENUCLEATE CELLS

Weeks	Control			10^{-6} molar			10^{-4} molar		
	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12	%	*p*H 5	*p*H 12
0	0	0 2	0 05						
4	10–16	0 23	0 11	4–16	0 2	0 09	0	0 25	0 06
8	16–8	0 3	0 12	5–30	0 4	0 13	0	0 3	0 06

These results show the same morphological changes as those described by Thimann and Beth[4] and Zetsche[7] The principal features are that the greatest enhancement of stalk growth and cap formation take place with different concentrations of kinetin Cap formation is, however, always linked with the appearance of alkaline phosphatase.

Kinetin is probably not normally present in *Acetabularia* because added kinetin causes the caps to form on shorter stalks Thimann and Beth[4] similarly concluded that indole acetic acid was not a native auxin Tandler[12] has described the presence of crystals in the central vacuole which are indole derivatives It was shown by the following experiment that this material was probably not an auxin *Acetabularia* plants, 25 mm long, were homogenized and the cell sap was added, in various proportions, to dishes of cells measuring 10 mm and 20 mm Growth was then followed for 6 weeks During this time there was no obvious difference between control cultures and the treated cells

It is unlikely that the phosphatases are closely associated with the chloroplasts, as shown by the following experiment Cell sap was collected from five hundred cells with caps and was then layered on a gradient of 20 per cent to 40 per cent to 60 per cent sucrose After centrifugation, all the chloroplasts were in the 40 per cent layer, but the phosphatase enzymes were on top of the 20 per cent layer There was no phosphatase activity at all in the chloroplast fraction

Both kinetin and TIB exert specific actions on *Acetabularia* and there is no evidence of bizarre changes in morphology or in enzyme formation This suggests that there are specific mechanisms which are modulated by these compounds These processes which cause stalk elongation or cap formation and synthesis of alkaline phosphatase are probably controlled by separate mechanisms in the cytoplasm and the extent of their activities is regulated, to differing degrees, by the presence or absence of inducers such as kinetin or indole acetic acid

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[1] Miller, C O , *Rev Plant Physiol* ,12, 395 (1961)
[2] Fawcett, C H , *Rev Plant Physiol* ,12, 345 (1961)
[3] Phinney, D , and West, A , *Handbuch der Pflanzenphysiologie* (edit by Ruhland, W), 14, 1157 (Springer-Verlag, Berlin, 1961)
[4] Thimann, K V , and Beth, K , *Nature* 183, 946 (1959)
[5] Brachet, J , *Exp Cell Res* , suppl , 6, 78 (1959)
[6] de Vitry, F , *Protoplasma*, 55, 313 (1962)
[7] Zetsche, K , *Planta*, 59, 624 (1963)
[8] Spencer T , and Harris, H , *Biochem J* , 91, 282 (1964)
[9] Hammerling, J , *Arch Protistenk* , 97, 7 (1944)
[10] Hammerling, J , *Ann Rev Plant Physiol* , 14, 65 (1963)
[11] Werz, G , *Brookhaven Symposia in Biology*, 18, 185 (1965)
[12] Tandler, C J , *Planta*, 59, 91 (1962)

Deficiency of Erythrocyte Nicotinamide Adenine Dinucleotide Nucleosidase (NADase) Activity in the Negro

It has been shown that haemolysates from newborn infants differ from those of adults in that UDPgalactose-4-epimerase (epimerase) is active in the absence of exogenous nicotinamide adenine dinucleotide (NAD) (ref 1). The apparent NAD-independent epimerase activity results from low or absent NADase activity in the haemolysates from newborns[2]

Among a group of control samples from adults, epimerase activity independent of exogenous NAD was found in the haemolysate of one individual, a Negro NADase activity was absent from erythrocytes A preliminary study has shown that reduced or absent erythrocyte NADase activity is common among adult Negroes and that the deficiency may be genetically determined

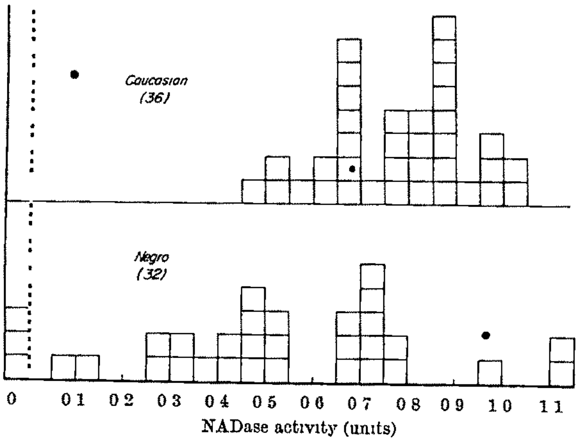

Fig 1 Erythrocyte NADase activity

Haemolysates were prepared from washed erythrocytes as previously described[3] Assay of NADase activity involved incubation in the presence of NAD, followed by determination of the residual NAD concentration In the initial incubation, the reaction mixture consisted of 0 5 μmoles of NAD, 100 μmoles of glycine buffer (*p*H 8 7) and 0 5 ml. of 50 per cent haemolysate in a total volume of 0 8 ml. Incubation was carried out in duplicate for 60 min at 37° C in 3 ml 'Lusteroid' tubes At the end of the incubation period, 2 ml of cold 0 6 normal perchloric acid was added The tubes were stoppered, mixed on a cyclomixer for 20 sec and held in an ice bath for at least 10 min

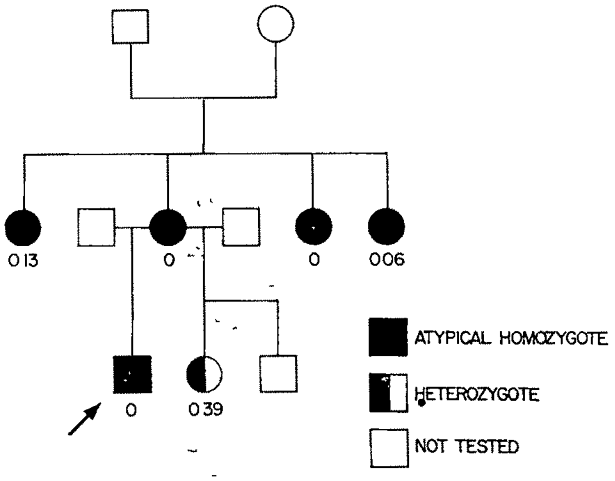

Fig 2 Erythrocyte NADase activity in a Negro family (family *G*) Presumed genotype indicated

NAD was determined according to the method of Minakami *et al* [4] The mixture was centrifuged in the cold at 10,000 r p m for 10 min The supernate was decanted and centrifuged once more at 3,000 r p m at room temperature for 10 min For removal of perchlorate from a 1 ml sample of the supernate, 0 1 ml of 1 molar triethanolamine hydrochloride and 0 1 ml of 5 molar K_2CO_3 were added After 30 min in an ice bath, the precipitate formed was removed by centrifugation for 10 min at 3,000 r p m The concentration of NAD in the supernate was determined by coupling to alcohol oxidation in the presence of an excess of alcohol dehydrogenase The initial concentration of NAD was established on the basis of unincubated control tubes to which perchloric acid had been added at zero time

The activity of haemolysate NADase was expressed as μmoles of NAD disappearing/h/ml of packed red blood cells[5] The validity of the assay was substantiated in a number of instances by simultaneous determination of NAD by the specific cyanide method of Ciotti and Kaplan[6]

The distribution of values found for erythrocyte NADase activity of unrelated healthy adults, both Caucasian and Negro, is shown in Fig 1 The activity range for Negroes spreads more widely than that for Caucasians In three of the thirty-two Negroes no activity was demonstrable

The distribution of values in the Negro sample suggests three groupings For the present purpose, we have arbitrarily designated individuals with values more than 0 65 U as normal homozygotes, those with values less than 0 15 U as atypical homozygotes and those with intermediate values (0 25–0 55 U) as heterozygotes It is

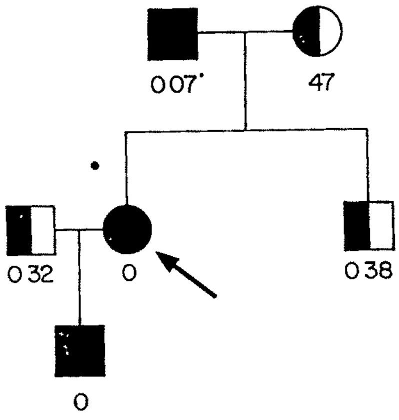

Fig. 3. Negro family *R*.

on this basis that presumed genotypes have been indicated in the pedigrees of the two Negro families so far studied.

The family of the original propositus is presented in Fig. 2. Erythrocyte NADase values were very low or absent for his mother and for each of his three maternal aunts. His half-sister was found to have an intermediate value (0.39 U). In the second family (Fig. 3), very low or absent erythrocyte NADase activity was found for the propositus, for her father and for her son; values for her mother, brother and husband were intermediate (0.47, 0.38 and 0.32 U, respectively). The distribution of activity among family members, particularly in the second family, suggests autosomal transmission.

The present study is a preliminary one, but the data indicate that the pattern of distribution of values among the Negroes so far examined is markedly different from that among the Caucasians.

The function of NADase in the red cell is not known, and significance of the present results in terms of the erythrocyte is uncertain. In the newborn Caucasian infant, erythrocyte NADase activity is low to absent, but normal adult levels are reached within the first year of life[2]. Burton has reported a similar finding for the enzyme in the developing rat brain[7]. Multiple forms of NADase have been demonstrated in mammalian tissues[8]. The availability of erythrocytes with altered NADase activity may provide a basis for establishing the function of this enzyme in the red cell.

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[1] Ng, W. G., Donnell, G. N., Hodgman, J. E., and Bergren, W. R., *Nature*, **214**, 283 (1967).
[2] Bergren, W. R., Ng, W. G., and Donnell, G. N., *Pediatrics*, **40**, 136 (1967).
[3] Ng, W. G., Donnell, G. N., and Bergren, W. R., *J. Lab. Clin. Med.*, **66**, 115 (1965).
[4] Minakami, S., Suzuki, C., Saito, T., and Yoshikawa, H., *J. Biochem.*, **58**, 543 (1965).
[5] Hofmann, E., and Noll, F., *Acta Biol. Med. Germ.*, **6**, 1 (1961).
[6] Ciotti, M. M., and Kaplan, N. O., *Methods in Enzymology*, **3**, 890 (1957).
[7] Burton, R. M., *J. Neurochem.*, **2**, 15 (1957).
[8] Swislocki, N. I., Kalish, M. I., Chasalow, F. I., and Kaplan, N. O., *J. Biol. Chem.*, **242**, 1089 (1967).

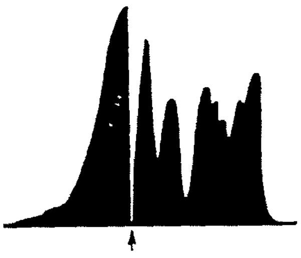
Proteins and Haemoglobins of Salmon–Trout Hybrids

SPECIMENS of the salmon (*Salmo salar*)–sea trout (*S. trutta*) which were developed by Dr D. J. Piggins of the Salmon Research Trust were obtained from a small lake at Galmoylestown, near Mullingar, County Westmeath. The eye lens, muscle and serum proteins and haemoglobins of these F_2 2 yr old (2+) specimens were compared with those of adult salmon from the River Lee, County Cork, and with those of 2+ specimens of brown trout (*S. trutta*) from the Fanure Fish Farm, Roscrea, County Tipperary.

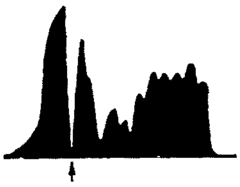
The proteins of the eye lens were separated electrophoretically in agar gel on microscope slides with phosphate buffer *p*H 7.3 at 5 m amp per slide for 40 min using the bath described by Koch *et al.*[2] and the technique of Scheidegger[3]. Muscle extracts were prepared from the middle of the body near the dorsal fin and electrophoresis was carried out in polyacrylamide gel[4] using 0.05 molar veronal buffer at *p*H 8.6 at 30 m amp for 120 min. Electrophoresis of serum proteins was also carried out in polyacrylamide gel. The electrophoretograms were scanned in the automatic fully integrated 'Chromoscan' recorder using a slit width of 1/20 mm, a 3 : 1 gear ratio and cam 5–034 *A*. The 'Chromoscan' patterns have been reduced in the figures to one-quarter the original size.

The haemoglobins were analysed using two different supporting media; micro starch gel and micro agar gel. For starch gel studies, the haemolysates were made up in *tris*-hydrochloric acid buffer at *p*H 8.2: some fractions migrated towards the anode and others migrated towards the cathode from the point of application. The technique used was the micro starch gel method of Koch, Bergstrom and Evans[5]. With the micro agar gel technique, we used the bath described by Koch *et al.*[2] and the method of Scheidegger[3]. In agar gel all the haemoglobin fractions travelled towards the negative pole.

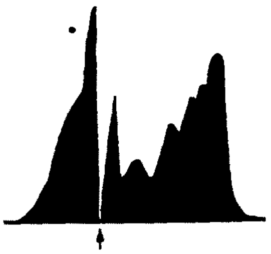
In Fig. 1 is shown the electrophoretic pattern of the lens proteins. The salmon shows at least eight components, the trout at least seven and the hybrid a minimum of ten. The hybrid pattern is not therefore like the parental patterns, nor does it seem to be a simple mixture of the two parental proteins. This is in contrast to what we have found in the case of the bream (*Abramis brama*)–



Lens salmon

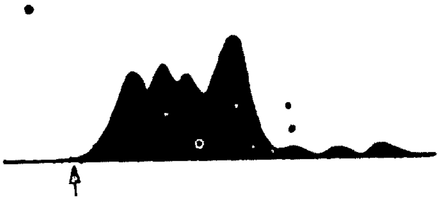


Lens salmon–trout hybrid

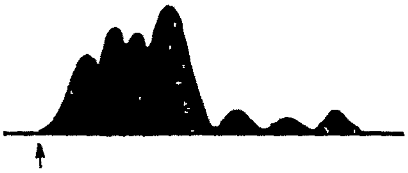


Lens brown trout 2+

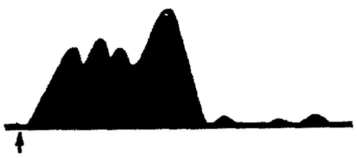
Fig. 1. Electrophoretic patterns of the eye lens proteins of salmon, trout and the salmon–trout hybrid.



Muscle salmon



Muscle salmon–trout hybrid



Muscle brown trout 2+

Fig 2 Electrophoretic patterns of the muscle proteins of salmon, trout and the salmon–trout hybrid

rudd (*Leuciscus erythrophthalmus*) F_1 hybrid whose pattern resembles the parental patterns which themselves are very similar These results are now in preparation.

In Fig 2 are shown the muscle protein patterns of the three fishes There is relatively little difference between the patterns of the two parental species, and the hybrid pattern is also very much like those of the parents Rabaey[6] using agar gel electrophoresis found that closely related species had similar patterns, although Connell[7] using the Tiselius method found that patterns of related species seemed to be as dissimilar as those of unrelated species. Greenberg and Kopac[8] found that in the genus *Xiphophorus* (using paper electrophoresis with a phosphate buffer at pH 6 5) hybrid forms showed characteristics of both parental species

The electrophoretic patterns of the serum proteins of the salmon, the sea trout and their F_2 hybrid are shown in Fig 3 It will be seen that the hybrid pattern is more like that of the trout than that of the salmon and does not seem to be a simple mixture of the parental patterns one new hybrid serum protein fraction does, however, occur. This is similar to our finding, the literature on which is now being prepared, that in the rudd–bream hybrid, the hybrid serum protein pattern resembles that of the bream. Sanders[9] in a study of three trout species and their hybrids, found that the hybrids gave serum protein electrophoretic patterns characteristic of one of the parental types Nyman[10] studied a roach (*Leuciscus rutilus*)–rudd hybrid, the splake, a hybrid between eastern brook trout *Salvelinus fontinalis* and the lake trout (*S manaycush*) and the bröding, a hybrid between *S fontinalis* and the char (*S alpinus*) In the serum protein patterns the roach–rudd hybrid showed perfect summation, the splake pattern was indistinguishable from *S fontinalis* and in the broding "not every band system is a summation of the parental patterns".

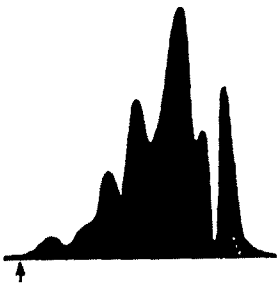
Nyman[11] reported that the F_1 salmon–brown trout hybrid serum protein pattern is a simple combination of the proteins of both parents He later[12] noted that some of these proteins seem to be determined by the same genes in both parental species and only one band is present in the F_1 hybrid in a concentration equal to that in the parents He found that in the F_2 generation, selection occurs against the salmon proteins of the serum and the kidney, liver and serum esterases, but others have shown that this is not true of the blood groups[13]

It may be seen from Fig 4 that no new hybrid haemoglobin was found using the starch gel technique, the hybrid pattern is not, however, just a mixture of the two parental patterns On both the anode and the cathode side, only four of the five salmon fractions are present in the hybrid and the C_1, C_2 and C_4 fractions of the trout do not occur in the hybrid The micro agar gel technique also shows that there is an overall reduction in the number of fractions in the hybrid, as seen in Fig. 5 Koch *et al* have shown that the relative quantities of the components, which at pH 8 2 migrate to the anode, decrease as the salmon and sea trout grow.

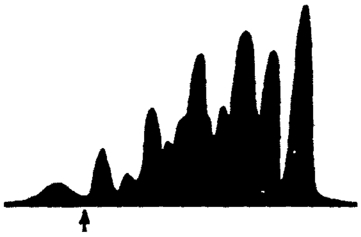
Sick, Frydenberg and Nielsen[14] studied the haemoglobin patterns of the plaice (*Pleuronectes platessa*) and the flounder (*Platichthys flesus*) and their natural hybrids Nearly all the hybrids (thirty-three out of thirty-four) showed the same haemoglobin pattern which clearly differed from that obtained by running an *in vitro* mixture of haemoglobins from the two parent species The authors explained that the presence of new haemoglobins in the hybrid was evidence for the occurrence of at least two different sub-units in the tetrameric haemoglobin molecules of the parental species The single deviant pattern found among the 327 individuals which were studied was recognizable morphologically as a hybrid which could have been the result of a mating between two hybrids or between a hybrid and one of the parent species

Manwell *et al.*[15] noted that there was little evidence for the formation of any new hybrid protein in various animals. These authors studied the haemoglobins of some hybrid birds and fishes to investigate the possibility that new haemoglobins might arise in hybrids from recombinations of the different polypeptide chains and be significant in explaining some aspects of hybrid vigour

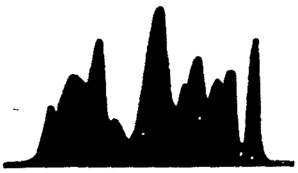
In a study of four species of centrarchid fishes, it was found[12] that in most cases the F_1 hybrids yielded a



Serum salmon normal



Serum salmon–trout hybrid



Serum brown trout 2+

Fig 3 Electrophoretic patterns of the serum proteins of salmon, trout and the salmon–trout hybrid

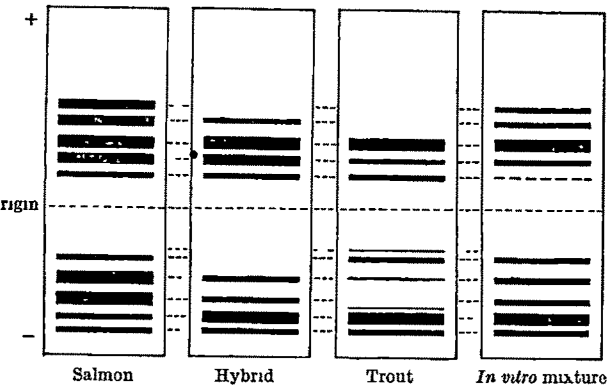


Fig 4 Haemoglobin patterns of salmon, trout, salmon–trout hybrid and an *in vitro* mixture of salmon and trout blood in starch gels

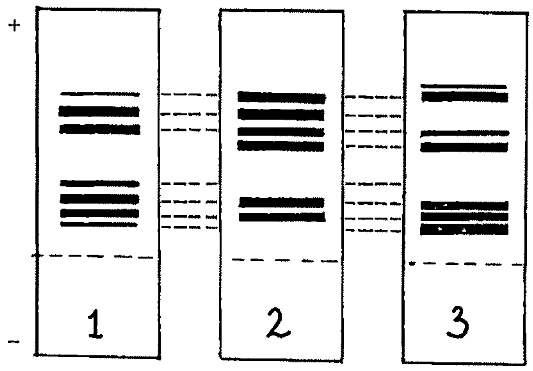


Fig 5 Haemoglobin patterns of salmon (1), trout (3) and salmon–trout hybrid (2) in agar gels C_1 and C_2 of the hybrid pattern correspond to the C_2 and C_3 of the salmon and trout patterns The C_3 component of the hybrid has a migration rate similar to the C_4 of the trout, while the C_5 fraction of the hybrid has comparable components in C_5 of the salmon and C_5 of the trout

haemoglobin pattern which was identical to that obtained by simply mixing the haemoglobins of the two parents In some of these crosses, however, the electrophoretic properties of 25–40 per cent of F_1 hybrids were different from those of the parental species

"Artificial hybridization" of haemoglobins from the small mouth bass *Micropterus dolomieu* and the large mouth bass *M salmoides* was achieved by mixing haemolysates and leaving the mixture in pure carbon monoxide for several days The new haemoglobins which were produced seem to be identical with those of the red-eyed bass *M coosae* and the spotted bass *M punctulatus* which, it is suggested, may have evolved from hybrids of the large-mouth and small-mouth bass Hybrid fishes do not seem to have any unique haemoglobin peptides, which again suggests that polypeptide chain recombination rather than biosynthetic interaction is responsible for the appearance of new hybrid haemoglobins Manwell *et al*[15] have shown that in some hybrid fishes there is better blood gas transport by the new haemoglobin, thus providing an understanding of the nature of hybrid vigour at the molecular level[15]

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[1] Piggins, D J , *Ann Rep Salm Res Trust, Ire , 1964*, 27 (1965)
[2] Koch, H J , Bergstròm, E , and Evans, J , *Medel Vlaam Acad Kl Wet*, 26, 1 (1964)
[3] Scheideggar, J J , *Intern Arch Allergy appl Immunol* , 7, 103 (1955)
[4] Raymond, S , and Weintraub, L , *Science*, 130, 711 (1959)
[5] Koch, H J , Bergstrom, E , and Evans, J , *Laxforskningsinstitutet Meddelande*, 6, 1 (1964)
[6] Rabaey, *Protides Biol Fluids*, 12, 273 (1964)
[7] Connell, J J , *Biochem J* , 55, 378 (1953)
[8] Greenberg, S S , and Kopac, M J , *Physiol Zool* , 38, 149 (1965)
[9] Sanders, B G , and Leone, C A , *Taxonomic Biochemistry and Serology* (New York, 1964)
[10] Nyman, L , *Laxforskningsinstitutet Meddelande*, 13, 1 (1965)
[11] Nyman, L , *Swed Salmon Res Inst Rep LFI Medd* , 3, 1 (1966)
[12] Nyman, L , *Rep Inst Freshwat Res Drottningholm*, 47, 5 (1967)
[13] Alabaster, J S , and Durbin, F J , *Rep Salm Res Trust Ire* , 38 (1964)
[14] Sick, K , Frydenberg, O , and Nielsen, J T , *Nature*, 198, 712 (1963)
[15] Manwell, C , Baker, A , and Childers, W , *Comp Biochem Physiol* , 10, 103 (1963)

Vitamin B_{12} Content of Aqueous Humour

THE vitamin B_{12} content of the aqueous humour of the eye does not seem to have been estimated before Samples of aqueous humour and blood were obtained at operation in sixteen patients having cataract extraction, the serum and aqueous concentrations of vitamin B_{12} were determined by biological assay using *Euglena gracilis* The results are presented in Table 1. It is interesting that the average value (29 9 pg/ml) is of the same order as that found in cerebiospinal fluid (21 pg/ml) by Kidd *et al*[1]

There was no significant statistical correlation between the concentrations in the blood and the aqueous (r= 0 097, $P \gg 0{\cdot}10$), but it must be admitted that the method of assay is not very accurate at low concentiation It is also emphasized that all the patients had cataracts and so most were elderly It does not seem justified to risk paracentesis and removal of aqueous humour from normal human eyes in order to obtain more "physiological" values Samples of aqueous humour were obtained through a cannula and dry-sterilized syringe in the early stages of the operation—before it, several instillations of guttae 2 per cent homatropine and cocaine 4 per cent hydrochloric acid and often 10 per cent phenylephrine had been given, and also a retrobulbar injection of 1·5 per cent xylocaine with adrenaline 1 200,000 Sparine (25 mg) was given by mouth on the evening before operation, 1 h before the operation 50 mg of pethidine with 50 mg of phenergan were injected intramuscularly Great care was taken to reduce contamination of the cannula with blood from the operative field to an absolute minimum, it was probably negligible

It is possible that abnormally low values of vitamin B_{12} in the aqueous humour could be found in patients suffering from "tobacco amblyopia", in view of the low concentrations of serum vitamin B_{12} found in this disease[2] and the improvement following treatment with vitamin B_{12} (refs 2 and 3) It is unlikely that there will be an opportunity to obtain samples of aqueous humour from patients with that disease, but the data available in normal patients were further analysed according to the smoking habits of the subjects The mean aqueous concentrations of vitamin B_{12} were 20 2 pg/ml for the smokers and 34 3 pg/ml for the non-smokers (t=1 12, P>0 10) The difference is not regarded as being more than suggestive, but it will be seen that the five smokers showed aqueous

Table 1 AQUEOUS B_{12} DETERMINED BY *Euglena* ASSAY

Number	Age	Sex	Smoking habits	Serum B_{12} (pg/ml)	Aq hum B_{12} (pg/ml)
1	87	F	—	420	58
2	67	F	—	224	32
3	58	M	40/day	434	29
4	63	M	—	269	49
5	75	F	—	339	42
6	73	F	—	194	35
7	87	F	—	313	12
8	52	F	10/day	93	14
9	75	F	—	123	60
10	48	M	Heavy pipe	248	15
11	71	F	—	135	29
12	53	F	10/day	161	28
13	87	M	Heavy pipe	128	15
14	87	M	—	252	14
15	74	F	—	454	18
16	72	F	—	564	28

Ranges, 12–60 pg/ml Crude mean, 29 9 pg/ml concentrations of B_{12} of less than 30 pg/ml , whereas eight of the eleven non-smokers had concentrations greater than 25 pg/ml If confirmed, this observation may be relevant in the elucidation of the pathogenesis of tobacco amblyopia

The underlying reason for this investigation, apart from intrinsic interest, was the suggestion (mentioned by Phillips and McKenzie[4]) that the mechanism of tobacco amblyopia, or any toxic amblyopia, is a toxic effect on the retina of, say, cyanide or other toxin, which enters the eye with the aqueous humour and affects the retina, especially the macular area, from its vitreous surface. There is evidence for a flow of fluid from vitreous to retinal blood vessels and through retina to choroid[5] This might explain why the retina is so selectively affected by certain toxins, the blood-aqueous barrier is not so high or so selective as the blood–retina barrier Similar considerations might apply to the blood–cerebrospinal fluid barrier and blood–brain barrier The response of the levels of aqueous B_{12} to injected hydroxocobalamin and cyanocobalamin is being studied, together with thiocyanate levels

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[1] Kidd, H M , Gould, C E G , and Thomas, J W , *Canad Med Assoc J* , 88 876 (1963)
[2] Heaton, J M , McCormick, A J A , and Freeman, A G , *Lancet*, ii, 286 (1958)
[3] Chisholm, I A , Bronte-Stewart, J , and Foulds, W S , *Lancet*, ii, 450 (1967)
[4] Phillips, C I , and McKenzie, A D , *Brain* (in the press)
[5] da Cunha-Vaz, J G , and Maurice, D M , *J Physiol* , 191, 467 (1967)

Iron Binding Properties of Saliva

Gastric juice of healthy people contains a high molecular weight iron binding protein "gastroferrin"[1] A role for the gastric iron binding protein in the regulation of iron absorption has been proposed[2,3], and alterations in its production have been described in clinical conditions of iron deficiency[3] and iron overload[2] The gastric juice obtained for the studies previously reported was free of saliva. Normally, however, swallowed saliva contributes substantially to the total volume of secretions present in the stomach It was therefore decided to investigate the possible iron binding properties of this secretion The effect of acid-peptic digestion on the iron binding properties of saliva was also studied because of the possibility that hydrochloric acid and pepsin secreted by the gastric mucosa react with the saliva present in the stomach.

Whole saliva was collected from thirty-five normal subjects who expectorated into a beaker Saliva was found to possess marked iron binding ability when this was measured by a standard iron-59 solubility test previously described[4] Iron binding capacity varied from 0 03 to 0 15 mg of Fe^{+++}/ml of saliva (mean 0 08 mg of Fe^{+++}/ml)

In the next set of experiments, saliva which had been labelled with iron-59 was resolved by molecular sieving through a column of 'Sephadex *G*-200' The relative protein content of the eluted fractions was determined by optical density measurements at 280 mμ and the iron content by measurement of the gamma activity of the iron-59 label A constant elution pattern of two protein peaks was obtained, a totally excluded peak of molecular weight greater than 200,000 and a peak of lower molecular weight Both peaks contained bound iron-59 (Fig 1) In contrast, iron binding in gastric juice occurs only in the high molecular weight fraction[1]

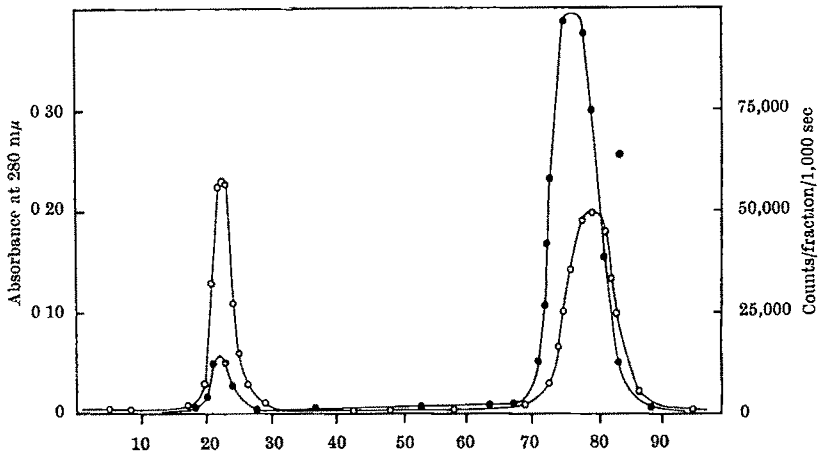

Fig 1 Filtration on 'Sephadex *G*-200' of whole human saliva labelled with iron-59 Sample volume was 3 ml Column dimensions were 2 5 × 40 cm Buffer, physiological saline buffered to *p*H 8 0 with 0 02 molar HCl/NH_4OH Fractions (3 5 ml) were collected at a flow rate of about 30 ml /h ○, UV absorbance, ●, radioactivity

When saliva was labelled with iron-59 and subjected to digestion with 0 02 per cent w/v pepsin in 0 1 molar HCl for 1 h at 37 5° C, the protein content of the high molecular weight fraction was almost abolished The absolute amount of iron-59 bound to this fraction was, however, not significantly altered The high molecular weight iron binding substance in saliva is therefore resistant to acid-peptic digestion The second fraction of lower molecular weight increased after acid-peptic digestion and also retained its iron binding activity

Experiments with polyacrylamide disc gel electrophoresis[5] of saliva labelled with iron-59 using standard 7 5 per cent polyacrylamide gel columns and *tris*-glycine buffer, *p*H 8 3, showed that iron binding occurred in the slow moving components These components have been shown by other workers to contain glycoproteins[6].

The total amount of high molecular weight iron binding substance secreted in saliva in 24 h is sufficient to bind approximately one tenth as much iron as can be bound by the estimated 24 h secretion of gastroferrin. The high molecular weight iron binding substance in saliva might have a function in health and disease similar to that of gastroferrin, both because of its molecular weight and because it is resistant to acid-peptic digestion

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[1] Davis, P S , Luke, C G , and Deller, D J , *Nature*, **214**, 1126 (1967)
[2] Davis, P S , Luke, C G , and Deller, D J , *Lancet*, ii, 1431 (1966)
[3] Luke, C G , Davis, P S , and Deller, D J , *Lancet*, i, 926 (1967)
[4] Davis, P S , *Proc Austral Assoc Clin Biochem* , 1, 190 (1965)
[5] Davis, B J , and Ornstein, L , *Disc Electrophoresis* (Distillation Products Industries, Rochester, USA, 1961)
[6] Caldwell, R C , and Pigman, W , *Arch Biochem Biophys* , 110, 91 (1965)

PHYSIOLOGY

Effects of Prostaglandins on Human Bronchial Muscle

INTRAVENOUS administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increases bronchial resistance in the cat[1] and air overflow volume in the Konzett–Rossler preparation of guinea-pig lungs *in vivo*[2] These effects suggest but do not prove that $PGF_{2\alpha}$ contracts bronchial muscle They might be caused by changes in fluid distribution in the lungs Main[3] and Horton and Main[4] showed that prostaglandins E_1, E_2, E_3, $F_{1\alpha}$ and $F_{2\alpha}$ relax cat isolated trachea Because of the uncertainty about the action of $PGF_{2\alpha}$ on tracheobronchial muscle and because this prostaglandin and PGE_2 have been found in human lungs[5,6], where they may affect the smooth muscle of the bronchial tree, we studied their effects on human isolated bronchial muscle We also studied the effects of PGE_1 and of mixtures of PGE_1 or PGE_2 with $PGF_{2\alpha}$ on this smooth muscle preparation

Pieces of bronchus (1–3 mm in diameter) were cut spirally and suspended in Krebs–Henseleit solution at 37° C in a 25 ml bath aerated with 5 per cent carbon dioxide in oxygen Fresh solutions of test substances in 0 9 per cent w/v sodium chloride in water were added to the bath for 3–6 min To measure contraction isotonically, a weighted thread from the muscle was passed over a pulley wheel, which was fixed coaxially to the spindle of a variable rotary transformer (Elliott) The output from the transformer was recorded on a self-balancing servo-potentiometric pen recorder (Rikadenki) Fuller details of this method will be described elsewhere[7]

After a variable latency of up to 1 min, during which the muscle sometimes relaxed slightly, $PGF_{2\alpha}$ (0 8–800 ng/ml) contracted human bronchial muscle, the response increasing with increasing dose Comparable doses of PGE_1 relaxed the muscle PGE_2 usually relaxed it, but was rather less active than PGE_1 When mixed with $PGF_{2\alpha}$, either prostaglandin E_1 or E_2 reduced the contraction caused by $PGF_{2\alpha}$ (Fig 1) Neither atropine (1 μg/ml) nor mepyramine (1 μg/ml) lessened the response to $PGF_{2\alpha}$, so the prostaglandin did not act by stimulating cholinergic nerves or by liberating histamine

The slow reacting substance produced in anaphylaxis of isolated lung (SRS-A) is not yet chemically purified and identified, but has been described as an acid with some lipid characteristics[8] and might therefore be related to the prostaglandins Because both SRS-A (refs 2 and 9) and $PGF_{2\alpha}$ contract human bronchial muscle, we tested whether cross-tachyphylaxis could be induced between them, using SRS-A prepared as previously described[2] Tachyphylaxis to either SRS-A or $PGF_{2\alpha}$ could be induced by prolonging by a factor of about ten the time of exposure of the muscle to a high dose of the substance Pieces of muscle made insensitive to $PGF_{2\alpha}$ responded to SRS-A (Fig 2) and vice versa The absence of cross-tachyphylaxis confirms the conclusion reached on other grounds[2], that these two substances from lung are distinct After tachyphylaxis had been induced to $PGF_{2\alpha}$, prostaglandins E_1 and E_2 continued to relax the bronchial muscle (Fig 2)

In these experiments, $PGF_{2\alpha}$ contracted, whereas PGE_1 and PGE_2 relaxed, human isolated bronchial muscle $PGF_{2\alpha}$ occurs in human lungs[5,6] and it may be concerned in conditions involving bronchoconstriction, such as bronchial asthma PGE_2 is also present in human lungs[6], so a functional relationship between the contractant $PGF_{2\alpha}$ and the relaxant PGE_2 seems possible

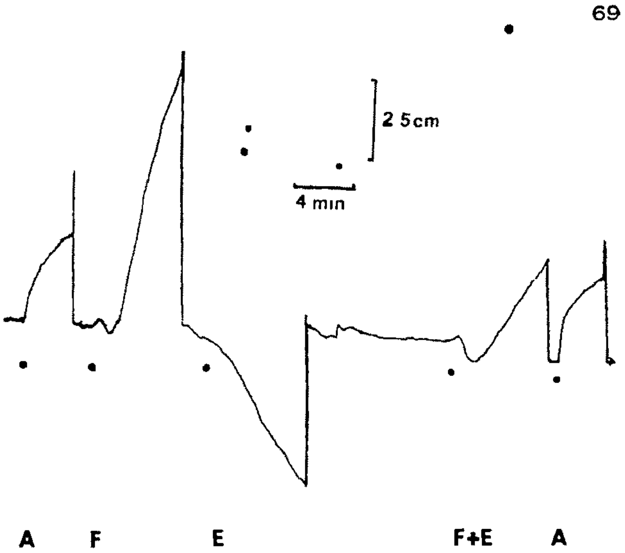

Fig 1 Human isolated bronchial muscle in a 25 ml bath Responses to prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_1 (PGE_1) and to a mixture of the two A, 10 μg of acetylcholine, F, 20 μg of $PGF_{2\alpha}$, E, 10 μg of PGE_1, ●, drug added Recording magnification × 40

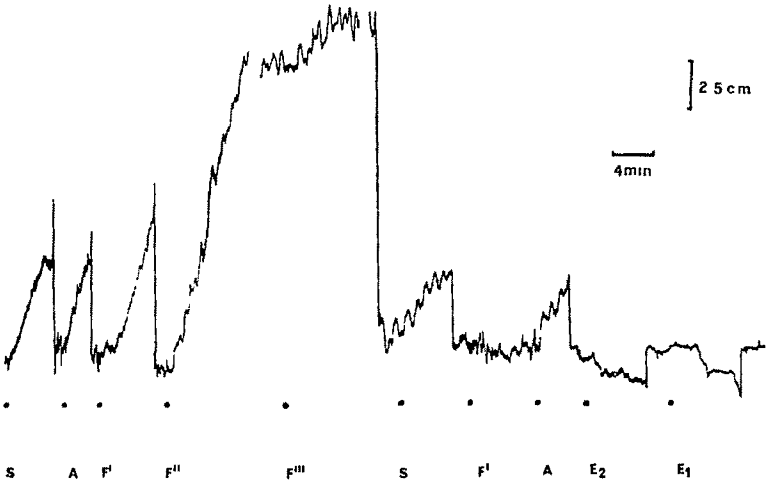

Fig 2 Human isolated bronchial muscle in a 25 ml bath Development of tachyphylaxis to $PGF_{2\alpha}$ without loss of response to slow reacting substance in anaphylaxis (SRS-A) or to PGE_1 or PGE_2 During the first break in the record, exposure to 2 μg $PGF_{2\alpha}$ continued for 5 min, during the second break in the record, exposure to 20 μg $PGF_{2\alpha}$ continued for 15 min S, 0 25 mg of SRS-A, A, 1 μg of acetylcholine, F', 1 μg, F'', 2 μg and F''', 20 μg of $PGF_{2\alpha}$, E_2, 0 5 μg PGE_2, and E_1, 0 5 μg of PGE_1, other details as in Fig 1

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[1] Anggård, E , and Bergström, S , *Arch Physiol Scand* , 58, 1 (1963)

[2] Berrv, P A , and Collier, H O J , *Brit J Pharmacol Chemother* , 23, 201 (1964)

[3] Main, I H M , *Brit J Pharmacol Chemother* , 22, 511 (1964)

[4] Horton, E W , and Main, I H M , *Brit J Pharmacol Chemother* , 24, 470 (1965)

[5] Anggård, E , *Biochem Pharmacol* , 14, 1507 (1965)

[6] Karim, S M M , Sandler, M , and Williams, E D , *Brit J Pharmacol Chemother* , 31, 340 (1967)

[7] Christianson, A H , and Sweatman, W J F , *Brit J Pharmacol Chemother* (in the press)

[8] Brocklehurst, W E , *Biochem Pharmac* , 12, 431 (1963)

[9] Brocklehurst, W E , *J Physiol* , 120, 16*P* (1953)

New Test for Biological Identification of Bradykinin

In 1961, Boisonnas, Guttmann and Jaquenoud[1] first synthesized bradykinin and other syntheses have since been recorded[2-4] In these studies the contracting effect of this substance on the isolated uterus of a rat or the ileum of a guinea-pig has, among other tests, been taken as evidence of identity with bradykinin which was isolated from plasma, and as a measure of its biological activity As will be shown here, however, this sole criterion may not suffice to characterize the peptide

Recently, we determined the biological identity and activity of bradykinin which was synthesized by Fridkin, Patchornik and Katchalsky according to a new method (presented at the International Symposium on the Pharmacology of Hormonal Polypeptides and Proteins, Milan, September 14, 1967, results to be published) The ileum of a guinea-pig which was suspended in a 5 ml organ bath of Tyrode solution at 35° C was used as the test object Contractions were recorded on a kymograph by means of a frontal writing lever Drugs were allowed to act for 1 min and then the preparation was washed twice The time cycle was 5 min Solutions of all substances were made up in saline Bradykinin Sandoz and a sample prepared by Sakakibara and Inukai[4] served as reference compounds

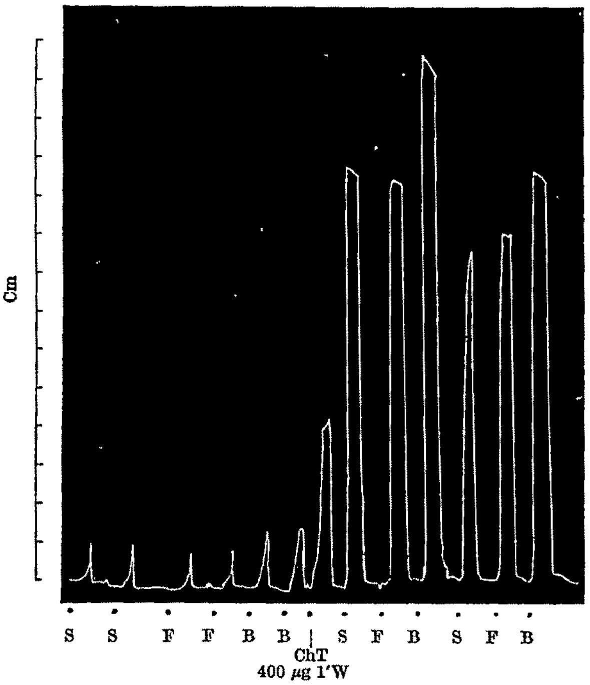

Fig 1 Responses of the isolated ileum of a guinea-pig to three different specimens of bradykinin (20 µg), namely that of Sakakibara and Inukai (S), Fridkin, Patchornik and Katchalsky (F) and Sandoz (B) After each contraction the preparation was washed twice Note that after the presence of 400 µg of chymotrypsin for 1 min (ChT 400 µg 1'W), responses were about ten times higher than that of control

The material which was obtained at the final step of the new synthesis caused contraction of the isolated gut in doses of 7 to 15 ng The magnitude of the responses closely compared with those elicited by corresponding amounts of bradykinin Sandoz When 500 µg of chymotrypsin was introduced into the bath, however, the subsequent contractions elicited by the test compound did not increase but in fact decreased In contrast, those provoked by bradykinin Sandoz were five times higher than those of the control These experiments indicated that the test substance was not identical with the reference substance Re-examination of the material revealed that the *p*-nitrobenzyl group which was attached to the terminal arginine had not been removed after treatment with hydrofluoric acid[5] The material was therefore reduced in the presence of palladium and barium sulphate, it was purified in a column of 'Amberlite *IRC*-50' and lyophilized The product was tested on the isolated ileum in doses of 5 to 20 ng and elicited responses similar to those of the two reference bradykinins Furthermore, after the presence of chymotrypsin, all three compounds behaved identically they elicited contractions which were eight to ten times larger than those of control Thus the identity of all three specimens of the peptide could be established with certainty Fig 1 illustrates a typical experiment

This work supports a previous suggestion[6] on the usefulness of employing chymotrypsin as a specific sensitizer of the isolated ileum of a guinea-pig for the biological identification of bradykinin It should be remembered that no specific antagonist of bradykinin is at present available Moreover, it can also be inferred that the present biological test for identification of bradykinin, which consists of the disappearance of its contracting activity on smooth muscle on incubation with chymotrypsin, might be misleading In the present work, for example, Phe-Arg bond of the *p*-nitrobenzyl blocked bradykinin could readily be split by chymotrypsin, rendering it inactive on smooth muscle, even though it is not identical with the pure peptide

I thank Dr M Fridkin for providing me with the bradykinin which was prepared by Sakakibara and Inukai

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[1] Boisonnas, R A , Guttmann, S , and Jaquenoud, P A , *Helv Chim Acta*, **43**, 1349 (1960)
[2] Nicolaides, E D , Dewald, H A , and Craft, M K , *Ann NY Acad Sci* , **104**, 15 (1963)
[3] Merrifield, R B , *Biochemistry*, **3**, 1385 (1964)
[4] Sakakibara, S , and Inukai, N , *Bull Chem Soc Japan*, **39**, 1567 (1966)
[5] Sakakibara, S , Shimonishi, Y , Okada, M , and Kishida, Y , in *Peptides* (edit by Beyerman, H C , Van de Linde, A , and Van den Brink, W M) (North-Holland Publ Co , Amsterdam, 1967)
[6] Edery, H , *Brit J Pharmacol Chemother* , **24**, 485 (1965)

Changes in Iron Metabolism in Natives of 13,000 ft. brought down to Sea Level

Iron metabolism in natives of high altitudes (14,900 ft) decreased when they were brought down to sea level[1,2] The values for the plasma iron and the plasma iron turnover rate obtained by Huff *et al* [1] were higher than those reported by other workers, but this may have been caused by storage and shipment of the samples

In experiments carried out in the north of Argentina from 1960 to 1963, we studied the iron metabolism of high altitude dwellers during the first 13 days after descent to sea level We had a greater number of volunteers and a more homogeneous population than in previous work Thirty-two high school students born and living at 13,000 ft (Mina aguilar, Argentina) served as volunteers None had ever worked in the mines and all played football or basketball Chest X-rays and physical and laboratory examinations confirmed their good health The average weight was 58 kg, height, 164 cm, and age, 18 yr Erythrokinetic studies were performed at high altitudes and afterwards the volunteers were flown to sea level. On days 3, 8, 10 and 13 after the descent the ferrokinetic measurements were repeated. Daily reticulocyte counts were performed throughout the experiment.

The plasma iron turnover (P.I.T.) rate was determined by the method of Huff *et al.*[3], and circulating red cell mass and blood volume by the method of Donohue *et al.*[4]. The plasma volume was computed as an average of the extrapolating method of Huff *et al.*[3] and from the blood volume and peripheral haematocrit[4]. The serum iron level was measured by Schales's technique[5].

At high altitudes, haematocrits, reticulocytes, blood volume and P.I.T. rate were lower than those found previously[1,2] because the measurements were taken at 13,000 as against 14,900 ft and because the volunteers were younger.

Table 1. OBSERVATIONS IN NATIVES OF 13,000 FT BROUGHT DOWN TO SEA LEVEL

	High altitude	Sea level Day 3	Day 8	Day 10	Day 13
No. cases	32	9	12	7	4
Haematocrits (%)	53 ± 0.7*	53.5 ± 0.8	50.5 ± 0.6	51.0 ± 1.0	50.5 ± 0.7
Plasma iron (μg %)	100 ± 8	120 ± 15	138 ± 15	129 ± 6	123 ± 14
Plasma volume (ml./kg)	38 ± 1.8	39 ± 1.6	44 ± 1.8	41 ± 6.1	43 ± 3.2
Red cell volume (ml./kg)	36 ± 2.2	37 ± 1.8	35 ± 3.1	35 ± 2.1	38 ± 2.3
$T\frac{1}{2}$ ^{59}Fe (min)	69 ± 4	145 ± 21	148 ± 14	329 ± 72	343 ± 78
P.I.T. rate (mg iron/day/kg)	0.58 ± 0.14	0.40 ± 0.14	0.38 ± 0.09	0.20 ± 0.11	0.18 ± 0.03

P.I.T. = plasma iron turnover.
* Standard error.

Table 1 shows a 20–30 per cent rise in plasma iron during the sojourn at sea level. P.I.T. rate decreased, and iron-59 clearance increased gradually, reaching six times the high altitude values, between days 10 and 13 at sea level. Red cell volume remained constant throughout the period, with an increase in plasma volume and consequently a decrease in haematocrit. Reticulocytes (Table 2) relate fairly well with the changes in P.I.T. rate.

Table 2. RETICULOCYTE COUNTS IN NATIVES OF 13,000 FT BROUGHT DOWN TO SEA LEVEL

High altitude 0.7 ± 0.1

Sea level

Day 1	0.4 ± 0.06	Day 7	0.2 ± 0.08
Day 2	0.5 ± 0.1	Day 8	0.2 ± 0.02
Day 3	0.2 ± 0.07	Day 9	0.1 ± 0.03
Day 4	0.3 ± 0.09	Day 10	0.1 ± 0.02
Day 5	0.3 ± 0.04	Day 11	0.2 ± 0.01
Day 6	0.3 ± 0.05	Day 12	0.2 ± 0.02
		Day 13	0.1 ± 0.02

Our results show a relationship between hypoxia and erythrokinesis in permanent dwellers at 13,000 ft. Red cell production was inhibited for the first 2 weeks when the polycythaemic volunteers were brought down to sea level.

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[1] Huff, R. L., Lawrence, J. H., Siri, W. E., Wasserman, L. R., and Hennessy, T. G., *Medicine*, 30, 197 (1951).
[2] Revnafarje, C., Lozano, R., and Valdivieso, J., *Blood*, 14, 433 (1959).
[3] Huff, R. L., Hennessv, T. G., Austin, R. E., Garcia, J. F., Roberts, B. M., and Lawrence, J. H., *J. Clin. Invest.*, 29, 1041 (1950).
[4] Donohue, D. M., Motulsky, A. G., Giblett, E. R., Pirzio-Biroli, G., Viranvvatti, V., and Finch, C. A., *Brit. J. Haematol.*, 1, 249 (1955).
[5] Schales, M., *Clinical Manual* (Klett-Summerson, Denver, 1955).

Prostaglandin Content of Particulate and Supernatant Fractions of Rabbit Brain Homogenates

WHOLE rabbit brains were homogenized in 0.32 molar sucrose solution, and subcellular fractions were prepared by centrifugation by the method of Gray and Whittaker[1]. Prostaglandins were extracted from the homogenates and from the various fractions by solvent partition and further purified by chromatography[2].

Extracts of rabbit brain homogenates showed prostaglandin-like activity when assayed biologically on the rat isolated fundal strip preparation. On silicic acid column chromatography, two main peaks of biological activity were detected, corresponding to the elution rate of pure prostaglandins E and F respectively. On thin-layer chromatography using plates of silica gel impregnated with silver nitrate, zones corresponding to prostaglandins E_2 and $F_{2\alpha}$ contained more than 90 per cent of the biological activity, confirming the report of Ambache, Brummer, Rose and Whiting[3]. The homogenates were estimated to contain 500 ng prostaglandin $F_{2\alpha}$ and 75 ng prostaglandin E_2/g of brain tissue. When the brain was macerated or homogenized in 96 per cent aqueous ethanol instead of sucrose, similar amounts of prostaglandins were recovered. There was no evidence in our experimental conditions for inactivation or new synthesis in the sucrose homogenates as reported by other workers[4].

In each of four experiments the final supernatant after centrifuging at 100,000*g* (4° C for 2 h) contained between 80 and 90 per cent of the total prostaglandin activity of the homogenates. Substances with chromatographic and biological properties identical to prostaglandins E_2 and $F_{2\alpha}$ were identified in the supernatant fraction, which also contained 80 per cent of the total potassium present in the whole homogenate.

The combined particulate fractions also contained the two prostaglandins. Between 5 and 10 per cent of the biological activity of the whole homogenate was in the fraction *P*2 which, according to electron microscopic evidence[1], would contain mitochondria and nerve endings. Because the amounts of prostaglandin in the *P*2 fraction were so small, further fractionation by sucrose gradient technique has not so far been feasible. The remainder of the prostaglandin-like activity of the particulate fraction was distributed between *P*1 (nuclei and cell debris), *P*3 (microsomes) and *P*4 (ribosomes and post-microsomal fractions).

The combined particulate fraction was treated in three ways in an attempt to release any bound prostaglandin. One sample was frozen and thawed ten times, another was incubated at *p*H 3 and 60° C for 1 h, and a third sample was suspended in water before extraction with ethyl acetate at *p*H 3. None yielded any additional prostaglandin.

As a control, the succinate dehydrogenase contents of the particulate and supernatant fractions were compared with those of the original homogenate. All the enzyme activity of the homogenate was recovered from the particulate fraction.

It is unlikely that the prostaglandin content of the particulate fractions was the result of contamination by supernatant, because washing with sucrose and recentrifugation did not reduce the amount recovered. But the possibility that the prostaglandin recovered from the supernatant originated from a very labile store in one of the particulate fractions cannot be excluded.

Our results differ from those of Kataoka, Ramwell and Jessup[5], who located 30–40 per cent of the prostaglandins in rat cerebral cortex in nerve ending fractions. The results from both laboratories point to a wide distribution of prostaglandins throughout subcellular compartments.

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[1] Gray, E G , and Whittaker, V P , *J Anat* , **96**, 79 (1962)
[2] Horton, E W , and Main, I H M , *Brit J Pharmacol Chemother* , **30**, 582 (1967)
[3] Ambache, N , Brummer, H C , Rose, J G , and Whiting, J , *J Physiol* , **185**, 77 (1966)
[4] Wolfe, L S , Coceani, F , and Pace-Asciak, C , *Proc Second Nobel Symposium on Prostaglandins* (Almqvist and Wicksell, Uppsala, 1967)
[5] Kataoka, K , Ramwell, P W , and Jessup, S , *Science*, **157**, 1187 (1967)

Modification of Uricaemia and the Excretion of Uric Acid Nitrogen by an Enzyme of Fungal Origin

TREATMENT of hyperuricaemia and gout is aimed at either synthesis or elimination of uric acid Previous attempts at *in situ* destruction of uric acid have remained experimental because of imperfections in the enzymes The therapeutic use of a new uricase or urate oxidase derived from *Aspergillus flavus* has been studied

Urate oxidase was first used in animals in 1949 (ref 1), it transformed uric acid into allantoin An enzyme derived from animals was partly successful in human therapy[2] The activity of these preparations lasted for several hours only and numerous daily injections were necessary

Laboureur and Langlois[3] isolated a urate oxidase from a selected strain of *Aspergillus flavus* which is sufficiently pure and which possesses a high specific activity in animals Its antigenic potential is moderate, toxicity is negligible. It could be produced in industrial quantities

Our work to verify in man the results obtained in animal studies (M Brunaud, personal communication) was conducted on sixty-one hospitalized patients, who all received a stabilized purine diet They were divided into three groups normal, hyperuricaemics, and patients with gout Uricaemia was measured using a modified Liddle[4] enzymatic technique Urate oxidase derived from *Aspergillus flavus* was used for the assay The buffer was at *p*H 8 5 and readings were taken at 285 mµ The solutions injected were sterilized and prepared by a patented process (Clin-Byla Laboratories) and were assayed at 500 units/l Uric acid in the urine was assayed by the same method Urinary allantoin was measured by the method of Gros and Raveux[5] it was separated from uric acid by passage through an anion exchange resin column. Then alkaline hydrolysis converted allantoin into allantoic acid Acid hydrolysis degraded the allantoic acid into urea and glyoxylic acid After ferrocyanide treatment in a strongly acid medium, the phenylhydrazone of glyoxylic acid was oxidized and the optical density of the resulting solution was read at 518 mµ A unit of urate oxidase activity was defined as the quantity of enzyme capable of causing the disappearance of 50 per cent of the substrate (100 µg/5 ml) in 10 min at 30° C at *p*H 8 5

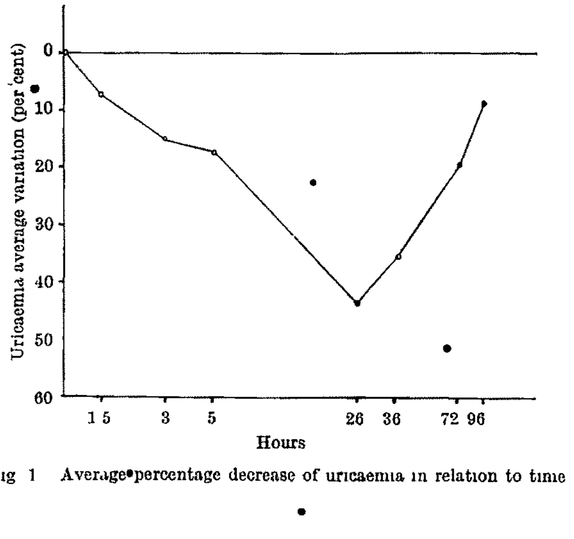

Fig 1 Average percentage decrease of uricaemia in relation to time

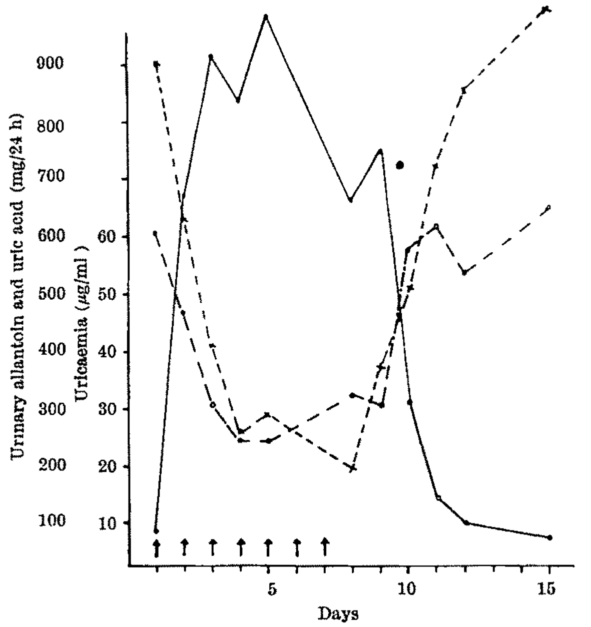

Fig 2 Uricaemia, urinary uric acid and urinary allantoin in a hyperuricaemic patient after seven daily injections (arrows) of 800 U of urate oxidase ●——●, Urinary allantoin, × – – – ×, urinary uric acid, O– –O, uricaemia

Uric acid metabolism in animals used in pharmacodynamic studies is very different from that of man It was therefore difficult to determine active human dosages and methods of administration

Intravenous injections were given at 24 h intervals in order to study variation in uricaemia in relation to various experimental doses The average decrease in uricaemia was for 125 U, 4 8 per cent, for 250 U, 0 per cent, for 500 U, 12 6 per cent, and for 800 U, 40 2 per cent It seems that the activity of the product is manifest above the 500 U level. Its effectiveness is clear at 800 U

The decrease of uricaemia 24 h after intramuscular, intravenous and *per os* administration of 500 U and 800 U doses of enzyme was compared (Table 1)

The efficacy was identical when the enzyme was given intravenously and intramuscularly *Per os* administration was ineffective

We studied the variation of uricaemia in nine patients at different intervals after an intravenous dose of 800 U (Fig 1) Uricaemia decreased quickly during the first 5 h after injection The average decrease was at a maximum

Table 1 URICAEMIA AFTER ADMINISTRATION OF ENZYME BY INTRAVENOUS (I V) AND INTRAMUSCULAR (I M) INJECTION

	500 U		800 U	
	I V	I M	I V	I M
Number of cases	5	6	13	5
Average control uricaemia, (µg/l)	56 8	48	52 2	44 4
Average 24 h post administration uricaemia, (µg/l)	49 6	41 5	31 2	27
Decrease (per cent)	12 6	13 5	40 2	39 2

after 24 h, was still apparent after 48 h, then rose slowly. There was still some enzyme activity after 96 h.

The average variation in urinary uric acid and allantoin in three patients after a single 800 U intravenous dose is shown in Table 2. Urinary uric acid fell during the first 24 h period and a considerable decrease was maintained for 72 h. Urinary allantoin exceeded the control concentration by six or seven times. This demonstrates the mechanism by which urate oxidase destroys uric acid.

After observing the metabolic effects of a single dose of enzyme we studied the uric acid and allantoin modifications after 800 U/day for a test period of 4 or 5 days. Three normal subjects and two hyperuricaemic patients were studied. Table 3 presents data from two normouricaemic subjects after a series of four or five 800 U intravenous injections. The effect on uricaemia, urinary uric acid, and urinary allantoin was recorded for 11 days after the last injection. Fig. 2 is a graphic representation of the same parameters in a hyperuricaemic subject.

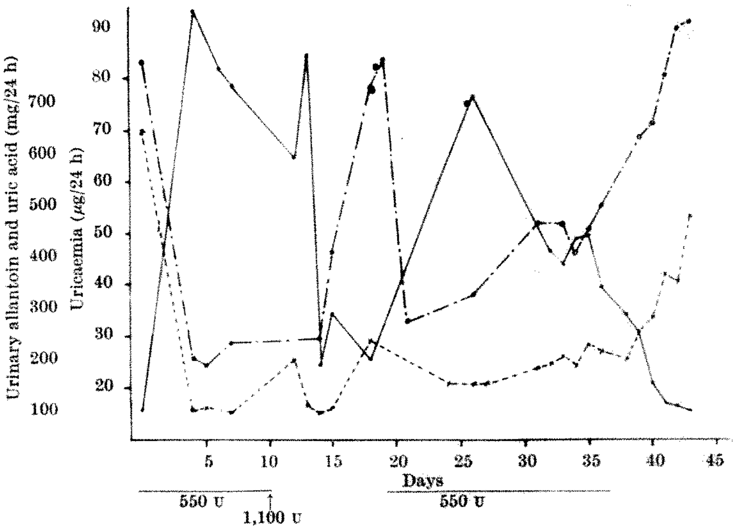

Fig. 3. Uricaemia, urinary uric acid and urinary allantoin in a gouty patient treated daily with urate oxidase. ●——●, Urinary allantoin; × - - - ×, urinary uric acid; ○— · —○, uricaemia.

Uricaemia and urinary uric acid and allantoin decreased in both normal and hyperuricaemic subjects. Uricaemia increased to a level greater than the control 4–7 days after the end of the injection period. Uricuria increased concomitantly with the decreased uricaemia. The urinary uric acid concentration continued to rise even after the uricaemia started to normalize. This asynchronism was constantly observed, suggesting an eventual kidney involvement. The ratio of uricuria to uricaemia is greater than that before treatment even when the uricaemia is at its lowest level. The ratio does not fall after the end of the treatment, so it seems that the enzyme improves the elimination of urinary uric acid.

Table 2. VARIATION IN URINARY URIC ACID AND ALLANTOIN AFTER A SINGLE 800 U INTRAVENOUS DOSE. AVERAGE FOR THREE PATIENTS

	Urinary uric acid				Urinary allantoin			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Variation (%)	−45	−33	−33	−19	+681	+609	+150	+120

Three typical acute gout attacks were interrupted 24 h after daily intravenous injections of 800 U were begun. Four patients with tophaceous gout were given daily injections of 550 U of the enzyme. This dose was selected in order to avoid a very high urinary allantoin concentration and its precipitation. The results were similar in all cases. Graphs of two cases are shown in Figs. 3 and 4. A decrease in uricaemia was observed which was less marked at the end of the treatment.

There was a parallel decrease in urinary uric acid and an elevation of urinary allantoin (up to ten times control concentrations). A later increase in uricaemia was noted, but analyses were not continued long enough after treatment to record the eventual increase in urinary uric acid. The ratio of uricuria to uricaemia was constantly lowered. The urinary elimination of uric acid seems to be a function of the uricaemia concentration in the gouty patients. It is improbable that the enzyme has a different renal action in normal and gouty subjects.

Fundal urate oxidase is negligibly toxic. We observed an immediate allergic reaction in only one of the sixty-one patients injected with urate oxidase for the first time. He had been undergoing treatment for a chronic mycosis and had symptoms of urticaria, oedema of the eyelids, status asthmaticus and dyspnoea. An immuno-electrophoretic study of the patient's serum was negative. Intradermal tests with the enzyme revealed an early tissular sensitization reaction. No disturbances were found in blood nitrogen, blood sugar, differential blood count, blood cholesterol, phosphatases, transaminases, protein electrophoresis or urea clearance in patients treated for long periods.

Two patients with tophaceous gout had attacks, the first immediately after cessation of treatment and the second on the tenth day. Two gouty patients with diagnosed interstitial nephritis had increased urea values which varied from one day to the next. This variation could be attributed to the treatment.

These observations affirm the hypouricaemiant activity of fungal urate oxidase. The conversion of uric acid to allantoin, the presumed mode of action, is confirmed by the presence of increased urinary concentrations of

Table 3. VARIATIONS OF URICAEMIA, URINARY URIC ACID, AND URINARY ALLANTOIN IN TWO NORMAL SUBJECTS AFTER FOUR OR FIVE 800μ URATE OXIDASE INJECTIONS

		Subject 1					Subject 2		
Time (days)	Dose (U/day)	Uricaemia (μg/ml.)	Urinary uric acid (mg/day)	Urinary allantoin (mg/day)	Time (days)	Dose (U/day)	Uricaemia (μg/ml.)	Urinary uric acid (mg/day)	Urinary allantoin (mg/day)
0	800	46·39	230·21	18·43	0	800	39·94	207·36	9·87
1	800	28·26	19·15	337·77	1	800	14·67	256·25	395·11
2	800	16·16	140·74	369·78	2	800	10·2	93·43	438·70
3	800	15·7	148·32	386·32	3	800	—	62·45	365·90
4	800	—	—	—	4		5·65	84·60	424·30
5		17·55	156·19	441·70	5		8·30	—	—
6		25·20	162·51	272·07	6		13·96	73·01	296·88
7		37·22	166·78	174·07	7		25·42	121·20	290·62
8		43·60	207·46	112·82	8		38·20	156·55	114·60
9		51·00	307·37	78·72	9		43·62	162·45	83·48
10		50·00	371·28	85·63	10		—	—	—
11		—	—	—	11		45·35	188·69	41·23
12		48·18	406·32	41·78	12		44·30	251·45	28·34
13		46·30	415·08	63·20	13		34·34	236·21	24·05
14		33·51	429·65	96·02	14		35·01	499·90	62·06
15		41·1	363·25	73·15	15		35·16	383·21	86·42
16		44·5	301·68	40·42	16				

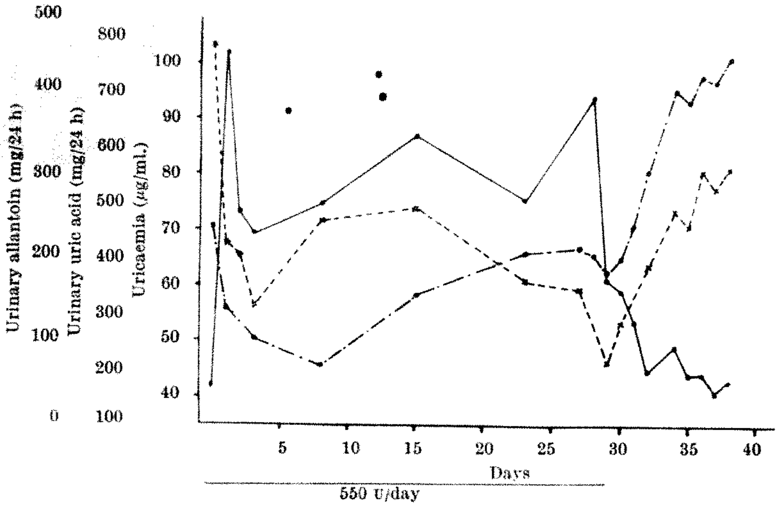

Fig. 4. Uricaemia, urinary uric acid and urinary allantoin in a gouty patient treated daily with urate oxidase. ●——●, Urinary allantoin; × --- ×, urinary uric acid; ○—·—○, uricaemia.

allantoin. Moreover, the number of uric acid molecules eliminated, either directly or by conversion into allantoin, was increased by an average of four times the pretreatment concentration. This confirms the increase in elimination of purine bases.

A possible renal effect should be investigated. Does the mass action effect of the reduced uricaemia bring about an increased uric acid synthesis from purine bases? The softening of the tophaceous deposits during the treatment suggests that the uratic reserves are preferentially mobilized. Prolonged treatment brings about a slight reduction in urate oxidase activity in normal as well as in gouty subjects. This decrease is probably caused by an antigenic effect observed in immuno-electrophoretic serum studies. The antigenic effect appears to be very labile: iterative treatments give comparable results throughout.

The only practical medication capable of destroying uric acid *in situ* is fungal urate oxidase; its use in the management of gout and hyperuricaemias is indisputable. Urate oxidase alone will probably not be able to solve all problems, but by association with uric acid eliminators or inhibitors of uric acid synthesis, with further clinical studies therapy could be developed.

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[1] Altman, K. I., Smull, K., and Barron, E. S. G., *Arch. Biochem.*, **21**, 158 (1949).
[2] London, M., and Hudson, P. B., *Science*, **125**, 937 (1957).
[3] Laboureur, P., and Langlois, C., *C.R. Acad. Sci.*, **264**, 2244 (1967).
[4] Liddle, L., Seegmuller, J. E., and Laster, L., *Lab. Clin. Med.*, **54**, 903 (1959).
[5] Gros, P., and Raveux, P., *Bull. Soc. Chim. France* (in the press).

Sickling Phenomenon in Sheep

IN 1840 Gulliver[1] described spindle and crescent shaped red cells in the blood of deer. Since then sickling of the erythrocytes of man and deer has become a well recognized and documented phenomenon[2–3]. It is important in man because of its association with sickle cell disease which afflicts about 8 per cent of American Negroes and up to 45 per cent of some African tribes. The disease is caused by the presence in the red cells of an abnormal haemoglobin, haemoglobin S. It was the first disease to be shown to result from genetic influence at the molecular level.

Among domestic animals, sickling has so far been reported in only one species. Holman and Dew[4] reported the appearance of elliptical corpuscles in films prepared from the blood of young goats. These blood films bore some resemblance to those of sickle-cell anaemia, but the phenomenon was restricted in the goat to the period between 1 month and 3 months of age. These workers quoted a personal communication from Dr H. Lehmann, who established that the sickling in the goat was similar to that occurring in deer in that it was dependent on *p*H and could be removed by lowering the *p*H to 6 (ref. 5).

Sickling of red cells has been observed in two of the three breeds of British sheep so far examined. The red cells of sequestrened blood samples from Soay sheep in sealed coverslip-slide preparations developed peculiar oat and fusiform shapes. Samples from thirty-nine Soay sheep have been examined and the red cells in thirty-four of these developed spindle, crescent, holly leaf and matchstick forms. Usually mixtures of varying proportions of the sickled forms that have been reported in the blood of man and deer were seen (Figs. 1–3). In samples from another four sheep the erythrocytes developed more bizarre forms, some of which resembled the outstretched hand (Fig. 4). These changes, best seen when using dark-ground or phase contrast illumination, appeared in 1–18 h in preparations kept at room temperature. The blood from one Soay sheep did not sickle in these conditions.

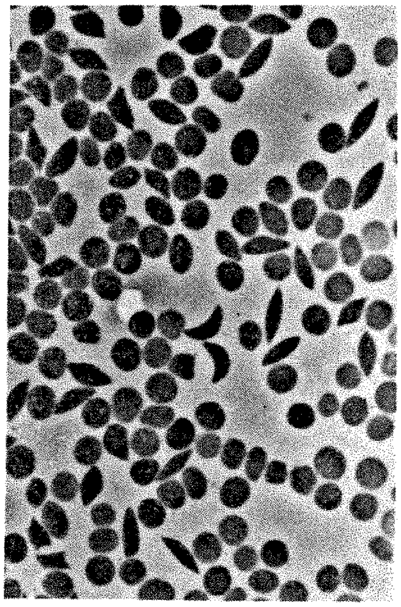

Fig. 1. Early spindle and crescent forms.

The phenomenon has also been seen in twelve out of twelve blood samples collected from recently weaned Swaledale lambs and in three out of nine adult Swaledale sheep from another, old established flock on the North Yorkshire moors. Nothing like sickling was seen in samples collected from twelve Clun Forest ewes apart from the development in four of these, in 24–48 h, of intracorpuscular crystallization of haemoglobin which did not distort the cell outline. An investigation of the incidence of sickling in other breeds is being carried out.

Although the exact physico-chemical conditions conducive to sickling of sheep erythrocytes have not yet been determined, there are indications of a similarity to the conditions necessary for *in vitro* sickling of deer cells. Sickling in sheep cells appears in whole blood saturated with oxygen and in suspensions prepared by diluting whole blood with an equal volume of 0·1 normal Sørensen's phosphate buffer ranging from *p*H 7·4 to *p*H 8·6 (very markedly at *p*H 8·6) but not at *p*H below 7·0. It can be reversed by the addition of a solution of sodium metabisulphite.

There is a marked degree of heterogeneity of haemoglobin in deer blood and sickling in this species can be attributed to the presence within the red cell of a specific haemoglobin component. It is dependent on the percentage oxygen saturation as well as the *p*H of the whole blood or suspending buffer[3,6]. Studies are now being carried out on the electrophoretic pattern of haemolysates prepared from the blood of sickling sheep.

There does not seem to be any correlation between the potassium content of the red cells and the propensity for sickling. In twenty-one Soay blood samples which sickled, eight were in the high red cell potassium category (mean value of 77 ± 2·4 m.equiv./l.) and thirteen were of the low potassium type (mean value 25·4 ± 3·5 m.equiv./l.). Neither do there seem to be any abnormal haematological changes associated wih the phenomenon. Mean values for packed cell volume and haemoglobin concentration were 41·5 ± 3·06 per cent and 13·9 ± 0·26 g/100 ml., respectively. Further studies to ascertain the fragility and thus life span of the red cells in affected sheep are indicated.

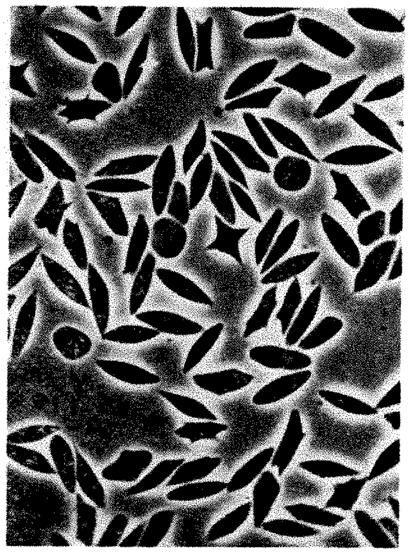
Fig. 2. Mainly spindle and few star and holly leaf forms.

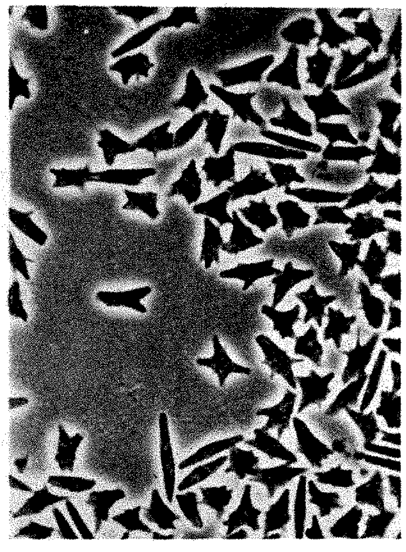
Fig. 3. Mixture of holly leaf and matchstick forms.

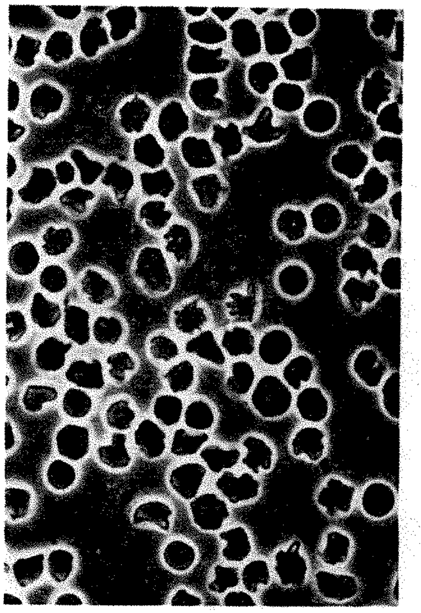
Fig. 4. Bizarre forms; central cell depicting shape of outstretched hand.

Although O'Roke[7] thought that sickle cell anaemia could be a factor in the causation of winter losses of deer in Michigan, a recent report[8] claims that the phenomenon in deer is innocuous because neither haemolytic anaemia nor periodic vascular occlusion occurs, and that the condition should be referred to as "pseudosickling". Nevertheless there may be an association between sickling and the dramatic "crashes" that periodically afflict the Soay population on the Isle of Hirta[9].

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[1] Gulliver, O., *Proc. Zool. Soc., Lond.*, **17**, 325 (1840).

[2] Harris, J. W., *The Red Cells* (Harvard University Press, Cambridge, Massachusetts, 1963).

[3] Kitchen, H., Putnam, F. W., and Taylor, W. A., *Science*, **144**, 1237 (1964).

[4] Holman, H. H., and Dew, S. M., *Res. Vet. Sci.*, **5**, 274 (1964).

[5] Undritz, E., Betke, K., and Lehmann, H., *Nature*, **187**, 333 (1960).

[6] Weisberger, A. S., *Proc. Soc. Exp. Biol. and Med.*, **117**, 276 (1964).

[7] O'Roke, E. C., *Proc. Soc. Exp. Biol. and Med.*, **34**, 738 (1936).

[8] Whitten, C. F., *Brit. J. Haematol.*, **13**, 650 (1967).

[9] Boyd, J. M., Doney, J. M., Gunn, R. G., and Jewell, P. A., *Proc. Zool. Soc. Lond.*, **142**, Pt. 1 (1964).

$p\mathrm{O}_2$ Levels in the Placental Circulation of the Mare and Ewe

PREVIOUS work on the oxygen tension in different parts of the placental circulation has been confined almost entirely to sheep and goats, in which the $p\mathrm{O}_2$ gradient between the umbilical and uterine circulations is high[1] and large changes in foetal $p\mathrm{O}_2$ levels are difficult to produce[2-4]. Thus foetal hypoxia occurs only after drastic reduction in maternal arterial blood $p\mathrm{O}_2$, and attempts to raise the foetal $p\mathrm{O}_2$ have met with little success despite the administration of pure oxygen to the mother[2,4]. Even when the foetus is supplied with oxygen by other means, the $p\mathrm{O}_2$ gradient between uterine and umbilical vessels is still present[3].

We have now examined the position in the pregnant mare in which the vascular supply to the diffuse epitheliochorial placenta is very different from that of ruminants. Much of the previous work on the sheep involved removal of the foetus from the uterus and, because it now seems that this procedure may alter foetal oxygen consumption and umbilical blood flow[5], the foetus was kept *in utero* in the present experiments. Measurements of blood oxygen tension in uterine and umbilical vessels were made in four ponies towards the end of pregnancy during different levels of maternal oxygen intake; the results obtained were compared with a similar series of observations on six ewes. All animals were anaesthetized with sodium pentobarbitone and the umbilical and uterine vessels were catheterized using the technique developed by Barron and his colleagues[6] for the ewe. In the mares much longer lengths (15–25 in.) of wider bore tubing (outer diameter, 0·060 in.) were threaded into the main vessels through tributaries exposed by a small incision in the uterine wall. In both species the foetus was undisturbed until the end of the experiment when the position of each catheter was identified. Changes in maternal arterial $p\mathrm{O}_2$ were produced by altering the percentage of oxygen in the inspired air and a period of 10–20 min was allowed at a given level of oxygen before simultaneous blood samples were drawn from the umbilical and uterine vessels for $p\mathrm{O}_2$ analysis. In the ewes, maternal arterial $p\mathrm{O}_2$ was monitored continuously while in the mares the $p\mathrm{O}_2$ in individual samples was measured every 5–10 min. Blood $p\mathrm{CO}_2$ and $p\mathrm{H}$ levels were also determined during these experiments. The methods for measurement of $p\mathrm{O}_2$, $p\mathrm{CO}_2$ and $p\mathrm{H}$ have been described previously[2].

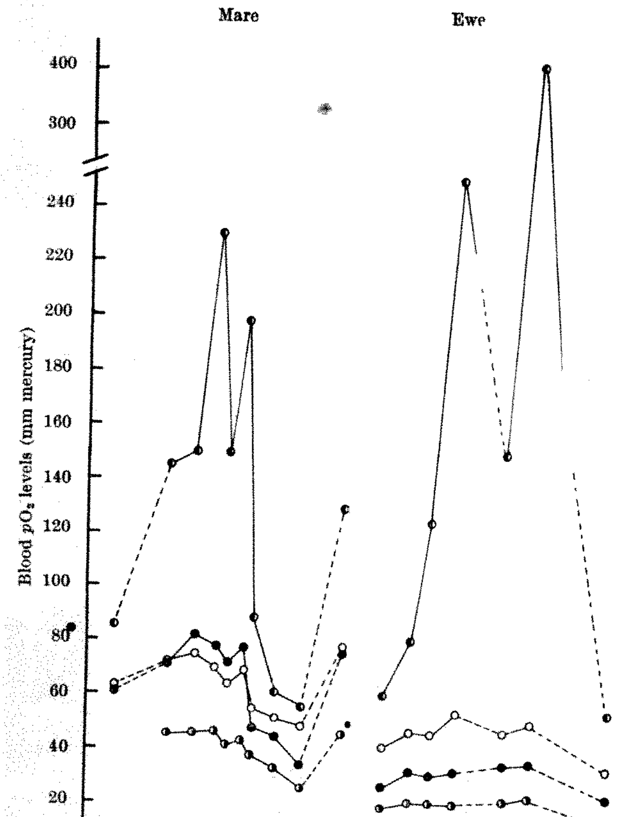

Fig. 1. The changes in $p\mathrm{O}_2$ in the umbilical vein (●), umbilical artery (◐) and uterine vein (○) at different levels of maternal arterial $p\mathrm{O}_2$ (◑) in the mare and the ewe. Each point shows the $p\mathrm{O}_2$ attained in the umbilical and uterine vessels after a period of 10–20 min at a given level of maternal oxygen intake.

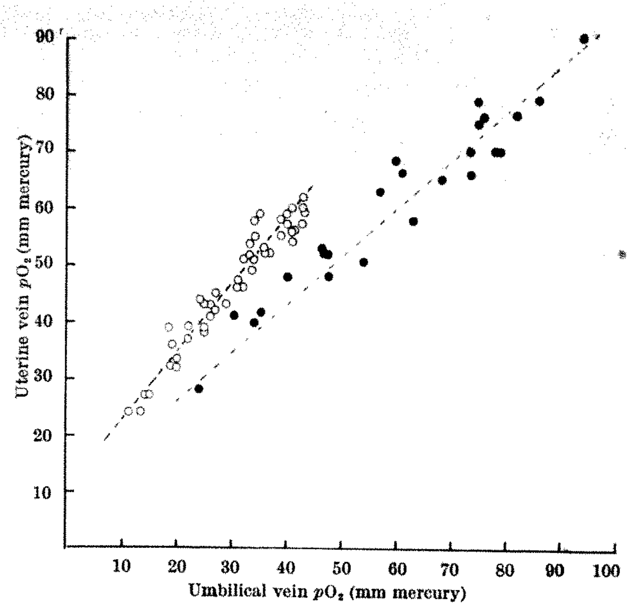

Fig. 2. The relation between the $p\mathrm{O}_2$ in the umbilical and uterine veins in the mare (●) and ewe (○). The values were obtained at maternal arterial $p\mathrm{O}_2$ levels ranging from 30–400 mm of mercury in the ewe (six animals) and 46–230 mm of mercury in the mare (four animals). Regression lines were fitted by the method of least squares – – – – ; the difference between the two regression coefficients $b = 1{\cdot}15 \pm 0{\cdot}036$ (ewes) and $b = 0{\cdot}80 \pm 0{\cdot}054$ (mares) is highly significant ($P < 0{\cdot}001$).

A marked difference between the mare and the ewe was evident even at normal maternal arterial $p\mathrm{O}_2$ levels (80–110 mm of mercury). In the sheep, the mean level for uterine vein $p\mathrm{O}_2$ was $50{\cdot}3 \pm 1{\cdot}63$ mm of mercury and the corresponding figure for the umbilical vein is $33{\cdot}9 \pm 1{\cdot}27$ mm of mercury ($n = 12$); these values were almost identical with those obtained in conscious ewes with indwelling catheters (our unpublished observations). In the mare, on the other hand, the $p\mathrm{O}_2$ levels in the two vessels were very similar (uterine vein: $61{\cdot}3 \pm 2{\cdot}94$ mm of mercury; umbilical vein: $57{\cdot}4 \pm 3{\cdot}26$ mm of mercury ($n = 7$)) and were far higher than any found in the ruminant. The contrast between these two species was enhanced when the effects of changes in maternal oxygenation were compared. The results from two such experiments are given in Fig. 1 and show the extent of the $p\mathrm{O}_2$ changes in the placental vessels when the maternal arterial $p\mathrm{O}_2$ is altered. In the sheep the changes in uterine and umbilical vein $p\mathrm{O}_2$ were very small compared with those in maternal arterial $p\mathrm{O}_2$, whereas in the mare umbilical vein $p\mathrm{O}_2$ exceeded that in the uterine vein when maternal arterial $p\mathrm{O}_2$ is high and the reverse occurred during maternal hypoxia. Thus the uniform gradient between the umbilical and uterine veins in the ewe[3] does not seem to be present in the mare. This is further illustrated in Fig. 2 in which the data from all experiments have been plotted to show the relation between umbilical and uterine vein $p\mathrm{O}_2$ in both species over a wide range of values.

There seems little doubt, even without the aid of statistics, that the slopes and positions of the two curves are very different. The results for the ewes were remarkably uniform with no values higher than 45 mm of mercury for the umbilical vein nor higher than 62 mm of mercury for the uterine vein, despite the fact that some of the ewes breathed 100 per cent oxygen for 0·5–1 h. In the mare the range of values was much greater because the pO_2 levels in both umbilical and uterine veins followed the different levels of maternal arterial oxygenation more closely. The difference between the slopes of the two regression lines, fitted by the method of least squares, was highly significant ($P < 0{\cdot}001$). That for the ewes was slightly greater than 1 ($b = 1{\cdot}15 \pm 0{\cdot}036$); thus the pO_2 difference between the umbilical and uterine veins was lowest (13 mm of mercury) at hypoxic levels and highest (18 mm of mercury) as the pO_2 in each became maximal. The data for the mares fitted a line with a slope of less than 1 ($b = 0{\cdot}80 \pm 0{\cdot}054$), so that in this species the pO_2 gradient between the two vessels decreased as pO_2 rose; at high levels the uterine vein pO_2 was less than that of the umbilical vein and the point at which the pO_2 in the two vessels was equal was 60 mm of mercury.

The very large and relatively unchanging gradient in pO_2 between foetal and maternal vessels in the sheep has been ascribed to the relative impermeability of the tissues (five to six layers) which separate the foetal and maternal blood[1,7], to the high degree of oxygen consumption by the placenta itself[3] and also to the possibility of shunts within the foetal umbilical circulation[3,7]. Such explanations, however, might equally well apply to the equine placenta, where six layers also separate the foetal and maternal blood, and there is no reason to suppose that oxygen is not used by this tissue to the same extent as in the ewe. Yet, in the mare, blood leaving the placenta on the foetal side is virtually in equilibrium with that leaving it on the maternal side. A relatively simple anatomical explanation for the apparent difference in oxygen transfer in the two species has recently been advanced by Steven[8]. He found that the maternal capillary networks which supply the areas of placental exchange are arranged very differently in the two species. Those of the mare are small networks supplied solely by maternal arteries, whereas those of the ewe are large and their extensive arterial blood supply is diluted to a varying degree with capillary blood which has already undergone some degree of exchange, so that most foetal villi in the sheep would seem to be exposed to a lower maternal pO_2 than those of the mare. The low and relatively constant umbilical venous oxygen pressure of the foetal lamb and the higher, more variable level in the foetal foal may therefore each reflect their particular maternal vascular arrangement.

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[1] Barron, D. H., *Yale J. Biol. Med.*, **24**, 169 (1951).

[2] Comline, R. S., Silver, I. A., and Silver, M., *J. Physiol.*, **178**, 211 (1965).

[3] Campbell, A. G. M., Dawes, G. S., Fishman, A. P., Hyman, A. I., and James, G. B., *J. Physiol.*, **182**, 439 (1966).

[4] Parker, H. R., and Purves, M., *Quart. J. Exp. Physiol.*, **52**, 205 (1967).

[5] Meschia, G., Cotter, J. R., Makowski, E. L., and Barron, D. H., *Quart. J. Exp. Physiol.*, **52**, 1 (1967).

[6] Meschia, G., Cotter, J. R., Breachnach, C. S., and Barron, D. H., *Quart. J. Exp. Physiol.*, **50**, 185 (1965).

[7] Metcalfe, J., Moll, W., Bartels, H., Hilpert, P., and Parer, J. T., *Circulation Res.*, **16**, 95 (1965).

[8] Steven, D. H., *J. Physiol.* (in the press).

Effects of Noradrenaline and Adrenaline on Oxygen Consumption Rate and Arterial Blood Pressure in the Newborn Pig

Moore and Underwood[1] showed that subcutaneous injection of noradrenaline in the newborn kitten produced a marked increase in metabolic rate, whereas adrenaline in the same dose was relatively inactive. A similar stimulation of metabolism in the newborn animal after the injection of noradrenaline was observed in the rabbit and rat[2] and in the human infant[3]. Subsequent work on the newborn rabbit showed the probable relation between the effect of noradrenaline and the activity of brown fat. Local heat production in brown fat is increased by the infusion of noradrenaline[4]. When the brown fat is excised, the rise in oxygen consumption during infusion is greatly reduced[5]. These observations suggested that the effect of noradrenaline should be investigated in the newborn pig, which seems to have no brown fat, and only about 1 per cent total fat in the body at birth[6]. The amount increases rapidly to reach about 10 per cent at 1 week of age[7].

Observations were made on male and female Large White piglets. The animals used were 1–23 days old and had body weights of 1·16–4·45 kg.

The rate of oxygen consumption was measured on conscious animals resting individually in a chamber of about 45 l. capacity. The chamber was situated in a controlled temperature room, which was kept at 30° C for experiments on animals less than 1 week old, and at 27° C for the older animals. The internal chamber temperature was approximately 1° C higher, so that the pigs were resting at about 2° C below their critical temperatures, which would be 33°–34° C for the newborn animals and about 30° C for the older ones. Rectal temperatures were maintained between 38·5° and 39·5° C. The rate of oxygen consumption was determined in the open circuit system using a Noyons diaferometer (Kipp and Zonen, Holland). The resting rate was measured for 0·5–1 h in the conscious animals. Then either adrenaline or noradrenaline was injected subcutaneously in a dose of 600 μg of amine base (0·1 per cent solution in 0·9 per cent saline)/kg body weight. The rate of oxygen consumption was determined again during a period of at least 1 h. At least 2 h were allowed to elapse before the second amine was injected, to allow time for the oxygen consumption rate to return to the original level. The effect of either drug on oxygen consumption could then be assessed on a preceding control period.

Fig. 1 shows that adrenaline produces an increase in oxygen consumption from the first day after birth, and that the effect persists in the older animals. Noradrenaline has no effect during the first week, and a relatively small effect in the second week.

The solutions of the amines were acid (pH $\simeq$ 4), and so sodium chloride solution brought to comparable acidity with hydrochloric acid, but devoid of amines, was injected into some pigs; there was no consistent effect on oxygen consumption rate. Injections of normal saline at pH 7 had no effect on oxygen consumption.

Arterial blood pressure responses were measured on a mercury manometer connected to a carotid artery cannula, in pigs anaesthetized with sodium pentobarbital administered intraperitoneally (40 mg/kg). For these measurements, the animal was partially immersed in a water bath held at 35° C and rectal temperature was maintained at 37°–38° C. The femoral vein was cannulated for injection of the drug. Doses of adrenaline and noradrenaline base, ranging between 0·1 and 0·5 μg/kg, were injected rapidly in volumes of 0·2 ml. of normal saline into the femoral vein. The effect on the response to amines of a beta-blocker, pronethalol, was determined in four pigs.

During the first 2 weeks after birth, the arterial blood pressure in anaesthetized pigs was found to be between 60 and 70 mm of mercury. The level in pigs at 3 weeks of age was about 100 mm of mercury. Fig. 2 shows that the pressor effect of noradrenaline was always greater than that of adrenaline during the first 3 weeks, a result opposite to the relative effects of the amines on oxygen consumption. Pronethalol, a beta blocking agent, had no effect on the response of blood pressure to noradrenaline, but it apparently potentiated the response to adrenaline. This is to be expected because if pronethalol blocked β-receptors, which are normally depressor, it would convert adrenaline from a mixed pressor/depressor to a pressor agent which would act, like noradrenaline, only on α-receptors.

In the conditions of these experiments, noradrenaline does not stimulate the metabolic rate in the newborn pig, although an effect appears after the first post-natal week. Adrenaline, however, has an effect which is apparent from birth. In this respect the pig is different from some other species and the difference might be related to the pig's lack of body fat in the immediate post-natal period. This possibility is supported by the increased metabolic rate produced by noradrenaline at 8 days of age, by which time the pig has formed a considerable amount of body fat. The results suggest that the mediation of noradrenaline cannot be invoked to explain any part of the vigorous metabolic response to cold of newborn pig. But the release of adrenaline could be very important for this response. The newborn pig relies principally on carbohydrate metabolism for energy production[8].

Responses to noradrenaline and adrenaline in newborn pigs bear comparison with those observed in newly hatched chicks[9]. In the chick, noradrenaline (300 μg/kg) causes a slight increase in oxygen consumption after hatching, but has little or no effect 1–3 weeks later. A similar dose of adrenaline results in a 30 per cent increase in metabolic rate in 1 day old chicks, an effect which remains demonstrable 1–3 weeks later. No brown fat has been found in the chick, and it appears that the chick, like the newborn pig, uses carbohydrate in producing the rise in metabolic rate which takes place in response to a cold environment.

The arterial blood pressure of 3 week old pigs has been found to be considerably greater than that observed during the first week. Both noradrenaline and adrenaline produce marked pressor responses during this period, the effect of noradrenaline always being larger than that caused by a similar dose of adrenaline. These findings are comparable with those reported in kittens[10] and in newborn rabbits[11].

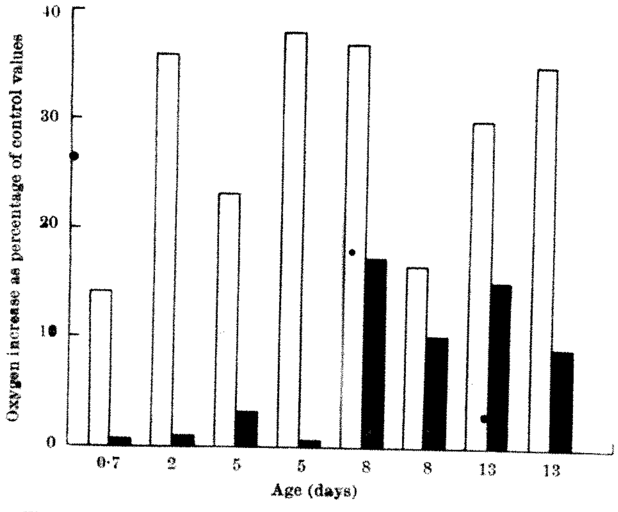

Fig. 1. The effects of 600 μg/kg of adrenaline (□) or noradrenaline (■), given subcutaneously, on oxygen consumption rate in pigs up to 13 days of age.

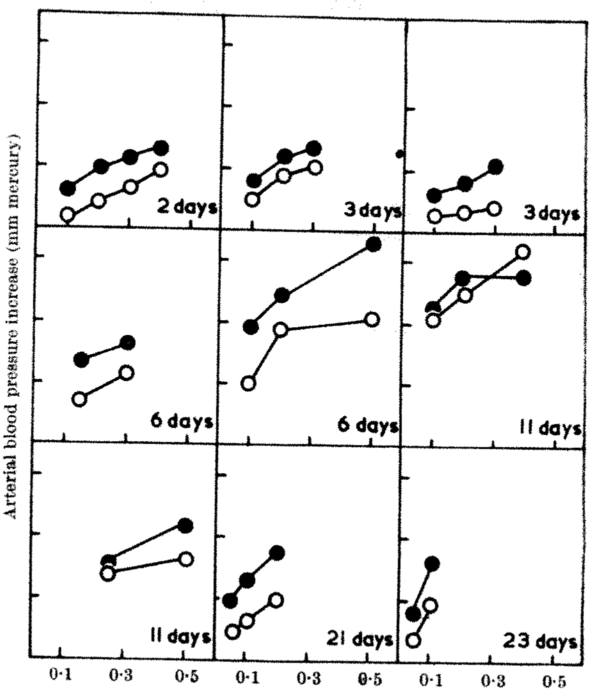

Fig. 2. The effects of adrenaline (○) and noradrenaline (●) given intravenously on arterial blood pressure in anaesthetized pigs up to 23 days of age.

Variations between species in the relative magnitude of the effects of noradrenaline and adrenaline on metabolic rate do not exist in their effects on arterial blood pressure. In the newborn pig, noradrenaline acts powerfully on the cardiovascular system, so that its lack of metabolic effect cannot be attributed to inactivation by enzymes, for example. The metabolic effect of noradrenaline, unlike that of adrenaline, may depend on the presence of brown fat. In its absence noradrenaline will, on this view, have no effect on metabolic rate.

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[1] Moore, R. E., and Underwood, Mary C., *J. Physiol.*, **150**, 13*P* (1960).
[2] Moore, R. E., and Underwood, Mary C., *J. Physiol.*, **168**, 290 (1963).
[3] Karlberg, P., Moore, R. E., and Oliver, T. K., *Acta Paediat., Stockh.*, **51**, 284 (1962).
[4] Dawkins, M. J. R., and Hull, D., *J. Physiol.*, **172**, 216 (1964).
[5] Hull, D., and Segall, M. M., *J. Physiol.*, **175**, 58*P* (1964).
[6] Widdowson, E. M., *Nature*, **166**, 626 (1950).
[7] Brooks, C. C., Fontenot, J. P., Vipperman, P. E., Thomas, H. R., and Graham, P. P., *J. Anim. Sci.*, **23**, 1022 (1964).
[8] McCance, R. A., and Widdowson, E. M., *J. Physiol.*, **147**, 124 (1959).
[9] Freeman, B. M., *Comp. Biochem. Physiol.*, **18**, 369 (1966).
[10] Hutchinson, E. A., Percival, C. J., and Young, M., *Quart. J. Exp. Physiol.*, **47**, 201 (1962).
[11] Dawes, G. S., Handler, J. J., and Mott, J. C., *J. Physiol.*, **139**, 123 (1957).

Respiration in Myocardium

The qO_2 or oxygen uptake of a tissue is considered to be a reflexion of the metabolic energy produced through substrate catabolism to support its functional activities. It is usually accepted that the total *in vivo* oxygen consumption is directly related to ATP production as the

Table 1. OXYGEN CONSUMPTION OF THE ARRESTED RAT HEART
(ml. of O_2/min/g dry weight)

Experiment	Control	Oligomycin (5 μg/ml.) +	Cyanide (2·5 mmolar) +	Epinephrine (0·1 μg/ml.) +	Octanoate (2·5 mmolar)	*n*
1	0·136 ± 0·006 →	→	0·032 ± 0·003			6
2		0·128 ± 0·003 →	0·026 ± 0·002			6
3			0·034 ± 0·006 →	0·039 ± 0·008		6
4			0·022 ± 0·004 →	→	0·028 ± 0·002	6

Hearts were perfused with buffer containing 16 mmolar potassium with the additions shown. After 10–15 min, the qO_2 was determined and the second condition was applied to the same heart with a second determination of qO_2 after 10 min. Results are expressed as the mean ± *S.E.M.*

sole energy transfer and storage mechanism for endergonic processes[1]. Energy balance calculations in biology make use of this assumption, which is based on the known *in vitro* efficiencies of these processes. Recently biochemists have shown that certain non-mitochondrial enzyme systems assimilate or "take up" oxygen[2] and that respiration without phosphorylation is probably essential to many of the biological functions of mitochondria[3]. The relative contribution of non-phosphorylating and non-mitochondrial oxygen uptake to total respiration *in vivo* has been studied in the isolated perfused rat heart. Oligomycin and cyanide were used as inhibitors to distinguish mitochondrial but non-phosphorylating respiration from non-mitochondrial respiration.

Hearts were removed from 250 g white male Wistar rats (fed *ad libitum*) and perfused as described previously[4-6]. To eliminate "mechanical" contributions to qO_2 in studies of the basal "metabolic" qO_2, 16 mmolar potassium was used for cardiac arrest[6]. Oxygen consumption (ml. of O_2/min/g dry weight) was calculated from the arteriovenous pO_2 difference determined in an oxygen electrode. All determinations were made after stabilization for 10 min at the beginning of perfusion and 10 min after experimental intervention. In this preparation 2·5 mmolar sodium cyanide provided maximal qO_2 inhibition and oligomycin (5 μg/ml.) was used to inhibit oxidative phosphorylation[6].

Though the oxygen consumption of the rat heart *in vivo* is not known, an extrapolation might be made from *in vitro* perfusion systems in which pressure and/or volume work are performed. Values equivalent to 1·0 ml. of O_2/min/g dry weight have been obtained by Dr Howard E. Morgan (personal communication) and Dr Lionel Opie[7]. This value was selected as an estimate of the qO_2 of the working heart. The Langendorff heart preparation I used beats without performance of external work, and decreased the value to 0·5 ml. of O_2/min/g dry weight[4-6]. When all mechanical activity was eliminated by potassium arrest, the qO_2 fell to approximately 0·15 ml. of O_2/min/g dry weight, the basal "metabolic" oxygen consumption of rat heart[5,6] (Table 1).

Eighty-five per cent of *in vivo* respiration in muscle is related to mechanical functions, which are supported by ATP, and an equally high portion of respiration must result in the production of high energy phosphate. Oligomycin is a potent inhibitor of oxidative phosphorylation in isolated mitochondria; any other respiration is not linked in an obligatory fashion to phosphorylation[8,9]. The effects in intact myocardium are analogous to those in mitochondria because there is a decrease in ATP (ref. 6), an inhibition of phosphorus-32 incorporation into ATP and an increase in DPNH fluorescence[10].

The dependence of the mechanical activities of myocardium on ATP has been demonstrated by a rapid arrest and decrease in ATP and qO_2 on exposure to oligomycin[6]. The greater portion of the arrested or basal qO_2 was not inhibited by oligomycin (Table 1)[6,10]. Non-phosphorylating respiration accounts for 85–90 per cent of the basal qO_2 (ref. 10). If the absolute value is constant, it could account for about 13 per cent of *in vivo* myocardial respiration. In fat cells, not dependent on ATP production to support mechanical activity, the portion of respiration insensitive to oligomycin was found to be 45 per cent (unpublished results from this laboratory). Though the energy produced is not stored as high energy phosphorus and might be considered as "waste", the high energy intermediates generated[3] may donate their energy to cellular processes capable of using it[3,11-16]. The efficiency or "coupling" of such an energy supply is not known but may be significant in certain pathological states[17].

The reduction of oxygen by mitochondria, whether or not it is accompanied by phosphorylation, may not be the only mechanism in the intact cell. Microsomes are capable of oxygen utilization and in isolated systems have the kinetic ability to accommodate rates of electron flow equivalent to those of mitochondria[2]. This is respiration unaccompanied by ATP formation and would be classified as a part of oligomycin-insensitive respiration of the intact cell.

Cyanide, an inhibitor of mitochondrial cytochrome oxidase, can be used to eliminate the oxygen uptake associated with mitochondrial activity. A definition of the respiration remaining in the intact heart is therefore "non-mitochondrial", but not necessarily microsomal. I found a remaining respiration of 0·022 ± 0·004 to 0·036 ± 0·006 ml. of O_2/min/g dry weight in the presence of cyanide (Table 1). This accounts for only 3 per cent of the total qO_2 of the working heart but approximately 20 per cent of the resting or the oligomycin-insensitive respiration (0·026 of 0·128, Table 1). Approximately 20 per cent of respiration of perfused rat liver, another system without the ATP requirement of contractile function, has been similarly defined as non-mitochondrial[18]. Epinephrine and octanoate, which increase the level of non-phosphorylating respiration in the rat heart[6,10], did not change the qO_2 in the presence of cyanide (Table 1); their effect is most likely to stimulate mitochondrial non-phosphorylating respiration.

The heart uses 87 per cent of its respiratory activity for ATP production, 85 per cent being directly related to contractility. The remaining oxygen uptake is associated with non-phosphorylating mitochondrial and microsomal activities the nature, stoichiometry and efficiency of which are not clear.

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[1] White, A., Handler, P., and Smith, E., *Principles of Biochemistry*, chap. 16, 17 (McGraw Hill, New York, 1964).

[2] Siekvitz, P., *Fed. Proc.*, **24**, 1153 (1965).

[3] *Control of Energy Metabolism* (edit. by Chance, B.) (Academic Press, New York, 1965).

[4] Morgan, H. E., Henderson, M. J., Regen, D. M., and Park, C. P., *J. Biol. Chem.*, **236**, 253 (1961).

[5] Challoner, D. R., and Steinberg, D., *Amer. J. Physiol.*, **210**, 280 (1966).

[6] Challoner, D. R., and Steinberg, D., *Amer. J. Physiol.*, **211**, 897 (1966).

[7] Opie, L. H., *J. Physiol.*, **180**, 529 (1965).

[8] Huijing, F., and Slater, E. C., *J. Biochem. Tokyo*, **49**, 493 (1961).

[9] Lardy, H. A., Johnson, D., and McMurry, W. C., *Biochemistry*, **3**, 1961 (1964).

[10] Challoner, D. R., *Amer. J. Physiol.* (in the press).

[11] Van Rossum, G. D., *Biochim. Biophys. Acta*, **82**, 556 (1964).

[12] Wojtczak, L. H., Zaluska, L. H., and Drahota, Z., *Biochim. Biophys. Acta*, **98**, 8 (1965).

[13] Beattie, D. S., and Basford, R. E., *J. Biol. Chem.*, **241**, 1412 (1966).

[14] Kroon, A. M., *Biochim. Biophys. Acta*, **72**, 391 (1963).

[15] Etnster, L., and Lee, C. P., *Ann. Rev. Biochem.*, **33**, 779 (1964).

[16] Azzone, G. F., and Azzi, A., *Proc. US Nat. Acad. Sci.*, **53**, 1984 (1965).

[17] Challoner, D. R., *Lancet*, ii, 681 (1966).

[18] Scholz, R., and Bucher, T., in *Control of Energy Metabolism* (edit. by Chance, B.), 393 (Academic Press, New York, 1965).

Reduction of Response Time for Potential in Salt Bridge Reference Electrodes for Electrophysiology

SATURATED or 3 molar potassium chloride salt bridges connected to a calomel half cell or a silver–silver chloride electrode are used extensively in electrochemistry and electrophysiology as reference electrodes for potential measurements. The 3 molar potassium chloride salt bridge electrode is used in electrophysiology primarily because the liquid junction potential from the salt bridge to physiological solutions is only a few millivolts and varies little at equilibrium when in different physiological solutions. Derivations for this liquid junction potential have been obtained[1-3].

The equilibration time for potentials in salt bridge reference electrodes is particularly important in electrophysiological studies where the dependence of the cell membrane potential on various ions is being measured. With cylindrical salt bridge reference electrodes (Fig. 1*a*) it has been found that long-lasting transient potentials of several millivolts can occur in certain situations when substitutions or changes are made in the physiological saline. In experiments where the membrane potential of crayfish axons in physiological saline was being measured, the transient change in potential at the 3 molar potassium chloride salt bridge reference electrode amounted to almost 4 mV when external chloride was changed from 240 to 130 mmolar[4]. The chloride was changed by one to one substitution with glucuronate or propionate. This potential artefact is considerable because it is measured electrically in series with the potential change of the cell membrane, which itself is usually only a few millivolts. The elimination and understanding of this transient error in potential in reference electrodes is therefore important in electrophysiology.

The transient potential change at the reference salt bridge electrode arises from the following factors. The concentrations in a cylindrical salt bridge, or any other salt bridge where diffusion is in one dimension, never acquire a steady state distribution. After a long equilibration time of a 3 molar potassium chloride-filled salt bridge in one solution, the concentrations at the cylinder tip approach that of the external solution and the concentrations approach the initial 3 molar potassium chloride concentration only a considerable distance into the cylinder. Consequently, with a change in the external solution, two liquid junction potentials appear. The first is the original liquid junction potential which is distributed over the extended boundary between the first external solution and the 3 molar potassium chloride in the cylinder. The second liquid junction potential is at the cylinder tip between the new solution outside and the first solution trapped or still held in the cylinder. The response time of the salt bridge reference electrode is the overall diffusion time necessary for a single liquid junction potential to be produced. This can be hours. If there are large liquid junction potentials between exchanged external solutions, large transient potential artefacts appear. This artefact arises as a consequence of one dimensional diffusion in the cylindrical salt bridge, so the diameter of the cylinder is not important in the response time.

One solution to this problem has been to use a flowing 3 molar potassium chloride junction[5]. This approach is usually impractical for physiological studies where solutions must often be retained and any leakage of potassium into the external saline would cause undesired physiological effects. Another approach would be to use a reference electrode the liquid junction of which consists of a small hole in a thin impermeable membrane which separates a large volume of 3 molar potassium chloride (in agar to prevent bulk fluid flow) and the physiological saline. The response time for voltage at the reference electrode would then depend on the time required for a steady state ion distribution to appear across this thin hole. Another successful method is to use a conical reference electrode of small tip diameter and large solid angle taper[4] (Fig. 1*b*).

The development described here is of the perforated membrane salt bridge reference electrode (Fig. 1*c*). This electrode consists of a large cylinder with an impermeable thin plastic membrane (0·001 cm thick, polyvinylidine chloride, polyester, polyethylene, etc.) glued with epoxy cement to one end. A small hole, about 0·02 cm in diameter, is made by puncturing the centre of the membrane with a small insect pin (No. 00) heated by attaching it to an electric soldering iron. The electrode is filled with 3 molar potassium chloride and 3 per cent agar to prevent fluid flow. A silver–silver chloride electrode or calomel cell is then connected to the 3 molar potassium chloride in the cylinder which completes the reference electrode. When immersed in crayfish saline, the electrical impedance of this perforated membrane electrode (with silver–silver chloride connexion) has been less than 750 ohms, which is satisfactory for grounding most physiological solutions. Fig. 1 shows a comparison of the response times of potential for a cylindrical, conical and a perforated membrane salt bridge reference electrode when external chloride is changed. The time for 90 per cent response (measured to final steady state potential) for the perforated membrane electrode has been faster than 0·5 sec and may be faster because the measurement was limited by the time necessary to change solutions and the response time of the silver–silver chloride electrode. The conical reference electrode performed almost as rapidly as the perforated membrane electrode. But the cylindrical reference electrode did not acquire a steady state potential even after many minutes. Voltage readings with cylindrical reference electrodes are therefore much less reliable in situations where there are changing physio-

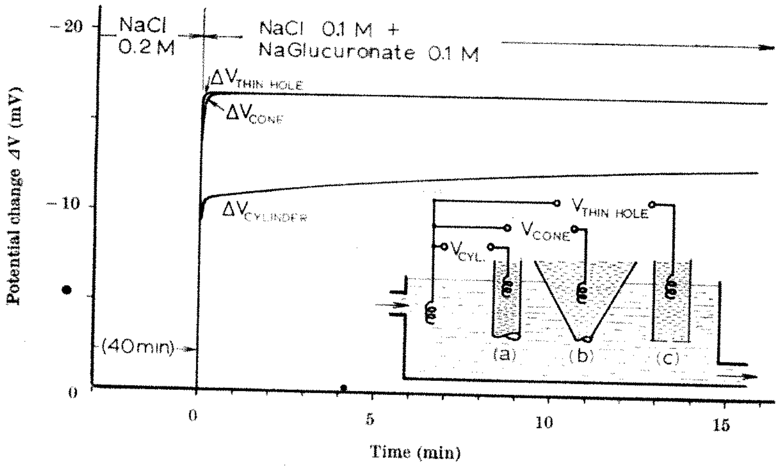

Fig. 1. The time course of potential for a cylindrical, conical and a perforated membrane salt bridge reference electrode referred to an Ag–AgCl electrode. The electrodes were placed in 0·2 molar NaCl for 40 min before the solution was suddenly changed ($t = 0$) to 0·1 molar NaCl and 0·1 molar Na glucuronate in order to change chloride from 0·2 to 0·1 molar without changing total concentrations or activity coefficients. The conical and perforated membrane electrodes became rapidly stable in potential within several seconds. Even after 15 min the cylindrical electrode had not acquired a steady state. The cylindrical salt bridge electrode was 0·1 cm in diameter. Cylinder diameter was unimportant in these comparisons. The conical salt bridge electrode has a 0·02 cm tip diameter and the taper subtended an angle of 40° until the diameter reached 0·2 cm, after which the electrode was cylindrical. The perforated membrane electrode consisted of a 0·5 cm diameter cylinder covered with a 0·001 cm polyester membrane ('Mylar', 'Dacron', 'Terylene') with a 0·02 cm diameter hole. All electrodes were filled with 3 molar KCl and 3 per cent agar.

logical solutions. An effective increase in reliability is obtained with a small hole perforated membrane reference electrode.

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[1] Planck, M., *Ann. Physik.*, **30**, 161 (1890); **40**, 561 (1890).

[2] Henderson, P., *J. Physik. Chem.*, **59**, 118 (1907); **63**, 325 (1900).

[3] MacInnes, D. A., *The Principles of Electrochemistry*, 220, 461 (Dover, New York, 1961).

[4] Strickholm, A., and Wallin, G., *J. Gen. Physiol.*, **50**, 1929 (1967).

[5] Lamb, A. B., and Larson, A. T., *J. Amer. Chem. Soc.*, **43**, 229 (1920).

Evoked Potentials reflecting Interocular and Monocular Suppression

It is well known that averaged visually evoked potentials (EPs) from the human occipital scalp show correlations with a number of subjective features of perception; but recent attempts to find EP correlates of the phenomenon of image suppression, as in interocular rivalry, seem to have yielded conflicting results[1–4]. In most of these experiments contrasting stimuli to the two eyes were allowed to set up spontaneously fluctuating rivalry, and the subject was required to press a key to indicate which stimulus was dominant at any time. There are, however, situations in which the perceptual dominance of one stimulus over another is stable. In the two experiments reported here, perceptual suppression of one stimulus by another in such stable situations has been found to be clearly accompanied by correlated changes in the averaged EPs.

The stimulus conditions used were dictated by an interest in a quite different phenomenon, and the discovery of EP correlates of image suppression was accidental. When a randomly patterned stimulus ("visual noise") is followed by a blank flash optically superimposed on the same area of the visual field, curious perceptual anomalies, and corresponding anomalies in the EP, occur at repetition rates of the order of 10–15/sec, which are not seen if the patterned and blank fields are presented to different eyes[5]. At lower rates of the order of 2/sec, these anomalies disappear; but two kinds of suppression phenomena are still observed.

(1) When the blank field, *B*, is flash-presented simultaneously to the same eye or eyes as the noise-patterned field, *N*, it suppresses perception of the patterned field, which becomes gradually visible as the number of milliseconds between *B* and *N* is increased. The critical interval depends on relative brightness, and in the present case was between 10 and 20 msec. This suppression of one stimulus by another presented to the same eye is termed "perceptual blanking".

(2) When *N* is presented to one eye and *B* to the other, perception of *B* is normally suppressed over a wide range of time intervals between *B* and *N*, and there are no perceptual effects of changing the timing or brightness of *B* over several tens of msec and several log units, respectively. This phenomenon is referred to as "interocular suppression".

In the present experiments, the EPs in response to single pairs of noise-patterned and blank stimuli presented approximately twice/sec, were recorded (by conventional averaging methods) with varying time intervals of the order of 0–50 msec between noise and blank, and with the two stimuli presented to either the same or different eyes. The two stimuli (*N* and *B*) were back-projected on separate screens and optically superimposed by means of a half silvered plane mirror, after optical polarization in orthogonal planes. Two pairs of polaroid spectacles were provided (fixed to the main framework, not worn) with polarizing orientations chosen to enable the two stimuli to be presented either (*a*) each to separate eyes or (*b*) both to the same eye or eyes at roughly half intensity.

Repetition intervals of about 0·5 sec were found long enough to prevent significant overlap between successive EPs, and the responses were averaged over runs of 60 to 105 presentations lasting from 0·5–1 min. Every run was repeated and the two results were superimposed on the cathode ray tube screen before photographing, to give an indication of their statistical significance. Two orthogonal bipolar recording placements were used, just to the left of the occipital midline, the lowest electrode being 1 cm left of the inion (Fig. 1).

Typical evoked responses to presentation of noise-patterned, *N*, and blank, *B*, flashes alone, at intervals of 573 msec, were recorded in the same subject on a separate occasion and are shown for reference in Fig. 1. *N* and *B* in this case were matched for subjective brightness (0·1 log ft. Lambert). Each trace is a superposition of the results of two successive runs of 105 responses each, indicating the run to run consistency obtainable. The upper of each pair of traces represents the average evoked potential, *L*, parallel to the midline; the lower represents the potential, *T*, transverse to it. It will be seen that although the longitudinal components are similar, the transverse components are strikingly different for patterned and blank stimulation; and the lowest pair, recorded 10 min later than the first but in the same conditions, indicates that this difference is also consistent. Its principal feature lies in the contrast between the rapid downward deflexion in response to "noise", and the slower upward deflexion at the same latency in response to "blank". These records were taken with binocular view and without polaroid, to give maximum signal to noise ratio. They have been found to be repeatable at any time in the same subject. Although bipolar, they reveal a similar contrast to that observed by Rietveld *et al.*[6] between the monopolar responses to checker-patterned and blank fields.

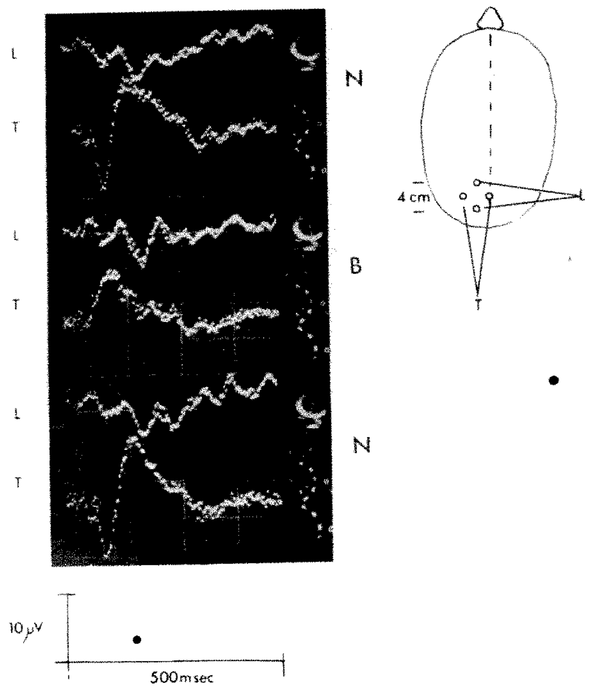

Fig. 1. Typical averaged responses to 105 exposures of noise-patterned, *N*, and blank, *B*, stimuli. Bipolar potentials recorded, *L*, parallel, *T*, transverse to midline. Each trace shows the results of two successive runs superimposed to indicate run-to-run consistency. Repeatability of *N* responses after an interval of 10 min may be checked by comparing upper and lower *N* records.

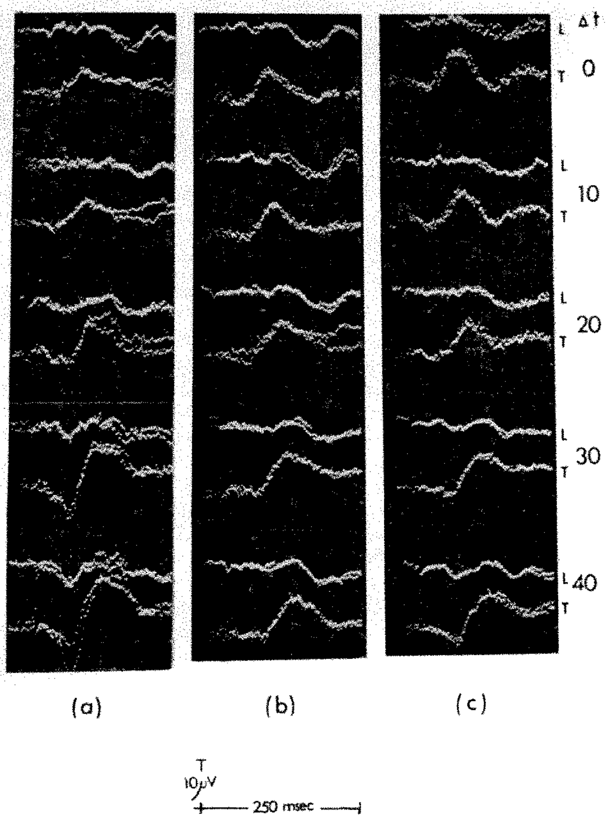

Fig. 2. *a*, Effects of increasing time interval, Δt, between N and B during normal binocular viewing. Note change of time-scale from Fig. 1. Each trace shows two successive results superposed. *b* and *c*, The same for left-eye and right-eye viewing, respectively.

Figs. 2*a*–*c* show the effect on the evoked potentials of increasing the time interval, Δt, between noise-patterned and blank flashes during normal binocular and monocular viewing. A repetition interval of 473 msec was used, and the blank flashes were adjusted to be subjectively 0·7 log units brighter than the noise. At low values of Δt, the transverse EP in all three cases shows the typical upward response to B (compare Fig. 1 B noting the difference in time scale), and the subject reports masking of the noise pattern. As Δt increases above 20 msec, the noise pattern becomes progressively more visible subjectively, and the EP correspondingly shows the initial downward deflexion (compare Fig. 1 N) evoked by N. With monocular viewing (Figs. 2*b* and *c*) the downward deflexions are much less pronounced, but the general trend (especially with right-eye viewing) is similar.

When the noise-patterned field is flashed to one eye and the blank to the other, suppression caused by interocular rivalry is observed, with the opposite effect; the blank field is rendered invisible by the noise-patterned field. The corresponding changes in the transverse EP are illustrated in Fig. 3. The longitudinal component, as before, showed no significant changes and has been omitted for clarity.

The first two rows show typical responses to N and B, respectively in left, L, or right, R, eyes, with $\Delta t = 0$, 30, 90 msec. The third row shows clearly how simultaneous presentation of N and B to opposite eyes gives the downward deflexion characteristic of N, with suppression of the upward "B" deflexion. With binocular viewing of N and B together, the responses in the fourth row of Fig. 3 were obtained, as in Fig. 2.

The contrast between third and fourth rows shows that a noise flash in one eye renders the transverse EP insensitive to the timing as well as the brightness of a blank flash in the other. With normal binocular viewing of N and B, blanking of the downward "N" component can be seen at $\Delta t = 0$, when the N stimulus is perceptually "blanked" by B, but not at $\Delta t = 30$ and 90 msec, when both stimuli are seen. The upward "B" component is present throughout. With dichoptic viewing of the same stimuli (third row), the downward "N" response is present at all values of Δt, the form of the EP shows no trace of the "B" response, and there is no significant change with Δt. This tallies with subjective experience, which is also quite insensitive to the brightness or timing of B in one eye (over this range) provided that N is presented only to the other eye.

These results seem to give definite evidence of EP changes correlated with two kinds of subjective suppression of one stimulus by another. Their detailed implications will be discussed elsewhere, but they suggest that whatever switching mechanisms are involved in the perceptual blanking and the interocular suppression

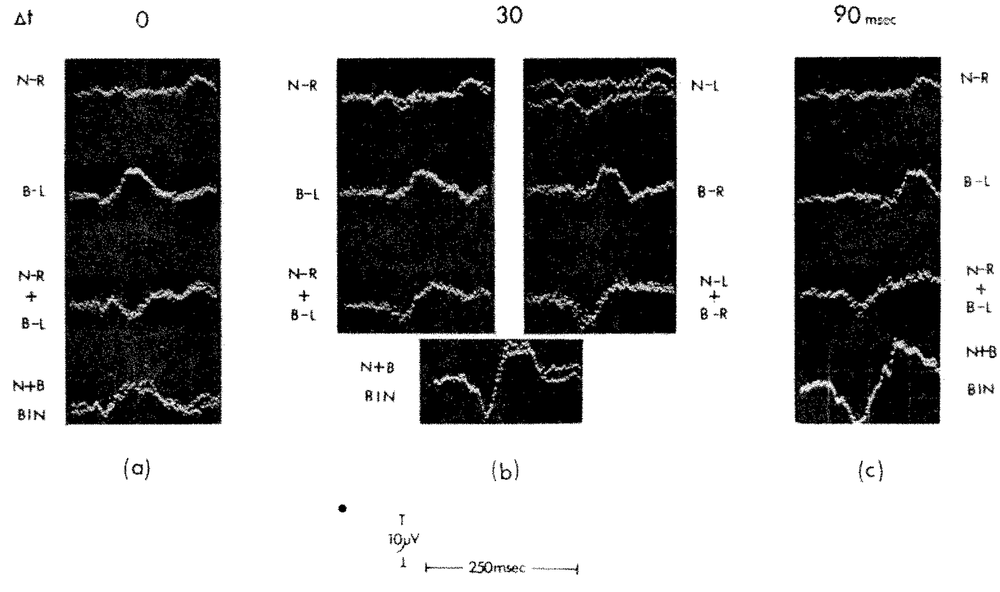

Fig. 3. Comparison of monocular (first two rows), dichoptic (third row) and binocular (fourth row) conditions: *a*, with $\Delta t = 0$; *b*, with $\Delta t = 30$ msec; *c*, with $\Delta t = 90$ msec. Note clear suppression of upward "B" response with dichoptic but not with binocular viewing. L = Left eye, R = right eye, N = noise, B = blank. Each picture shows two successive results superposed.

observed, they lie peripheral to the origin of the pattern-specific component of the EP. It is interesting to note that combination of the EPs to blank and noise is approximately linear when both stimuli are applied to the same retina, with a relative time interval of 30 msec or above. As this was also the condition in which both stimuli were subjectively visible, it suggests that the cerebral EP generating mechanisms activated by blank and patterned stimuli are largely independent. This would be expected if patterned stimulation excites in man a cortical population which is specifically contour-sensitive, analogous to those found in cat and monkey[7]. It might also explain some of the negative findings by other investigators using patternless fields.

These results may have some bearing on the recent finding by Lehmann and Fender[8] that the average EP to a blank flash was reduced according to the amount of structure presented (steadily, not flashed) to the other eye. They report that "spontaneous fading" occurred, but rely on earlier negative findings to discount its effect on the EP. If, however, what they observed occasionally with a steady pattern was the kind of interocular suppression that happens predictably with a flashed pattern, nothing more might be needed to explain a reduction in the average EP.

The reported lack of correlation between EPs and the fading of stabilized retinal images[9] would not discount this possibility, unless such fading were known to depend on the same mechanism as interocular suppression. The perceptual differences between the two phenomena make this unlikely.

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[1] Lansing, R. W., *Science*, **146**, 1325 (1964).

[2] Donchin, E., and Lindsley, D. B., *Electroenceph. Clin. Neurophysiol.*, **19**, 325 (1965).

[3] Cobb, W. A., Ettlinger, G., and Morton, H. B., in *The Evoked Potentials* (edit. by Cobb, W. A., and Morocutti, C.), *Electroenceph. Clin. Neurophysiol.*, suppl. 26 (1967) (in the press).

[4] Riggs, L. A., and Whittle, P., *Vis. Res.*, **7**, 441 (1967).

[5] MacKay, D. M., *J. Gen. Psychol.*, **72**, 181 (1965). MacKay, D. M., and Fiorentini, Adriana, *Nature*, **209**, 787 (1966).

[6] Rietveld, W. J., Tordoir, W. E. M., Hagenouw. J. R. B., Lubbers, J. A., and Spoor, Th., *Acta Physiol. Pharmacol. Neerl.* (in the press).

[7] Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **28**, 229 (1965).

[8] Lehmann, D., and Fender, D. H., *Nature*, **215**, 204 (1967).

[9] Lehmann, D., Beeler, G. W., and Fender, D. H., *Electroencephal. Clin. Neurophysiol.*, **22**, 136 (1967).

Antagonism of Presynaptic Inhibition in the Cuneate Nucleus by Picrotoxin

AN afferent volley in a cutaneous forelimb nerve produces on the surface of the cuneate nucleus a characteristic electrical response. This consists of a brief initial spike potential indicating the arrival of the primary afferent volley, followed by a negative wave (N wave) lasting about 4–5 msec and a prolonged positivity (P wave) reaching a maximum in 20–30 msec and lasting for about 200 msec[1]. Similarly, N and P waves result from electrical stimulation of the contralateral sensorimotor cortex[2]. The N wave produced by the afferent stimulus is thought to be caused by synaptically induced depolarization of the cuneate cells by the ascending volley in the dorsal column[3]. Systematic investigation of the P waves produced by cutaneous volleys and by cortical stimulation led Andersen *et al.*[3] to suggest that both are caused by prolonged depolarization of cuneate tract fibres close to their synaptic terminals in the cuneate nucleus. This suggestion was further supported by excitability testing of these presynaptic fibres at their terminals and along their length, and by intracellular recording from these fibres[4]. With the technique of extracellular single cell recording, Jabbur and Towe[5-8] had shown earlier that the sensorimotor cortex (primarily the contralateral) produced excitatory and inhibitory influences on cuneate and gracile neurones. With the additional technique of antidromic activation of dorsal column neurones after medial lemniscal stimulation[9,10], two types of neurones have been identified. These are direct projection neurones, or dorsal column-thalamic relay neurones, which are depressed by antecedent descending volleys from the sensorimotor cortex, and interneurones, through which the cortex exerts presynaptic (and sometimes post-synaptic[11]) inhibition on the afferent inflow passing through the dorsal column nuclei. There is histological evidence for the presence of these corticofugal projections[12].

Presynaptic inhibition has been reported to be reduced by the convulsive drug picrotoxin in doses which do not appreciably interfere with postsynaptic inhibition[13]. Strychnine, on the other hand, can block postsynaptic inhibition without altering presynaptic inhibition[13]. If the P wave produced in the cuneate nucleus by peripheral and by cortical stimulation is a reflexion of afferent terminal presynaptic depolarization, as proposed originally[3], then it would be expected to be sensitive to picrotoxin. Similarly, cortical inhibition of the response of cuneothalamic neurones to peripheral stimulation would be reduced by picrotoxin, if this inhibition were mediated, at least in part, presynaptically.

Cats were anaesthetized with α-chloralose (60 mg/kg) or pentobarbital sodium (36 mg/kg) and immobilized with a muscle relaxant (gallamine triethiodide or decamethonium bromide). The cuneate nucleus was exposed for recording gross potentials with a silver ball surface electrode placed about 3 mm below the obex where the largest N and P waves are usually recorded[3]. The contralateral sensorimotor cortex was also exposed and a pool of warm paraffin oil was made around it. Bipolar platinum electrodes were used to stimulate the forearm area of the pericruciate region. Stimulating needle electrodes were inserted into the ipsilateral forepaw. Above-threshold stimuli of 0·1 msec duration were applied peripherally, whereas a single pulse lasting 0·04–0·08 msec, or a train of four such pulses at a frequency of 312/sec, was applied to the cortex. A master unit drove both stimulating channels at 1 c.p.s. Typical responses to peripheral stimulation and to cortical stimulation are depicted in Fig. 1 *A*1 and *B*1, respectively.

The effects of 0·2–1·0 mg/kg of picrotoxin, administered intravenously, appeared within 4–5 min. Gradual reduction in the size of the P wave was observed, and in 10–20 min the higher doses resulted in almost complete disappearance of the P wave with little or no reduction in the size of the N wave. At the peak of action, the previously positive wave gave way to prolonged negative discharges. Both peripherally and cortically evoked P waves were depressed. Reduction in the size of the P wave could also be produced by local instillation of a few drops of 0·1 per cent picrotoxin on the surface of the cuneate nucleus (Fig. 1). All these changes could be effectively reversed by intravenous administration of small doses of pentobarbital sodium (10–15 mg/kg). Intravenous injection of 0·1 mg/kg of strychnine sulphate failed to depress the P wave; instead it frequently increased it in size. Preliminary experiments with single unit extracellular recording from cuneothalamic neurones indicate that inhibition by conditioning contralateral cortical stimulation can also be reduced by picrotoxin, whether administered intravenously or applied locally.

These results support the hypothesis that the P wave recorded in the cuneate nucleus, whether produced by

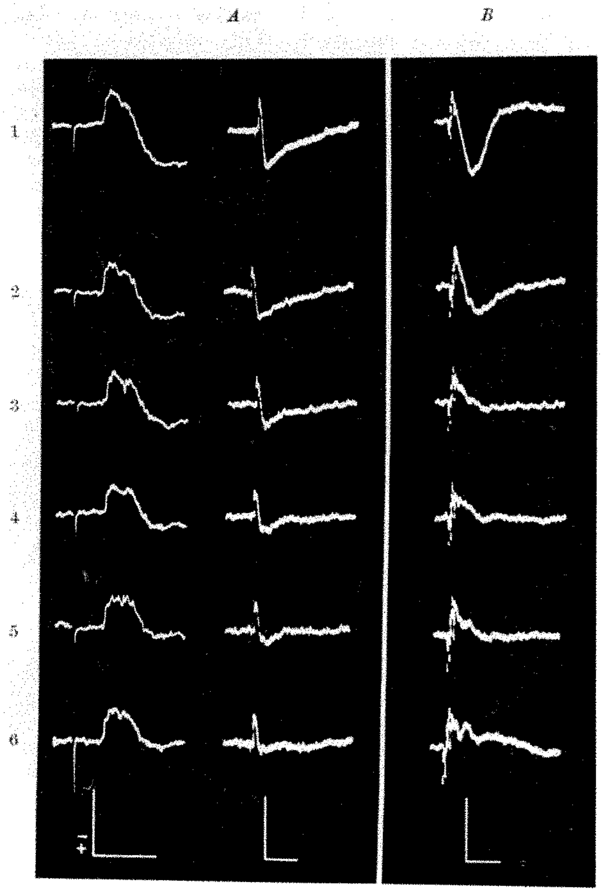

Fig. 1. The effect of local application of picrotoxin on cuneate responses. *A*, Cuneate potentials recorded after ipsilateral forepaw stimulation at two different sweep speeds, showing the *N* wave (left) and the *N* and *P* waves (right). *B*, Cuneate potentials recorded after a train of four pulses delivered to the forearm area of the contralateral sensorimotor cortex. (1) Control; (2–6) 5, 13, 22, 33 and 43 min after local application of a few drops of 0·1 per cent picrotoxin. Calibration: *A* (left), 200 μV and 10 msec.; *A* (right) and *B*, 200 μV and 50 msec.

cutaneous or by contralateral sensorimotor cortical stimulation, is indicative of dorsal column fibre depolarization or presynaptic inhibition.

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[1] Therman, P. O., *J. Neurophysiol.*, **4**, 153 (1941).
[2] Magni, F., Melzack, R., Moruzzi, G., and Smith, C. J., *Arch. Ital. Biol.*, **97**, 357 (1959).
[3] Andersen, P., Eccles, J. C., Schmidt, R. F., and Yokota, T., *J. Neurophysiol.*, **27**, 78 (1964).
[4] Andersen, P., Eccles, J. C., Schmidt, R. F., and Yokota, T., *J. Neurophysiol.*, **27**, 92 (1964).
[5] Jabbur, S. J., and Towe, A. L., *Fed. Proc.*, **18**, 73 (1959).
[6] Jabbur, S. J., and Towe, A. L., *Science*, **132**, 547 (1960).
[7] Towe, A. L., and Jabbur, S. J., *J. Neurophysiol.*, **24**, 488 (1961).
[8] Jabbur, S. J., and Towe, A. L., *J. Neurophysiol.*, **24**, 499 (1961).
[9] Gordon, G., and Jukes, M. G. M., *J. Physiol.*, **173**, 291 (1964).
[10] Andersen, P., Eccles, J. C., Schmidt, R. F., and Yokota, T., *J. Neurophysiol.*, **27**, 1080 (1964).
[11] Andersen, P., Eccles, J. C., Oshima, T., and Schmidt, R. F., *J. Neurophysiol.*, **27**, 1096 (1964).
[12] Kuypers, H. G. J. M., and Tuerk, J. D., *J. Anat.*, **98**, 143 (1964).
[13] Eccles, J. C., Schmidt, R. F., and Willis, W. D., *J. Physiol.*, **168**, 500 (1963).

Convulsant Action of Polyphenols

IN 1936 Bacq[1] demonstrated that, in addition to sensitizing certain smooth muscles of the cat to stimulation of sympathetic nerves and to injected adrenaline, some polyhydroxylic phenols also possessed convulsant activity. The sensitization was originally attributed to the antioxidant properties of the phenolic compounds. More recent studies, however, have indicated that polyphenols augment the peripheral actions of adrenaline by competitive inhibition of catechol-*O*-methyl transferase (COMT) (refs. 2–4). It has also been suggested that the central effects of polyphenols may be caused by a similar mechanism[5]. Our study was undertaken to investigate the possible role of adrenergic mechanisms in the central effects of these compounds.

Pyrogallol has been shown to be more potent than catechol as an inhibitor of cerebral COMT, both *in vitro*[6,7] and *in vivo*[8], and should therefore be a more powerful convulsive agent if COMT inhibition is involved in the central action of the hydroxy benzenes. The convulsant activities of catechol and pyrogallol, together with those of phenol and 2,3,4-trihydroxy acetophenone, were determined using female mice of an inbred strain, weighing 18–22 g. The compounds were injected intraperitoneally and the doses which induced convulsions in 50 per cent of the animals (CD_{50}) were determined using the method of Weil[9] with groups of ten mice.

The results (Table 1) show that pyrogallol and 2,3,4-trihydroxy acetophenone, substances reported to be among the most potent inhibitors of cerebral COMT (ref. 8), are much less active convulsants than catechol. In fact, the convulsions elicited by pyrogallol occurred only after a sustained period of depression and were always accompanied by distinct cyanosis, so the possibility that these were anoxic in origin cannot be excluded. Phenol, on the other hand, although not methylated by COMT (ref. 10), produced convulsions of rapid onset, which were very similar to those induced by catechol.

Table 1. CONVULSANT ACTIVITY OF CERTAIN PHENOLIC COMPOUNDS IN MICE

Compound	CD_{50} mmole/kg intraperitoneally (95% confidence limits)
Phenol	1·04 (0·93–1·17)
Catechol (1,2-dihydroxybenzene)	0·37 (0·35–0·41)
Pyrogallol (1,2,3-trihydroxybenzene)	5·71 (4·07–7·93)
2,3,4-trihydroxy acetophenone	> 8·0

Despite the fact that the *in vivo* inhibition of cerebral COMT by pyrogallol is well documented[8,11] it has been suggested that pyrogallol may not be able to pass the blood–brain barrier because the levels of catecholamine in the brain are altered by this compound only when it is administered intracerebrally[12]. Preliminary experiments in our laboratory, however, using a simple ferric chloride colorimetric reaction, have shown that pyrogallol injected intraperitoneally into mice readily enters the brain.

Further evidence to demonstrate the potent convulsant action of catechol in comparison with that of pyrogallol was obtained by applying the compounds directly to the spinal cord of frogs. Decerebrate frogs were anaesthetized with urethane (1·5 g/kg) to prevent spontaneous movements. Perfusion of the neuraxis was carried out by means of a needle inserted into the cord at the junction of the urostyle with the ninth vertebra and the electromyogram was recorded with needle electrodes inserted into the hypoglossal muscles. Results from one experiment are shown in Fig. 1.

The electromyogram showed no change in activity when the perfusate was changed from ordinary frog Ringer to Ringer containing pyrogallol (10 mg/ml., Fig. 1*a*) whereas a large increase in activity occurred when the perfusate contained catechol (10 mg/ml., Fig. 1*b*). Furthermore, in experiments using rats, it was found that when injected intracisternally, catechol (0·4 mg) elicited myoclonic twitches of the neck and ears whereas

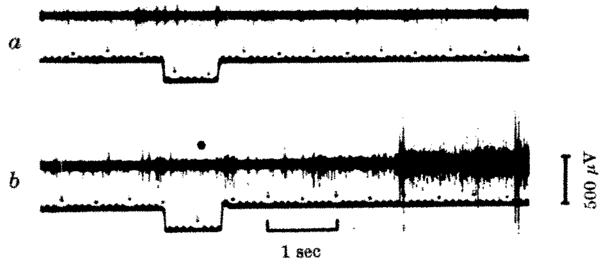

Fig. 1. Activity recorded from needle electrodes in the hypoglossal muscles of a decerebrated anaesthetized frog. A downward deflexion of the time scale indicates a change in the perfusing fluid from Ringer's solution to Ringer's solution containing 1,2,3-trihydroxybenzene (pyrogallol, 10 mg/ml.) in (*a*) and 1,2-dihydroxybenzene (catechol, 10 mg/ml.) in (*b*).

pyrogallol injected by this route had no effect in doses of up to 2·5 mg. Clearly, therefore, pyrogallol possesses little excitatory activity on the central nervous system when compared with catechol.

Further evidence that the central excitatory effects of catechol are unlikely to be the result of an adrenergic mechanism is given in Table 2. Reserpine in doses which deplete the brain of catecholamines[13] and chlorpromazine, a central adrenaline antagonist[14], both failed to antagonize the convulsive activity of catechol.

Table 2. EFFECT OF RESERPINE AND CHLORPROMAZINE ON CONVULSIONS IN MICE INDUCED BY CATECHOL

Pre-treatment			
Drug	Dose	Time administered prior to catechol	Catechol CD_{50} mmole/kg (95% confidence limits)
Saline		2 h	0·36 (0·32–0·41)
Reserpine	10 mg/kg	6 h	0·34 (0·31–0·38)
Chlorpromazine	20 mg/kg	2 h	0·37 (0·34–0·41)

In conclusion, therefore, the central effects of catechol and pyrogallol are unrelated to their relative activities as inhibitory agents of COMT and the convulsant activity of catechol is unlikely to be mediated by central adrenergic mechanisms. The mechanisms involved in the central excitatory actions of catechol and other polyhydroxic phenols are now being investigated.

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[1] Bacq, Z. M., *Arch. Intern. Physiol.*, **42**, 340 (1936).
[2] Bacq, Z. M., Grosselin, L., Dresse, A., and Renson, J., *Science*, **130**, 452 (1959).
[3] Axelrod, J., and Laroche, M. J., *Science*, **130**, 800 (1959).
[4] Udenfriend, S., Greveling, C. R., Osaki, M., and Witkop, B., *Arch. Biochem. Biophys.*, **84**, 249 (1959).
[5] Bacq, Z. M., Schlag, J., Falherbe, J., and Chaillet, G., *CR Acad. Sci.*, **249**, 2839 (1959).
[6] Ross, S. B., and Haljasmaa, O., *Acta Pharmacol. Toxicol.*, **21**, 205 (1964).
[7] Abbs, E. T., Broadly, K. J., and Roberts, D. J., *Biochem. Pharmacol.*, **16**, 279 (1967).
[8] Ross, S. B., and Haljasmaa, O., *Acta Pharmacol. Toxicol.*, **21**, 215 (1964).
[9] Weil, Carol S., *Biometrics*, **4**, 249 (1952).
[10] Axelrod, J., and Tomchick, R., *J. Biol. Chem.*, **233**, 702 (1958).
[11] Crout, J. R., Crevelin, G., and Udenfriend, S., *J. Pharmacol. Exp. Therap.*, **132**, 269 (1961).
[12] Masami, M., Hirosh, Y., and Reiji, I., *Biochem. Pharmacol.*, **11**, 1109 (1962).
[13] Shore, P. A., *Pharmacol. Rev.*, **14**, 531 (1962).
[14] Brodie, B. B., Spector, S., and Shore, P. A., *Pharmacol. Rev.*, **11**, 548 (1959).

New Glandular Structure in the Kidney of *Clarias batrachus* (Linn.)

DURING a study of the kidney and associated structures in Indian fishes, I have come across a peculiar feature, as yet undescribed, in the mesonephros of the air-breathing teleost, *Clarias batrachus* (Linn.). In this species a spheroidal multicellular gland is present inside the renal mass, adjacent to the extension of the caudal vein (Fig. 1). It is surrounded by a delicate investing membrane and is constituted by the close folding of the epithelial layer so as to form a number of semi-parallel ridges of columnar gland-cells separated by narrow interspaces. The chink-like lumen of the gland opens, by a minute orifice into a space surrounding it, and this periglandular space discharges, by a funnel-shaped aperture, into a duct situated on one side of the gland (Fig. 2).

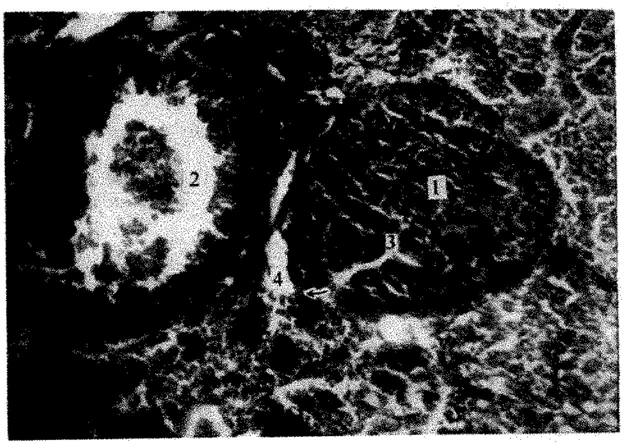

Fig. 1. Part of a transverse section of the mesonephros of *Clarias batrachus* showing its position inside the kidney. (× 900.) 1, Intra-mesonephric gland; 2, extension of the caudal vein inside the renal mass; 3, chink-like lumen of the intra-mesonephric gland; 4, periglandular space; the arrow indicates the opening of the intra-mesonephric gland into the periglandular space.

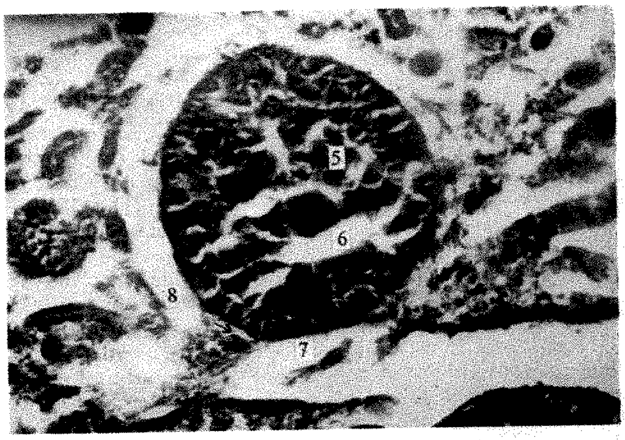

Fig. 2. Part of a transverse section of the mesonephros of *Clarias batrachus* showing the structure of the intra-mesonephric gland and the opening of the periglandular space into a duct. (× 900.) 5, One of the glandular ridges; 6, an interspace between the ridges; 7, a duct; 8, the periglandular space; the arrow shows the opening of the periglandular space into the duct.

It is evident from this description that the gland is a definitive structure. Its situation, disposition and histological details show that it is different from the adrenal inter-renal and other glands so far described in connexion with the kidney. It may be designated as the "intra-mesonephric gland". Further investigations particularly on its histochemistry and function are in progress.

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Effect of Haemoglobin on Experimental Infections with *Pasteurella septica* and *Escherichia coli*

BOTH haematin hydrochloride and ferric ammonium citrate can abolish the protective effect of immune serum in experimental infections with *Pasteurella septica* in mice[1]. Ferric ammonium citrate has the same effect with *Clostridium welchii* Type *A* infections in guinea-pigs[2]. The importance of these observations depends on the fact that iron can abolish the bacteriostatic effect of sera against *Cl. welchii in vitro*[3]. Because iron compounds seem to have such similar effects both *in vitro* and *in vivo*, it seems quite likely that the bacteriostatic action of serum may play an important part in resistance to bacterial infections.

We find that homologous lysed red cells as well as heterologous crystalline haemoglobin are effective in abolishing passive immunity to *P. septica*. Homologous lysed red cells and crystalline haemoglobin also enhance the virulence of *Escherichia coli* (type 0111*B*4) in normal guinea-pigs.

Red cells were prepared from blood withdrawn from the tails of anaesthetized mice; they were separated by centrifugation, washed three times with normal saline and lysed by the addition of approximately 5 volumes of distilled water. The cell stroma was removed by centrifugation and the supernatant fluid sterilized by filtration through a Seitz *EK* pad. The haemoglobin in the filtered solution was measured colorimetrically by the carboxyhaemoglobin (Haldane) method. Lysed guinea-pig cells were prepared in the same way as lysed mouse cells except that the blood was removed by cardiac puncture from animals anaesthetized with ether. Chromatographically homogeneous haemoglobin was isolated from human blood by the method of Allen *et al.*[4] and the solution sterilized by filtration through a cellulose ester membrane (Oxo, Ltd., London). Crystalline human haemoglobin was prepared from the same sample of blood by the method of Drabkin[5]. Two samples of crystalline horse haemoglobin were used (twice recrystallized grade *A*, Calbiochem; and Koch-Light Laboratories). The haemoglobin solutions were sterilized by filtration through a Seitz *EK* pad. The lysed red cells and the haemoglobin solutions were mixed with the bacteria immediately before intraperitoneal injection.

The methods of passive immunization, and infection with *P. septica* (strain *CN* 3449, Wellcome Research Laboratories), were the same as those described previously[1]. For all the experiments the dose of bacteria injected intraperitoneally varied between $1{\cdot}5 \times 10^5$ and $2{\cdot}9 \times 10^6$, but in any individual experiment the treated and control groups received identical numbers of organisms. The effects of lysed mouse red cells (equivalent to 7–28 mg haemoglobin) and the purified haemoglobins on passive immunity against *P. septica* are shown in Table 1. None of these compounds was toxic to normal mice.

E. coli strain 0111*B*4 was passaged in guinea-pigs by the same technique used for mice, but the lethal dose ($> 3 \times 10^7$ organisms) for guinea-pigs weighing 250–300 g remained relatively high. For the experiments with lysed red cells and haemoglobin shown in Table 2, $2{\cdot}5$ to $6{\cdot}6 \times 10^4$ *E. coli* were injected intraperitoneally. Neither the lysed cells nor the haemoglobin solution was toxic to normal guinea-pigs.

Table 1. EFFECT OF LYSED MOUSE RED CELLS AND PURIFIED HAEMOGLOBINS ON INFECTION BY *P. septica* (STRAIN *CN* 3449) IN PASSIVELY IMMUNIZED MICE

Haemoglobin intraperitoneally with *P. septica*	Amount of haemoglobin (mg)	Number of mice	Deaths (5 days)	Deaths (%)
Nil	Nil	327*	34	10
Lysed mouse red cells	7–28	60	58	97
Human haemoglobin: crystalline	7	10	10	100
Human haemoglobin: chromatographically pure	7	10	10	100
Horse haemoglobin: crystalline sample 1	28	10	10	100
Horse haemoglobin: crystalline sample 2	28	10	10	100

* Including controls from other experiments.

Table 2. EFFECT OF LYSED GUINEA-PIG RED CELLS AND CRYSTALLINE HAEMOGLOBIN ON INFECTION BY *E. coli* (STRAIN 0111*B*4) IN NORMAL GUINEA-PIGS

Haemoglobin intraperitoneally with *E. coli*	Amount of haemoglobin (mg)	Number of guinea-pigs	Deaths (3 days)	Deaths (%)
Nil	Nil	25	0	0
Lysed guinea-pig red cells	166–236	30	29	97
Lysed guinea-pig red cells	50	15	8	53
Human haemoglobin: crystalline	135	10	9	90

It is clear that homologous lysed red cells and a variety of purified haemoglobins can be effective in abolishing passive immunity to *P. septica* in mice, and enhance the virulence of *E. coli* in normal guinea-pigs. The mechanism of these reactions needs further investigation, but for the moment we have assumed, in the case of *P. septica* at least, that the reason for the effectiveness of these compounds is connected with the ability of the bacteria to acquire iron from the haemoglobin molecule. This is based on the observation that ferric ammonium citrate, a relatively simple iron compound, produces the same effect as haematin and haemoglobin. Whether the presence of free haemoglobin in wounds or infected tissue might play a part in encouraging bacterial infection is a matter for speculation, but it seems to be a possibility.

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[1] Bullen, J. J., Rogers, H. J., and Cushnie, G. H., *Nature*, **214**, 515 (1967).

[2] Bullen, J. J., Cushnie, G. H., and Rogers, H. J., *Immunology*, **12**, 303 (1967).

[3] Rogers, H. J., *Immunology*, **12**, 285 (1967).

[4] Allen, D. W., Schroeder, W. A., and Balog, J., *J. Amer. Chem. Soc.*, **80** 1628 (1958).

[5] Drabkin, D. L., *Arch. Biochem.*, **21**, 224 (1949).

Mechanism of Closure of the Aortic Valve

THE human aortic valve consists of three cusps made of relatively inelastic, muscle-free material about 0·15 mm thick. It opens and shuts about once a second, and withstands a pressure difference of 100 mm of mercury when closed. It usually functions for 70 yr without failure, and works so efficiently that very little blood is regurgitated at each pulse. In order to support this large

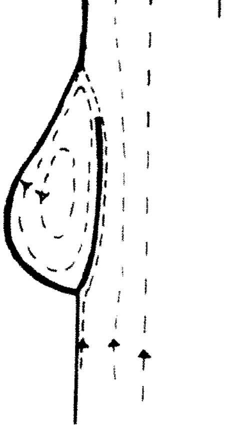

Fig. 1. Vortex formation in a sinus.

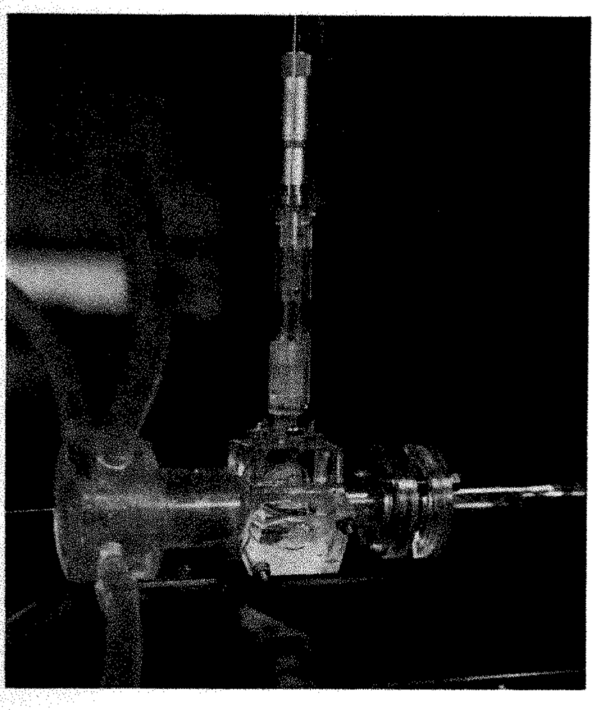

Fig. 2. Photograph of model valve in a tube of diameter 2·5 cm. A thin-film velocity probe is in the sinus, and is clamped in a traversing mechanism. The flow is from right to left.

pressure difference, the cusps must close simultaneously in all operating conditions and should not touch the wall of the aorta, for considerable reversed flow would then be required to close the valve. This action suggests a fluid dynamic control mechanism which positions the cusps away from the wall of the aorta, so that the slightest reversed flow will close the valve.

Such a mechanism is a vortex trapped between each cusp and its sinus (a bulge in the aortic wall). The vortices derive their energy from the forward going stream (Fig. 1), with the streamline along the cusp striking the distal end of the sinus in a stagnation point. This streamline divides, half reversing into the sinus and generating a vortex, the other half following the wall of the aorta distal to the valve. There is a phase difference between the main stream and the vortex; the vortex persists after peak systole, dominates the flow in the main stream and starts to close the valve. The vortices produce a thrust on both the cusp and the sinus wall, and the closure of the cusps is thus steady and synchronized. Leonardo da Vinci[1] correctly predicted the formation of vortices between the cusp and its sinus, and appreciated that these would help to close the valve, but thought the vortices were shed and featured in the dissipation of the kinetic energy of the fluid, for he was unaware of the circulation of blood.

To see if the trapped-vortex mechanism was an adequate explanation, a copy of a human aortic valve (Fig. 2), with rigid sinuses and aorta, was made in 'Perspex'. The cusps were made of nylon net impregnated with silicone rubber, and these were similar in thickness, smoothness, strength, shape and elasticity to physiological cusps. Two sets of identical cusps were made; one was fitted into a 'Perspex' tube with sinuses, the other into a tube with no sinuses. Both valves were tested statically for leakage, and were then tested dynamically in a pulsatile-flow rig, both together in series, and separately. The rig consisted of a constant head device for steady flow, together with a piston driven by a variable amplitude, variable frequency shaker which could produce sine waves of frequencies up to 10 c.p.s. The steady flow could be set at any value up to 9 l./min, and the shaker set to superimpose a suitable sine wave on the flow. With the flow at 3 l./min, and a pulse rate of 1 c.p.s., the valve with sinuses closed with about 4 per cent regurgitation (and some of this was caused by displacement of fluid by the cusps rather than leakage), but the valve without sinuses would not move, the cusps aligning themselves with the vessel wall. Not until the reversed flow had reached 23 per cent of the forward flow would this valve shut. Unlike the valve with open sinuses, this valve's cusps remained on the wall until it was violently shut by rapid reversed flow. The same results obtained at both greater and lesser flow rates.

In the valve with open sinuses, high energy vortices could be seen trapped between the sinuses and the cusps; peak velocity in the sinuses occurred after peak systole and the vortex persisted through the closure phase. These instantaneous velocities were measured with a heated thin-film gauge fitted into a needle of 1 mm diameter, and were shown to reach about two-thirds of the peak systolic velocity in the aorta. Apart from producing a thrust normal to the cusp (unlike the axial pressure gradient) and initiating valve closure, the vortices would also scour out the sinuses and prevent the formation of thrombi behind the cusps in blood flow.

Dr Grant de J. Lee of the Radcliffe Infirmary, Oxford, has used this velocity probe in animals, and the traces show that the flow is laminar (except possibly very briefly at peak systole). The flow in the model valve was laminar at 5 l./min, and thus might form the basis for a design for an artificial valve which does not cause haemolysis.

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[1] Keele, K. D., *Leonardo da Vinci on Movement of the Heart and Blood*, 81 (Harvey and Blythe, Ltd., London, 1952).

IMMUNOLOGY

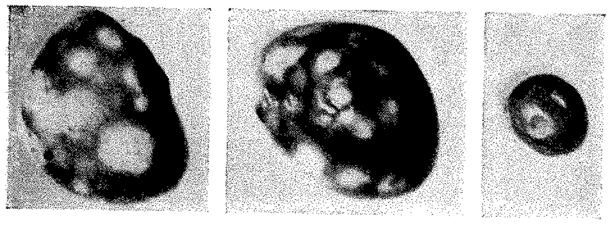
Cellular Immune Competence of Spleen Bursa and Thymus Cells

IN the chicken there are two central lymphoid organs, the thymus and the bursa of Fabricius. Recognition of the roles of these two organs in the ontogeny of immunological responsiveness permits definition of two functionally and morphologically distinct lymphoid cell lines[1-4]. The avian thymus, like the mammalian thymus, is essential to the normal development of a population of small lymphocytes and cellular immunity, while the bursa of Fabricius plays a similar part in the ontogeny of the plasma cell line and humoral immunity[4]. In addition, the thymus may contribute towards full development of humoral immune responsiveness[4-6]. A bursa equivalent has not been identified in all species, but evidence indicates that lymphoid tissues associated with organized gut exercise these functions in rabbits. These lymphoid tissues include Peyer's patches, sacculus rotundus and appendix[3,7].

While the functions of the thymus and bursa are different, the organs show similarities of development and lympho-epithelial morphology which seem to characterize central lymphoid tissues. Both are composed of follicles of lymphoid tissue with cortical and medullary organization[1], develop independently of antigenic stimulation[8], and possess relatively few antibody synthesizing cells[9]. Bursal lymphocytes differ from thymus lymphocytes in that the former are larger and contain a high RNA/DNA ratio and an abundance of polyribosomes[2,10]. In an effort to detect possible differences in the intrinsic immunological competence of bursal and thymus lymphocytes, we have measured the graft versus host activities of these cells. The data obtained are in accord with the hypothesis of compartmental differentiation of two separate lymphoid cell lines and afford further characterization of the avian thymus and bursa of Fabricius.

In this investigation, the immunological competence of bursa, spleen and thymus cells was estimated by a method similar to that used by Simonsen[11]. With this assay system the degree of splenomegaly produced by a given number of lymphoid cells is an index of their cellular immune potential[4]. Donor organs were removed from exsanguinated, 10 week old Arbor White Rock donors and placed in chilled beakers. Bursae were cut in half and swirled in saline to remove the slime which usually coats their epithelial surface. To reduce bacterial contamination, each organ was rinsed in saline containing penicillin (300 U/ml.) and streptomycin (300 μg/ml.). The tissues were transferred to Petri dishes containing a 40 mesh stainless steel screen and 10 ml. of chilled Eagle's basal medium, penicillin (100 U/ml.) and streptomycin (100 μg/ml.). Crude cell suspensions were prepared by rubbing the organs against the screens. These preparations were transferred to test tubes, and the non-cellular debris and clumped cells were allowed to settle for 15 min. Cells remaining in the supernatant were washed three times by centrifugation and resuspended in fresh medium. The number of viable cells was determined by counting in a haemocytometer those cells which were unstained after 10 min of exposure to 'Erythrosin-*B*' (0·2 mg/ml.) in balanced salt solution. As a control, some cells were killed by heating at 56° C for 1 h. Convenient numbers of viable or heat killed cells were injected into an allantoic vein of 14 day old Ghostley White Leghorn embryos. Five days later, the gross pathology as well as spleen weights and body weights of injected embryos were observed and recorded.

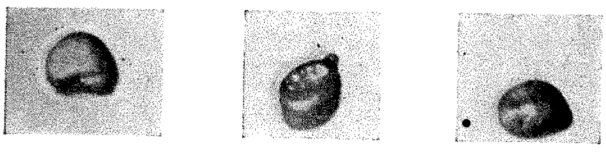
The spleens shown in the upper portion of Fig. 1 are representative of those seen in embryos which received 10^6 viable spleen, bursa or thymus cells. Those in the lower portion came from embryos injected with heat killed cells. The white lesions and splenomegaly are the results of graft versus host reactions and can be taken as evidence of the cellular immune competence of the donor cells. Spleen and thymus cells appear competent, while bursal cells fail to elicit the reactions. The degree of splenomegaly induced by injections of $4·0 \times 10^5$ viable bursa, spleen or thymus cells, shown in Table 1, indicated that spleen and thymus, but not bursa, produced significant splenomegaly. Splenic cells have a greater capacity than thymus cells to elicit graft versus host reactions; heat killed cells did not elicit significant splenomegaly.



Viable spleen cells

Viable thymus cells

Viable bursa cells



Heat killed spleen cells

Heat killed thymus cells

Heat killed bursa cells

Fig. 1. Spleens of 19 day chick embryos injected 5 days previously with 10^6 viable or heat killed lymphoid cells prepared from the spleen, thymus or bursa of 10 week old White Rock pullets.

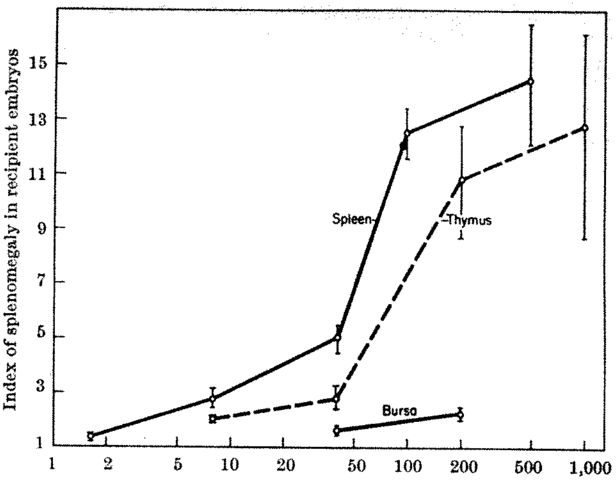


Fig. 2. Relative graft versus host activity of lymphoid cells of the thymus, spleen and bursa of Fabricius. Vertical bars indicate standard errors.

To determine whether the differences were attributable to differences in the ratio of competent to non-competent cells in the spleen and thymus or to an intrinsic difference in immunological activity per cell, we injected different numbers of cells from each organ. It was reasoned that if the cells from the three organs were intrinsically different in degrees of competence the slopes of the dose-response curves would be different. If, on the other hand, the differences previously mentioned were caused only by the ratio of competent to non-competent cells in a given organ, the slopes of the dose-response curves should be parallel. The indexes of splenomegaly are plotted against the logarithm of the number of donor cells as previously carried out by Sosin *et al.* for mice[12]. Our results, summarized in Fig. 2, show that the spleen has five times more capacity to elicit graft versus host reactions than does the thymus, but the slopes of the dose-response curves are similar. This finding indicates that the specific activity of avian spleen and thymus lymphocytes is the same. Variability inherent in the assay, however, might not permit the detection of small differences. Perhaps even more significant is the relative inactivity of bursal cells. Bursal cells elicited some splenomegaly, but because this was detected only with very large numbers of donor cells, the response could be attributed to unavoidable contamination with blood cells.

The absence or relative infrequence of graft versus host inducing cells in the bursa of Fabricius as compared with the thymus parallels previous findings showing that the thymus but not the bursa functions in the ontogeny of cellular immunity[4,13,14]. A question which remains unanswered concerns the role of the thymus in the ontogeny of humoral immunity. Thymectomy of very young animals (avian and mammalian) has been shown to decrease humoral immune responsiveness to some antigens in later life[4–6,15–18]. One explanation for this effect is that some of the lymphoid cells of the bursa are derived

Table 1. GRAFT VERSUS HOST ACTIVITY OF SPLEEN, THYMUS AND BURSA LYMPHOID CELLS

Treatment*	No. of recipients with necrotic spleen lesions / No. of recipients	Mean index of splenomegaly ± standard error	*P*
4×10^5 Viable spleen cells	9/10	5·4 ± 0·8	< 0·01
4×10^5 Viable thymus cells	8/10	3·4 ± 0·6	< 0·01
4×10^5 Viable bursa cells	0/9	1·3 ± 0·1	> 0·05
4×10^5 Heated spleen cells	0/10	1·0 ± 0·1	> 0·05
4×10^5 Heated thymus cells	0/10	1·1 ± 0·1	> 0·05
4×10^5 Heated bursa cells	0/10	1·3 ± 0·2	> 0·05
Uninjected control	0/10	1·0 ± 0·1	

Index of splenomegaly, $\frac{\text{spleen wt}}{\text{body wt}}$ (test recipient) ÷ $\frac{\text{spleen wt}}{\text{body wt}}$ (uninjected control).

P, Confidence interval obtained by simple analysis of variance. Each group was compared separately with the uninjected control group.

* Cells prepared from the spleen, thymus or bursa of 10–13 week old White Rock donors were injected into 14 day old White Leghorn embryos. Recipients were killed 5 days later, and spleen and body weights and gross spleen appearance were recorded.

from the thymus. This hypothesis now seems less likely because lymphoid cells derived from thymus would be expected to be capable of the graft versus host reaction. It is not clear whether this effect of thymectomy is a direct result of the absence of the thymic function or an indirect result of depression of bursal differentiation as a consequence of lack of vigorous thymus-dependent defence mechanisms[19,20]. Alternatively, thymus could influence bursal cells or the bursa-dependent population by humoral means without providing cells directly to the bursa, or a population of lymphoid cells may migrate directionally from the bursa to the thymus as previously suggested for embryonic and young adult chickens[21].

These findings may also be relevant to future attempts at restoring the immunoglobulin-producing system of cells in immunoglobulin deficient patients. It has been shown earlier that bursal cells can restore this cell system in agammaglobulinaemic chickens. Cells of the bursa-equivalent should have similar functional capabilities and should not produce untoward graft versus host disease in recipients. Thus host compatibility would be the only limiting requirement in such restoration.

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Received November 9, 1967.

[1] Good, R. A., Gabrielsen, A. E., Peterson, R. D. A., and Cooper, M. D., *Trans. and Studies of the College of Physicians of Philadelphia*, **33**, 180 (1966).

[2] Cooper, M. D., Gabrielsen, A. E., and Good, R. A., *Ann. Rev. Med.*, **18**, 113 (1967).

[3] Cooper, M. D., Perey, D. Y., McKneally, M. F., Gabrielsen, A. E., Sutherland, D. E., and Good, R. A., *Lancet*, i, 1388 (1966).

[4] Cooper, M. D., Peterson, R. D., South, M. A., and Good, R. A., *J. Exp. Med.*, **123**, 75 (1966).

[5] Humphrey, J. H., Parrott, D. M. V., and East, J., *Immunol.*, **1**, 419 (1964).

[6] Pinnas, J. L., and Fitch, F. W., *Intern. Arch. Aller. and Exp. Immunol.*, **30**, 217 (1966).

[7] Sutherland, D. E. R., Archer, O. K., and Good, R. A., *Proc. Soc. Exp. Biol. and Med.*, **115**, 673 (1964).

[8] Thorbecke, G. J., Gordon, H. A., Wostman, B., Wagner, M., and Reyniers, J. A., *J. Infect. Dis.*, **101**, 237 (1957).

[9] Dent, P. B., and Good, R. A., *Nature*, **207**, 491 (1965).

[10] Clawson, C. C., Cooper, M. D., and Good, R. A., *Lab. Invest.*, **16**, 407 (1967).

[11] Simonsen, M. T., *Acta Pathol. et Microbiol. Scand.*, **40**, 480 (1957).

[12] Sosin, H., Hilgard, H., and Martinez, C., *J. Immunol.*, **96**, 189 (1966).

[13] Jankovic, B. D., and Isvaneski, M., *Intern. Arch. Allerg. Appl. Immunol.*, **23**, 188 (1963).

[14] Perey, D. Y., Cooper, M. D., and Good, R. A., *Transplantation*, **5**, 615 (1967).

[15] Miller, J. F. A. P., *Proc. Prog. Soc.*, London, **156**, 415 (1962).

[16] Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., and Papermaster, B. W., *J. Exp. Med.*, **116**, 773 (1962).

[17] Jankovic, B. D., Waksman, B. H., and Arnason, B. G., *J. Exp. Med.*, **116**, 159 (1962).

[18] Cooper, M. D., Peterson, R. D. A., and Good, R. A., *Nature*, **205**, 143 (1965).

[19] Fichtelius, K. E., Louvell, G., and Philipsson, L., *Acta Pathol. et Microbiol. Scand.*, **51**, 81 (1961).

[20] Cooper, M. D., Chase, H. P., Lowman, J. T., Krivit, W., and Good, R. A., *Amer. J. Med.* (in the press).

[21] Cain, W. A., Weidanz, W. P., and Cooper, M. D., *Fed. Proc.*, **26**, 571 (1967

Japanese Haemoglobin Variant

RECENT haemoglobin surveys in the south-western area of Japan have revealed a great variety of abnormal haemoglobins in Japanese individuals despite the rarity of haemoglobinopathy. In Japan, at least thirteen abnormal haemoglobins have been identified by elucidation of amino-acid substitution, and similar studies are now in progress on ten other variants.

As part of an extensive screening programme for abnormal haemoglobins in Japanese individuals, some two thousand patients who visited the Yamaguchi Prefectural Central Hospital in Hofu City, Yamaguchi Prefecture, have been tested by agar gel electrophoresis (pH 7·0 and 8·6)[1] since 1964. An abnormal haemoglobin was discovered in only one of them—a 55 year old woman who came to the hospital because of abdominal discomfort and who was diagnosed, by means of X-ray study and gastrocamera, to be suffering from gastritis. The red blood cell count was 400×10^4, haematocrit was 31·3 per cent, reticulocyte count was 1·3 per cent and white blood cells amounted to 6,500 with normal differential count. Her only child did not carry the abnormal haemoglobin and her parents were dead. When the haemolysate was subjected to agar gel electrophoresis at pH 8·6, the abnormal haemoglobin separated as a distinct band which was situated more towards the anode than the normal Hb A band, but it was inseparable from Hb A at pH 7·0. Its electrophoretic mobility in starch gel electrophoresis (pH 8·6)[2] suggested that it had two extra negative charges per molecule (Fig. 1). Hb F content of the haemolysate was 0·91 per cent by Singer's method[3]. Solubility of the haemolysate in the deoxygenated form[4] was normal. This fast-moving abnormal haemoglobin was named Hb Hofu, after the name of the town where the propositus lived. Hb Hofu content of the haemolysate was 50·6 per cent; this was determined by scanning ($\lambda = 570$ mμ) the agar gel after electrophoresis.

Pure Hb Hofu was prepared by starch block electrophoresis[5] at pH 8·6. Its β chain anomaly was demonstrated by a hybridization test with canine haemoglobin[6], in which the migration during starch gel electrophoresis at pH 8·6 of the hybrid molecule, $\alpha_2^{\mathrm{Can}}\ \beta_2^{\mathrm{Hofu}}$, was faster than the corresponding hybrid, $\alpha_2^{\mathrm{Can}}\ \beta_2^{\mathrm{A}}$, while the migration of $\alpha_2^{\mathrm{Hofu}}\ \beta_2^{\mathrm{Can}}$ was equal to that of $\alpha_2^{\mathrm{A}}\ \beta_2^{\mathrm{Can}}$. Paper electrophoresis (pH 8·6) of globin in 7 molar urea[7] also revealed the fast-moving β chain anomaly.

Globin was prepared from the purified Hb Hofu by Anson–Mirsky's technique[8] and the β chain was separated from the α chain by sodium ion gradient chromatography in 8 molar urea on carboxymethyl cellulose column[9]. The β chain was aminoethylated with ethyleneimine[10] before it was digested with trypsin. Fingerprints were made according to Ingram's[11] and Baglioni's[12] techniques. An abnormal spot which was located more towards the anode than the β Tp-1 spot, and which occupied the position of

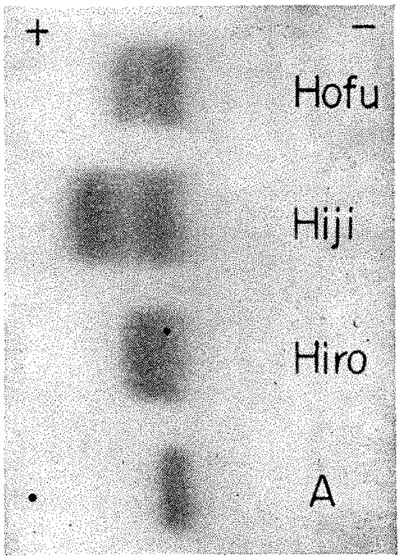

Fig. 1. Starch gel electrophoresis (pH 8·6) of the haemolysate. Hofu, Hb Hofu; Hiji, Hb Hijiyama ($\alpha_2^{\mathrm{A}}\beta_2^{120\ \mathrm{Lys}\rightarrow\mathrm{Glu}}$); Hiro, Hb Hiroshima ($\alpha^{\mathrm{A}}\ \beta_2^{143\ \mathrm{His}\rightarrow\mathrm{Asp\ or\ Asn}}$); A, normal adult haemolysate.

Table 1. AMINO-ACID COMPOSITON (EXPRESSED BY MOLAR RATIO) OF THE ABNORMAL SPOT*

Abnormal spot:	Lys	Thr	Pro	Ala	Val	Tyr	Phe	Glu
Estimated	1·04	0·97	2·23	1·91	0·11	+	0·93	3·98
Nearest integer	1	1	2	2	0	+	1	4
Expected for β Tp-13	1	1	2	2	1	1	1	3

* The abnormal spot was consistently positive for tyrosine in a specific coloration test.

α Tp-4, was clearly visible (Fig. 2). The β Tp-13 spot was present but was much weaker than the other spots in ninhydrin coloration. The abnormal spot was cut out from the fingerprints; the peptide was eluted into 6 normal hydrochloric acid and digested at 110° C for 20 h in a vacuum-sealed tube. The amino-acids of the acid hydrolysate were quantitatively analysed[13] by the Yanagimoto amino-acid analyser (Table 1). The amino-acid constituent of the abnormal peptide completely coincided with β Tp-13

121 122 123 124 125 126 127 128 129 130 131 132
Glu–Phe–Thr–Pro–Pro–Val–Gln–Ala–Ala–Tyr–Gln–Lys

except for the absence of Val and the presence of an extra Glu (ref. 14). It was therefore presumed that the abnormal peptide spot was derived from β Tp-13 and that Glu or Gln had substituted for Val β 126. Because an amino-acid substitution of Gln for Val would not cause any change in either the electric charge of the molecule or the sub-unit, nor in the migration towards the anode of the β Tp-13 spot, Glu (not Gln) seemed to be the most probable substitute for Val. The faint spot coincident with the normal β Tp-13 spot was regarded as a derivative from a contaminating normal β chain. We conclude therefore that Hb Hofu is a new abnormal haemoglobin which can be expressed by the formula $\alpha_2^A \beta_2^{126\ \mathrm{Glu}}$.

Amino-acid substitution of Glu for Val, the reverse of Hb S, has been known in Hb $\mathrm{M_{Milwaukee\text{-}1}}$ (ref. 15) and Hb New York[16], but Hb Hofu will be the first case in which a substitution of the 126th amino-acid of the β chain was discovered. Hb Hofu seems to be a very rare abnormal haemoglobin which is not associated with the clinical symptoms.

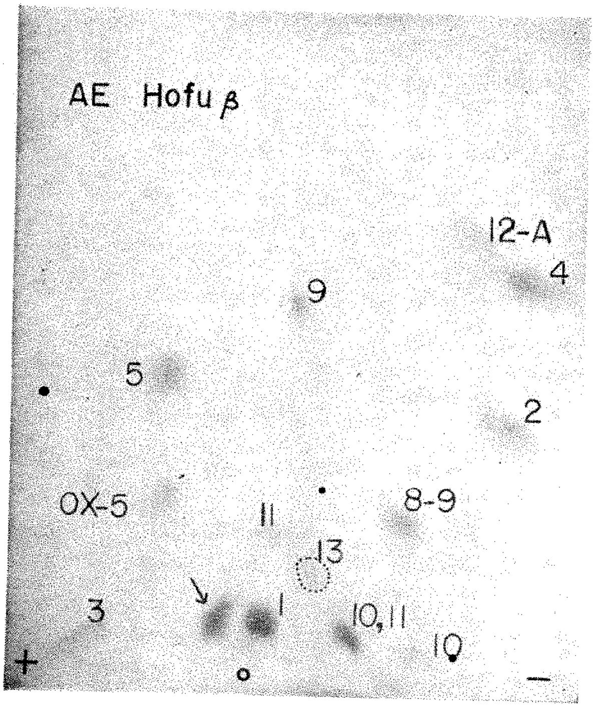

Fig. 2. Fingerprint of the aminoethylated β chain of Hb Hofu (by Baglioni's method). Numerals refer to β Tp numbers. The abnormal spot is indicated by an arrow. It is considerably biased from the position of the corresponding normal β Tp-13 spot (encircled by dotted line) toward the anode and the bottom.

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[1] Shibata, S., and Iuchi, I., *Acta Haem. J.*, **24**, 51 (1961).
[2] Smithies, O., *Adv. Protein Chem.*, **14**, 65 (1959).
[3] Singer, K., Chernoff, A. I., and Singer, L., *Blood*, **6**, 413 (1951).
[4] Goldberg, C. A. J., *Clin. Chem.*, **4**, 146 (1958).
[5] Ueda, S., *Jap. J. Clin. Hemat.*, **3**, 26 (1958).
[6] Shibata, S., Iuchi, I., Ueda, S., Miyaji, T., and Takeda, I., *Acta Haem. Jap.*, **25**, 675 (1962).
[7] Take, T., *J. Biochem.*, **49**, 206 (1961).
[8] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, **13**, 469 (1930).
[9] Clegg, J. S., Naughton, M. A., and Weatherall, D. J., *J. Mol. Biol.*, **19**, 91 (1966).
[10] Jones, R. T., *Cold Spr. Harb. Symp. Quant. Biol.*, **24**, 297 (1964).
[11] Ingram, V. M., *Biochim. Biophys. Acta*, **28**, 539 (1958).
[12] Baglioni, C., *Biochim. Biophys. Acta*, **48**, 392 (1961).
[13] Piez, K. A., and Morris, L., *Anal. Biochem.*, **1**, 187 (1960).
[14] Braunitzer, G., Hilse, K., Rudloff, V., and Hilschmann, N., *Adv. Protein Chem.*, **19**, 1 (1964).
[15] Gerald, P. S., and Efion, M. L., *Proc. US Nat. Acad. Sci.*, **47**, 1758 (1961).
[16] Ranney, H. M., Jacobs, A. S., and Nagel, R. L., *Nature*, **213**, 876 (1967).

BIOLOGY

Mycological Aspects of Diseased Atlantic Salmon

As part of the research programme on fish disease mentioned before[1], we have undertaken an investigation to establish the role of fungi in the disease termed ulcerative dermal necrosis (UDN) which affects freshwater fish, particularly salmon. The fact that fungi are associated in some way with this disease is indisputable; indeed, diseased fish in the rivers are recognized chiefly by the presence of white fungoid patches.

We found that the disease pattern is as follows: initially the disease becomes apparent as grey-white bruise-like areas on the surface of the fish. These areas then develop fungal growth which is obvious to the naked eye, with subsequent haemorrhagic ulcers usually developing at each site. The musculature is often exposed by these ulcerous lesions. Similar autopsy findings in eels are reported by Hoshina and Ookubo[2]. The fungus appears as an off-white raised excrescence usually covering the lesions. In colour the diseased areas vary from off-white to dark brown depending on how much blood or silt has been deposited in the fungal mycelium.

While there is a dearth of literature on fish mycopathology, nevertheless certain work[2–5] has established the pathogenicity of fungi towards freshwater fish. It is a popular belief that one fungus only is found on fish—"fish-mould" has become synonymous with the fungus *Saprolegnia*. Investigation shows that many fungi are involved in disease of fish, mostly representatives of the family *Saprolegniaceae*. Apart from various species of the genus *Saprolegnia*, members of the genera *Achlya*, *Dictyuchus* and *Aphanomyces* are listed in the literature mentioned here as lethal parasites of fish. The parasitic potential of the family is exemplified by the crayfish plague fungus, *Aphanomyces astaci*, which Unestam[6] reports as causing 100 per cent mortality in attacked populations of *Astacus astacus*. Unestam believes that this species of crayfish faces extinction in Europe because of the severity of the attack. Fungal activity obviously plays a prominent part in ulcerative dermal necrosis whether as (*a*) a lethal primary parasite, (*b*) a secondary invader of already debilitated tissue and the eventual killer, or (*c*) merely as a saprophyte or scavenger of dead tissue. In view of the fact that there is no general agreement as to the cause of the disease, to dismiss the fungal infection as secondary (ref. 7 and numerous press reports) is to us an unacceptable premise.

As part of the present investigation the general mycoflora of healthy and diseased fish was surveyed. Since 1966, some ninety salmon (*Salmo salar*), smolts and adults and a more limited number of brown trout (*Salmo trutta*), fry and adults, have been examined in detail. Diseased salmon were obtained from the rivers Shannon, Liffey, Lee, Blackwater (Cork) and Dargle; diseased trout from the Lagan and Dargle. Healthy salmon were obtained from the rivers Boyne and Owenea. Where possible fish were taken directly from the water to polythene sleeving and transported immediately to the laboratory. Due caution was exercised where contamination from, for example, soil was suspected.

The fungi most commonly isolated from the surface of healthy and diseased fish, apart from lesions, include species of *Penicillium*, *Fusarium*, *Mucor* and *Trichoderma*, together with numerous pycnidial fungi, yeasts and unidentified imperfect types. Several hundred isolates now await specific identification. None of these fungi was visible macroscopically. It has not been established whether they are present as actively growing mycelia or merely as spores adhering to the surface mucus. Most of these fungi are non-aquatic and are presumably present in rivers as aliens from soil and air[8]. The mycoflora is not constant from one fish to another and it has been difficult to establish whether a fish surface supports a recognizable fungal community. From results obtained so far, it seems that there is no constant mycoflora associated with fish surfaces but rather that its composition varies considerably from one individual to another and from river to river, largely depending on environment, for example the extent of river pollution or flooding. Considering the specialized demands of many aquatic phycomycetes it is not surprising that so few have been isolated in this survey.

Saprolegnia alone has been isolated constantly from the lesions of diseased fish. Not only has it been isolated externally but also internally from the musculature, to a depth of 1·5 cm, and occasionally elsewhere other than from visibly diseased areas. This fungus has been isolated at all observable stages of the disease, even from the "white patches"[7] of the presumed pre-mycotic stage. With few exceptions the *Saprolegnia* isolates share one feature in common, that is the absence of sexual reproduction. This character together with the type of sporangial branching (cymose) and parasitic nature on fish would until recent times be sufficient evidence for their common identification as *Saprolegnia parasitica* Coker (Coker)[9,10]. Certain strains of this fungus, however, given suitable conditions and sufficient time—in some instances up to a year—have been reported as forming sexual organs[5]. For the positive identification of this species it is now considered necessary to observe the characteristics of these structures[11]. Experiments, using sterols, aimed at inducing the initiation of a sexual stage in our isolates have not been successful.

The reluctance of these isolates to reproduce sexually has led to the suggestion that heterothallism may be operative in the genus. A programme of pairing the isolates has been undertaken to investigate this possibility. So far most of the paired cultures have not shown any complementary response. In one case, however, sexual structures were formed at the interface of two asexual isolates. This has given impetus to more intensive research on the possible existence of heterothallism in the genus *Saprolegnia*. Although the tardiness of our isolates to produce oogonia suggests that they are *Saprolegnia parasitica*, a definite identification is still outstanding pending further evidence of the formation of a sexual stage. Our tentative identification of these isolates as *Saprolegnia parasitica* is particularly significant. Of all the saprolegniacious fungi known to attack fish, *Saprolegnia parasitica* is the one most frequently reported as a fish parasite. Tiffney[3], Hoshina and Ookubo[2] and Vishniac and Nigrelli[4] have shown experimentally that this species can act as a lethal primary parasite of healthy fish.

The spread of the disease is most marked during periods of low water temperature and, conversely, with increasing water temperatures there seems to be a diminution of the disease. A relationship between low water temperature and parasitic potential of *Saprolegnia* has been considered for some time as a possible important factor governing epidemics of fungus disease in fish. Thus, Hoshina and Ookubo[2] observed the cessation of fungus epidemics in eels as water temperatures approached 18° C. We have obtained similar evidence from a recent outbreak of mycoses at the Mullingar fish rearing station where several hundred brown trout fry died within days. Two fungi, *Saprolegnia ferax* and a species of *Pythium*, were isolated from these fish. It seems to be significant in connexion with this outbreak that the water temperature dropped from 16° to 10° C (Ryan, personal communication) in the week before the onset of the disease. Accordingly, the apparent correlation between the spread of fungus in ulcerative dermal necrosis and water temperatures merits investigation.

It has frequently been suggested that freshwater fungi cannot live in salt water, but we found that *Saprolegnia parasitica* (culture obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland) has a high salinity tolerance. The growth of *S. parasitica* in a range of concentrations of sodium chloride and seawater was examined by the method of Scott and Warren[12]. Using colony area as a criterion, growth in a solution of 3·5 per cent sodium chloride was half, and in 100 per cent sterile seawater two thirds, that obtained in controls. It is emphasized that in these particular experiments optimal growth conditions were provided for the fungus, which experienced no competition from the usual biota of seawater. Reference is made to Te Strake[13], who found that members of the Saprolegniaceae could grow and sporulate in brackish waters if temperature and nutritional levels were suitable. She also emphasized the futility of habitat nomenclature, such as "fresh water" fungi, when "fresh water" fungi given appropriate conditions can become "brackish water" forms. Vishniac and Nigrelli[4] have suggested that the Saprolegniaceae may parasitize fish of brackish water. Salmon recently returned from the sea, as evidenced by the presence of sea-lice on the fish, have been taken from estuarine waters and have shown symptoms of ulcerative dermal necrosis; *Saprolegnia* has been isolated from these fish. It is therefore possible that the fungus could colonize salmon immediately they entered the river estuary.

Inoculation experiments now in progress in this laboratory are expected to furnish definitive information about the role of *Saprolegnia* in ulcerative dermal necrosis. On the basis of existing evidence, it seems improbable that it functions merely as a scavenger. It is more reasonable to suggest that the fungus is the primary parasite in ulcerative dermal necrosis or alternatively that an extremely close relationship exists between an unknown primary parasite and *Saprolegnia*.

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[1] Editorial comment, *Nature*, **215**, 141 (1967).
[2] Hoshina, T., and Ookubo, N., *J. Tokyo Univ. of Fish.*, **42**, 1 (1956).
[3] Tiffney, W. N., *Mycologia*, **31**, 310 (1939).
[4] Vishniac, H. S., and Nigrelli, R. F., *Zoologica*, **42**, 131 (1957).
[5] Scott, W. W., *Developments in Industrial Microbiology*, **5**, 109 (1964).
[6] Unestam, T., *Physiol. Plant*, **18**, 483 (1965).
[7] Mulcahy, M. F., *Nature*, **215**, 143 (1967).
[8] Willoughby, L. G., *J. Ecol.*, **50**, 733 (1962).
[9] Coker, W. C., *The Saprolegniaceae* (University of North Carolina Press, 1923).
[10] Coker, W. C., and Matthews, V. D., *North American Flora*, **2**, 20 (1937).
[11] Seymour, R. L., thesis, Virginia Polytechnic Institute (1966).
[12] Scott, W. W., and Warren, C. O., *Tech. Bull.*, 171 (Virginia Agricultural Experimental Station, 1964).
[13] Te Strake, D., *Phyton, Intern. J. Exp. Bot.*, **12**, 147 (1959).

Prey Selection: Oddity or Conspicuousness ?

PIELOWSKI has shown that goshawks (*Accipiter gentilis*) select as prey pigeons (*Columba livia*) which differ in colour from most pigeons which are present in Polish forests[1,2]. Salt[3] has shown how these and other experimental results fit rather well into the generalization that predators preferentially capture prey animals which are odd, or which differ in some way from most prey. As Pielowski noted, however, it is probably easier for a goshawk to fix its attention on the odd-appearing member of a flock, and it is possible that the goshawks were merely taking conspicuous individuals out of the flock. Selection for conspicuous prey has been amply verified by experimentation[4-8]. I have attempted to determine the parts played by oddity and conspicuousness in prey selection.

A tamed hawk (*Falco sparverius*) preyed on laboratory mice which were either white or which had been dyed grey. In each experiment, nine mice of one colour and one of the other were presented on a table which was coloured to match one or the other colours of mice. Each of the two colour combinations of mice was replicated fifty times on each of the two colours of background, giving a total of two hundred experiments.

Experimental series *A* and *B*, in which the background was grey, clearly show that the hawk selected conspicuous, but not odd, prey (Table 1). In view of these results, I suggest a reconsideration of the generalization that odd prey is preferentially selected by predators. In series *C* and *D*, where grey mice were conspicuous, prey selection did not differ from random. I suggest that this is a result of a conflict in preference for (*a*) white and (*b*) conspicuousness. The hawk took a significantly longer time (*t* test, 95 per cent level) to select a mouse in series *C* and *D* than it did in series *A* and *B* (Table 1), which can be considered as evidence for conflict. I cannot explain this unexpected preference for white mice other than by suggesting that the hawk may have become accustomed to preying on white mice in series *A* and *B*. The hawk may thus have formed a "specific searching image"[9] in series *A* and *B* and maintained it throughout series *C* and *D*. Further experiments are in progress.

Table 1. PREY SELECTION EXPERIMENTS

Series	No. of experiments	Mice presented in each experiment		Background	Mice captured		P*	Mean reaction time (sec)	Standard error
		Grey	White		Grey	White			
A	50	9	1	Grey	37	13	0·0002	261	44·2
B	50	1	9	Grey	0	50	0·03	230	44·1
C	50	9	1	White	44	6	0·30	374	41·0
D	50	1	9	White	6	44	0·30	394	47·3

* Probability of this result occurring if prey selection were random (chi-square test).

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[1] Pielowski, Z., *Bull. Acad. Pol. Sci. Ser. Sci. Biol.*, **7**, 401 (1959).
[2] Pielowski, Z., *Ekologia Polska* (*A*), Tom IX, No. 11 (1961).
[3] Salt, G. W.. *Ecol. Monog.*, **37**, 113 (1967).
[4] Sumner, F. B., *Proc. US Nat. Acad. Sci.*, **20**, 559 (1934).
[5] Sumner, F. B., *Proc. US Nat. Acad. Sci.*, **21**, 345 (1935)
[6] Sumner, F. B., *Amer. Nat.*, **69**, 245 (1935).
[7] Isely, F. B., *Ecology*, **19**, 370 (1938).
[8] Dice, L. R., *Contrib. Lab. Vert. Biol. Univ. Mich.*, *Ann Arbor*, No. 34 (1947).
[9] Tinbergen, L., *Arch. Neer. Zool.*, **13**, 265 (1960).

Pigmentation in the Radial Nerve of *Diadema antillarum*

RECENT examination by phase-contrast microscopy of teased, fresh or macerated radial nerve, or of frozen sections in *Diadema antillarum*, has shown the presence of reddish cells that are neither amoebocytes nor chromatophores, but which resemble neurones in their general form (Figs. 1 and 2).

The presence of a reddish pigment in the radial nerves of this echinoid is of special interest because in experimental conditions the nerve can serve as a photoreceptor in the characteristic shadow response[1-2]. Photosensitivity is maximal between 455 and 460 mμ (ref. 3), and the pigment, when extracted in acidified diethyl ether, absorbs maximally in the visible range at 465 mμ. Absorption of both visible and ultraviolet radiation corresponds closely with that of the reddish pigment which can be extracted from the skin[4] and which is now known to be echinochrome *A*. The near coincidence of these maxima coupled with the presence of the pigment in the photosensitive skin as well as in the radial nerve has caused speculation that the pigment may be involved in photoreception[5,6]. Our recent discovery in the radial nerve of similarly pigmented cells which resemble neurones therefore reinforces the idea.

Caution has already been urged, however[7]. Most of the red pigment in the radial nerve is in the ubiquitous amoebocytes or in their disorganized remains, and the pigmented presumptive neurones are rare. On the other hand, photoreceptors for the shadow response seem to be widespread in the nerve and it is unlikely that amoebocytes are directly involved. Again, previous determinations of spectral absorption have relied entirely on extracted

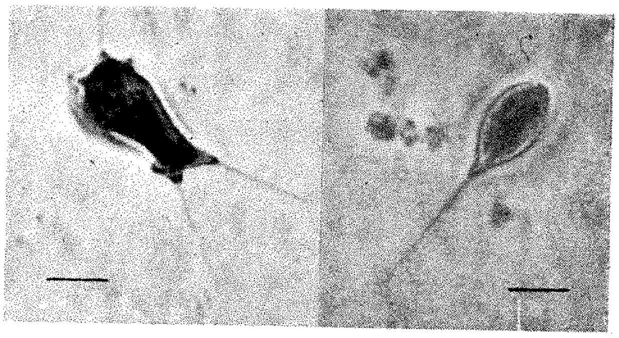

Fig. 1 Fig. 2

Figs. 1 and 2. Two of the pigmented structures which have been interpreted as neurones in the living radial nerve of *Diadema*, which were examined by microspectrophotometer. Scales: Fig. 1, 5·3 mμ; Fig. 2, 4·6 mμ.

pigment. Determination of the absorption spectrum of the presumed neuronal pigment *in situ* is therefore crucial and this has now been achieved by microspectrophotometry. Absorption is maximal between 540 and 560 mμ, so that whatever the precise nature and function of the presumptive neurones could be, they are unlikely to be involved as receptors in the shadow response.

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[1] Yoshida, M., and Millott, N., *Experientia*, **15**, 13 (1959).

[2] Takahashi, K., *Nature*, **201**, 1343 (1964).

[3] Yoshida, M., and Millott, N., *J. Exp. Biol.*, **37**, 390 (1960).

[4] Millott, N., *Proc. Zool. Soc. Lond.*, **129**, 263 (1957).

[5] Thomson, R. H., *Quinones: Structure and Distribution*, in *Comparative Biochemistry* (edit. by Florkin, M., and Mason, H. S.), **3** (Academic Press, London, 1962).

[6] Yoshida, M., *Photosensitivity*, in *Physiology of Echinodermata* (edit. by Boolootian, R. A.) (Interscience, New York, 1966).

[7] Millott, N., in *Light as an Ecological Factor* (edit. by Bainbridge, R., Evans, G. C., and Rackham, O.), British Ecological Society Symposium No. 6 (Blackwell, Oxford, 1966).

Vision Index for Isopod Crustacea related to Latitude and Depth

THERE has been no systematic study of the distribution of eye-bearing and blind benthic crustaceans in relation to the depth of the sea at which they occur. It has, however, been known for many years[1] that many deep-sea isopods are blind and it has also been known that most intertidal species have well developed eyes[2]. We have examined the bathymetric range of many species of isopods in order to determine the correlation between presence or absence of eyes, latitude, depth and light penetration. To do this we have developed a visual or ocular index.

The ocular index (*O.I.*) can be defined as the percentage of species of isopods in the total isopod fauna which have eyes. In order to provide an expression which can be related to any latitude, the 50 per cent level of species which have eyes can be defined as an ocular index of 50 ($O.I._{50}$).

The data on which this study is based come from collections off Beaufort, North Carolina (35° N.) (collections made by R/V Eastward, NSF grant), off Lima, Peru (10° S.) (collections made by the R/V Anton Bruun, Cruise 11), and in the Antarctic (60° S.) (collections made by USNS Eltanin). Added data were compiled from the sub-Antarctic[3,4] and the Arctic[1,5,6] and for the tropics[7-11]. The number of benthic species of isopods which were utilized in the determination of ocular index is given in Table 1 with reference to five different latitudes and seven different levels of depth. The total number of organisms involved in this study exceeds fifteen thousand, but Table 1 records only the number of species.

Table 1. NUMBER OF SPECIES OF ISOPODS AND PERCENTAGE WITH EYES IN RELATION TO DEPTH AND LATITUDE

	65° N.		35° N.		10° N.		15° S.		70° S.	
	No. spp.	%	No. spp.	%	No. spp.	%	No. spp.	%	No. spp.	%
Intertidal 0–2 m	12	100	32	97	42	100	37	100	—	—
Subtidal 20 m	17	65	26	93	5	100	12	100	7	71
Shelf 40–200 m	50	46	25	92	9	100	31	97	31	58
Slope (*u) 200–1,000 m	69	17	13	69	—	73*	7	71	25	36
Slope (m) 1,000–2,000 m	43	8	9	0	5	20	8	25	32	13
Slope (L) 2,000–4,000 m	45	2	23	1	16	0	49	0	30	3
Abyss 4,000–6,000 m	3	0	8	0	9	0	9	0	22	0

* Asterisk denotes the point determined from the curve.

Except perhaps for absence of pigment, the absence of eyes in a species of marine isopods does not correlate with other changes in external morphology. Absence of eyes seems to be a frequent change among the species of many genera. Good examples of this can be found in the typically shallow water genera *Serolis*, *Janira*, *Munna*, *Cirolana*, and so on, which have eye-bearing as well as eyeless species. In contrast, all known species of typically abyssal genera (*Haploniscus*, *Mesosignum*, *Macrostylis*) lack eyes.

The proportion of eyeless to eye-bearing isopods usually shows an increase with depth. A comparison of the data from various latitudes shows two general patterns. The first is a high latitude or polar curve in which the percentage of eye-bearing species shows an immediate drop from the shelf to the great depths (Fig. 1). In this case, the $O.I._{50}$ is located at shallow depths between 100 and 500 m. The second pattern (Fig. 1) is exhibited between latitudes 35° N. and 15° S. where there is no abrupt decrease in eye-bearing species in the shallow waters (100–500 m). Instead there is a more or less gradual decline in eye-bearing species with depth until no eye-bearing species are present. The data suggest the $O.I._{50}$ is found at progressively greater depths as latitude decreases. For example, the $O.I._{50}$ at 35° N. is at 1,280 m. This striking picture of equatorial submergence of the $O.I._{50}$ is shown in Fig. 2, where the $O.I._{50}$ data are plotted against latitude.

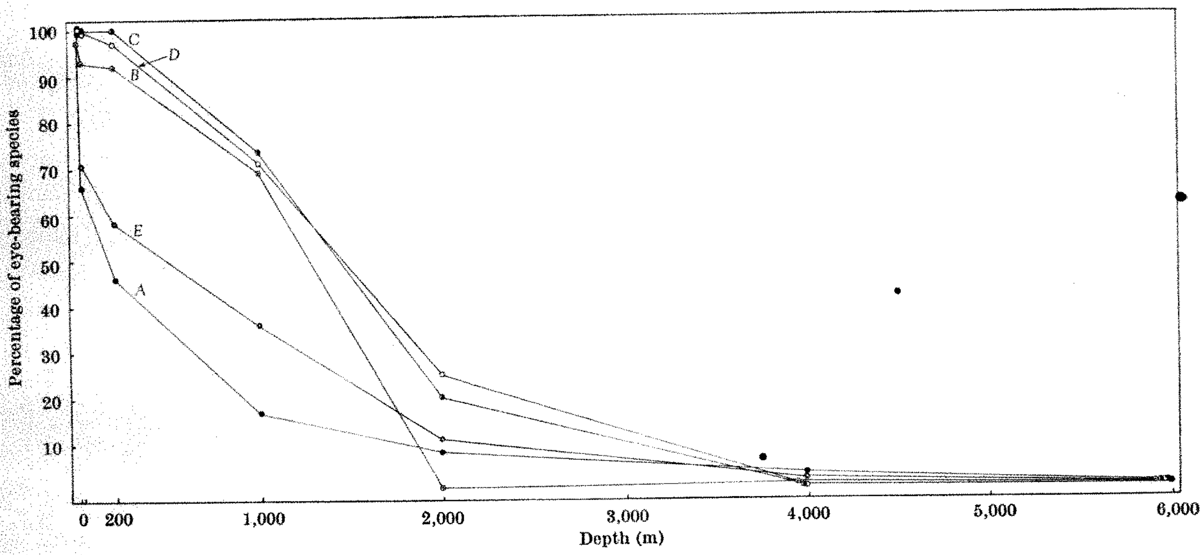

Fig. 1. Percentage of eye-bearing isopod species in relation to latitude and depth: ●, *A*, 65° N.; ○, *B*, 35° N.; ◑, *C*, 10° N.; ○, *D*, 15° S.; and **O**, *E*, 70° S.

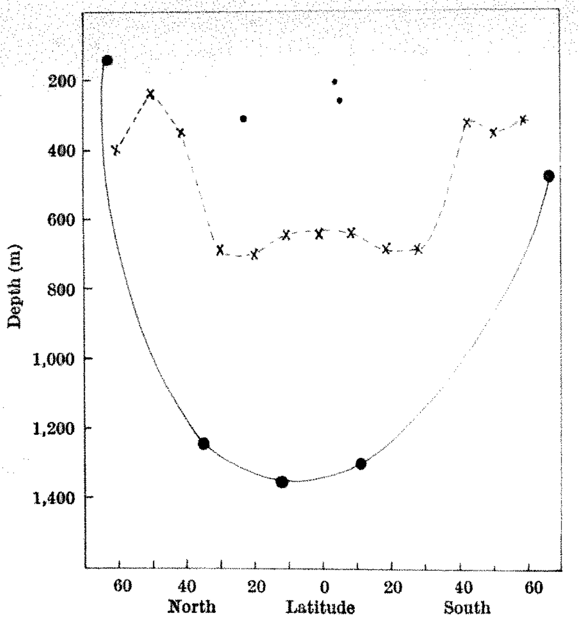

Fig. 2. $O.I._{50}$ for marine isopod species in relation to depth and latitude, as derived from the distribution of vertical light extinction coefficient of surface water (after Neuman and Pierson[12]). Curve with dotted line represents light penetration north to south along 40° W. in the Atlantic.

Several factors could be associated with the phenomenon of the equatorial submergence of the $O.I._{50}$. One might believe that variations in depth of penetration of visible light with latitude would show a direct relationship to the $O.I._{50}$. Indeed, the shape of the two curves is similar with visible light penetrating to lesser depths in polar regions and to greater depths in low latitudes. (At high latitudes the penetration of light is obviously minimal during winter months and maximal during summer.) The vertical extinction coefficient of light in surface water at 50° N. and 50° S. is greater than four, but is less than two in the Sargasso Sea[12]. Penetrating daylight is visible to the human eye down to depths of about 700 m in the Sargasso Sea, but the $O.I._{50}$ is located at 1,300 m or about twice the maximum depth of penetration of light. Light may be important and indeed probably is, but its relation to the $O.I._{50}$ is not clear. It is clear that a sizable proportion of the species have eyes well below the penetration of visible light. The answer could lie in the evolutionary mechanisms which are related to the ecology and dispersion of the various species in relation to visible light. It could be assumed that species which live in the light and which have eyes have an advantage, while those without eyes are at a disadvantage and should be eliminated through natural selection. Selective mechanisms could account for the fact that the $O.I._{50}$ is well below the penetration of visible light. What eye-bearing species living in deep water see, and how shallow species without eyes survive, could be contingent on many ecological and evolutionary factors such as mutation rates, the intensity of competition, and the dispersion capacities of the various species, and so on. The possible role of environment bioluminescence might be looked into.

Another parameter which might influence the tropical or equatorial submergence of the $O.I._{50}$ is temperature. Ekman[13] has described and discussed the equatorial submergence of marine animals. Species living in shallow water at high latitudes often inhabit deeper water nearer the equator. Ekman states "submergence is simply a consequence of the animals' reactions to temperature. Cold water animals must seek colder, deeper water layers in regions with warm surface water . . .". But this explanation, Ekman admits, presupposes a certain degree of stenothermy in the organisms in question. Ekman's explanation of submergence may be valid. Precisely what direct effect the latitudinal variations in temperature can have on the presence or absence of eyes is not clear because the curve for the $O.I._{50}$ does not follow a given isotherm from the poles to the tropics. Ekman[13] was dealing with a submergence by populations of the same species in his discussion of tropical submergence. In our data which are at present being prepared on the benthic isopods of the Peru–Chile Trench, more than fifty different species are involved in 80–90 per cent of the tabulations and therefore the submergence by individual species that Ekman describes as tropical submergence cannot explain the submergence of the $O.I._{50}$. This latter figure applies to the isopod population as a whole at a given depth and latitude.

The abrupt drop in percentage of species which bear eyes in polar regions could result simply from the high proportion of typically abyssal genera which occur in the shallow water (100–500 m) of polar latitudes (Fig. 3). The data show that blind abyssal genera are found in shallow water in the polar regions, but only in deeper water at lower latitudes. This phenomenon we call polar emergence of abyssal genera.

There have been many attempts to define the upper and lower bathymetric limits of the abyssal zone[14] in the oceans. Most definitions are based on arbitrary depths of convenience for tabulation rather than on biological criteria. It should be noted (Table 1) that the $O.I._0$ (all species blind) occurs between 4,000 and 6,000 m at all latitudes. The upper depth limit of 4,000 m coincides closely with Vinogradova's[15] average upper limit of the abyssal zone at 3,600 m. Between 2,000 and 4,000 m, 2–3 per cent of the isopod species have eyes at the poles, whereas at intermediate latitudes the eye-bearing percentage is 0·1 (Table 1). Thus the eye-bearing species

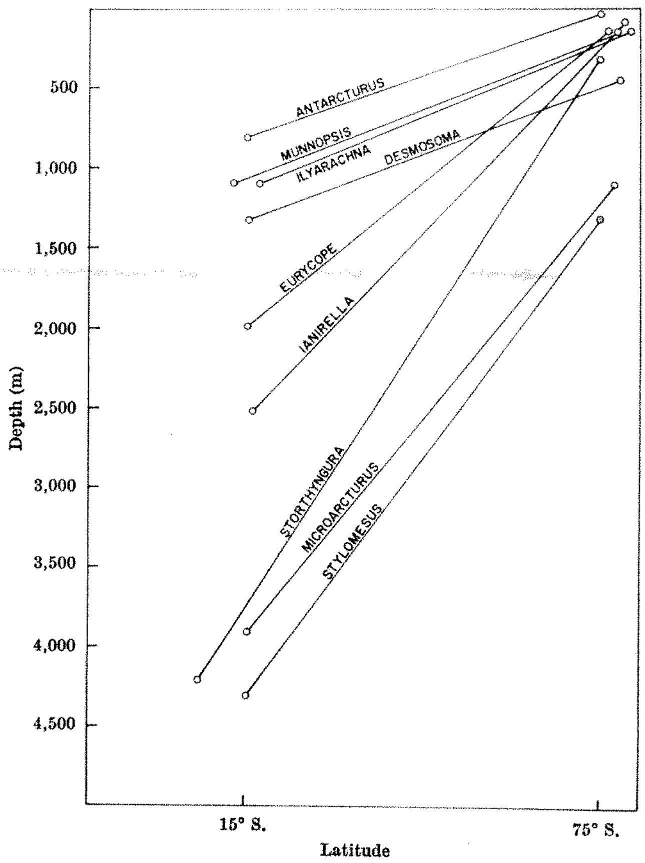

Fig. 3. Minimum level of bathymetric distribution of isopod genera at lat. 15° S. and 70° S., with a demonstration of polar emergence of abyssal genera.

obviously show a greater depth penetration at the poles than at intermediate latitudes. This phenomenon occurs in parallel with the polar emergence of typical abyssal genera. These phenomena coincide with water temperatures which are not more than 3° C above zero and show no relationship to light penetration.

An equatorial submergence of species which bear eyes has been well documented in large collections of marine Isopoda. Conclusive correlations between this phenomenon and visible light penetration into the sea are not, however, apparent because many eye-bearing species occur far below the limits of visible light. The direct effects of temperature are even less clear.

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[1] Hansen, H. J., *The Danish Ingolf-Expedition*, **3**, 10262 (1916).
[2] Richardson, H., *Proc. US Nat. Mus.*, **27**, 1 (1904).
[3] Nordenstam, A., *The Swedish Antarctic Expedition*, **3**, 1 (1933).
[4] Menzies, R. J., *Reports of the Lund Univ. Chile Expedition*, **42**, 1 (1962).
[5] Sars, G. O., *Crustacea of Norway*, **2**, 41 (1899).
[6] Hult, J., *Zoologiska Bidrag Fran Uppsala*, **21**, 1 (1941).
[7] Menzies, R. J., *Vema Research Series*, **1**, 79 (1962).
[8] Moore, H. F., *Bull. US Comm. Fish and Fisheries*, **2**, 161 (1902).
[9] Richardson, H., *Bull. US Nat. Mus.*, **54**, 10727 (1905).
[10] Menzies, R. J., and Glynn, P., *Handbook on the Common Marine Isopods of Puerto Rico* (in the press).
[11] Menzies, R. J., *Breviora*, **163**, 1 (1956).
[12] Neuman, G., and Pierson, jun., W., *Principles of Physical Oceanography* (Prentice Hall 65, 1966).
[13] Ekman, S., *Zoogeography of the Sea*, 415 (Sidgwick and Jackson, Ltd., London, 1953).
[14] Menzies, R. J., *Ocean. Mar. Biol. Ann. Rev.*, **3**, 195 (1965).
[15] Vinogradova, N., *Deep-Sea Res.*, **8**, 245 (1962).

Co-ordinated Neuromuscular Activity in Insect Spiracles at Sub-zero Temperatures

DIAPAUSING pupae of some saturniid moths overwinter in an environment in which temperatures may drop to −30° C. In such conditions of cold and low humidity there are opportunities for pupae to desiccate; however, this does not happen. The primary sites of pupal water loss are seven pairs of functional spiracles. We have examined the functional behaviour of these spiracles at sub-zero temperatures. Apart from the interest this might have for students of insect physiology, it seemed of general interest to observe the effects of sub-zero temperatures on co-ordinated neuromuscular activity, and on the sensitivity of the spiracular control system to carbon dioxide and oxygen. The experiments also examine the question of pupal water loss at low temperatures.

The opening of each spiracle is regulated by a spiracular valve, moved by a single closer muscle which is opposed by an "elastic" ligament. The muscle is innervated by a nerve from the central nervous system[1]. Gas exchange across the spiracles has been shown to be discontinuous from +25° to 0° C. (A. P. Brockway and H. A. Schneiderman, in preparation).

Discontinuous respiration is characterized by a series of cyclical events which are causally connected[2]. Metabolic carbon dioxide is stored in the pupa, and released periodically in bursts. A burst is initiated when intratracheal pCO_2 reaches a triggering threshold. During a burst the spiracular valves open widely, and the intratracheal barometric pressure remains at atmospheric. Following a burst the spiracular valves constrict, and intratracheal barometric pressure falls as the tracheal oxygen is removed by the tissues. Intratracheal pCO_2 begins to rise and pO_2 falls steadily. When the pO_2 falls to a certain level the valves begin to open and close continually or "flutter". Intratracheal pressure rises and falls in "microcycles" throughout the flutter period. The flutter period is terminated when the intratracheal pCO_2 again reaches the triggering threshold and the valves open widely.

Prolonged valve opening is a response of the spiracular closer muscle to high carbon dioxide concentrations or to anoxia; this response persists after the closer muscle has been denervated[3]. Fluttering of the valves, on the other hand, ceases after muscle denervation[3,4], and is controlled by the pO_2 in the tracheae supplying the segmental ganglia[4]. Thus flutter indicates the continuing response of the central nervous system to oxygen.

Discontinuous respiration has been suggested as a mechanism which enables these pupae to conserve water[5,6]. Indeed, it has been shown that at 25° C the spiracular valves are open no more than 5 to 8 per cent of the entire respiratory cycle[7], and at lower temperatures they must open still less. This enables the pupae to conserve water.

How do the spiracles behave at the sub-zero temperatures experienced by the pupae? Does the co-ordinated neuromuscular activity, necessary for discontinuous respiration, persist? If discontinuous respiration does not continue, are there other adaptations which limit pupal water loss?

To answer these questions, a diapausing *Hyalophora cecropia* pupa was placed in a constant temperature chamber which could be adjusted to temperatures between +20° and −20° C ± 0·5°. Intratracheal pressure and movements of one spiracular valve were recorded simultaneously at five degree intervals. Pupal water loss was determined at −2° and −16° C.

The pupa was connected to a pressure transducer with a polyethylene cannula, and intratracheal pressure changes were recorded automatically[7,8]. The pupal tracheal system could be opened to atmospheric pressure by remote control, using a system of solenoid control valves.

To record spiracular movements, we employed a method described recently[4]. The cuticular covering of the fourth right abdominal spiracle was removed to expose the spiracular valve. The exposed valve was then covered with a transparent plastic window to prevent the valve from drying out. Movements of the valve were observed through an ocular micrometer and recorded. Gas exchange through the spiracle was blocked permanently by the window. Gas exchange, however, occurred through the corresponding spiracle on the left side; the behaviour of such paired spiracles has been shown to be co-ordinated[7].

In the experimental conditions pupae were cooled slowly (≤ 0·5°/min). Between −5° and −10° C the pupae froze, and when cut with a knife they appeared to be solid throughout.

At −5° C cyclical pressure changes, microcycles, and intratracheal vacuums, characteristic of discontinuous respiration, persisted throughout 286 h of continuous recording. The frequency of valve movements decreased from 32/min at +20° to 1/min at 0° C; similar results have been reported for Cecropia and for several other insects (refs. 9–11, and Brockway and Schneiderman, in preparation). At −5° C movements occurred from one every 2 min to one an hour; however, there were intervals of several hours during which no movements were observed. Most valve movements occurred slowly at −5° C, but nonetheless the changed position of the spiracular valve could be recorded easily. At some ambient temperature between −5° and −10° C discontinuous respiration ceased; the valves closed and they remained closed thereafter when the temperature was decreased to $-15°$ and $-20°$ C. The valves were not hermetically sealed, however, because intratracheal pressure remained atmospheric throughout 270 h of continuous recording at $-15°$ and $-20°$ C. Other data show that oxygen uptake by frozen Cecropia pupae continues even at sub-zero temperatures ($<0{\cdot}1$ $\text{mm}^3/\text{g}^{-1}$ h^{-1} at approximately $-12°$ C)[12]. Had the valves been hermetically sealed the intratracheal pressure records would have indicated steadily decreasing intratracheal pressure as a result of the removal of tracheal oxygen by metabolizing tissues.

The results up to this point show that at $-5°$ C: (1) spiracular valves move; (2) the cyclical nature of the spiracular movements indicates that the responses to oxygen and carbon dioxide persist; (3) the occurrence of flutter and the responses to oxygen indicate that neuromuscular co-ordination persists.

Additional evidence that the spiracular apparatus continues to respond to intratracheal gas changes at $-5°$ C comes from experiments in which the tracheal system was perfused with oxygen and carbon dioxide in various concentrations by a method previously described[4]. At $+25°$ C, perfusion with 7 per cent carbon dioxide in air causes the spiracles to open; perfusion with 3 per cent oxygen in nitrogen causes them to open as a result of anoxia; 8 per cent oxygen plus nitrogen causes them to close. Perfusing the tracheal system of a pupa at $-5°$ C with a mixture of 20 per cent carbon dioxide in air or with 0·5 per cent oxygen in nitrogen caused the spiracular valves to open; the valves could then be closed by perfusing the tracheal system with air. At $-10°$ C the valves failed to open in response to intratracheal perfusions of up to 50 per cent carbon dioxide. Moreover, if the valves were open at $-10°$ C then they could not be made to close by perfusions of up to 100 per cent oxygen. This is shown by the results of the following experiment. The ambient temperature was lowered from $-5°$ to $-10°$ C while the valves were in an open position. After the pupa had equilibrated at $-10°$ C for 12 h air was perfused intratracheally. The valves remained open and did not constrict even when 100 per cent oxygen was perfused through the tracheal system. Seven hours after the ambient temperature was increased again to $-5°$ C, however, valves did constrict in response to an air perfusion.

Further evidence that at temperatures below $-5°$ C the spiracular apparatus does not respond to carbon dioxide comes from studies of pupal water loss at $-2°$ and $-16°$ C. At the end of one week in air, at about 0 per cent relative humidity at $-2°$ and $-16°$ C, two groups of pupae lost an average of $1{\cdot}29 \pm 0{\cdot}56$ (*S.D.*) and $0{\cdot}65 \pm 0{\cdot}29$ per cent of their original weights, respectively. The pupae, which had been kept at $-16°$ C, were warmed to room temperature, exposed to a mixture of 20 per cent carbon dioxide in air to open all spiracular valves, and then, with the valves still partly open, were chilled again at $-16°$ C for 1 week with the spiracles open. The average weight loss of the pupae with partly open spiracles at $-16°$ C was $1{\cdot}44 \pm 0{\cdot}65$ per cent. This weight loss was more than twice the average weight loss in air at $-16°$ C when the spiracles were constricted. A second group of pupae, which in air at $-16°$ C had lost an average of $0{\cdot}40 \pm 0{\cdot}20$ per cent of their original weights, was exposed to a mixture of 20 per cent carbon dioxide in air at $-16°$ C. After 1 week the average weight loss was $0{\cdot}22 \pm 0{\cdot}13$ per cent of the average weights before the exposure to 20 per cent carbon dioxide. Apparently at $-16°$ C the spiracles did not respond to carbon dioxide.

These experiments show that down to at least $-5°$ C (but not at $-10°$ C) diapausing Cecropia pupae continue to respire discontinuously (thus conserving water), and their spiracular muscles and some nerves remain functional. The spiracular muscle continues to respond to carbon dioxide, the central nervous system continues to respond to oxygen, and at least paired spiracles remain co-ordinated. At some temperature between $-5°$ and $-10°$ C pupae freeze in our experimental conditions. Determinations of pupal water loss at $-16°$ C indicate that normally spiracular valves freeze in a constricted position but intratracheal pressure records show that valves are not hermetically sealed. At temperatures below $-5°$ C gas exchange must occur by simple diffusion past constricted valves; thus water loss is kept low. The spiracles continue to serve the insect even at very low temperatures at which there is no nervous or muscular control of spiracular function because conditions in nature normally ensure that the spiracular valves freeze in the constricted position.

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[1] Beckel, W. E., *Proc. Tenth Intern. Cong. Entomol.*, **2**, 87 (1958).
[2] Levy, R. I., and Schneiderman, H. A., *J. Insect Physiol.*, **12**, 465 (1966).
[3] Beckel, W. E., and Schneiderman, H. A., *Science*, **126**, 352 (1957).
[4] Burkett, B. N., and Schneiderman, H. A., *Science*, **156**, 1604 (1967).
[5] Buck, J., in *Physiological Triggers* (edit. by Bullock, T. H.), 72 (Amer. Physiol. Soc., 1957).
[6] Buck, J., *Proc. Tenth Intern. Cong. Entomol.*, **2**, 339 (1958).
[7] Brockway, A. P., and Schneiderman, H. A., *J. Insect Physiol.*, **13**, 1413 (1967).
[8] Schneiderman, H. A., and Schechter, A. N., *J. Insect Physiol.*, **12**, 1143 (1966).
[9] Hazelhoff, E. H., thesis, Univ. Utrecht (1926).
[10] Punt, A., *Physiol. Comp. Oecol.*, **2**, 59 (1950).
[11] Wigglesworth, V. B., *Proc. Roy. Soc.*, B, **118**, 397 (1935).
[12] Kanwisher, J. W., as cited by Asahina, E., in *Cryobiology* (edit. by Meryman, H. T.), 451 (Academic Press, 1966).

Possibility of Investigating Movement in a Group of Ants by the Mössbauer Effect

THE probability of recoil-free resonance absorption of gamma-quanta depends on the relative velocities of source and absorber[1,2]. The velocity distribution of moving absorbers can be deduced from the form of the resonance line. In principle this is possible when the velocities are within the range of the Mössbauer spectrometer. In practice they should have a resonance width close to that of stationary absorbers because otherwise their line can become so broad as to cause considerable difficulty[3].

We have used the Mössbauer effect to investigate movement in a group of ants. These are large enough to carry measurable amounts of a resonance absorbing substance, and small enough so that a considerable number of them can be placed between the source of radiation and the detector; this is necessary for statistical reliability.

We used a Mössbauer spectrometer with a source of radiation of constant velocity. The velocity was adjusted smoothly from 0 to 40 mm/sec by means of a 10-rotation helical potentiometer. The counts for positive and negative velocities are collected in separate counters for every cycle of movement of the source. Stannic oxide was used as a resonance absorber. We summed the counts from those velocities which were equal in absolute value and differed only in their sign. The effect observed at room temperature can be expressed as $\varepsilon_0 = \dfrac{N_\infty - N_0}{N_\infty}$ where N_0 is the number of counts at zero velocity, and N_∞ is the number of counts far from resonance and which goes up 20 per cent to 30 per cent, depending on the radio-

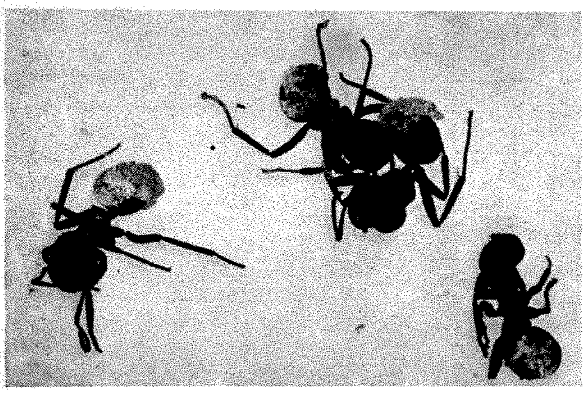

Fig. 1. Photograph of ant "marked" with stannic oxide.

active purity of the source. The relatively small temperature dependence of ε_0 (refs. 4 and 5) is a particularly valuable property of stannic oxide. Its chemical inertness, its stability in air, and the fact that it takes in no moisture and does not decompose, are also very important. The gamma-quanta, with 23·8 keV energy, were detected with a thallium doped crystal of sodium iodide 1 mm thick, in front of which was placed a palladium filter to eliminate X-rays.

We used *Formica pratensis*, De Geer, an ant which is widespread in Bulgaria. Worker ants, which we used, can reach 9 to 11 mm in length. They move quickly and are aggressive; when in danger they stand up and bend their abdomen, from which they eject formic acid.

The respiratory system, as in all insects, consists of tracheae, which open on to the lower part of the abdomen and also partly onto the thorax. The stigmae can open and close, or be permanently open[6], depending on the proportion of oxygen and carbon dioxide in the air. The abdomen makes "telescopic" movements, during which the individual segments merge into one another. The abdomen can also expand slightly, inflate and shrink. Ants are never completely still. The body, and in particular the abdomen, vibrates irrespective of respiratory movements. Metabolism and the demand for oxygen increase when the temperature deviates from the optimum[6].

The upper part of the abdomen of each ant was pasted with "BΦ 2" glue (made in USSR), and finely ground stannic oxide was immediately sprayed onto the glue. The marked ants (Fig. 1) were placed in an insectarium with mesh walls which ensured aeration without which ants would die in a few minutes because the atmosphere was saturated with glue vapour.

Fifty ants were placed in several aluminium cuvettes with mesh bases, stacked on top of each other with 2·3 ants/cm^2. When placed in the cuvette the ants spray formic acid and the scent only disappeared after air had been blown through the cuvette. The cuvettes were placed in a double-walled metal vessel, with water at a pre-determined temperature between the walls. A stream of air, which was previously moistened (100 per cent by passing it through water at the temperature of the thermostat), was passed through the cylinder formed by the cuvettes. The air input was measured by an anemometer. To kill the ants, to measure the "natural width" of the absorber, the water was replaced by ether. The ants stopped moving in 1 to 2 min.

In our experiments so far we have only studied the influence of temperature and, partly, of the air input, on the shape of the resonance line. Changes in temperature affect the band width considerably (Fig. 2). For a better comparison of the spectra on the abscissa of the graph in Fig. 2 the value of $\varepsilon_v/\varepsilon_0$ has been included, where ε_v is the effect at velocity v. Fig. 3 shows the dependence of the semi-width resonance line on the temperature band for two series of tests. In both cases ants were taken from the same nest, but on different dates. The dot placed above zero temperature refers to dead ants, and in this case is equal to "frozen". Attention should be drawn to the fact that for the broadening of the resonance absorption line only that component of the speed of the separate "absorbers" in the direction of the beam of gamma-photons is important.

With increasing temperature the dots characteristically begin to fluctuate outside the limits of the most probable deviation and the instruments do not seem to work in a stable manner. The greater the temperature the stronger is the effect (Fig. 2). After the death of the ants the instruments become "stable" at once. We believe that this is caused by ants which crawl at the higher temperature, and change the geometry of the experiment. Fluctuations cease when the value is taken from several counts for each velocity, alternated with measurements at zero velocity; the effect is determined by the difference of the count rates summed for each group of measurements. This is how the resonance line shown on Fig. 4 was obtained. Obviously the best method for further measurements would be to work with a source the velocity of which changed triangularly with time with the spectrum displayed on a multi-channel analyser[1,2].

Resonance curves for ants at the same temperature, but with a constant inflow of air (cm^3/min) as well as with no inflow of air, are given on Fig. 5. In the second case there is a slight expansion, probably caused by the in-

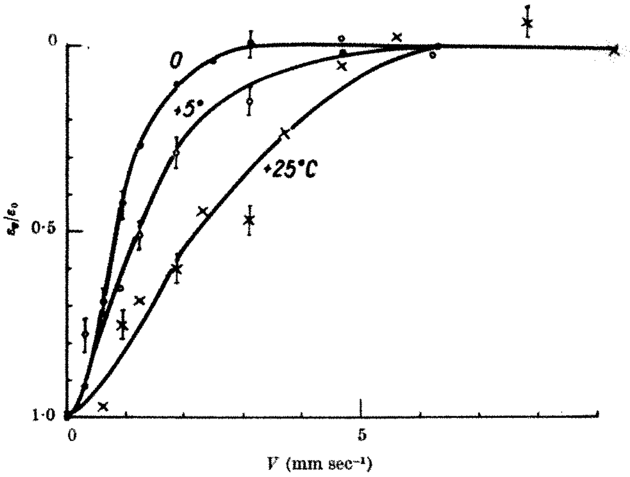

Fig. 2. Curves of resonance absorption of ants at various temperatures. The "0" curve refers to dead ants after the completion of measurements. 270 ants were killed.

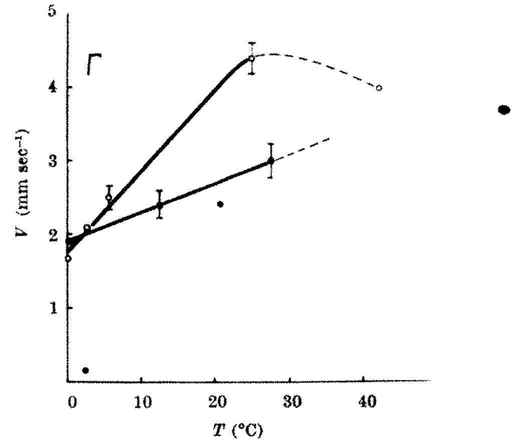

Fig. 3. Dependence of the semi-width of absorption line for ants at various temperatures. The dots at 0° C correspond to dead ants. The two sets of measurements were made on: upper curve, May 9, 1966, 270 ants; lower curve, June 24, 1966, 450 ants.

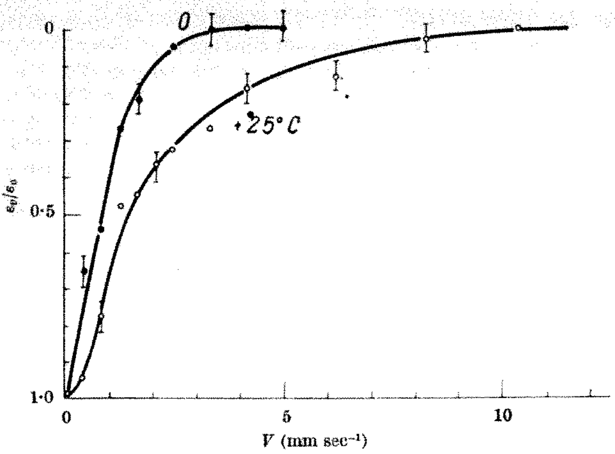

Fig. 4. Curves for resonance absorption. Ten measurements of 20 sec at each speed, alternating with the same number of measurements at zero velocity, were made. The lower curve refers to ants at +25° C, and for comparison the upper curve shows the resonance absorption for the same ants when dead. 450 ants were used.

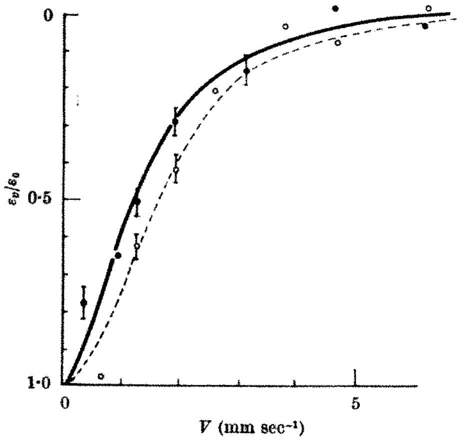

Fig. 5. Curves for resonance absorption of ants at +5·5° C. Upper curve in an air stream of 600 cm³/sec, and lower curve with no air stream.

crease in periodicity cycles of the breathing movements. When measurement is continuous, for example, for more than one or two days, and in particular at high temperatures (over 25° C), the ants begin to die, making a "background" or "sediment" of immobile filters.

Broadening of resonance should be regarded as a consequence of breathing movements and flickering of the abdomen. Ants move very quickly, up to 3–5 cm/sec, and the result is out of the scope of our measurement. The resonance absorption of these velocities is too weak and very good statistics are necessary to outline the resonance line.

We think that these first results show that the Mössbauer effect can be used to measure changes in the reactions of organisms under the influence of various factors. The laws governing the movement of individual insects can be tested by other methods, for example, still and cine photography, but the Mössbauer effect makes it possible to examine changes in the average quadratic speed of a large number of insects. Many measurements can be made in a short time, and the behaviour of organisms from various taxa can be compared.

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[1] Frauenfelder, G., in *The Mössbauer Effect* (Atomizdat, Moscow, 1964).
[2] Wertheim, G. K., in *Mössbauer Effect, Principles and Applications* (Academic Press, New York and London, 1964).
[3] Shpinel, V. S., *Izvestia ANSSR* (*Power and Automation*), No. 4, 71 (1962).
[4] Delyagin, N. N., Shpinel, V. S., and Bruykhanov, V. A., *Zhurn. Eksp. Tior. Fiz.*, **41**, 1374 (1961).
[5] Sklarevsky, V. V., Samoylov, B. N., and Stepanov, E. P., *Zhurn. Eksp. Tior. Fiz.*, **40**, 1874 (1961).
[6] Chauvin, R., in *Physiologie de l'Insecte* (Paris, 1949).

CYTOLOGY

Changes in Membrane Impedance associated with a Cortical Contraction in the Egg of *Xenopus laevis*

Attention has recently been drawn to the concept of the cell membrane as a transducer of external stimuli, mediating between environmental effectors and cytoplasmic responses by altering the surface potential (refs. 1 and 2, and our own results which are in preparation). Alteration of the potential profile through the membrane,

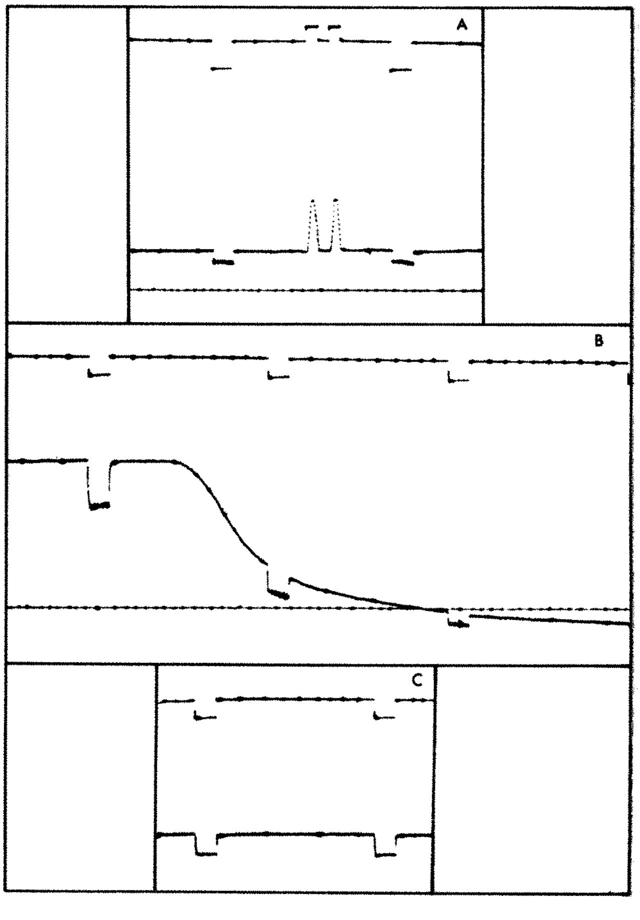

Fig. 1. Changes in electrical potential and cell membrane impedance in a two cell embryo preceding contraction in response to local application of ribonuclease to the animal pole, in the presence of divalent cations. Voltage pulses for current determination appear on the uppermost trace; those for resistance measurements appear as downward displacements of the middle trace while the lowest trace (not shown in *C*) is a constant reference potential. Time runs from left to right in each insert. Speed: normal, 0·5 cm/sec. *A*, 1 mV calibration on the current trace and 10 mV calibration in 1 mV increments on the resistance trace prior to penetration of the egg. *B*, After penetration of both electrodes, the resistance pulse was seen to be larger. Immediately before the addition of RNase, membrane impedance was calculated to be 420 kΩ and the measured potential (–) 20 mV. On adding RNase both fell steeply. The potential became positive (+12 mV) and the impedance was reduced to a few kΩ. Contraction occurred. *C*, Spontaneous recovery after a further 10 min partially restored potential and impedance to (–) 8 mV and 120 kΩ, respectively. The contraction was subsequently seen to have relaxed. Note: current amplifier gain was altered between *A* and *B*.

resulting from a change in electrostatic surface potential caused by changes in external ionic strength, adsorption of polyions or the close approach of cell surfaces, could lead to cytoplasmic reactions[1]. Changes in membrane field might affect the conformation of membrane macromolecules, leading to the presentation of new groups at the cytoplasmic interface. Alternatively, changes in permeability of the membrane might follow conformational changes, or else might occur in their absence. Any of these mechanisms could provide the cytoplasm with information about the external environment on which it might act accordingly. The possibility of co-operative phenomena in membranes, envisaged by Changeux and his colleagues[3,4], is of great interest in this respect,

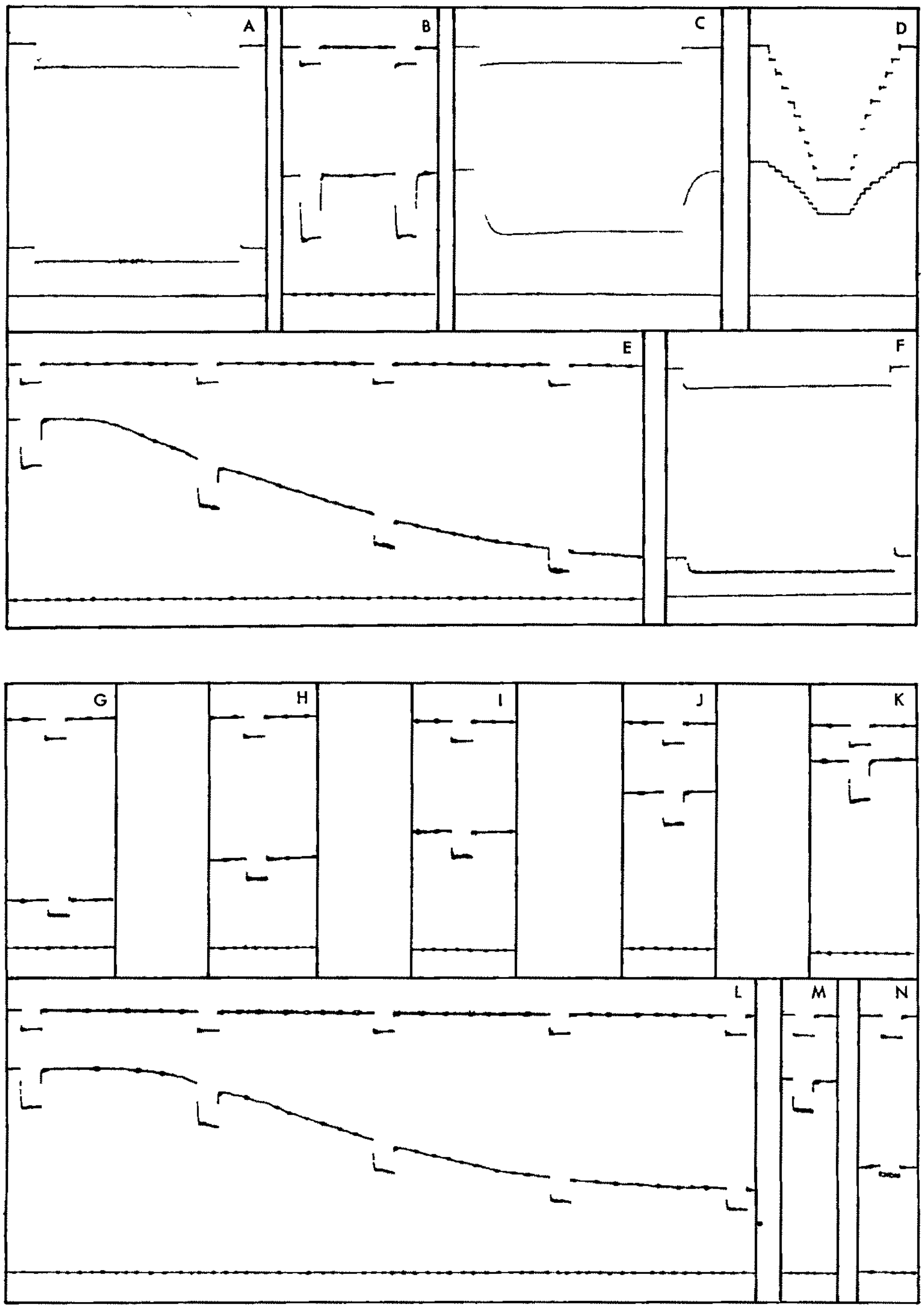

Fig. 2. Electrical potential and impedance changes in a four cell embryo without contractile response, caused by the action of local application of ribonuclease to the cell surface, in medium free of divalent cations. Voltage pulses for current measurement are recorded on the uppermost trace, while the lowest trace is a constant reference potential. Time runs from left to right in each insert. Speed: normal, 0.5 cm/sec, fast 5 cm/sec. *A*, Fast pulse taken with both electrodes outside the cell. *B*, On penetration with both electrodes, a negative potential and increased resistance were recorded. Membrane impedance was calculated to be 430 kΩ. *C*, A fast pulse showed capacitative rise and fall. *D*, 10 mV calibration in 1 mV steps. *E*, A few minutes later the potential had increased. Local addition of 1.0 per cent RNase at the animal pole of the egg resulted in a fall of the potential to zero and the impedance was reduced to a few kΩ. No contraction occurred. *F*, A fast pulse taken immediately after *E* was seen to have lost its capacitative shape almost completely (compare *C*). *G* to *K*, Spontaneous recovery of both potential and impedance over a period of about 6.5 min. The former rose to its original value and the latter recovered to 360 kΩ. *L*, A second application of RNase sent the potential and impedance down to (–) 9 mV and 59 kΩ respectively. No contraction took place. *M*, Spontaneous recovery after 6 min. Potential, (–) 18 mV, impedance, 270 kΩ. *N*, After electrode withdrawal the pulse was reduced and the zero had shifted about 10 mV in a positive direction.

especially because the stimulus for co-operative change might be a change in membrane electrostatic field[5]

A new system for experimental investigation of these concepts involves a cortical contractile response by the eggs and early cleavage stages of *Xenopus laevis* to surface-adsorbed polyions and ionic detergents (results in preparation) When 0·1 per cent solutions of poly-L-lysine of molecular weight 2,600, 50,000 or 250,000, or ribonuclease, both of which are polycationic for a wide *p*H range, were applied locally by means of a fine pipette near the surface membrane of fertilized eggs or early cleavage stages, from which the jelly and vitelline membrane had been removed, a localized non-propagated contraction occurred after a delay of 1–2 min in normal Steinberg's medium The same phenomenon followed similar application of the cationic detergent hexadecyl trimethylammonium bromide at 0·5 mmolar and also the anion detergent sodium dodecyl sulphate at 1·0 mmolar Nor ionic detergents 'Triton *X*-100' and 'Pluronic *F*68' wer ineffective even at 1 per cent Electron microscopy regions which had been thus treated shows that the su face membrane is thrown into extensive folds as a resu of contraction of the subjacent cortex, which is chara terized in the animal pole by local accumulation of pi ment granules There is no evidence that the membra undergoes active contraction

Reversible relaxation of the contraction induced b poly-L-lysine was obtained, with a few minutes' delay, b bathing contracted eggs in 0·1 per cent sodium poly- glutamate (molecular weight 68,000) or sodium hepari at around neutral *p*H Replacement by normal Stein berg's medium even after 30 min resulted in local r

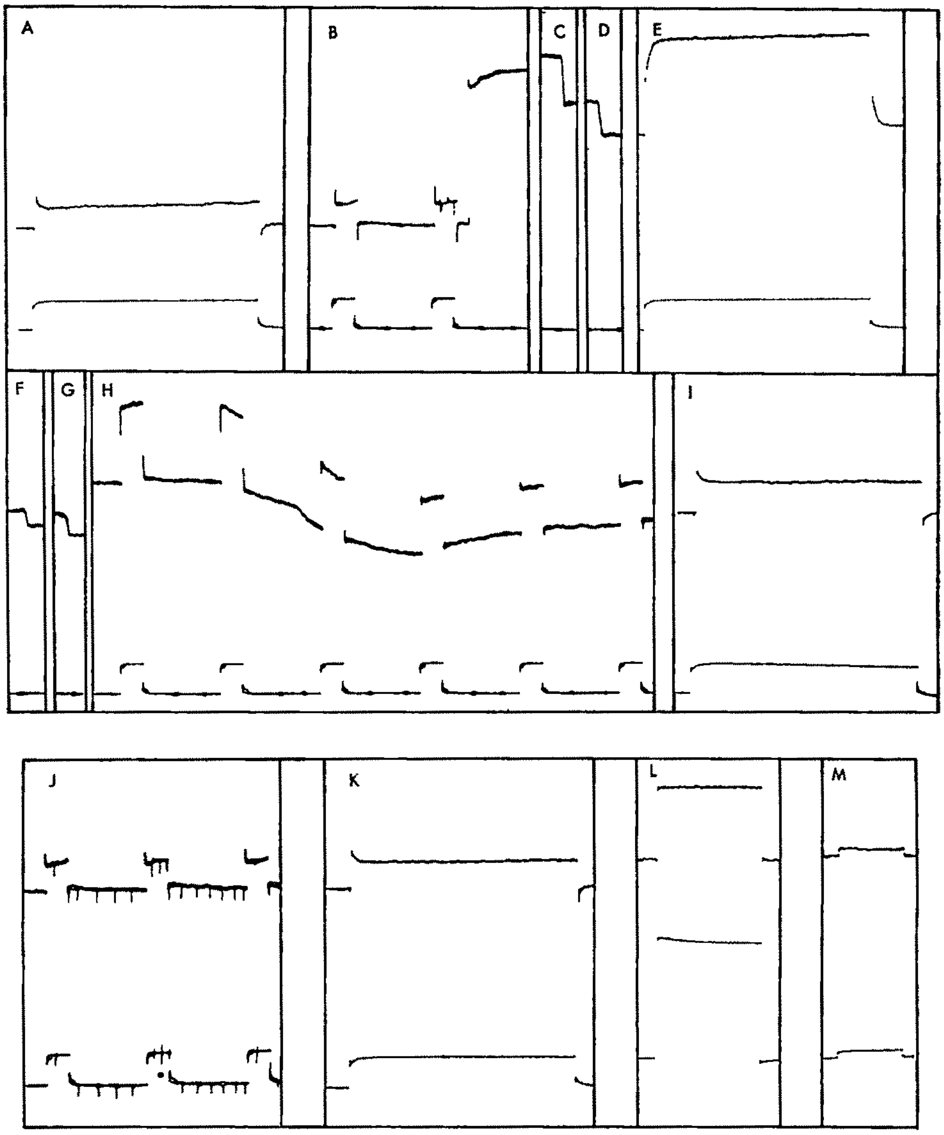

Fig 3 Changes in electrical potential and membrane impedance associated with the action of polylysine on a two cell stage embryo, first in the absence of divalent cations and then after their addition The upper trace records potential and the periodic upward displacements give resistance data The lower trace records current pulses Time runs from left to right in each insert Speed normal 0·5 cm/sec, fast, 5 cm/sec *A*, Square pulse before penetration of recording electrode *B*, Penetration of recording electrode A negative potential of (−) 22 mV was recorded *C* and *D*, Potential trace was shifted to accommodate impedance pulses *E* Capacitative fast pulse membrane impedance was calculated to be 320 $k\Omega$ *F* and *G* Potential trace shifted again during flushing out of the electrode bath with divalent cation-free Steinberg's medium The measured potential fell 6 mV *H*, After washing for 6 min the membrane impedance had fallen to 210 $k\Omega$ though the pulse was still capacitative 0·1 per cent polylysine was added locally to a region of the animal pole between the first and second pulse of this insert The potential underwent a temporary change of (+) 10 mV, but the impedance fell and continued falling to a few $k\Omega$ There was no sign of contraction in the egg *I*, A fast pulse showed negligible capacitance and little impedance *J*, Three min after the addition of polylysine the electrode bath was replenished with ordinary Steinberg's medium Drop-wise addition produced 'spikes" on the record *K*, Without further change in electrical characteristics of the membrane (compare *I*) contraction occurred within 1 min of adding Steinberg's medium *L*, 10 mV calibration. *M*, 1 mV calibration

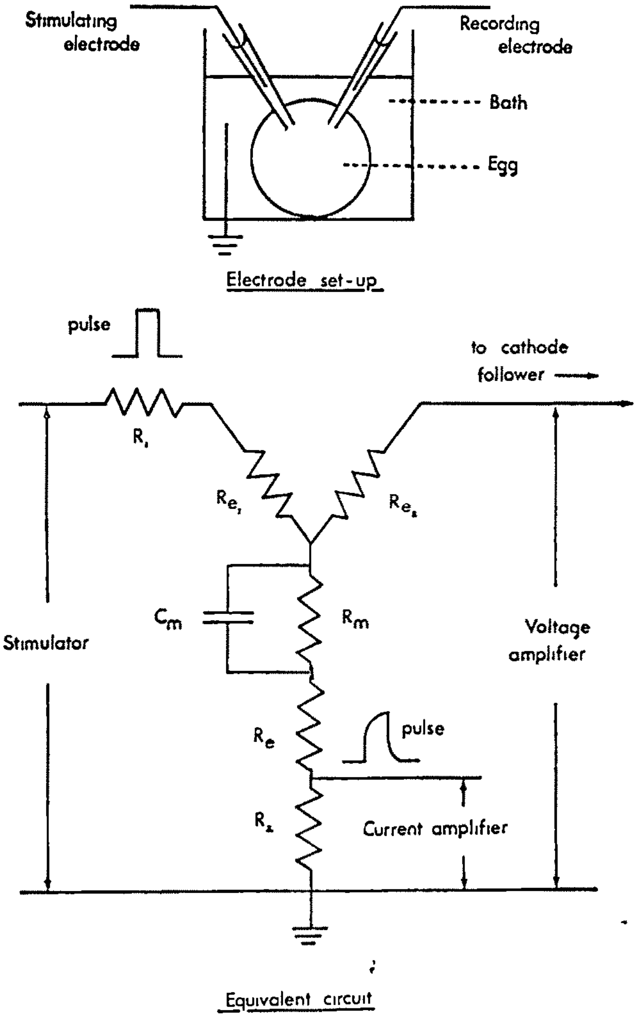

Fig 4 The upper diagram shows the electrode arrangement used in experiments on *Xenopus laevis* eggs and the lower diagram gives an outline of the circuit R_{e_1} is the resistance of the stimulating electrode, R_{e_2} the resistance of the recording electrode and R_e that of the indifferent electrode R_m is the resistance of the cell membrane and C_m its capacitance R_1 is the output resistance of the stimulator, 10^9 Ω R_2 is a resistance of 10^5 Ω across which current is measured Characteristic pulse profiles before and after the current passes through the cell membrane are indicated

contraction of the fully relaxed region, suggesting that the polycation was not eluted from the cell surface by the polyanion Exposure to 0 5–2 0 molar sodium chloride caused relaxation, probably without removal of bound polycation

Using a recording technique which employs two intracellular glass microelectrodes[8] filled with 3 molar potassium chloride (Fig 4), untreated two or four cell stages were found to have impedances (R_m) in the range 250 to 450 kΩ If the surface is taken to be 0 04 cm², an R_m of 350 kΩ would give a unit area resistance of 14 kΩ cm² Recorded potentials which were not corrected for changes in microelectrode tip potential inside the cell were usually between – 20 and – 30 mV in normal Steinberg's solution Potential and impedance vary during early cleavages (unpublished results of Palmer) and for this reason care was taken to avoid measurements on dividing cells Following local applications of ribonuclease near the cell surface, the potential which was measured fell and sometimes reversed to positive values, while the membrane impedance invariably fell to below 50 kΩ within a few seconds Visible contraction followed in a minute or two Poly-L-lysine also reduced the impedance, but the potential underwent a temporary change followed by a return to normal Rapid recovery of impedance and potential did not occur after poly-L-lysine treatment alone, but spontaneous recovery accompanied by relaxation followed about 8 min after ribonuclease treatment (Fig 1) The addition of 0 1 per cent poly-L-glutamate caused slow relaxation of a region which was previously contracted by poly-L-lysine during the course of several minutes, and this was preceded by restoration of membrane impedance but with little change in the potential The polyanion alone had almost no effect on the impedance or potential of untreated cells.

In the absence of external divalent cations with or without 3 mmolar di-sodium ethylene diaminetetraacetate (EDTA), neither ribonuclease nor poly-L-lysine induced contraction, although the membrane impedance fell as before and the potential changed characteristically (Fig 2) Addition of normal Steinberg's medium containing 0 34 mmolar Ca^{++} and 0 83 mmolar Mg^{++} was attended, however, by a rapid contraction at the site of polyion application without further change in membrane impedance or potential (Fig 3)

The events leading to contraction seem to fall into a sequence (1) a change in the membrane in response to adsorbed charged molecules, (2) ion movements in response to changed membrane properties, and (3) cytoplasmic contraction, the mechanism of which is not considered in any detail here. From the work of Nevo, de Vries and Katchalsky[7] there is good evidence that poly-L-lysine binds to the outer surface of the red cell without penetrating the membrane Electron microscopic examination of eggs which were treated with poly-L-lysine reveals a thick dense layer, 200–300 Å on the surface, which is not present in untreated eggs This layer probably represents poly-L-lysine adsorbed to the surface coat Ionic detergents could adsorb at the lipid–water interface within the membrane Adsorption of charged molecules which alter the surface potential is therefore believed to be the first step in the sequence A fall in membrane impedance followed immediately, indicating increased membrane permeability, though to which ions is uncertain The direction of the accompanying potential change after ribonuclease treatment suggests increased sodium permeability No cortical contraction follows unless divalent cations are present in the external medium Passage of these ions into the cytoplasm may be directly responsible for activating a contractile apparatus in the cortex, but this has not yet been directly demonstrated An alternative explanation may be that divalent cations are released from combining sites on the inner surface of the membrane in response to changes in the membrane as a result of the adsorption of charged molecules in the presence of external divalent cations

The membrane changes preceding relaxation could be initiated by restoration of the surface potential Salt would act thus, by reducing the positive surface potential caused by adsorbed polycation towards zero by virtue of increased ionic strength Relaxation in Steinberg's medium, which contains 0 06 molar sodium chloride, following contraction which was induced by ribonuclease, is probably caused by salt elution of the protein which is less strongly electrostatically bound than poly-L-lysine The relaxation induced by polyanions can be interpreted as a result of their reversible bonding to surface-bound polycations, thus reducing the net positive charge and potential near the cell surface Sequestering of divalent cations in solution by polyanions could play some part in the process, though divalent cation removal *per se* would increase membrane permeability and cannot explain the increased membrane impedance which precedes relaxation

In conclusion, the observations which have been made may be tentatively interpreted as follows An increased negative or positive surface potential triggers a change in the state of the membrane which is characterized by a fall in impedance which is reversed on restoration of the surface potential towards a smaller absolute value The cytoplasmic contraction which is dependent on divalent cations and which follows reduction of membrane impedance could reflect an influx of divalent cations

It is tempting to speculate on the apparently analogous events in muscle where release of calcium from the sarcoplasmic reticulum in response to membrane depolarization[10] is believed to initiate myofibrillar contraction Both systems could involve movements of divalent cations through a membrane the state of permeability of which is a function of its electric field Cellular motile responses of *Amoeba proteus* to polyions and salts have been examined with the microelectrode technique and the results, which also suggest that changes in potential of the surface membrane can control cytoplasmic events, will be reported in detail elsewhere (results in preparation)

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[1] Gingell, D , *J Theoret Biol* ,17, 3 (1967)
[2] Wolpert, L , and Gingell, D , *Symp Soc Exp Biol* ,22 (1968) (in the press)
[3] Changeux, J P , Thiery, J Tung, Y , and Kittel, C , *Proc US Nat Acad. Sci* , 57, 335 (1967)
[4] Changeux, J P , and Thiery, J , *J Theoret Biol* (in the press)
[5] Hill, T L , *Proc US Nat Acad Sci* , 58, 111 (1967)
[6] Ito, S , and Hori, N , *J Gen Physiol* , 49, 1019 (1966)
[7] Nevo, A., de Vries, A , and Katchalsky, A , *Biochim Biophys Acta*, 17, 536 (1955)
[8] Dollander, A , *Arch Anat Strasbourg*, 44, 93 (1962)
[9] Loewenstein W R *Ann NY Acad Sci* ,137, 441 (1966)
[10] Podolsky, R J , *Symp Soc Exp Biol* ,22 (1968) (in the press)

MICROBIOLOGY

Skin Heterogenizing Virus

WE have reported previously[1-3] that some murine transplantable tumours contain an agent capable of inducing a strong transplantation antigen in the skin of mice with tumours The skin was regularly rejected both in syngeneic grafting and in autotransplantation We call this phenomenon heterogenization or allogenization of skin under the effect of tumour[1-3] Skin grafts taken from mice which had rejected skin grafts obtained from the tumour-bearing donors were also rejected in syngeneic transplantation We also demonstrated the transfer of skin heterogenization by means of skin grafting in eight successive passages

Heterogenization of the skin has now been obtained by injecting tumour homogenate Our experimental data show that the skin heterogenizing agent is a new virus, possibly the first representative of a new oligocystronal group of viruses

In our experiments, sarcoma *K*-237 of *C57BL/6 Jax* mice was used The tumour strain and the experimental technique were partially described before[1,2]

For homogenates, the tumour pieces were cleaned from the necrotic areas and ground with glass for 30 min at 4° C The homogenous mass was then diluted to 10 per cent with Hanks solution (*p*H 7 2) The basic material used throughout the experiments was the supernatant fluid obtained after 10 per cent homogenate had been centrifuged for 30 min at 1,500*g* (later called supernatant 1) For differential centrifugation the following speeds were used stage 1, 3,000*g* or 10,000*g*; stage 2, 80,000*g* or 130,000*g* The sediments were resuspended with the initial volume of Hanks solution, the supernatants were used for further centrifugation

To study the thermostability, supernatant 1 was heated for 30 min at different temperatures in a water bath or kept at −20° C or −70° C for 24 h

Ether sensitivity was determined by adding an equal part of diethyl ether and incubating at 4° C overnight The ether was then removed, and the remainder was evaporated in a Petri dish at room temperature

The ten-fold dilutions, using Hanks solution, were used for agent dose titration The presence of skin heterogenizing agent in different materials was tested as follows Donor mice, adult *C57BL/6 Jax* females, received the materials in 0 5 ml volumes, both subcutaneously and intraperitoneally, in a total volume of 1 ml to each mouse The skin of these mice was checked by syngeneic grafting, carried out 3 weeks after inoculation

The presence of the virus in the sera and lymphoid organs of the tumour-bearing mice was also investigated For this purpose, donor mice were injected with 0 15 ml of pooled serum both intraperitoneally and intravenously. The same injection route was used for a mixture of spleen and lymph nodes cells, the total amount being 250×10^6 cells/mouse The control materials were injected in the same way The establishment of heterogenization was tested

It can be seen from Table 1 that the skin heterogenizing agent is present both in tumour homogenates and in lymphoid cells of tumour-bearing mice, but not in the pooled serum where it is possibly neutralized by antibodies The agent is etheroresistant, inactivated at 60° C and only partially sedimented at 130,000*g* Furthermore, only partial sedimentation was obtained even after centrifugation at 226,000*g* for 6 h The properties of the agent indicate that it resembles a very small virus which is resistant to ether The virus dose in tumour supernatant 1 was higher than 10^{10} particles/ml of the heterogenization producing mature virus (not indicated in Table 1)

Table 1 PROPERTIES OF THE SKIN HETEROGENIZING VIRUS

C57BL/6J donors inoculated with	Rejection of syngeneic skin grafts in normal recipients
Pieces of the tumours, *K*-237, used for preparation of homogenates	24/24*
Serum pool from tumour-bearing mice	0/4
Mixture of lymphoid organ cells from tumour-bearing mice	8/8
Supernatant 1	28/32
Supernatant 1 heated for 30 min at 45° C	13/16
Supernatant 1 heated for 30 min at 60° C	1/11
Supernatant 1 frozen for 24 h at −20° C	8/8
Supernatant 1 frozen for 24 h at −70° C	8/8
Supernatant 1 treated with ether	8/8
Supernatant after centrifugation of supernatant 1 for 30 min at 3,000*g* or 10,000*g*	26/29
Sediment after centrifugation of supernatant 1 for 30 min at 3,000*g* or 10,000*g*	0/4
Supernatant after centrifugation of supernatant 1 for 60 min at 130 000*g*	6/7
Sediment after centrifugation of supernatant 1 for 60 min at 130,000*g*	3/7

* Numerator, number of mice with grafts rejected, denominator, total number of recipients

The possibility of specific virus neutralization was investigated in the following experiment The donors of immune serum were *C57BL/6 Jax* female mice which rejected heterogenized skin A month after the average end-point of rejection, these mice were injected with 1 ml of supernatant 1 once a week for 3 weeks

Blood was taken from the orbital sinus of donor mice 1 week after the last injection The pooled serum, from six mice, was heated at 56° C for 1 h in a water bath; the sera of the control normal *C57BL/6 Jax* mice and *BALB/c* mice was prepared in the same way The sera were diluted 1 2, 1 20, 1 200 and 1 2,000 The supernatant served as a source of virus (infectious titre being higher than 10^{10}) It was admixed with equal volumes of corresponding dilutions of tested sera and incubated at

Fig. 2 Fig. 3 Fig. 4 Fig. 5

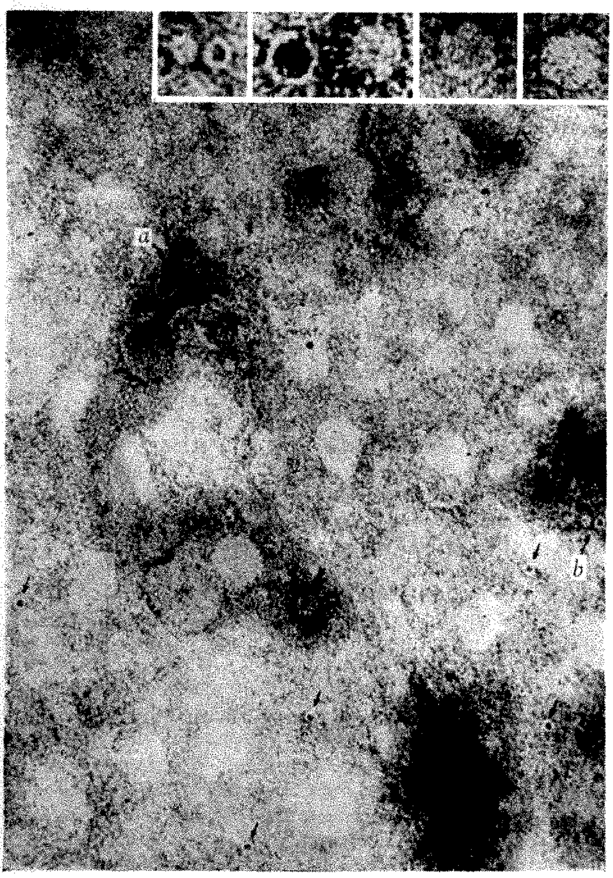

Fig. 1

Fig. 1. Two kinds of viral particles (× c. 212,960).

Fig. 2. Type *b* particles (× c. 555,550).

Fig. 3. Type *a* particles (× c. 555,550).

Fig. 4. Type *a* particle (× c. 620,370).

Fig. 5. Type *a* particle (× c. 620,370).

37° C for 1 h. Then the groups of *C57BL/6 Jax* mice were inoculated and the establishment of heterogenization was tested as usual.

The data in Table 2 show that the specific neutralization of skin heterogenizing virus occurred. The immune sera employed here must contain large amounts of neutralizing antibody because the virus dose was higher than 10^{10}.

Table 2. NEUTRALIZATION OF THE SKIN HETEROGENIZING VIRUS

Donors of sera	Reciprocal to serum dilution 2	20	200	2,000
C57BL/6 Jax immune	0/8*	0/5	3/6	4/5
C57BL/6 Jax normal	4/6	4/4	4/4	—
BALB/c normal	6/6	6/6	6/6	—
Hanks solution	8/8	—	—	—

* As in Table 1.

Passage of heterogenization from one mouse to another by skin grafting showed that not only tumour cells but also the skin cells produced the mature virus. The persistence of "new" transplantation antigen, as well as the virus producing capacity of skin cells, was maintained in the infected mice for more than 4 months. The virus was also present in skin homogenates free of living cells.

The simultaneous induction of strong transplantation antigen, and the production of virus in large quantities, indicates a quite original mechanism of interaction between virus and cell in this experimental system. A quantitative study of virus behaviour is now in progress. The *C57BL/6 Jax* and *BALB/c* mice, which were inoculated by different routes with the supernatant 1 when they were born, had not produced any noticeable pathological changes after 8 months of observation.

A resuspended pellet from supernatant 1, after centrifugation at 80,000*g* for 1 h, was used for electron microscopy. The preparation was contrasted with 2–4 per cent phosphotungsten sodium solution *p*H 7·0. The electron microscope, *JEM*-7, was used at instrumental magnifications of × 50,000, × 70,000 and × 100,000.

Two kinds of complete, hollow (empty) virus-like particles were found in the preparations, and were designated as type *a* and *b* particles (see Figs. 1–5). The particles exhibit cubic symmetry of the icosahedral type.

The diameter of type *a* particles was 165 to 175 Å, the thickness of the walls of the hollow particles being not more than 30 Å. The diameter of the internal cavity of these particles was about 100–115 Å and the volume was about 1,000,000 $Å^3$. The external diameter of type *b* particles was 75 to 85 Å, the thickness of the walls of the hollow particles was about 20 Å, the internal cavity diameter was 35–45 Å, and the volume was about 60,000 $Å^3$ (Fig. 6).

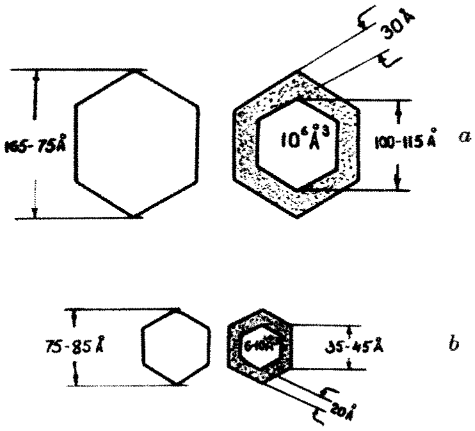

Fig. 6. Viral particles scheme.

These results confirm our previous suggestion that skin heterogenization is produced by a virus[1-3]. This virus seems to be etheroresistant and very small. It reproduces in the tumour and skin cells, and is present at least in the lymphatic organs of tumour-bearing animals. The only evident sign of infection is the induction of new antigen in the skin, which makes it incompatible in autologous and syngeneic grafting.

In this respect it seems important that virus particles were found in sarcoma *K*-237 homogenate with the help of electron microscopy. In view of the high magnification employed, these particles may be considered to be present in high concentrations (see Fig. 1). This fact correlates in an indirect way with the high biological titre of the heterogenizing virus in the homogenate.

Previously one of us[4-6] emphasized that "cell heterogenization in respect of the whole organism" is a basic feature of interaction between cell and virus. There may be a whole world of viruses which cause the diseases based on the cell heterogenization phenomenon. Some of these diseases are referred to as autoimmune rheumatic fever, the nature of others, such as pemphigus and psoriasis, is not known very well.

Possibly this skin heterogenizing virus is the first representative of a large group of viruses which are inductors of autoimmune diseases or syngeneic tissue compatibility conflicts. These viruses are preliminarily designated as rheumoviruses.

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[1] Svet-Moldavsky, G. J., Mkheidze, D. M., and Liozner, A. L., *Nature*, **214**, 693 (1967).
[2] Svet-Moldavsky, G. J., Mkheidze, D. M., and Liozner, A. L., *J. Nat. Cancer Inst.*, **38**, 933 (1967).
[3] Svet-Moldavsky, G. J., Mkheidze, D. M., and Liozner, A. L., *Rep. Acad. Sci. USSR*, **174**, 243 (1967) (in Russian).
[4] Svet-Moldavsky, G. J., *Proc. Second All-Union Conf. Oncologists*, Leningrad, 1958, 114 (1959) (in Russian).
[5] Svet-Moldavsky, G. J., *Acta Virol.*, **5**, 167 (1961).
[6] Hamburg, V. P., and Svet-Moldavsky, G. J., *Nature*, **203**, 772 (1964).

GENERAL

Holographic Model of Temporal Recall

How do we manage to recognize immediately, and to recall without effort, familiar words, sentences and other behavioural sequences? I suggest that we employ a system which is the temporal analogue of optical holography[1-3]. The analogy between holography and visual memory has already attracted attention in the literature, but this is not quite the thought that I shall pursue here; rather I shall consider the time dimension, because any perceptual event must certainly be associated with cerebral happenings which are extended not only in space but in time. If a system of this kind were indeed used by the brain, not only would some familiar phenomena of perception be naturally explained, but the cells or cell clusters responsible for laying down sensory information should have a certain property which might be accessible to experimental test.

The system which I have in mind is most easily described with the aid of a concrete example. Imagine a bank of resonators, rather like the strings of a piano. Their resonant frequencies ν_k are supposed to be evenly distributed over a certain frequency band, with a spacing equal to μ, and the bandwidth of each resonator will be assumed equal to the same constant μ. Each resonator is supposed to be coupled to the input signal $f(t)$ according to the equation

$$\ddot{x}_k + (4\pi\mu)\dot{x}_k + 4\pi^2(\mu^2 + \nu_k^2)x_k = A_k f(t)$$

where x_k denotes the displacement of the kth resonator, and A_k is a coupling constant. Then it can be shown that the total response of the bank, conveniently defined as

$$g(t) = \sum_k(\dot{x}_k + 2\pi\mu x_k)$$

will be proportional to $f(t)$ provided (*a*) that the coupling coefficients A_k are all equal; (*b*) that $f(t)$ contains no frequencies lying outside the range of the bank and (*c*) that μ is sufficiently small—that the resonators are sufficiently densely distributed over the frequency range.

I now imagine that after the passage of a signal $f(t)$, the coupling constant of each resonator is capable of being altered by an amount proportional to the work recently done on it by the signal, that is,

$$\Delta A_k = \lambda\int_{-\infty}^{0} f(t)\dot{x}_k(t)e^{4\pi\mu t}\mathrm{d}t$$

where the quantity λ is supposed to be the same for each resonator. If the signal consisted of a pulse $f_1(t)$ followed immediately by another signal $f_2(t)$, then it can be shown that if the coupling constants are subsequently adjusted as shown, a later input of the same pulse will immediately evoke a response $g_2(t)$ proportional to $f_2(t)$. This phenomenon is the mathematical analogue of the following holographic observation: a collimated laser beam is split into two parts; one is directly incident on a photographic plate, and the other falls on an object, the light from which is also incident on the plate. The plate is developed and replaced by its positive print, the hologram; the object is removed, and the hologram is illuminated with the first part of the same laser beam. An observer looking through the hologram then beholds the absent object in its original position. The mathematical analogy is not, however, quite complete, whereas the hologram records the square of the Fourier transform of the wavefront leaving the object, the bank of resonators records the square of the Laplace transform of $f(t)$, because to record the Fourier transform would require a violation of causality.

This result can be generalized in two stages. First, it can be shown that if a signal $f(t)$ is arbitrarily partitioned into an earlier part f_1 and a later part f_2, then after the bank has "memorized" the whole signal, the input of f_1 will evoke a more or less distinct "echo" of g_2, immediately afterwards. The distinctness of the echo will be greater the more closely the temporal autocorrelation function of $f_1(t)$ approximates to a delta function at the origin.

Second, the bank may be used for memorizing, at widely different times, a number of different signals $f^{(n)}$, $n = 1, 2, 3, \ldots$ If these signals are so different that their mutual correlations can be neglected, then the later input of $f_1^{(m)}$ will evoke a more or less distinct echo of $g_2^{(m)}$ uncontaminated by any of the other signals that have also been recorded in the bank.

If the bank is used, in this way, for recording a number of different signals, there will obviously be a limit to the length of any one signal that can be recorded. This limit is, in fact, the reciprocal of the frequency spacing μ. But the bank can be made to regenerate signals of much greater length by connecting it to a regenerative feedback mechanism such that when the response g_n appears the signal f_n is fed back into the bank. Then, if the signals (f_1 then f_2), (f_2 then f_3), and so on, have all been recorded, the input of f_2 will cause the bank to re-create the long sequence (g_2 then g_3 then g_4 then g_5 and so on).

The resemblance of such behaviour to that of memory is sufficiently striking for one to wonder whether the brain may not employ systems of this general kind for recording temporal sequences such as those which are involved in speech and kinaesthetically controlled movement. If so, perhaps groups of cells could be detected which have the property of becoming more responsive to particular frequencies the more they are stimulated at those frequencies. Cells are already known which display an accurately sinusoidal metabolism, and have been tentatively identified as "biological clocks"[4]. Could such cells be vitally involved in the processes of temporal recall?

I thank Dr A. Moscowitz, Dr L. Harmon and Dr B. Julesz for helpful criticism, and the Bell Telephone Laboratories for their hospitality in the course of this work.

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[1] Gabor, D., *Nature*, **161**, 777 (1948).
[2] Gabor, D., *Proc. Roy. Soc.*, A, **197**, 454 (1949).
[3] Julesz, B., and Pennington, K., *J. Opt. Soc. Amer.*, **55**, 604 (1965).
[4] Chance, B., Pye, K., and Higgins, J. J., *IEEE Spectrum*, **4**, No. 8, 79 (1967).

BOOK REVIEWS

SEALS

Seals of the World

By Gavin Maxwell, with John Stidworthy and David Williams (World Wildlife Series No 2) Pp xii+151+ 16 plates (London Constable and Co , Ltd , 1967) 45*s* net

Seals have been much before the public in England during the past few years owing to the conflict between the interests of fishermen, to whom they are pests, and of others to whom they are endearing creatures An attempt to reduce the numbers of the grey seal on some parts of our coasts has led to much ill-informed protest, but it is surely better that public opinion, however misguided, should be anxious that our fauna be preserved than indifferent to its destruction The publication of this book under the auspices of the World Wildlife Fund which is to receive "part of the proceeds" from its sale seems to be timed to take advantage of this feeling to whip up support for its propaganda

Seals have for thousands of years been of the greatest economic importance to the people who live near the shores of the northern oceans of the world, they yield food, oil, furs, hides, ivory and other useful products So long as seals were cropped for subsistence by people on the peasant level of life, all was well, but when, around the beginning of the nineteenth century, big business realized the wealth awaiting the gathering, an era of almost incredible destruction started Although seals are aquatic mammals they have not, as have the whales, completely emancipated themselves from the land, or ice-fields, to which they must resort to give birth to their young and nurse them through the first few weeks of life It is when they temporarily leave the sea and congregate in large herds that they are vulnerable to commerce

Local exploitation of seals, mainly for their oil, was carried out on a small scale in different parts of the world as European exploration advanced, but the devastation came at the turn of the eighteenth century when fortunes were made from fur seals, especially in the southern hemisphere, and in a couple of decades the animals were practically exterminated Throughout the nineteenth century the remnants were spasmodically hunted down, until early in the twentieth century even occasional raids no longer paid expenses It has taken half a century for the populations to begin showing signs of recovery Events in the north Pacific took a similar course, but were halted at a rather earlier time The sea lions and fur seals of South America, the elephant seals of the sub-Antarctic, and the fur seals of the north Pacific are now cropped rationally—an easy thing to do with polygamous species that have an excess of males The ice-field breeding seals of the north Atlantic, such as the harp, bearded and other seals, are, however, still over-fished although some measures for their conservation have been taken The history of sealing is disgraceful, but at the eleventh hour before the final disappearance of the animals reason is beginning to prevail, and the prospects of preserving the world populations of these scientifically fascinating animals, and of taking a crop of useful products from them for the benefit of mankind, seem to be brighter

Apart from their economic value seals are of the greatest interest to zoologists—their highly adapted anatomy, and the innumerable peculiarities of their physiology, have long attracted attention, and provide a host of problems for study by observation and experiment that will keep enthusiasts at work for many years The recently discovered use of sound echo-location in some species has opened yet another field of investigation—and now SCUBA divers are able to meet seals in their own element, even below the ice-fields of the Antarctic wastes

This book gives a short general account of seals, and of man's dealing with them, it then gives an account of each species in turn under headings such as distribution, appearance, habits, reproduction and so on It concludes with a list of the species and a short bibliography The author claims no originality in the work for which his two assistants ransacked the literature in search of material They have been industrious, and have collected most of the important facts about seals, and they have not copied over many of their predecessors' mistakes, though they have missed some of the latest discoveries There are a few misprints such as "deputations of sealing vessels" for "depredations" The interesting photographic illustrations show twenty-eight of the thirty-two species It is not clear, however, why this book should have been compiled, for a first-class book with the same title, written by Judith E King who has spent years in original research on seals, was published by the British Museum (Natural History) only three years ago at less than a quarter of the price of this volume L Harrison Matthews

ANOTHER JOURNAL

Biochemical Genetics

Vol 1, No 1, June 1967 Edited by Charles R Shaw Pp 1–71 Annual subscription (4 issues) $18 Outside US and Canada add $1 80 for postage and handling (New York Plenum Publishing Corporation, 1967)

Any new scientific journal is at present a mixed blessing library budgets are usually stretched to the limit, and the reading capacity of every research worker had already reached saturation some twenty years ago

In this light a new quarterly by the title *Biochemical Genetics* is unlikely to be welcome on the usual grounds of filling a non-existing gap

This said, what are the mitigating circumstances ? First, the proposed field of the journal states its primary interest in the biochemical genetics of diploid organisms Presumably this means organisms other than *Escherichia coli* and phage T_4 or, more generally, eukaryotes This is welcome, but it may lead to class consciousness and inferiority complexes Second, the editorial board includes a number of very distinguished people Third, the first number includes some good specialized papers To quote a few, one on polymorphism for alcohol dehydrogenases in maize, one on a new assay for galactose-urydyltransferase in cultured human cells, and one on ATP levels in erythrocytes of Negro and white Americans

Among the articles of general interest, there is one by Fitch and Margoliash on a method for estimating the number of invariant amino-acid coding positions in a gene

On the whole the first number is good, and if we have to be plagued by—and subscribe to—yet another journal, let us wish it as good a continuation G Pontecorvo

IN THE SUB-ANTARCTIC

Sub-Antarctic Sanctuary

Summertime on Macquarie Island By Mary E Gillham Pp 223+32 photographs (London Victor Gollancz, 1967.) 45*s*.

Macquarie Island is one of nine truly sub-Antarctic oceanic islands, scattered around the Southern Hemisphere between latitudes 50° and 60° south It is of volcanic origin, and was heavily glaciated at the height of the Quaternary ice ages Its meagre flora, vegetation of tussock grassland, damp moorland and feld-mark, sparse land fauna and enormous breeding colonies of sea birds and seals are typical of the oceanic sub-Antarctic. Soon after discovery, in 1810, the island's stock of fur seals was virtually exterminated by sealers and for a long period thereafter the elephant seals and penguins were exploited for oil. Sealers not only made great inroads into the native bird and mammal populations, but imported rabbits, cats and the New Zealand weka, and these species have had an equally devastating effect on the ecosystem The rabbits have undermined the vegetation on coastal slopes and caused widespread erosion, while cats and rats, jointly or separately, are thought to have exterminated the island's endemic flightless landrail and destroyed colonies of the smaller sea birds Altogether, Macquarie Island was in a sorry state in 1933, when it was declared a sanctuary for its surviving wild life even since then, its management has not been uniformly enlightened Sheep, pigs, chickens and goats have been deliberately imported by the Australian National Antarctic Research Expedition which has maintained a scientific station at Macquarie since 1948, and while the goats were later destroyed, the sheep (which appear to serve no useful purpose) continued, in 1960, when the author of this book visited the island, to damage the vegetation in the one area of the island not previously infested with rabbits.

The efforts of the Australian authorities to protect Macquarie have largely been successful in allowing the recovery of penguin, albatross and seal populations. there is little hope of restoration of the overall oceanic island ecosystem to the level to be seen in the less damaged sub-Antarctic islands of Iles Crozet, Prince Edward Island or the outliers of South Georgia At the same time, the island does provide, in its bird and mammal populations, an impressive wild life spectacle, it has given opportunities for some outstanding long-term research on penguin and elephant seal biology and population dynamics, and even if imperfect, it is the most effective sanctuary, so far established, in the sub-Antarctic zone

Dr Mary Gillham's book does not come up to the claims made for it on its dust cover It is not a "must" for the specialist because of the information it gives about current research programmes Most of the scientific detail within it will already be known to the specialist from other sources, and what is new is written in so anecdotal a style that its basis and authority cannot be checked The book, in fact, is a fluently written piece of popular natural history, resulting from a few weeks' visit in midsummer As would be expected from so experienced a plant ecologist, Dr Gillham's notes on plant distribution and succession—for example, in abandoned elephant seal wallow grounds or old penguin colonies—are penetrating and of real interest. Her account of zoological topics and of southern circumpolar ecology and biogeography as a whole is inevitably less first-hand and less authoritative, contains a sprinkling of minor errors and doubtful generalizations, and is occasionally spoiled by emotional anti-scavenger and anti-predator bias The book, taken by and large, is, however, an entertaining addition to the growing library of sub-Antarctic popular natural history It has good photographs, rather less satisfactory pen-and-ink drawings, a valuable bibliography, appendices and index, but is spoiled by the omission of an adequate map M W. HOLDGATE

REFLECTIVE ZOOLOGY

Invertebrate Structure and Function

By E J W Barrington Pp x+549 (London Thomas Nelson and Sons, Ltd , 1967) 60*s* net

THE study of the organization of the invertebrates has long formed a major constituent of many university courses in zoology The undergraduate frequently receives instruction on invertebrate structure and classification and also on comparative physiology, and has to knit this information together into a moderately coherent whole Professor Barrington's book attempts to bring about such a synthesis: it is intended, as he says, "that the book may serve as an accompaniment to the more general and reflective parts of a university zoology course; as a companion for the seminars, the tutorials and the essay writing"

The chapters are grouped together into six parts There is an introductory section on the general features of living systems, followed by five chapters on movement, six on aspects of metabolism, four on information and co-ordination, three on reproduction and life histories and finally three on intraspecific and interspecific relationships

The treatment of movement concentrates mainly on the anatomical organization of muscular systems, without much analysis of the means whereby propulsive forces are applied to the substratum or the medium. The hydrostatic skeleton is examined in a wide variety of different animals, but the role of geodesic connective tissue fibres in the body wall is not really made very clear The section on aspects of metabolism deals comprehensively and interestingly with feeding methods, but the treatment of respiration, excretion and osmoregulation is rather brief The chapter on sense organs contains some errors of detail no single vertebrate eye contains rhodopsin, iodopsin, porphyropsin and cyanopsin, the axons of crustacean stretch receptors are not myelinated (as indicated in Fig 14–1) and the caption to Fig. 14–5 is incorrect The two chapters on nervous systems are mainly concerned with the relation between structure and behaviour The author's explanations of how nervous systems work, in terms of the properties of their individual neurones, are vague and misleading; it is incorrect to suggest that, at the cellular level, inhibition is the opposite of facilitation The next chapter examines the organization of endocrine systems in some detail and provides a useful synthesis of structural and functional approaches The last six chapters deal with a variety of topics in reproduction, development and interrelationships between animals, in a way which is interesting and frequently illuminating, this part of the book is, I think, of a much higher standard than much of the preceding material The illustrations are numerous, clear and well set out. There is a selected bibliography at the end of the book.

Apart from these points of detail, however, there is a more fundamental criticism that can be made of this book. The subject matter is largely concerned with physiology and functional morphology; both of these are essentially experimental sciences, and the problems which occur in them are to be solved by experimental methods A study of these subjects should surely, therefore, include some treatment of what experiments have been done and how the evidence so acquired should be assessed It is not sufficient to feed a student of a scientific subject on a diet consisting almost entirely of hypotheses and conclusions: he must be brought into close contact with the imaginative processes involved in conceiving experiments and the rigorous processes of deduction required for their interpretation

If these criteria are to be applied, then Professor Barrington's book must be found wanting He presents the reader with a large number of conclusions, but only rarely does he give an adequate account of the evidence on which these conclusions are based Connected with this feature is a marked absence of the quantitative approach, both in the text and in his selection of illustrations, there are nearly three hundred diagrams, but only twelve of them are graphs or experimental records with quantitative axes For example, in the chapter on respiration we might reasonably expect to find, in a book of this nature, answers to some of the following questions What is the rate of oxygen uptake in any one particular animal? How can this be measured? What environmental factors affect it? Is oxygen uptake affected by the size of an animal? How much more oxygen is required by active animals? Do animals living in habitats with low oxygen concentrations show any anatomical adaptations for increasing their oxygen uptake? What does the dissociation curve of a respiratory pigment look like? None of these is dealt with Yet these are surely just the kinds of question that the attention of the student should be drawn towards, and especially so in his seminars, his tutorials and his essay writing D. J AIDLEY

BIRD'S EGG CHEMISTRY

Biochemistry of the Avian Embryo

A Quantitative Analysis of Prenatal Development By Alexis L Romanoff with the collaboration of Anastasia J Romanoff Pp xviii+398 (New York and London Interscience Publishers, a division of John Wiley and Sons, 1967) 156*s*

PROFESSOR ROMANOFF has long been an authority on the development of the avian embryo, and this, his latest production, is a valuable book which every embryologist, once he has seen it, will wish to have for frequent reference Compiled from an examination of more than 5,000 pertinent publications, the information is provided in an intelligible and palatable form, mostly by means of tables with adequate explanatory text and many figures Equally important are the large number of references and more than twenty pages of bibliography, where the reader may find more details and be able to investigate the biochemistry of his problem in depth Indeed, the book combines an introduction to chemical embryology and a reference manual for the advanced research worker in this field

It should be emphasized, however, that it is not a discourse on the biochemical processes occurring in the avian embryo, nor does it deal with the problem of differentiation and intercellular reactions On the contrary, it is more of an encyclopaedia of biochemical facts and figures relating to every component of intra- and extra-embryonic tissue or organ at frequent stages (usually every day) from the beginning of incubation to hatching Even those who have ceased to marvel at the transformation of the contents of a bird's egg into an independent viable organism will learn a great deal to their advantage and be grateful to the authors for the labour of collecting so much and presenting it in so little space.

It is with my tongue in my cheek that I direct attention to page 122 where the total blood volume within the chick egg on the eighteenth day is reported as reaching its peak of 2,900 ml Reference to the accompanying tables soon reveals that 2,900 mm³ is the intention—a more acceptable figure JOHN MCKENZIE

THYMIDINE IN CELLS

Thymidine Metabolism and Cell Kinetics

By J E Cleaver (North-Holland Research Monographs Frontiers of Biology Vol 6) Pp 259 (Amsterdam North-Holland Publishing Company, 1967) 86*s*.

THE introduction of the radioactive isotopes of carbon, phosphorus, sulphur and hydrogen into the biological sciences during the past two decades has permitted great advances in our understanding of the structure and function of living matter A significant factor in their use is that they can be incorporated by living tissues without giving rise to excessive disruption of metabolic processes by virtue of their radioactivity

Cleaver's monograph deals specifically with work done using tritium (H_3) labelled thymidine, a unique compound that is almost exclusively incorporated into cell DNA and therefore makes possible very specific marking of this important macromolecule Possible uses which come to mind are studies of DNA replication, chromosome and nuclear distribution, the cell cycle and population kinetics Early chapters in the book discuss methods of preparation and detection of the compound, as well as what is known about thymidine metabolism in various types of cell Experimental details are generally not included (they would, in any case, make the book unnecessarily complicated), but this is compensated for by a comprehensive bibliography The use of tritiated thymidine in following events during the cell cycle occupies the larger part of the book, including a treatise on the kinetics of defined cell populations The text is well supplemented with simplified diagrams, and those less mathematically minded are spared the complicated theory and assumptions involved in the use of radioactive thymidine until a final appendix.

Dr Cleaver is mainly concerned with mammalian cells, but does not ignore the results obtained from micro-organisms, plants and insects Such cell types especially have contributed to our knowledge of chromosome replication, the segregation patterns of chromatids after mitosis, and of cross-overs during meiosis In treating the cell cycle, the various phases of which are usually represented schematically by sectors on a clock face, the mammalian cell is used almost exclusively as a model A large number of different cell types have now been characterized with respect to the duration of each part of the cycle, both *in vitro* and *in vivo*, and it is of interest that the G_1 phase (between mitosis and DNA synthesis) has greater variability between different cell types than either the DNA replication phase (S) or the period following replication (G_2) The commonly held fallacy that tumour cells have a higher growth rate than non-malignant cells was exposed as such by comparing the respective cell cycles

In writing the book, Dr Cleaver has assumed the reader to have a certain fundamental knowledge of the Watson and Crick hypothesis, the mitotic cycle, and of the basic organization of cell nuclei. The application of tritiated thymidine to such problems, however, is fully discussed Though our understanding of chromosome structure and its mode of replication in diploid cells is less precisely known than in haploid unicellular organisms, models based on the latter are suggested for the more advanced cell types

The index might usefully have included those authors mentioned in the text It may also be that the immunologist will be a little hurt that the interesting mitotic events following antigenic challenge have been largely ignored These, however, are minor criticisms, and research workers in any way involved in the study of nuclear DNA are likely to find the monograph of invaluable assistance and a useful reference source

A. J. S. DAVIES

NON ANGELI SED AVES

Radar Ornithology

By Eric Eastwood Pp. xii+278+24 plates (London: Methuen and Co, Ltd, 1967) 75*s*. net.

To speak of a new science, as does the author, is surely an enthusiastic exaggeration A new technique has certainly been exploited in the past dozen years by those ornithologists who parasitically battened on to early-warning or airport radars It now has such an accepted place in bird migration studies that it is hard to appreciate the difficulties early users had in convincing radar technicians that many anomalous echoes on their screens, classified by some anonymous wit as "angels", really did have wings

Future practitioners may appreciate this book, with its reasonably clear enunciation of radar principles and practice and its summary of the few score of original papers that have appeared to date Until recently one might have wondered whether there would be many such people High power, long-range radar equipment is far too expensive for ornithologists and the showers of angels were an obscuring nuisance to the prime users of the sets Technical advances could soon have resulted in their elimination Modern aircraft, however, are showing an increased tendency to collide with birds, so a new and economically important interest has arisen as to where birds are flying A radar watch for migratory concentrations may therefore become a standard requirement for airport controllers But the majority of airstrikes are on military aircraft streaking along below radar cover, or on take-off and landing, so one should not, perhaps, have too high hopes that ornithologists of the future will have access to a regular mine of photographic records of migrations in progress

They will have to be dedicated men While the speeded up cine film of a radar display is alive and exciting, the individual frames are deadly dull, as witness the many plates adding substantially to the high cost of this book To the uninitiated they are just one lot of "damned dots" after another Nor are the attempts to represent them in diagrammatic form much happier Dozens of little maps sprinkled with tiny arrows give more the impression that hostilities have broken out in the Channel and that Harold is again in danger of personal disaster To be fair, radar studies of migration are not alone in having failed to achieve an attractive form of presentation The compilations of visual records from bird observatories, plastered with endless synoptic charts, have set a high standard of boredom Indeed, this may be the reason for one of the main lacks in this book, there is no thorough-going attempt to relate radar findings (many of which are not novel) with the information obtained by other methods of studying migration

Perhaps more emphasis should have been laid on the crucial weaknesses of the radar method, the inability to describe, other than in very broad categories, the numbers or the kinds of birds involved in the choirs of "angels" It is said that specific identification of the "signatures" of radar echoes may be possible for single birds at short distances Sadly, those seeking to follow up the reference to the (10 cm) radar detection of pulsations in the pectoral muscles of a small passerine will not find it published in the *Proceedings of the International Ornithological Congress* as specified (or anywhere else for that matter) The reference list also omits a number of relevant publications in ornithological journals, but does usefully give some papers in technological offerings that would not normally come the way of the ornithologist

The book is well produced and free from typographical mistakes G V T MATTHEWS

WAVEGUIDES IN THEORY

The Theory of Waveguides and Cavities

By R A Waldron Pp xiii + 120 (London Maclaren and Sons, Ltd , 1967) 32*s* net

Theory of Waveguides and Cavity Resonators

By Emile Argence and Théo Kahan Pp xv + 424 (London and Glasgow Blackie and Son, Ltd , 1967) 120*s* net

ALTHOUGH the two books which form the subject of this review bear almost the same title, this is practically the only common factor between them The first text, by Richard Waldron, has as its core a number of papers published by the author in 1961 It is essentially intended for research workers studying waveguide boundary value problems or the theory underlying the measurement of dielectric or ferrite properties using cavity resonators

In the introductory chapter the concept of the HSP (homogeneous–simple–perfect) waveguide is presented and its properties are reviewed Later the author proposes that a 1 1 correspondence exists between modes in non-HSP and HSP guides Although no general proof is given the proposition does appear to be borne out by experience In Chapter 3 the author argues strongly that in approaching a new non-HSP waveguide problem one should seek to identify modes with those of the HSP guide The fourth chapter sets out the author's views on mode nomenclature with illustrations drawn from the behaviour of circular waveguide containing an axial dielectric rod Chapter 5 attacks the concept of surface impedance when used in the determination of the complex propagation coefficient of a mode in a waveguide with imperfectly conducting walls Undoubtedly Waldron's point is valid, but it would be instructive to have a comparison made with an exact rather than perturbation solution of the non-HSP waveguide problem With present day computers this is quite feasible, and could form a valuable study Chapter 6 presents a critical derivation of the formula for the determination of the complex permittivity and permeability of a small sample introduced into a resonant cavity The next chapter extends perturbation theory to include waveguides After a derivation of the static expressions for fields in small ellipsoids, the final chapter makes a careful appraisal of cavity methods of measuring ferrite properties

Although Waldron's style of writing will not appeal to all, there is something to be said for "black and white" statements in a monograph of this kind Undoubtedly some readers will be stimulated to make their own examination of the problems described

The second book, a translation from the French, was specially prepared for science faculty students obliged to study on their own So far as British students are concerned, however, the treatment is such as to appeal principally to those graduates in mathematics or theoretical physics who have an interest in electromagnetic problems There is a nine chapter mathematical introduction which, in part, aids understanding of the sixteen chapters dealing with electromagnetic fields, waveguides and cavities which follow The introduction includes three quite substantial chapters on the theory of distribution which I did not find to be essential to my understanding of the main work of the book The section on the electromagnetic field contains an account of Mie's analysis of diffraction by a dielectric sphere and the integral equation method of Levine and Schoninger, applied to the same problem The section on waveguides commences with a treatment of plane wave propagation in a dispersive absorbing medium, which is followed by a review of basic circuit theory A general theory of propagation in waveguides is then considered and results for specific waveguides follow. If thus far a certain incoherence in treatment seems apparent, this impression is probably justified From the remaining chapters those describing the modal analysis of source excited waveguides, due to Felsen and Mascuritry, and the application of radiational methods are noteworthy The whole book really comprises a selection of topics in electromagnetic theory intended for the advanced student The treatment is essentially mathematical with rather sparse physical descriptions, however, the originality of approach may appeal to those having reasonable familiarity with the subject matter In places the presentation of important subjects is terse, whereas elsewhere a student with mathematical competence could easily follow the steps

P J B CLARRICOATS

TODAY'S RADIO TELESCOPES

Radioastronomical Methods of Antenna Measurements
By A D Kuz'min and A E Salomonovich Translated by K N Tirogoff Translation edited by Ernest Jacobs (Electrical Science a Series of Monographs and Texts) Pp xvi+182 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1966) $8 50

THE past ten years have witnessed an impressive growth of new telescopes which can explore the Universe by radio and radar One of the earliest of this generation of radio telescopes, the 250 ft diameter Mark I at Jodrell Bank, has now completed ten years of service in planetary, galactic and extragalactic studies It has since been joined by an international group of large fully or partially steerable telescopes including the 210 ft at Parkes, Australia, the 300 ft at Greenbank, USA, the 1,000 ft in Aricebo, Puerto Rico, and the 660 ft × 130 ft tiltable plane mirror at Nançay, France A new generation of telescopes capable of operating at very short wavelengths (2 cm or even shorter) has been put into service during the past two years Three such instruments 140–150 ft in diameter have been installed in eastern USA and Canada and already have been able to satisfy an upsurge of interest in the short wavelength emission from quasars and other compact radio galaxies and in the structure of galactic ionized hydrogen regions seen both in their continuum emission and in their recombination line emission Other large telescopes have been built by Russia and USA for tracking deep-space probes

Unfilled aperture telescopes with large spacings of their component collecting areas, and consequently higher resolution than the filled aperture telescopes, have also been developed during this period These include the 1 mile synthesis telescope at Cambridge, the 1 mile cross telescope at Molongo, Australia, and the 1 9 mile diameter ring telescope at Culgoora, Australia

Radio astronomy is an integral part of the broad front of astronomy and has provided a strong impetus to many branches of the subject, particularly galactic structure and topics relating to cosmology The quest for more sensitive and versatile telescopes to pursue these studies continues The Dutch are well on the way towards completing an ambitious synthesis system at Westerbork, while the German radio astronomers are preparing to build a 330 ft fully steerable telescope near Bonn with a grant from the Volkswagen Foundation In Great Britain a move has been made towards the construction of a 400 ft telescope to operate at wavelengths down to 20 cm or shorter when the Science Research Council decided to negotiate a contract for a final design study American radio astronomers have been given support by the Dicke committee of the National Science Foundation in their request for a large synthesis system consisting of eight 130 ft telescopes and for the resurfacing of the 1,000 ft Aricebo telescope to enable it to work down to 10 cm wavelengths

An important part of the commissioning procedure for any new telescope is the accurate calibration of the position of its electrical axis and of the telescope gain at various wavelengths The book *Radioastronomical Methods of Antenna Measurements* by A D Kuz'min and A E Salomonovich provides the background for such measurements These two radio astronomers from the Lebedev Physical Institute, Moscow, have first hand experience in this field with their 72 ft telescope which has a good performance at wavelengths down to 8 mm They discuss in detail the basic concepts of receiver design and calibration in addition to those of aerial gain measurement Their treatment, which is rigorous throughout, also includes an estimate of the effects of atmospheric refraction and absorption, the effects of finite time constant and beam width on the measurement of radio sources

A useful parameter in evaluating the performance of the combined aerial and receiver system when used to detect weak signals as in radio astronomy or communications operations is the ratio of aerial gain to system noise figure This concept, which is hinted at in the book, underlines the compromise which has to be struck between highest aerial gain of a given reflector and the consequent contribution to system noise by the aerial spillover With the advent of high performance low noise receivers operating at a few tens of degrees Kelvin input noise temperatures, this parameter is a fundamental factor in aerial design

This book has concentrated entirely on the calibration of filled aperture radio telescopes and has shown how radio sources can be used for this purpose There are related problems in setting up and calibrating non-filled aperture telescopes These instruments provide the ultimate in accurate angular location Now that very long baseline interferometers have been successfully established the process can be inverted to derive the interferometer baseline to a fraction of a wavelength from astronomical observations These measurements could be of geophysical importance

The translators have performed a valuable service in making this excellent book available in the English language They have included many explanatory footnotes to the text There are, however, traces of literal translation in some passages as well as many typographical errors in the text, including a misleading factor of 10^2 in Table 4 which gives the flux density of radio sources suggested for calibration purposes These are not major blemishes in a book which will be found useful to those working in radio astronomy and in communications systems R D DAVIES

ENGLISH FOR GERMANS

Dictionary of Science and Technology, English–German
Compiled and arranged by A F Dorian With the co-operation of L Herzbruch Pp 1,238 (Amsterdam, London and New York Elsevier Publishing Company, 1967) 180*s*

THIS English–German dictionary which, so the blurb says, includes reference to the most important terms from 128 different fields of science and technology, should be very useful to anyone having to translate technical English into German The German scientist reading a vitally important English paper, if he does not speak English already, which he probably does, should be pleased to have recourse to this single volume covering all branches of physical and biological science So should the translator busy rendering complex scientific English prose into German, or anyone else who needs to know the German equivalent of the many words which fill the seven centimetres between the covers of this book

Each word has an explanation of the branch of science from which it comes, together with its translation, for example tranquillizer [med] Beruhigungsmittel *n*, rudderhole [naut] Ruderstockoffnung *f*, hidradenitis [med] Schweissdrusenentzundung *f* There are also often explanations of the meaning of the words, for example mind [gen] (abstract term for any form of collective intelligence) Sinn *m*, Gemut *n*, hexachloroethane [chem] (a rubber accelerator and camphor substitute in celluloid manufacture) Hexachlorathan *n*, Perchlorathan *n* These explanations might be of more use to readers if they were in German, after all, the English translator is more likely to know what the English words mean than is the German scientist to be able to understand the explanation

Revised and enlarged editions of the dictionary are to follow every eight years to keep the work up to date, and the German–English volume will appear this year to complete a useful set MARY LINDLEY

University News :

Birmingham

Dr T P Whitehead has been appointed professor of clinical chemistry (part-time).

Glasgow

Mr W Bryan Jennett, a consultant neurosurgeon with the Institute of Neurological Science, has been appointed to the new chair of neurosurgery.

London

Professor K W Keohane, professor of physics at Chelsea College of Science and Technology and co-ordinator of the Nuffield Foundation Science Teaching Project, has been appointed to the chair of science education tenable at that college The title of professor of animal behaviour has been conferred on Dr J S Kennedy, in respect of his post at the Imperial College of Science and Technology

Reading

Dr G. W. Series, at present university lecturer in the University of Oxford, has been appointed to a chair of physics

Strathclyde

Dr Edward Eisner, of the Mechanical Research Department of Bell Telephone Laboratories Inc , New Jersey, has been appointed to the new chair of applied physics

Appointments

Dr L C F. Blackman has been appointed director-general of the British Coal Utilization Research Association

Mr J N R Jeffers, at present head of the Statistics Section of the Forestry Commission, has been appointed director of the Nature Conservancy's Merlewood Research Station at Grange-over-Sands, Lancashire

Mr Donald F Moore, formerly deputy federal co-ordinator for meteorological services and supporting research, has been appointed assistant administrator for plans and programmes of the Environmental Science Services Administration, US Department of Commerce

Meetings

Protein Structure and Function, January 22–23, University of Miami (Dr E L Chambers, University of Miami School of Medicine, P O Box 875, Biscayne Annex, Miami, Florida 33152).

Power Group Winter Meeting of the Institute of Electrical and Electronics Engineers, January 28–February 2, New York (Institute of Electrical and Electronics Engineers, 345 East 47th Street, New York, NY 10017)

Rheumatism, February 17–21, Bombay (Dr M M Desai, c/o Indian Rheumatism Association, Fateh Manzil, 457–459 Lamington Road, Bombay 4)

Fundamental Cancer Research, March 6–8, Houston (Dr Manley Mandel, Chief, Section of Molecular Biology, Department of Biology, M D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas 77025)

Modern Chemistry in Industry, March 11–14, Eastbourne (Society of Chemical Industry, 14 Belgrave Square, London, SW1)

New Approach to Machine Tools in Relation to the Components They Make, March 20, Institution of Mechanical Engineers (R S Glynn, Head of the Conference Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, SW1)

Thermodynamics and Fluid Mechanics, March 27–29 University of Bristol (Groups Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, SW1)

Heat Transfer and Fluid Dynamics of Near Critica Fluids, March 27–29, Bristol (The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1)

Surface Chemistry of Oxides, March 28–29, University of Nottingham (Dr G C Parfitt, Department of Chemistry University of Nottingham, Nottingham)

Thermophysical Properties, April 1–4, University of Maryland (Mr J R Moszynski, Symposium Chairman Department of Mechanical and Aerospace Engineering University of Delaware, Newark, Delaware 19711).

Cell Biology, April 4–5, Middlesex Hospital, London (Dr L M Franks, Secretary-Treasurer, British Society for Cell Biology, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC1)

Thermodynamics, April 8–10, Manchester (Dr H A Skinner, chairman, c/o University of Manchester, Oxford Road, Manchester 13)

Vacuum Congress, April 17–19, Reynold Building of the University of Manchester Institute of Science and Technology (Mr J Yarwood, Technical Secretary of the Congress, 47 Belgrave Square, London, SW1).

Genetics and Breeding of *Streptomyces*, May 31–June 2, Dubrovnik (Dr M Alačević, Secretary, Symposium on Genetics and Breeding of *Streptomyces*, Faculty of Technology, University of Zagreb, Zagreb, Pierottijeva 6/IV, Yugoslavia)

Photosynthesis Research, June 4–8, Freudenstadt (Black Forest) (Professor H Metzner, International Congress of Photosynthesis Research, c/o Institut fur Chemische Pflanzenphysiologie der Universität-Tubingen, 72 Tubingen, Germany)

Vacuum Microbalance Techniques, June 17–18, Technological University of Eindhoven (Pat Gaskins, Secretary, Seventh Conference on Vacuum Microbalance Techniques, 7500 Jefferson Street, Paramount, California 90723)

Precision Electromagnetic Measurements, June 25–28, Boulder Laboratories of the National Bureau of Standards (George Goulette, Bureau of Continuation Education, 328 University Memorial Center, University of Colorado, Boulder, Colorado 80302)

Erratum. In the communication "Taylor Vortices with Eccentric Rotating Cylinders" by J A Cole (*Nature*, 216, 1200, 1967) the reference mentioned in the first and eighth lines of the third paragraph and in the second line of the last paragraph should be No 3 and not No 2

Erratum. In the article "Site of Attachment of Retinal in Rhodopsin" by Deric Bownds (*Nature*, 216, 1178, 1967) the last sentence of the fifth paragraph should read "They contain rhodopsin in a large micelle with digitonin". In the seventh line of the seventh paragraph 100° should read 110°

Erratum In the communication "Density Distribution Analysis of Lymphocyte Populations' by Ken Shortman *et al* (*Nature*, 216, 1227, 1967) the first lines of the legends of Figs 4 and 5 should refer to cells which form haemolytic antibody and not haemolytic cells which form antibody In the following communication "Density Distribution Analysis of Antigen Sensitive Cells in the Rat" by J S Haskill (*Nature*, 216, 1229, 1967) the references should be altered so that the first paragraph ends "The technique is that of sedimentation to equilibrium in density gradients of bovine serum albumin[7,8]" In the rest of the communication references mentioned in the text should read 9 for 8, 10 for 9 and 11 for 10.

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 8

Institution of Electrical Engineers (at Savoy Place London, WC2), 5 30 p m—Discussion Meeting on "Domain Originated Functional itegrated Circuits 'DOFICS' ", opened by Mr C P Sandbank

Institution of Electrical Engineers (at Savoy Place, London, WC2), 5 30 p m—Discussion Meeting on "Logarithmically Periodic Aerials", pened by Mr M F Radford

Society of Chemical Industry, London Section (joint meeting with ie Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, W1), at 5 30 p m—Mr H W Vallender "Whither Detergents"

Monday, January 8—Tuesday, January 9

Society of Chemical Industry, Pesticides Group, and the Phyto-hemical Society (at the School of Pharmacy, Brunswick Square, London, VC1)—Symposium on "Plant Growth Regulators"

Tuesday, January 9

Chemical Society, Nucleotide Group (in the Chemistry Department, Jniversity of Birmingham), at 10 30 a m—Inaugural Meeting

Institution of Electrical Engineers (at Savoy Place, London, WC2), t 5 30 p m—Discussion Meeting on "Electrical Signals for Data Acquisition nd Transmission—What Form Should They Take?", opened by Mr R E ischbacher

Institution of Mechanical Engineers, Automobile Division and Ianipulative and Mechanical Handling Machinery Group (at 1 Bird-age Walk, Westminster, London, SW1) at 6 p m—Mr H C Cox and Mr E Norman "The Design of Automobile Door Latches"

Institution of the Rubber Industry, London Section (at the Eccleston Hotel, Victoria, London, SW1), at 7 p m—Mr G Lambsdale "Overcoming Communication Problems in Process Industries"

Tuesday, January 9—Thursday, January 11

Chemical Society (in the Physical Chemical Laboratory, South Parks Road, University of Oxford), at 9 a m daily—International Symposium on "Valence and Reactivity"

Wednesday, January 10

Mineralogical Society (in the Department of Geology and Mineralogy, The University, Parks Road, Oxford)—Meeting on "Choice of Methods in Geochemical Analysis"

Institute of Fuel, and the Institute of Marine Engineers (at 76 Mark Lane, London EC3)—Symposium on "Combustion in Marine Boilers" *

Geological Society of London (at Burlington House, Piccadilly, London, W1), at 5 p m—Mr J G Jones "Intraglacial Volcanoes of the Laugarvatn Region, South-West Iceland", Dr R C Selley "Near-Shore Marine and Continental Sediments of the Sirte Basin, Libya"

Institution of Electronic and Radio Engineers (at 8–9 Bedford Square, London, WC1), at 6 p m—Professor J Merhaut "Some Aspects of Electrostatic Loudspeakers"

Institution of Mechanical Engineers Applied Mechanics Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m—Discussion Meeting on "Current Problems in the Pressure Vessel Field"

Institute of Information Scientists (at Knightway House, 20 Soho square, London W1), at 6 15 p m—Dr A J Barrett "The Engineering Sciences Data Service"

Thursday, January 11

Institution of Electrical Engineers (at Savoy Place, London WC2), at 5 30 p m—Discussion Meeting on "Electrical Properties of Polyolefines", opened by Mr W Reddish

Institute of Petroleum (at 61 New Cavendish Street, London, W1), at 5 30 p m—Mr R G Henbest "Gas Turbines for Peak Load Generation"

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk Westminster, London, SW1), at 6 p m—Discussion Meeting on "Fuel Cells"

Friday, January 12

Chemical Society Protein Group (at the Geological Society, Burlington House, Piccadilly, London, W1), at 2 p m—Inaugural Meeting

Saturday, January 13

Bone and Tooth Society (at the Zoological Society, Regent's Park, London, NW1), at 10 a m—Meeting on "Comparative Bone Disease"

Monday, January 15

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m—Discussion Meeting on "Project Problems" opened by Mr A Draper

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Senior Lecturer in Ophthalmology—The Registrar, The University of Manchester, Manchester, 13 quoting Ref 192/67 (January 10)

Digital Computer Programmer (with previous programming experience and preferably mathematics to degree level)—The Principal, Lanchester College of Technology, Priory Street, Coventry (January 11)

Lecturer or Assistant Lecturer (preferably with research interests in some branch of operational research or computational statistics) in the Electronic Computing Laboratory—The Registrar and Secretary, The University Leeds, 2 quoting Ref 68/21/1 (January 12)

Head of the Department of Mathematics—Clerk to the Governing Body, Woolwich Polytechnic, Wellington Street, London, SE18 (January 19)

Senior Experimental Officer (with a degree or equivalent qualifications) in the Palaeoecology Laboratory, for duties which will include responsibility for the operation, maintenance and development of radiocarbon dating equipment—The Secretary to the Academic Council, The Queen's University, Belfast, Northern Ireland (January 20)

Lecturers or Assistant Lecturers in Biochemistry, Genetics, Plant Physiology, and Animal Behaviour—The Assistant Registrar (Establishment), The University of Sussex, Essex House, Falmer, Brighton, Bn1 9QQ, quoting Ref 856/5 (January 21)

Lecturer/Senior Lecturer (with an appropriate degree, training and experience in pasture agronomy, and preferably experience with tropical species) in Agriculture at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office) Marlborough House Pall Mall, London SW1 (London and Brisbane January 26)

Senior Lecturer/Lecturer in Organic Chemistry at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London January 30)

Assistant Lecturer/Lecturer (preferably with experience in enzymology and willing to develop investigations into the enzymology of lipids) in the Department of Biochemistry—The Registrar, The University, Liverpool quoting Ref RV/301 (January 31)

Lecturer in Civil Engineering (mainly for administrative duties) at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, January 31)

Lecturer in the Department of Experimental Pathology, Faculty of Medicine and Dentistry, University of Birmingham—The Assistant Registrar, Medical School, Birmingham, 14 (January 31)

Lecturer (with previous research and/or teaching experience in the field of physical chemistry and a Ph D or equivalent qualifications) in Physical Chemistry at the University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall London, SW1 (Australia and London January 31)

Research Fellow in Neurochemistry (Biochemistry) at the John Curtin School of Medical Research, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall London, SW1 (Australia and London, January 31)

Chair of Chemical Pathology at St Bartholomew's Hospital Medical College—The Academic Registrar (A), University of London, Senate House, London, WC1 (February 2)

Senior Lecturer or Lecturer in the Department of Zoology, University of Khartoum—The Secretary, The Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, WC1 (February 13)

Senior Lecturers or Lecturers (2) in (*a*) Modern Algebra, (*b*) Topology, or (*c*) Functional Analysis at the University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, WC1 (February 13)

Chair of Philosophy at the University of Tasmania—The Association of Commonwealth Universities (Branch Office) Marlborough House, Pall Mall, London, SW1 (Australia and London, February 19)

Lecturer (experimental or theoretical physicist with experience in nuclear structure research) in Nuclear Physics—The Registrar, King's College (University of London), Strand, London, WC2 (February 28)

Assistant or Associate Professor in Mammalogy—Professor H E. Welch, Head of Zoology, University of Manitoba, Winnipeg, Canada

Assistant Professor or Associate Professor to assume responsibility for the development and operation of undergraduate laboratories and lecture demonstrations—The Chairman, Physics Department, University of Alberta, Edmonton, Alberta Canada

Biochemist, Basic Grade (with an honours degree in chemistry or biochemistry or equivalent qualifications)—The Group Secretary, Wolverhampton Hospital Management Committee, Royal Hospital, Wolverhampton

Graduate Research Assistant (preferably with previous experience in the field of tissue culture) to work in an organ culture laboratory—The Secretary, St Mary's Hospital Medical School Paddington London W2

Lecturers and Assistant Lecturers in Mechanical Engineering—The Registrar The University of Manchester, Manchester, 13

Research Assistant for work on cell culture using foetal and adult haemopoietic tissue—Dr G D Pegrum, Department of Haematology, Charing Cross Hospital Medical School, 62–65 Chandos Place, London, WC2

Research Assistant in the Department of Medicine for work involving the investigation of metabolism of collagen in bone disease—Professor J. Anderson, Department of Medicine, King's College Hospital, Denmark Hill, London, SE5

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Royal Observatory, Edinburgh Publications—Vol 6, Nos 2 and 3 The Wavelength Dependence of Interstellar Obscuration of Wolf-Rayet Stars By V C Reddish Observations of Interstellar Reddening IV Results for Region in Cepheus By N Nandy Pp vi + 15–23 (Edinburgh and London H M Stationery Office 1967) 8*s* net [2010

National Research Development Corporation Eighteenth Annual Report and Statement of Accounts for the year ended 31st March 1967 together with the Comptroller and Auditor General's Report on the Accounts Pp 70 (London H M Stationery Office, 1967) 5*s* 9*d* [2310

Ministry of Overseas Development Report of the Anti-Locust Research Centre for 1966 Pp 56 (London H M Stationery Office, 1967) 7*s* net [2310

Drug Addiction Pp 32 (London Office of Health Economics 1967) 2*s* 6*d* [2310

Ministry of Agriculture, Fisheries and Food Bulletin No 151 A Manual of Carnation Production Pp vii + 158 + 8 plates (London H M Stationery Office, 1967) 12*s* 6*d* net [2310

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Museum School Services Prepared by the Group for Educational Services in Museums Edited by Francis W Cheetham Pp 48 (London The Museums Association, 1967) 4*s* 6*d* [2510

Philosophical Transactions of the Royal Society of London Series B Biological Sciences No 782, Vol 253 (26 October, 1967) The Visceral Nerves of *Octopus* By J Z Young Pp 1–22+plates 1–8 (London The Royal Society, 1967) 17*s* 6*d* , $2 60 [2610

The Wellcome Trust Its Origin and Functions Pp 20 (London The Wellcome Trust, 1967) [2610

Other Countries

AFOSR Research The Current Research Program and a Summary of Research Accomplishments Pp vii+231 (Arlington, Virginia Air Force Office of Scientific Research, 1967 Available from Clearinghouse for Federal Scientific and Technical Information, U S Department of Commerce, Springfield, Virginia) $3 [2010

Australia Commonwealth Scientific and Industrial Research Organization Bulletin 286 A World Review of Introduced Species of Termites By F J Gay Pp 88 Annual Report of the Division of Tribophysics, 1966–67 Pp 21 (Melbourne Commonwealth Scientific and Industrial Research Organization, 1967) [2310

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Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Fisheries and Oceanography, 1966–67 Pp 49 (Cronulla, N S W Commonwealth Scientific and Industrial Research Organization, 1967) [2316

India Council of Scientific and Industrial Research, Annual Report of the Central Building Research Institute, 1966 Pp viii+96 (Roorkee, U P Central Building Research Institute, 1967) [2310

United States Department of the Interior Geological Survey Bulletin 1063-K Geology of the Hot Springs Quadrangle, Fall River and Custer Counties, South Dakota By Don W Wolcott Pp iv+427–442+plate 18 Bulletin 1063-L Geology of the Cascade Springs Quadrangle, Fall River County, South Dakota, By Edwin V Post Pp v+443–504+plates 29–31 Bulletin 1244-I The Lincoln Creek Formation, Grays Harbor Basin, Southwestern Washington By Helen M Beikman, Weldon W Rau and Holly C Wagner Pp iii+11–14 $0 15 (Washington, D C Government Printing Office, 1967) [2310

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Pan American Health Organization Scientific Publication No 15 Patterns of Urban Mortality Report of the Inter-American Investigat of Mortality By Ruth Rice Puffer and G Wynne Griffith Pp xiii+35 (Washington, D C Pan-American Health Organization, Pan Americ Sanitary Bureau, Regional Office of the World Health Organizatio 1967) [25

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Bulletin of the Museum of Comparative Zoology, Harvard University Vol 136, No 1 The Theridiid Spider Fauna of Chile By Herbert W Levi Pp 1–20 Vol 136, No 2 Habitat Observations, Records, and New South American Theridiid Spiders (Araneae, Theridiidae) By Herbert W Levi Pp 21–38 Vol 136, No 3 The Tertiary Lorisiform Primates of Africa By George Gaylord Simpson Pp 39–62 Vol 136, No 4 The Ameghinos Localities for Early Cenozoic Mammals in Patagonia By George Gaylord Simpson Pp 63–76 Vol 136, No 5 A Study of the Effects of Expatriation on the Gonads of Two Myctophid Fishes in the North Atlantic Ocean By William T O'Day and Basil Nafpaktitis Pp 77–90 (Cambridge, Mass Harvard University, 1967) [3010

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The Senate of the Queen's University of Belfast invites applications for a Senior Lectureship or Lectureship in the Department of Pure and Applied Physics, School of Physics and Applied Mathematics, from October 1 1968 Applicants should have research experience (experimental or theoretical) in some branch of photon physics such as lasers, photo-electronics, image tubes, communications and information processing or physical optics Salary range for a Senior Lectureship is £2,605 to £3,415 plus contributory pension rights under the F.S S U and for a Lectureship £1,470 to £2,630 plus F S S U Initial placing on the salary scales will depend on qualifications and experience Applications should be received by February 5, 1968

Further particulars may be obtained from G R Cowie, M A, LL B J P, Secretary Queen's University, Belfast, BT7 1NN, Northern Ireland (25)

UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF ELECTRICAL ENGINEERING AND ELECTRONICS

EXPERIMENTAL OFFICER

Applicatons are invited for the post of Experimental Officer in Electronics The duties attached to this post involve the operation and maintenance of a low energy electron diffraction apparatus Candidates should have a good knowledge of physics and previous experience of the fields of high vacuum techniques or electronics circuits is desirable

Commencing salary according to qualifications and experience in the scale £920 to £1,525 per annum Federated Superannuation System for Universities

Application forms and conditions of appointment may be obtained from the Registrar of the Institute, Sackville Street, Manchester 1, to whom completed applications should be returned by February 29, 1968 (47)

THE DEPARTMENT OF BIOLOGY, UNIversity of Calgary, Calgary, Alberta, Canada has Graduate Student Assistantships available in the areas of Comparative Animal Physiology, Developmental Animal and Plant Biology, Environmental Biology, Genetics Microbiology, and Plant Taxonomy Graduate Teaching Assistantships range from $1,840 to $2 800 for eight months (September to April) Graduate Research Assistantships range from $2,400 to $2,800 for twelve months Fees are remitted and travel assistance to a maximum of $250 is available Students holding eight-month assistantships are eligible to compete for Inter-Session Bursaries ($1,000) for the summer period —Inquiries should be addressed to the Head of the Department well before February 15, 1968 Completed applications must be received by March 1, 1968 (17)

THE DEPARTMENT OF PHYSIOLOGY and Pharmacology, Ontario Veterinary College University of Guelph, Guelph, Ontario, Canada requires four faculty members to meet growing teaching and research needs Persons interested in the following positions are invited to send curriculum vitae, grade transcripts, list of publications, and special qualifications and interests to Dr H G Downie, Professor and Head of the Department

1 D V M (or equivalent) with Ph D in Pharmacology Duties include participation in the team approach to teaching undergraduate and graduate Pharmacology, and research in areas of own interest Rank Associate Professor

2 D V M (or equivalent) with Ph D in Pharmacology, Toxicology, or Biochemistry Duties include participation in the team approach by teaching undergraduate and graduate Toxicology and Pharmacology, administrative supervision of a veterinary toxicology testing laboratory, and research and development in forensic analysis Rank Associate Professor

3 D V M (or equivalent) with Master's degree in any related science Duties include participation in the team approach to teaching undergraduate Pharmacology The candidate would be expected to pursue graduate work at the Ph D level in Pharmacology Rank Lecturer

4 PHYSIOLOGIST with Ph D qualification Duties include instruction in Physiology to Physical Education undergraduates and participation in a laboratory course on Human Physiology Consideration will be given to those individuals having a special research interest in respiratory or cardiovascular physiology Rank Assistant Professor

Moving expenses up to $1 000 will be provided for each position All salaries are competitive with industry (16)

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Do the Universities have a Case?

Ever since the University Grants Committee was told two months ago what money there would be to spend in each of the next five academic years in Britain, individual universities have been complaining that they will not be able to make ends meet without serious and even crippling economies And on the face of things, of course, there is something in what they say. The rate of grant will be increased more or less in proportion with the increase in student population to be expected in the immediate future, but there is nothing to suggest that in the five years immediately ahead the universities will have the opportunity to make good some of the more glaring deficiencies of the present system—the lack of a research tradition in the newer institutions and the general skimping on postgraduate teaching which is now becoming commonplace Unfortunately, however, the universities are their own worst enemies when it comes to discussions of financial matters and in the past few weeks have foolishly and even wrongly declined to publish factual information about the prospects for the coming quinquennium, which is the only means by which the outside world can judge whether the complaints now being made are substantial and not just the public irritation of people whose budget applications have been cut down to size

The circumstances are these The University Grants Committee announces at the beginning of each quinquennium how much money it will have to spend on financing the universities, but it is for universities themselves to decide whether to make public their own share or prospective share On this occasion, the Universities of Oxford and Cambridge have made a clean breast of their prospects, partly no doubt because their forms of university administration make it necessary that information like this should be publicly conveyed to the academic body Elsewhere, however, universities behave as if this information is and should be secret to themselves Sometimes a vice-chancellor in making a public complaint will let slip the fact that the allocation for the coming year is 1 per cent, or 2 per cent, or x per cent greater than in the present Sometimes a university will agree that this information should be made available after it has been through the mill of discussion in the senate Sometimes universities behave as if they intend to keep the details secret for as long as possible in much the spirit that private individuals choose not to make public information about their bank accounts The absurdity, of course, is that the University Grants Committee will in due course make public the details of its allocations to all the universities In future, no doubt, the decision that the universities as a whole should be made publicly accountable will lead to the publication of much more detailed information about the working of universities, and about the methods by which they obtain and spend their money, than has ever been available in the past The universities may enjoy trying to shut the stable door, but they ought to know that the horse is certain to bolt in the end

There are several reasons why these policies are mistaken and should be changed For one thing, the financing of the universities has become a matter of immediate public importance and it is wrong that the University Grants Committee should seek to perpetuate the fiction that its dealings with individual universities are entirely private matters After all, the committee is the first to solicit public support or at least discussion at times when it feels that too little money is being spent on the development of the universities The time has also come when it should recognize that the country as a whole has a right to ask not merely whether the scale of the whole operation is right, but whether more detailed aspects of the policy on the universities are being handled properly Are some categories of universities getting too large or too small a share of the cake ? Is the University Grants Committee itself as zealous as it should be in trying to spend as much of its money as possible in those institutions which have a proven record for efficiency ? Is the geographical distribution of university money fair ? Are the colleges of advanced technology getting a fair crack of the whip ? The time to ask these questions is when decisions are being taken about the allocation of funds When the money has been spent, it is too late It is true that the University Grants Committee may well argue that a bald statement of how it proposes to allocate its funds may be misleading, for there may be considerable fluctuations from year to year in the cost of operating individual universities which arise not from broad issues of policy but from accidental causes, one university may have to equip a new laboratory, for example, and the cost may fall predominantly in one year or one quinquennium But this is not an argument for no information but, on the contrary, a reason why the announcement by the University Grants Committee of its plans for the coming quinquennium should be accompanied by a full statement of the reasons why its decisions have been made The cryptic character of its customary behaviour is merely one of the reasons why politicians and others have been provoked into believing that a much tighter method of controlling the universities is overdue

If the coyness of the University Grants Committee is mistaken, that of individual universities is downright self-destructive. For one thing, it is a means of hampering intelligent public discussion of the running of universities, both among the academics most directly concerned and in the outside world It is true, of course, that those who belong to the right committees will sooner or later be given detailed statements of how much or how little money there is to spend, but this is no substitute for the awareness among the teaching staffs of universities of the ways in which their operations are necessarily circumscribed by financial considerations. Internally, the consequences can be quite serious. What sense is there in universities' attempts to operate more efficiently and economically if those who will have to save the pennies are not kept fully informed? And how can academics be expected to form sensible opinions about the long-term strategy for the development of the university to which they belong if the details of now much money there is to spend are regarded as the privileged possession of a handful among them? Outside the universities, however, the consequences of this coyness are even more alarming. In effect, the universities by their pointless gentility are prevented from making the full-blooded claim on public sympathy which their objectives would justify.

What is to be done? The universities themselves could do a lot to help and, if the University Grants Committee is unwilling to make an intelligent comment on quinquennial allocations as these are made, the Committee of Vice-Chancellors and Principals could well take over that task. But in the long run, the real need is somehow to suppress the feeling among university administrators and even academics that there is some virtue in keeping silence about the ways in which money is spent. So long as universities were private institutions and so long as the numbers of people with whom they dealt were so small that their influence in the economic life of Britain could be neglected, this may have been a tenable position. But the need now is for a much more open conduct of affairs. Since it is public money which is being spent, the universities would be prudent to remember that if they do not voluntarily provide information which is of great public importance, matters may be taken out of their hands.

Public Transport should be Practicable

THIS looks like being a decade in which governments will be preoccupied with issues of public transport, at least in Britain and the United States. After many years of speculation and timid experiment in urban planning, it has now been borne in on a great many people that it is not feasible to plan the shape of cities without at the same time planning the transport networks by which people will move or seek to move from one place to another. In Britain, the issue has been made controversial as well as topical by the energetic way in which Mrs Barbara Castle, Minister of Transport for more than a year, has been reshaping the administrative structure of public transport. The starting point for the policies which will obviously flow freely in the years ahead is the White Paper *Public Transport and Traffic* (HMSO, 3*s* 9*d*). Even Mrs Castle's critics have been compelled to admit that this is a formidable and even admirable document. One of its striking and least assailable conclusions is that public transport should be organized on a local basis, not centrally, which will frequently mean the transfer of large parts of existing transport undertakings to local passenger transport authorities, but which will also on some occasions entail that bus companies at present operated by comparatively small local authorities will be swallowed up by larger units. One important innovation is that passenger transport authorities will be required to assume some responsibility for the pattern of railway services, particularly where these are widely used by commuters, and no doubt the time will come when these passenger transport authorities find themselves paying subventions to the central railway authority for the cost of maintaining public services which would otherwise be uneconomic. It is, of course, entirely sensible that the costs of subsidies like this should be felt directly at the local level at which they arise, and that machinery should exist for making comparisons between the social benefits that can be obtained by investing a sum of money in alternative means of transport. The doubts which Mrs Castle's scheme arouses are not doubts of principle but of practice. What prospect is there that the passenger transport authorities established at a local level will be able to seize the opportunities which transport technology now promises? How well will they be able to integrate their policies for the development of public transport with policies for the shape of urban planning under the control of other authorities? How far will they be able to rise above the parochial considerations which bedevil local government in Britain?

The trouble is that there are now a great many conflicting claims on the attention of those who would more effectively exploit the technology of modern transport. The Ministry of Transport itself has recently been acquiring a commanding expertise in the economics of transport systems, and there is obviously a great deal to be done in the improvement of the efficiency of transport by persuading the users of the transport system to make demands on it which can more easily be satisfied. The danger here, however, is that too great a concern for pricing systems may lead to policies which are aimed at "pricing the motor car off the roads" when the real objective should be to make the most efficient use of the transport system as a whole—trains, buses and private cars included. The force of this argument was well put recently by the Standing Joint Committee on the Buchanan Report which is being sponsored by the engineering institutions.

United States Science Examined

As this issue of *Nature* went to press, the OECD was beginning its confrontation with the United States authorities on the report about the condition of science in the United States which has been prepared by the team of examiners which has been at work on the problem for the best part of two years The study is the eighth to have been carried out in the series of studies of national science policies in the 21 member nations of the OECD, but the chances are great that the special circumstances of the United States will bring up questions which earlier reports in the series have not raised Prominent among these are likely to be questions related to what is sometimes called the technology gap—a cause known to be close to the heart of the OECD. Not merely is there likely to be a serious attempt to draw from American experience in the management of science and technology, but there is also likely to be some attempt to determine the wisdom of trying to follow in Europe some of the developments which have been prominent in the United States in recent years

The OECD report, which is the basis for the discussions being held in Paris, has been prepared partly by the secretariat of the OECD and partly by a panel which consists of Dr H G B Casimir, director of the Philips Research Laboratories at Eindhoven; Mr Theo Lefevre, a former prime minister of Belgium, M Pierre Masse, chairman of Electricité de France, and Professor C H Waddington of the University of Edinburgh A full summary of the review of United States Science Policy, together with an account of the confrontation in Paris, will appear in *Nature* next week.

Drugs' Dark Days

The United States pharmaceutical industry must be suffering from a bad case of nostalgia The good old days of the nineteen-fifties—when wonder drugs brought wondrous profits and gratitude from the public as well—are probably gone for ever Instead, the federal government is getting more and more inquisitive about the whole business of medicines how much they cost to make, whether they perform as advertised, whether those with melodious trade names are better healers than those with clumsy generic names Two Senate investigations are now in progress, one looking into possible abuses in the diet pill industry, another into the pricing policies of drug manufacturers The total government spending on drugs by federal, state and municipal authorities continues to increase—it is now $200 million a year—so that the authorities are now unlikely to relax their vigilance

The aura of suspicion created by Senate investigators was even blamed by an executive of one of the pharmaceutical firms found guilty last week for conspiring to control the production and sales of tetracycline Certainly the jury had a lot to contend with—patent law, chemical formulae, patent and anti-trust laws were all freely quoted during the trial Their finding that three companies—Charles Pfizer and Company, American Cyanamid Company and the Bristol Myers Company—are guilty of criminal conspiracy in monopolizing the manufacture of tetracycline until 1961 has shaken the industry The decision, if it is not reversed, opens the door to lawsuits from major purchasers of tetracycline from the defendants The City of New York and the State of Florida have already filed their suits in the hope of recovering the treble damages allowed by the Sherman Anti-Trust Act against companies convicted of monopoly The sums eventually paid back to irate customers could exceed those awarded a few years ago when some American electrical companies were convicted of price-fixing

The industry, not surprisingly, feels hard done by The big laboratories, as the argument goes, bear the costs of all the research and, as soon as they come up with something useful, smaller companies rush in, copy the medicine and sell it at cut-prices Are the better-known branded products more efficacious? The big companies say they are, and many doctors, including some from the American Defense Department, which has been buying low-priced tetracycline from Italy rather than the expensive domestic brands, agree The Food and Drug Administration set up a special committee to study the problem of efficacy and equivalence, and the job is harder than anyone had thought. After eighteen months, the committee has asked for more time

The debate has really just begun Last year in Congress Senator Long of Louisiana tried to make it mandatory for all public agencies in the medicine-buying business to buy drugs by their chemical rather than by their trade names He failed, but a vestige of his wish survived in the form of a directive to the Department of Health, Education and Welfare to ask all state governments to promise that the medicines that they were buying for people on relief rolls was purchased at the lowest possible cost At the very least, this means that more attention will be paid to the question of getting best buys The price differences between what are said to be equal quantities of the same compound are staggering The Senate is hearing that public agencies were charged $2 09 in one case and $160 in another for the same quantity of reserpine tablets, and the Justice Department's complaint in the tetracycline case was that antibiotic tablets costing $2 or $3 a hundred to produce were being sold to the customer at $51 a hundred

Meanwhile, the FDA is getting ready to make public the results of its inquiry into the efficacy of medicines sold in America between 1938 and 1962, when stricter standards were established The advance reports say that about 10 per cent of the old favourites in the medicine cabinet could be denounced as worthless and some may have to be withdrawn from sale

Computers More Expensive

International Business Machines last week announced increases in the cost of both buying and renting its computers in Britain Renting an IBM machine will cost 10 per cent more, while buying one will cost up to 10 per cent more The hiring of time at computer bureaux and data centres will go up by between 3 and 10 per cent, and maintenance charges will increase by 4 per cent These increases, a response to the devaluation of sterling, may well be duplicated by other computer companies which use a large proportion of imported parts—a group which includes every computer manufacturer in Britain, with the possible exception of International Computers and Tabulators

IBM has also taken the opportunity of announcing a new computer, as part of the 360 series The new model, the 360/25, falls between the smallest machine in the IBM range, the 360/20, and the next up, the 360/30 It will offer users of the 360/20 a chance to increase their computing power without having to learn a new language, and competitive pricing should help to encourage buyers These moves come at a time of unprecedented weakness for IBM in the British market—it has even been suggested that IBM's share of the British market has fallen below 30 per cent Whether or not this is true—IBM always refuses to divulge its market share—it is certainly true that Britain is proving a tougher market than some others Because IBM does most of its production for Europe in France and West Germany the effects of devaluation are likely to hit the company harder than others Honeywell, which produces computers at its factory in Scotland, will be cheered by devaluation ICT, on the other hand, still views the effects of devaluation with some suspicion Colonel A T Maxwell, chairman of the company, presenting the annual report for 1967, said that the effect of the measures was still not clear "We are inevitably faced with higher costs, but we shall so direct our affairs as to contain these as much as possible We are also seizing the opportunity of increasing our exports "

Only one other computer manufacturer, Burroughs, has so far announced price increases of its own, they will work out at an average of about 7·5 per cent So far, no other companies have followed IBM and Burroughs, but the betting is that some—perhaps including English Electric—will have to do so within the next few weeks

Policy for Research

The British Government will shortly be issuing a long-awaited White Paper on research policy At least one of the purposes of the White Paper will be to define ways in which the research establishments can help industry Because there is plenty of room for disagreement—and misunderstanding—between the ministry and the industries it is trying to assist, the Electronic Engineering Association has got in first by producing a memorandum which sets out its own views on what the relationship should be The report, which goes for discussion to the Development Council for the Electronics Industry next month, may differ in emphasis from the policy which the ministry must by now have agreed on So far, though, any clashes between the association and the ministry are hypothetical

The chief point which the report makes is that, when projects are jointly undertaken by government and industry, leadership should be very firmly in the hands of the industrial partners Captain R. A Villiers of the association points out that when research establishments were involved with military work they kept closely in touch with the military market, but the shift to civil work means that a new relationship with industry has to be worked out. He thinks that the ministry has much to learn by examining the records of other industrialized countries, which tend, among other things, to contract more work out to industry and to do less in the establishments The main part the establishments can play on their own, he thinks, is in more basic research work—like, for example, the work on oceanography at Harwell.

Another idea which Captain Villiers puts forward involves the actual transfer of people from the establishments to industry and vice versa Teams of people from the establishments should be able to join industrial firms, and teams from industry should be seconded to government establishments to pick up know-how To do this, he says, both sides must become more flexible about transferring pension rights But so far he seems confident that there is no clash of views between himself and the Government Others will await the White Paper before committing themselves so cheerfully.

Swiss Smelting

The Swiss Aluminium Company has submitted a tender to build an aluminium smelter in Britain The company says the smelter could be built in 2 5 years at a cost of between £18 and £20 million and a maximum capacity of 100,000 tons Other tenders have so far been made by Rio Tinto Zinc in conjunction with British Insulated Callender's Cables, by Alcan Aluminium of Canada and by British Aluminium British Aluminium is jointly owned by Reynolds Metal Company of the United States and by Tube Investments.

The high cost of electricity in Britain has hitherto prohibited the development of a native aluminium smelting industry of any size The decision by the British Government to invite tenders for a plant was made with the intention both of reducing imports and of creating new jobs in development areas. Tariffs being out of the question, the project is rendered commercially viable by the offer of cheap electricity and generous investment grants (see *Nature*, **216**, 109, 1967) The Board of Trade is prepared to grant these incentives to foreign and British companies without discrimination The Government envisages the construction of two plants with a total capacity of 240,000 tons a year It is hoped that the first will begin production in 1970 and the second in 1974

Sediments for Peace

The Parliamentary Group for World Government, which is based in London, seems well launched on its campaign to bring international law and order to the bottom of the deep oceans Earlier this week the group made public the membership of the committee which has been formed to study some of the problems of arranging that the resources of the deep oceans should be exploited "for the benefit of all mankind" The chairman of the committee is a barrister, Mr Maurice Bathurst, and the ordinary membership seems equally divided between lawyers, scientists and those with more general interests Lord Ritchie-Calder is perhaps most accurately described as the founder member of the movement, for he was one of the first to urge, a year ago, that there should be a United Nations agency empowered to exploit the resources of the oceans and to spend the proceeds on international peace-keeping forces Other members include Dr G E R Deacon, director of the National Institute of Oceanography, Commandant Cousteau, director of the Institut Oceanographique at Monaco, Dr T. Gaskell, of British Petroleum Ltd, and Mr Oscar Gugen of the World Underwater Federation Dr Thor Heyerdahl has promised his support to the committee, which has already met on two occasions and which is evidently prepared for a busy year ahead

By all accounts, the committee will have to divide its time between the discussion of general principles and the lobbying of other interested—or uninterested—parties. The response of the last General Assembly of the United Nations to the request of the Maltese delegation that there should be set up an agency to organize the exploitation of the ocean beds was to establish, on December 20, a sub-committee to study the problem. Advocates of the peaceful uses of ocean sediments are not sure whether this is merely a device for putting off a decision about the formation of an international agency, although it would have been unreasonable to expect that the General Assembly could create an agency which raises all kinds of new problems in international law, and which may be financially hazardous as well, without satisfying itself that there is a feasible task to be accomplished. Nevertheless, there are some, at least, who fear that a committee with more than a score of nations as members will serve as a splendid cloak behind which the nations which fancy their own chances in the exploitation of the sea will be able to block agreement.

At this stage, however, nobody seems inclined to make light of the problems. Strictly academic problems are formidable enough. How, for example, to distinguish between the continental shelf and the deep sea ? The committee has apparently given some thought to a measurement of the thickness of the crustal layer as a definition, though some oceanographers would no doubt have interesting reservations to make on that. There are also questions such as whether a treaty which reserved the right of ocean exploration to an international agency should cover the extraction of minerals from seawater as well. But technical problems are insignificant compared with the legal obstacles yet to be fully defined. Freedom of the high seas in the most literal sense is clearly in jeopardy. The question of what to do with any profits from international exploitation is obviously another teaser, although the advocates of an international agency seem at this stage more concerned that there should be profits of some kind than that there should be agreement in advance on the distribution of the profits between peace-keeping, aid for development and other good causes. The committee has a good point when it urges that if an international agency is to be formed at all this should be done before national activities have established awkward precedents. Plainly it is undismayed by the slow start there has been on ocean mining.

Unlucky Strike Again

Mr Robert L. Strickman, the inventor of a new type of cigarette filter, announced last week his intention of robbing Columbia University of the privilege of developing the filter. The university, to which most of the rights in the filter were assigned, seems likely to dispute this decision and an acrimonious battle through the courts may be in the offing.

Mr Strickman claims that his filter is much more effective than other types both in removing tar and nicotine and in allowing the taste of the tobacco to come through. Wishing to have the filter tested, developed and licensed by a non-profit institution, Mr Strickman offered the rights to Columbia University. In July of last year, Columbia conducted a press conference, the timing and manner of which were widely criticized, to announce its acceptance of Mr Strickman's offer (*Nature*, **215**, 339; 1967). The university was at that time about to launch a campaign for raising funds of $200 million, and the potential income from licensing the filter was undoubtedly a principal factor in its decision. Critics, however, questioned the propriety of an academic institution making money in this way, and pointed out the risk the university took in creating the impression that it condoned or thought innocuous the smoking of cigarettes.

The advisability of its decision apart, Columbia is clearly determined to put the filter through the most stringent tests it can devise. Here lies the point of disagreement with Mr Strickman, who considers that enough testing has been done and that Columbia should have already begun licensing the filter to cigarette manufacturers. Although Mr Strickman is pressing for the return of his rights, he still wishes Columbia to share in the profits from the sale of the filter. The question now is whether Columbia will be able to accept even that truncated connexion with the filter.

Underdeveloped Trade

The Overseas Development Institute has ventured into a new field with its guide to *The Less Developed Countries in World Trade* (ODI, 30*s*.), written by Michael Zammit Cutajar and Alison Franks and published this week. The institute has studied the two-way process of trade for the first time, having previously concentrated on the one-directional flow of official aid and private investment to less developed countries. The trading problems of the less developed countries were examined in detail at the three month United Nations Conference on Trade and Development (UNCTAD) in 1964, and a second UNCTAD conference on the subject is to begin in New Delhi on February 1. The institute, an independent non-government body which promotes wide development overseas, hopes that the study will provide background information on the trading problems of the underdeveloped countries which form the majority of UNCTAD. The UNCTAD secretariat has co-operated in the compiling of the guide, although the idea for it came from members of the institute.

Before giving world trade figures and details of the exports of less developed countries, the guide puts forward the fundamentals of economic theory to explain the need for trade. Development cannot be achieved efficiently by either trade or aid alone, and situations are described in which each is most appropriate.

In the 12 years to 1965, world trade has increased by 135 per cent, but during that time the share of the less developed countries has fallen by over 7 per cent, to less than 20 per cent of the total. When exports are divided into two groups—primary commodities and manufactures—the less developed countries are found to be dependent on the first group, which accounts for 86 per cent of their export earnings. Developed and less developed countries each export about 42 per cent of the world total of primary products, but when it comes to manufactures the development gap is clearly seen. Developed countries export 80 per cent of the world total, while the share of the less developed countries stands at 6 per cent. The guide discusses the current demand for primary products and gives details of international agreements which are in force. Eleven primary products, ranging from petrol and metals to tea and coffee, are discussed in detail in terms of the nature and importance of the product, countries of supply, importing areas and national production figures, and the state of the market. British, United States and Common Market policies for trade with the less developed countries are discussed with a mass of information on the trade of the countries concerned. The guide concludes with a résumé of the work of the UNCTAD board and committees, and makes three proposals—a cocoa agreement, a scheme for tariff preferences for less developed countries and a scheme for supplementary financial measures, which could well be important talking points at the New Delhi meeting next month.

No Change at Caltech?

THE annual report of the president of the California Institute of Technology for the academic year now past provides the usual detailed and absorbing account of the institute's recent achievements. Although, this year, Dr Lee DuBridge has much to say about the launching of the institute's public appeal for $85 million, his account of events does less than justice to the questions increasingly being asked among the faculty at Pasadena and their contemporaries elsewhere—is the pursuit of excellence, or pre-eminence, as Dr DuBridge calls it, in conflict with the educational needs of the undergraduates, now almost outnumbered by the faculty?

Dr Lee DuBridge, president of the California Institute of Technology.

Like many other institutions, the California Institute of Technology is the continuing victim of what is now called sophistication. Simply staying still costs an extra 6 or 7 per cent a year. The institute wants to spend some of the $85 million which it hopes to raise on new buildings, while some of it will be added to the endowment, which amounted to a comparatively modest $129 million in 1967. Within the running budget, which included expenditure of $30·35 million in 1967, federal research plays a prominent part. Thus the direct cost of federally sponsored research worked out at $11·22 million, compared with $9·24 for what is described as "instruction and research". In practice, the institute seems not too heavily dependent on any one agency for its funds—the US Navy provided $2·13 million and the US Air Force $1·74 million in 1967, with roughly comparable amounts from the Public Health Service ($3·21 million), the AEC ($2·69 million), the NASA ($2·17 million) and the NSF ($2·01 million). It should fare as well as any other institution if, in the months ahead, there is a sharp reduction of the scale on which federal funds are made available. Whether the institute will be affected indirectly by the straitened NASA budget in the years ahead is another matter, for annual expenditure at the Jet Propulsion Laboratory which is managed on behalf of federal agencies by the institute grew by less than 1 per cent (to $248 million) between 1966 and 1967, and could conceivably decline in the years immediately ahead.

Financial considerations have not, however, impeded the sustained if modest growth of the institute in the recent past. The faculty, for example, has grown from 515 to 600 in five years, with the number of full professors almost unchanged at 142 but with a rapid increase from 159 to 206 in the number of research fellows. The intake of undergraduates, by contrast, has been constant for some years at 200 or so, but outsiders will be a little surprised that the rate of application for entry is only five times as great. Drop-outs account for 39 per cent of the student body over a four year undergraduate course, though no doubt some of these people complete their undergraduate degrees elsewhere. No doubt the rate of dropping out will be taken merely as a proof that even the more rigorous processes of selection cannot be a cast-iron assurance of academic success. At Caltech, as the Swann Committee has found in Britain, however, academic success begets academic temperament. Thus the production of PhD degrees is running at about 100 a year, and has not yet been much affected by the increase of the graduate school from 660 to 770 in the past three years. In 1967, for example, 70 per cent of those graduating with PhD degrees found jobs at universities or at research institutions.

Decline of Bone Strontium

A CONTINUING decline of the concentration of radioactive strontium in human bones in the British Isles is recorded in the most recent of the reports on the subject by the Medical Research Council (*Assay of Strontium-90 in Human Bone*, HMSO, 3*s*.). The results are based on analyses carried out separately by the UK Atomic Energy Authority and by a group based on Scottish hospitals. Bone samples were obtained from the vertebrae of individuals dying between July and December 1966, and the results obtained by the two groups are shown in the following table.

	UKAEA		Glasgow	
	1965	1966	1965	1966
Newborn and stillborn	2·5	2·2	3·4	2·7
Under 5 years (excluding newborn)	6·7	5·1	8·2	6·5
6–23 months	9·1	6·2	11·0	8·3

This accords well with the measurements of the concentration of radioactive materials in the atmosphere and in rain which have now been published by the Health Physics and Medical Division of the AERE, Harwell. The report (HMSO, 7*s*.), which runs up to the middle of 1967, shows that the average concentration of strontium-90 in rainwater had decreased by roughly 40 per cent between the first half of 1966 and the first half of 1967. Because rainfall in 1967 was considerably less than in the previous year, the actual deposition of strontium-90 in the first half of 1967 was only 40 per cent of that deposited in the same period during the preceding year. By the end of 1966, it seems as if the rate of deposition of fresh strontium-90 had become comparable with and smaller than the rate of disappearance of deposited strontium-90 by means of radioactive decay, so that "for the first time", the "cumulative deposition began to decrease with time". The balance was reversed with the spring rains in 1967, but it is clear that an end is in sight to the accumulation of radioactivity in the soil.

All Quiet on the Quiet Sun

LIKE a great actress in later life, the organization of the International Years of the Quiet Sun is now well settled in the habit of making its final appearance. Formally, it is true, the great symposium at Imperial College last July marked the end of the period of co-ordinated or at least simultaneous observation. Now there has appeared the latest issue of *IQSY Notes*, the twenty-first, which is full of valedictory statements and which also contains the announcement that this issue will be the last. The next step is the publication of the *Annals of the IQSY*, which is being managed by the Royal Society. There will be admiration and even gratitude for the firm promise that the first of the eight or nine volumes in this series will appear early this year, and that publication of the whole series—which will include a record of the symposium last July—should be complete by the end of 1969. With luck, it should be possible to avoid overlap between the productions of the IQSY and of Dr Herbert Freeman's Commission on Solar and Terrestrial Physics, now in the process of gestation.

Although the latest *IQSY Notes* has no great surprises, it does provide some information about the financing of international operations like these. It is notable that the total cost of administering the IQSY for a period of 5½ years amounted to merely $173,000—a sum which had to cover the maintenance of the secretariat in London, the publication of manuals and the organization of meetings. The publication of the *Annals* is reckoned to cost a further $60,000. Most of the funds have been raised by voluntary contributions from the national committees of participating countries. The statement of the council goes out of its way to emphasize that "the payment of a contribution has never been a condition of adherence to the IQSY programme. Contributing and non-contributing committees have been treated exactly alike. . . . This was essential, since the prime objective of the scientific programme was to encourage the widest possible geographical participation in the programme. . . ." This decision was no doubt wise, for the list of contributors suggests that some participants have been rather slow in paying up. It is perhaps understandable that Taiwan and the Congo should not have paid after the first year or two. It is more surprising that Belgium, Finland, Israel, Rumania and Switzerland should not have contributed after a similar period. The Soviet Union had only paid two contributions out of six by October last year—a total of $10,000. Difficulties about the convertibility of the rouble (and of the rupee) are known to have been a complication in this connexion. But the IQSY seems to have managed well enough and, even if its scientific programme was inescapably a somewhat undramatic business, the organization may well have been able to establish standards of good management among international organizations.

More Medical Science

A SMALL reform in the organization of the British Medical Association seems likely to come about as a result of suggestions made by Dr Ronald Gibson, chairman of the council of the association. Dr Gibson has proposed that all the scientific and educational work done by the BMA should be brought under the umbrella of a central board. As well as the work of the present Committee on Medical Science, the board would provide for scientific and sociological work. But the hope expressed by Dr Gibson that the BMA could in this way "prove its right to be considered a forceful entity in the field of medical science" is hardly realistic. The proposal does not recommend any increase in the money which the BMA spends on research—last year it was £60,000—but simply concentrates on the reform of the committee structure. Nor is the suggestion particularly new—a similar idea was put forward some years ago. Dr Gibson has suggested that if the board is set up—a decision which the council of the BMA will have to take within the next few weeks—it might also sponsor some general sociological surveys. Topics put forward include drug addiction, the population explosion, nutrition, the stress diseases and the like.

There seems to be no reason why the council should not approve the suggestion, modest as it is. It may, however, put the BMA into a position where it could begin to influence medical research in Britain, given a greater financial outlay. A counterweight to the massive influence of the Medical Research Council would not be entirely unwelcome. The field where the BMA might be most influential, clinical research, has not so far had the same attention in the MRC as more basic studies, although this may change when the MRC Clinical Research Centre opens at Brent Hospital in 1970. In the BMA, the move reflects a feeling that the politics of medicine has in the past few years been allowed to obscure the more cerebral activities of the BMA. Having exhibited its taste and talent for political controversy, the argument runs, the BMA should now try to divorce itself somewhat from the image of a militant, if successful, trade union.

The American Medical Association itself turned to research for many of the same reasons several years ago. There was a feeling that the AMA had become too closely identified with the fight against the Medicare proposals, and too far detached from scientific research. But the move to support basic research, in the Institute for Biomedical Research at the AMA headquarters in Chicago, seems not to have been wholly successful. After only two years of existence, the institute is now to have its third director—George W. Beadle, the retiring president of the University of Chicago. (His predecessors were Dr Roy E. Ritts and Sir John Eccles.) The AMA has also decided to move the institute to a new site on the campus of the University of Chicago. Unless the BMA decides to spend a good deal more on research, however, it is unlikely to be troubled by problems such as these.

Doctors' Complaints

THE career structure of the medical profession in Britain is an important stimulus of the emigration of doctors and an effective deterrent to their return. This is one of the conclusions reached by an interview board sent by the Ministry of Health to the United States and Canada last October and which has now reported its findings. (*British Medical Journal*, i, 45; 1968.)

Between 1962 and 1964 the rate of emigration of British doctors to all countries was about 300 a year—more than one-sixth of the annual output of British medical schools in those years. This rate of emigration, which there is no reason to suppose will have diminished in subsequent years, was the goad to the board's departure. Having advertised its coming in various medical journals, it was able to interview 135 out of the 200 doctors who applied to it, with the purpose both of finding them jobs in Britain and of ascertaining why they had left and what impediments they saw to their return. Fifty of the doctors whom the board interviewed have already been offered, and have accepted, appointments in Britain and it is expected that more will do so.

The board discovered a fairly consistent picture of dissatisfaction with the National Health Service and its doings. General practitioners who in Britain had aspired to a specialist career gave as their reason for emigrating the lack of hospital facilities for doing major surgery and other specialized practice, and the waiting period for senior posts after the appropriate higher qualifications had been gained. Other reasons for emigrating were the paucity of senior academic posts in the United Kingdom and the relative scarcity of research funds. To the board's regret, it found that an emigrant's decision to leave was not always a purely personal one: "evidence of British consultants and professors advising and encouraging trainees to emigrate because of the uncertainty of their prospects and the rigidity of the National Health Service and academic structure in the United Kingdom was unfortunately forthcoming".

For those who wish to return to the United Kingdom, the most formidable obstacle is the insistence by the National Health Service that candidates attend an interview. If unsuccessful the candidate is left with his travelling expenses to pay, and if successful he is faced with removal costs of his dependants and effects as well. In this instance the board was empowered to reimburse such costs, which may account for much of its success in attracting doctors back. Other impediments to a return are the lack of information about jobs, the hostile attitude of the North American press, and its British element in particular, towards the National Health Service, and the lack of any accepted equivalence between the higher qualifications awarded in North America and in Britain.

One reason for the board's success may have been that it was less scrupulous in selecting candidates for interview than other interview boards have been. It recommends that the obstacles to return should be reduced, and that to diminish the rate of emigration in future the arrangements for career guidance of trainees should be improved and that more opportunities should be created for academic careers. The Minister of Health is now studying these recommendations.

The loss of 300 doctors a year is indeed serious, but should be seen in proportion to the numbers Britain gains from other countries. Last year almost 2,000 doctors from the Commonwealth alone applied to enter Britain. In 1965, the most recent year for which figures are available, there were 6,000 doctors working in National Health hospitals who had been born abroad—about 30 per cent of the total. Of general practitioners 2,600, or 12 per cent of the total, were born abroad. If every doctor were to practise in the country of his birth, Britain would not be the gainer. But this is not to decry any efforts that the ministry may now make to encourage the return of expatriates, and to allay the discontent of those who would follow them.

More on Asbestosis

THAT there are risks associated with asbestos is not now disputed. A review of the problem as it occurs in Britain has just been published as a memorandum by the Advisory Panel on Problems Arising from the Use of Asbestos (HMSO, 3*s*. 9*d*.). The advisory panel, set up in 1965 by HM Factory Inspectorate, is responsible for considering the use of asbestos in relation to the health of those directly or indirectly exposed to asbestos in their occupations. Over the whole country the panel estimates that about 20,000 persons are exposed to asbestos in the use or handling of the fibres. The figure is probably higher than this; people can easily be put at risk by working near to where asbestos is handled. The present Asbestos Industry Regulations, 1931, are applied to some 300 registered factories, but the report says that there are many other factories which use asbestos products and which do not come within the regulations. The Ministry of Labour is at present drawing up new regulations to cover a wider range of factories.

It was in the 1920s that it was first clearly recognized that asbestosis, a fibrotic condition of the lungs, is caused by occupational exposure to asbestos. Within a few years it was suggested that the disease might be complicated by the development of carcinoma of the lung, but an association was not generally accepted until the 1950s. Recently, there has been concern over the occurrence of mesothelial tumours of the pleura or peritoneum which appear to be causally related to asbestos exposure in many cases. Since 1966 mesothelioma has been a scheduled industrial disease.

The advisory panel concludes that there is an increasing incidence of asbestosis in the country. The most likely explanation for this increase is the greater use of asbestos and the greater numbers of people employed in the industry over the past 20 years. But the panel says that "there is no evidence that the overall attack rate has decreased in the industry over this period, although it has in some textile mills in which the dust conditions have been greatly improved". It appears that if the regulations are more widely and more stringently applied than at present, the number of cases should inevitably fall.

The panel indicates that crocidolite has a special significance in relation to mesothelial tumours. It recommends that other types of fibre should be substituted for crocidolite wherever possible, and, where this is impossible, special precautions should be taken to reduce the risks of inhaling the material. The panel admits that at the moment the level of exposure below which the risk may be negligible is not known. The quantity of crocidolite (blue asbestos) imported into the country is much less than the other varieties of asbestos, and it has remained at about 7,000 tons per year for the last few years. But time may show that other fibres are also implicated in the development of mesothelial tumours.

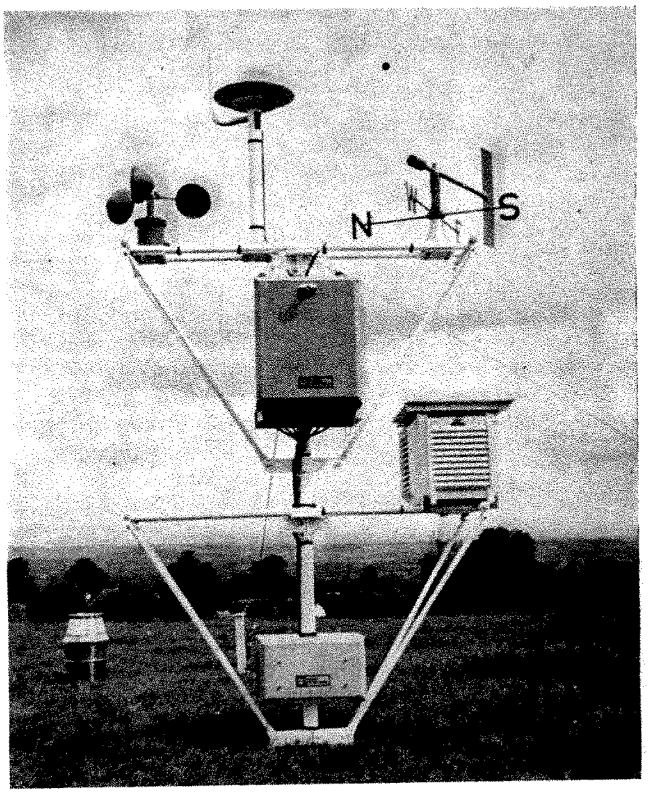
Finally, the report suggests that action should be taken to control the discharge of dust-laden air from asbestos factories and the dispersal of dust from waste dumps. It emphasizes, however, that the problem of mesothelioma in association with asbestos exposure should be kept in a proper perspective. Even though the incidence is increasing, the total number of cases of mesothelioma in Britain over several years is very small when compared with the annual total of deaths from cancers of the trachea, bronchus and lung.

Recording Weather

An automatic weather recording station which can operate unmanned for at least three months has been produced by the Plessey Company Ltd. This station, which can make and record more than 82,500 measurements in a three month period, is available from the Marine Systems Division of the Plessey Electronics Group at Ilford. The value of automatic weather stations was recognized by a recent report from the World Meteorological Organization (*Nature*, **215**, 1324; 1967). They are important because modern developments in weather forecasting require information about climate from all over the world—sometimes from uninhabited areas—and it is becoming increasingly hard to persuade amateur meteorologists to provide accurate data day by day. Professionals cost money, and so there should be a real market for automatic stations which can do the job reliably for long periods without attention.

The Plessey station is virtually self-contained and can monitor parameters such as rainfall, humidity, wind speed and direction, air, soil and water temperatures, barometric pressure, *p*H, solar radiation and water level and flow. The basic design was evolved in co-operation with the British Water Resources Board, the Meteorological Office and the National Physical Laboratory. By adding various instruments to the basic design, it has been possible to produce a family of stations including hydrometeorological, climatological, water level, water quality and recording rainfall stations. Each can be arranged for automatic operation, and the recorded data can be sent by telemetry to a control centre on demand.

Each station is supplied as a complete system with sensors, recorder, power supply, 'Stevenson' screens and a stayed aluminium mast which supports the sensors. The memory of the station samples up to eight sensors in turn, recording their outputs in ten-bit binary code on standard quarter-inch magnetic tape. It samples at intervals of 5, 10, 20, 30 or 60 minutes and is accurate to within 0·1 per cent.



Discomforts of Space

by Angela Croome

The next moves in manned spaceflight may be uncertain, but in both the United States and the USSR, background experiments for the longer flights of the future are going ahead. This month the McDonnell Douglas Corporation of California, contractors to the Office of Advanced Research and Technology of NASA (National Aeronautics and Space Administration), is to start on a two-month-long test of a closed system space chamber. The Soviet Union has recently completed a 70-day confinement experiment involving three men. American and Soviet trials have relevance both for survival in space and for the operation of manned submersibles under the sea.

In the case of the American closed life-support trial, four college students of the University of the City of Los Angeles are acting as experimental subjects. For a period of 60 days, they will exist on reclaimed water and oxygen from their own metabolic wastes. The test is believed to be the longest so far attempted under flight conditions using a closed water–oxygen loop. The closed water system consists of an evaporation unit, a charcoal filter and a condenser that reclaims moisture directly from the crew and from the air in the cabin. Oxygen will be reclaimed through a process of mixing hydrogen and carbon dioxide and then converting them to water and methane gas. The oxygen is recovered from the water by electrolysis and the methane gas is expelled. Atmospheric pressure within the cabin will be at 7 p.s.i. (half that at sea-level) and will consist of 3·1 p.s.i. oxygen and 3·9 p.s.i./nitrogen. The simulation of space conditions will not, of course, be complete, for the trial will take place on the ground where both men and system will operate under normal gravity.

The Soviet experiment has already taken place and lasted the full period of 70 days during which a medical doctor, an engineer and a journalist remained shut up together under cramped and uncomfortable conditions. The exact dimensions of the isolation chamber are not given, but it is described as small. All three subjects were thoroughly instrumented for the measurement of their physical condition during the test. Their regime was to fulfil a daily routine of work, eating, sleep and medical examination up till about the halfway stage when they switched to a "free" routine, doing as they pleased. The journalist of the group recorded reactions in his log at the end of the first three weeks: "Watch-keeping, dinner, medical examination, sleep—our life has acquired a feverish though monotonous rhythm. There is almost no free time left. But we are already beginning to feel exhausted. Stanislav [the doctor] is getting thinner, there is a stubble of beard on his cheeks and dark rings under his eyes. Leonard's eyes have become red and anxious. I cannot escape the feeling that some pungent fog is creeping into our tiny room. While gradually poisoning our brains, it makes us more intolerant towards one another".

But during the later period when there was no set routine, things seemed even worse. There was no longer a work schedule, but the men could pass the time as they chose—playing chess, reading or presumably sleeping. (Food preparation did not amount to much of a task as their diet was a typical space diet of dehydrates, concentrates, and tinned foods.) Now, they reported, time passed more slowly and nervous tension increased. Sleep was troubled and they had hallucinations of sound. They dreamt of music, singing and the noise of aircraft. None the less, no member of the group "deviated from the programme or complained"—complaint presumably being defined as a request for release from the experiment. The experimenter in charge comments that the trial was above all a test of character and willpower, but much useful information was gleaned.

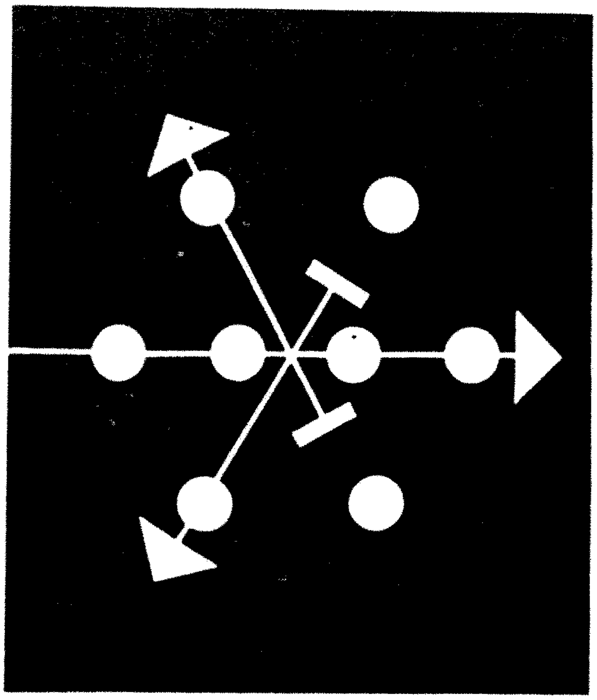
Mapping in Metric Units

One organization faced with a formidable task in changing to metric units is the Ordnance Survey. Among proposals which have so far been endorsed by the Joint Standing Committee in accomplishing this task are that heights will be shown in metres instead of feet on new and revised maps at 1 : 1,250 and 1 : 2,500 scales. Areas will be shown in hectares instead of acres on maps at 1 : 2,500 scale. Heights will be given in metres instead of feet on bench mark lists prepared from new levelling. Contours will be shown at a metric interval on new six-inch maps. Finally, the problems of changing the six-inch and one-inch maps to "rational" scales such as 1 : 10,000 and 1 : 50,000 will be reviewed. These proposals were circulated to 180 interested bodies, representing all classes of map users.

According to the annual report of the Survey for 1966–67 (HMSO, 7*s*. 6*d*.), in which these facts are published, the past year was marked by a change of ministry. In February the responsibility for the Ordnance Survey was passed from the War Office to the Minister of Housing and Local Government. Overall expenditure during the year was £5,715,000—about one-third of which was recovered by revenue from sales of maps and from royalties. On the whole, however, there was a falling off in demand for maps, which, the report suggests, is mainly due to the deferment of investment in development schemes in many parts of the country as a result of the Government's economic measures, together with an increase in the price of maps at certain scales. On March 31, the total number of staff was 4,090 non-industrial and 552 industrial compared with 4,008 and 540 a year earlier—a vigorous recruiting programme bringing in some 480 new staff.

Survey of the principal town areas on the scale of 1 : 1,250 is said in the report to be nearing completion and revision of the old 1 : 2,500 plans covering minor towns and rural areas has been speeded up, largely through the increased use of aerial photographs. Instead of the five-year re-levelling programme which was originally aimed at, subsidence areas will be levelled on a twenty-year cycle as in normal areas.

New Physics Symbol



The Institute of Physics and the Physical Society hopes that this symbol, when correctly placed on a red ground, will soon become familiar to anyone who has any acquaintance with physics. The symbol has been designed to accompany all kinds of information produced in connexion with the four-day Physics Exhibition opening at the Alexandra Palace on March 11. It is based on the weight diagram for the irreducible representation D^8 (1,1) of the $SU(3)$ symmetry which has been successful in bringing order to the classification of sub-atomic particles. Mr D. Walter, who is organizing the exhibition, first thought of basing the symbol on $SU(3)$ symmetry and, after advice from Imperial College, the final representation was drawn by Mr M. R. Preston of the Science Museum.

Letter to an Imaginary Soviet Scientist

The problems of cultural relations between countries which speak different languages are necessarily formidable, but the following letter suggests that these problems may sometimes be needlessly complicated.

Dear colleague,

The news of your election to the Soviet Academy has just reached us here, and I hasten to add my congratulations to those of your other friends and admirers. It does not seem fifteen years since we began to notice your name as the author of a series of brilliant contributions to our subject, and wondered whether you were as clever in conversation as you seemed in print. Nor does it seem as long as seven years since we actually met, and I learnt to value your cheerful, charming and provocative companionship. You deserve your honour, my dear friend, not only for your scientific work but for these human qualities which we have all appreciated on those few occasions when we have been able to have you with us in person.

Now that you have risen to this august rank you will, of course, have heavier administrative responsibilities—but you will also be able to play a larger part in the general direction of science in your country. Knowing you as I do, I can only feel that this will be beneficial both to the Soviet Union and to our subject throughout the world. The combination, within your person, of intellectual power and human judgment fits you almost uniquely for such a role.

Perhaps, therefore, you will excuse me, even on this especially happy occasion, if I mention something that has been worrying me from time to time and that has cast some small shadows over our friendship. It is not a matter that should stand between us personally—but I hope that you may exert your new-found authority to do something about it.

I allude to the extraordinary deficiencies, amounting sometimes to downright discourtesy, that hinder written communications and practical arrangements between Soviet scientists and ourselves. You must, I am sure, know the sort of thing I mean.

For example, a mutual friend arranged a conference here last December. Knowing the problems of "getting any Russians", he set to work in good time—or so he thought. In January he wrote to his opposite number in Moscow, an Academician, inviting him to come as a guest speaker. He also asked for the names of other good Russian scientists as possible invited speakers and participants. To this letter he received no answer. At the end of March, having nearly finalized the programme of review lectures, he wrote again to the Academician. Still no answer.

At this stage, he would have been justified in dropping all Russian names from the programme. However, being experienced in such business and genuinely wishing to hear at first hand about the work of the Moscow Institute, he wrote directly to the Presidium of the Academy and to the Ministry of Education, officially inviting six leading Russian scholars by name. This letter, sent in May, was answered in October, as follows "Dear Professor X, the Soviet delegation to your conference will consist of Y and Z. Please make arrangements for them to receive visas to enter your country."

Unfortunately, the two names mentioned in this brief and uncivil communication were not among those asked for. Indeed they were practically unknown to our friend, who could only assume that they were junior workers who had not yet risen to international prominence. He wrote back asking for more information, pointing out that he would not now be able to include these names in the official programme as invited speakers, but would welcome them nevertheless. He also set in motion the Foreign Office machinery for granting visas.

Again nothing seemed to happen until three days before the start of the conference, when a cable (in Russian) demanded accommodation for eight Russian scientists, including now the Academician himself. With tremendous energy, the organizing secretary wheedled six more rooms out of the local hotels, aroused the Foreign Office to action, and rearranged the programme. He even set graduate students awaiting at the airport to guide the party. But nobody came.

Finally, in the last two days of the meeting three Russians appeared, unannounced. One of them did not seem very familiar with the actual subject, but they all insisted on reading out their unscheduled communications. In the end, beer and vodka in the local pub resolved many inhibitions, and we soon became good friends. They were, indeed, full of apologies for any slight difficulty that may have been caused by the inefficiency of their bureaucracy—and once again we forgave them because, poor souls, it was not their fault that we had been put to so much trouble.

What puzzles me about such buffoonery (believe me, I do not exaggerate the sort of thing that often happens) is that it benefits nobody. If attendance at scientific conferences is valuable, then surely the most suitable Russian participants should have been chosen, by experts such as the Academician, and efficient arrangements made for their travel. Much expense and effort seems to have been wasted. And the international standing of Soviet science was not enhanced.

Well, of course, we too have our difficulties sometimes in persuading financial committees to make grants for travel to conferences, and in rearranging our university duties so as to get leave of absence: sometimes even nine months is an insufficient gestation period for such important administrative decisions. But at least the advice of expert scientists is followed. I know that it would not be fair to blame your Academician personally for this particular fiasco; but I cannot think of anyone else with the knowledge needed to take such decisions and to bear the responsibility for their success.

You must yourself have suffered through similar events, and do not need to be told by me that they are harmful to the Soviet Union and its scientific development. I can only say, as an outsider, that they really cause us a great deal of trouble, to the extent that many of us, in organizing international scientific meetings, may begin to feel that it is not worth the effort to try to get Russian participation. There is a limit, in terms of time and worry, to the price that we are prepared to pay for this commodity, however valuable it may be to us. I hope therefore that, in your new rank, you will press, through the Academy and other organs of the Soviet scientific community, for a great improvement in the practical management of all such matters.

Yet there is a more subtle aspect of relations between Russian and "western" scientists that cannot be improved by mere administrative reorganization. I have mentioned letters that were not answered, nay, not even acknowledged. Friends have told me of the rebuffs they have received in planning a visit to the Soviet Union. For example, polite requests to talk to such and such a scientist have been fobbed off or simply ignored. We all know of the custom of translating scholarly works into Russian, without consulting the author, despite the advantages that may be gained from his advice concerning printing errors. Are these and other incivilities always to be forgiven ?

Can it be that you and your colleagues are simply unfamiliar with our ways, just as we must be with yours ? Consider, for example, the general tone in which correspondence is conducted between established scientists in different countries. It is hard to characterize this tone specifically, but it is always courteous and friendly, whether formal or informal. From an unknown German or Italian, a letter might be written in the old-fashioned style of conventional respect; from an American friend it might be all on first-name terms, with chat about each other's family. In every case, the letter would be addressed as from person to person, without passing through the bureaux of organizations, academies, universities, or other institutions. The courtesies would be those normal between independent individuals, asking perhaps for advice or assistance, regretting in return the inability to give such help, conveying opinions or information, making practical arrangements, etc.

The point is that, however much we may be in fact employees of universities, government laboratories or industrial corporations, we still like to regard ourselves as persons of independent standing, capable of deciding for ourselves on all matters associated with our professional activities—what subjects to work on, when and what to publish, whom to meet and talk to, where to go for conferences and advanced instruction. I do not say that we are in fact quite as free as we like in all such questions; we must, indeed, perform our university duties and otherwise satisfy our directors of research and governing boards—but we are not mere functionaries. To fail to answer a letter from one such scholar to another is therefore something worse than administrative inefficiency—it is a personal insult. To act as if one scientist is interchangeable with another as the speaker in a conference is an attack on the personal standing of each one of us. To give meaning to our work, we need to preserve the illusion that we are contributing individually to the growth of knowledge and that each one of us deserves to be treated as the independent proprietor and cultivator of his own little cabbage patch in the land of learning.

You yourself, I know, have never offended in this way; all I ask now is that you try to explain to your colleagues the importance of such apparently small issues. Our mutual good relations depend on an implicit respect for each other's position by an explicit regard for his social conventions. An improvement at this level would be of far greater benefit than any number of cultural exchange agreements between our respective governments.

These benefits would not be merely a vague increase in "international understanding". It is now clearly established, by those who have studied the sociology of science, that the informal channels of communication between scholars are quite as important as the official organs of scientific publication. Information about new discoveries and new techniques diffuses very rapidly through our scholarly communities by means of letters, visits and personal contacts at conferences. To be cut off from such contacts is one of the main complaints of scientists in small countries, far from Europe and North America; it hampers their research at every level, seeming to make it always a little out of date. If we could unblock these channels between the Soviet scientific community and our own, then we should both benefit greatly; we should not have to learn, by painful experience, some of the things that you already know; your research would always start from the best possible basis of knowledge, including some of those subtle ideas that we have already conceived. I cannot emphasize this point too strongly to you, to your professional Russian colleagues, and to all those who have anything to do with the scientific development of your country. I know very well that there are obstacles to the opening of all doors between your scholarly community and our own; at least a general amelioration of the level of civility in personal communications would unlatch a few windows between us.

You will, I trust, forgive me for having spoken so bluntly. I have not the least complaint against the way that you have treated me over these many years. The role of the "candid friend" is dangerous and foolish in personal relations. But this is not, in the end, a question of the relationship between private individuals; it concerns whole communities, great nations and our common dedication to the pursuit of knowledge; to fail to speak our minds honestly is to betray our social responsibilities. Perhaps we too are unwittingly offending you by our behaviour; give us the opportunity to show our emotional maturity by accepting your criticism in a humble spirit.

Meanwhile, dear friend, we must make the best of our little lives within our chosen spheres. Once more let me congratulate you on the recognition that has crowned those past years of toil and effort and wish you many years of further success, the delights of yet more discoveries, and the fruits of wisdom and understanding.

Arrivederci : to the time when we meet again.

Yours ever,

JOHN ZIMAN

H. H. Wills Physics Laboratory,
University of Bristol.

NEWS AND VIEWS

What is to Happen about Fusion?

THESE are dog days for those who wish to see a rapid development of the kinds of devices which may in due course lead to the development of fusion reactors capable of extracting power from the thermonuclear fusion of light elements. In Britain, the laboratory at Culham will be running on progressively tighter budgets in the five years ahead, for the rate of expenditure is to be reduced at 20 per cent a year for each of the next five years. In the United States there has been a modest increase of expenditure in recent months, but the scale on which large experiments are undertaken in the United States will remain comparatively modest. Gone, for the time being at least, are the days when men working with machines such as that once known as NRX (at Los Alamos) were inclined to think that they would soon be able to accomplish the tricks which had earlier eluded the British machine called ZETA. Instead, everybody is taking a sober view of the problems that will have to be solved before thermonuclear power can become a reality. Plasma physics is the watchword, not fusion research. So long as this is properly appreciated, however, it is valuable to be reminded, now and again, of the potential benefits there would be if fusion reactors ever became a practical proposition.

At least the uncertainty over the scale of support for fusion does not seem to have demolished the confidence of its supporters. Workers at Culham and elsewhere are going ahead with research and even with engineering designs for full scale power reactors. Last month, Mr R. Carruthers from Culham described just such a study for a 2,100 MW (e) fusion power station which would, he said, cost substantially less than the advanced gas cooled reactors being built at Dungeness, and which would generate electricity 10 per cent more cheaply than a fast breeder reactor. Fuel costs would be negligible and there would be no problem of disposal of radioactive waste.

Although some may feel that it is optimistic indeed to design a reactor before the fundamental problems of plasma containment have been solved, there had until last week been little argument about the question of waste. On more than one occasion, indeed, supporters of fusion as a source of power have compared the messy results of fission reactors with the clean, waste-free designs for fusion reactors. The announcement last week by the European Nuclear Energy Agency that it has started to dump radioactive waste in the eastern Atlantic as a collaborative project involving five European countries brought home just how large the problems of disposal will have become by the end of the century. But now, in a paper in *Science* (**159**, 83; 1968) Dr Frank L. Parker of the Health Physics Division at the Oak Ridge National Laboratory in the United States has suggested that fusion reactors would indeed be a source of harmful waste, in the form of tritium lost by the reactor vessel. Dr Parker considers a reactor in which deuterium reacts with tritium —the same design as that described by Mr Carruthers —and quotes results obtained by Professor D. J. Rose at Massachusetts Institute of Technology. These figures, he says, show that for every tritium atom burned 1·15 atoms have to be produced. This means that 15 per cent of the tritium produced is lost, and this would be equivalent to $1\cdot1 \times 10^6$ curies of radioactivity a day for a 1,000 MW (e) station.

This calculation seems a little wild, and Mr Carruthers is undeterred by it. The fact that tritium is lost does not mean that it is all lost to the atmosphere. On the contrary, in a working reactor, most of the tritium would be recirculated. Some would be absorbed into the material of the reactor and a small but not negligible amount would be lost by decay. Although it would be important to ensure that tritium leakage was minimized, he says, the problem would not be as great as Dr Parker feared.

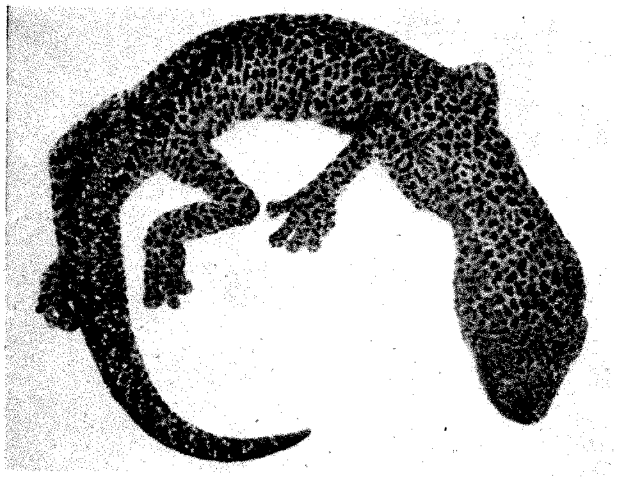
An Account of Geckos

GEKKONID lizards are perhaps best known for their vocalization and for their ability to climb smooth vertical surfaces by means of dilated areas—suction pads—on the ends of their digits. There is, however, much more to their biology than this. It is perhaps not so well known that a few forms have extensive skin folds on their bodies and tails which, when extended, enable the animal to glide from tree to tree. Some species are even known to excavate their own tunnels. The family indeed shows a wide range of structural and biological diversity, and it has adapted to most environmental extremes. An authoritative account of the biology of the family has been written by A. G. Kluge (*Bull. Amer. Mus. Nat. Hist.*, **135**, 1–60; 1967). In this paper, Dr Kluge also discusses the taxonomy and evolution of gekkonid lizards, and he proposes a new sub-familial classification which differs in some features from that suggested earlier by Underwood. In a more recent publication, with more specific appeal, Kluge now deals with the systematics, phylogeny and zoogeography of the genus *Diplodactylus* Gray (*Aust. J. Zool.*, **15**, 1007–1108; 1967).

The Gekkonidae are found on all the main land masses between the latitudes 50° N. and 50° S. The genera number 82, and some 650 species have been identified. Adults vary in total length from less than 40 mm to more than 350 mm. The digits are long and slender, angulate or straight to very dilated. Some species have webbed hands and feet, and there are some with sharp retractile claws. The "suction pads" consist of divided or undivided lamellae, each of which bears numerous microscopic, hook-like projections that appear to facilitate movement in trees.

Maderson (see *Nature*, **203**, 780–781; 1964) has suggested that this climbing mechanism is dependent on an electrostatic charge rather than on adhesion, but experimental work to support or reject the theory does not appear to have been carried out. Gekkonid tails, which are almost always autotomic (self-amputating), vary in size from one species to another; in some forms the tail becomes very swollen with fatty tissue, apparently serving as a food reservoir. The eyes of gekkonids must be one of their most striking features. The margins of the iris are very mobile and it seems as if this mobility has enabled members of the family to adapt to a wide range of light intensities. In most forms, the eyes are protected by a spectacle (brille) instead of movable eyelids. Apparently it is common for geckos to lick their spectacle with their tongues and so clean it in the absence of movable eyelids which normally perform this function.

Most gekkonids are nocturnal and arboreal in their habits, although there are exceptions; a few are cavernicolous, and some species actively feed and bask in the direct sunlight. They are primarily insect-eating, but the larger species are known to eat other lizards and even small birds and mammals.



Dorsal view of *Diplodactylus taenicauda* from Queensland. (Kluge, A. G., *Aust. J. Zool.*, **15**, 1967.)

Kluge reviews the fossil history of the family, and suggests an Upper Jurassic–Lower Cretaceous origin; he also suggests that the limbless lizards of the family Pygopodidae have evolved directly from the Gekkonidae. He does not agree with Underwood's classification of gekkonids which is based on the shape of the pupil; Kluge considers this classification unnatural because of the variability of diagnostic characters. Instead, he uses eighteen characters to group the 82 genera into four sub-families of equal rank: the Eublepharinae, Sphaerodactylinae, Diplodactylinae, and the Gekkoninae. The number of assemblages recognized and their names coincide with those of earlier studies. The difference lies in Kluge's definition of Eublepharinae and Sphaerodactylinae as sub-families. Underwood had Eublepharidae, Sphaerodactylinae and Gekkonidae as three families, with Gekkonidae subdivided into the sub-families Gekkoninae and Diplodactylinae. Kluge points out that although he has retained Gekkoninae and Diplodactylinae, their generic composition is very different from that in Underwood's groupings.

Kluge considers that not only do his four taxonomic groups agree with the morphological characters of gekkonids, but that they are consistent with present thought on zoogeography. It appears that south-east Asia was their place of origin. Kluge comments on the early evolution and dispersion of the different sub-families; the Diplodactylinae are thought to have dispersed from Asia towards Australia by way of the Indo-Australian Archipelago. It is postulated that the sub-family reached Australia by Palaeocene–Eocene times. Today the Diplodactylinae are restricted to the Australian region (Australia (excluding Tasmania), New Caledonia, Loyalty Islands and New Zealand). Kluge gives distribution maps for all the sub-families.

Molecules Constrained

from our Thermodynamics Correspondent

THE second instalment has now appeared in the *Proceedings of the Physical Society* (*Journal of Physics A*, **1**, 15; 1968) of the treatment by Professor S. F. Edwards of the consequences for statistical mechanics of the imposition of topological restraints on long-chain molecules. The long-term objective is to construct a theoretical framework for the statistical mechanics of assemblages of long-chain molecules. The first instalment of Professor Edwards's analysis (*Proc. Phys. Soc.*, **91**, 513; 1967) was largely confined to two-dimensional problems, where the imposition of restraints can be represented by the appearance within the array of molecules of an impenetrable point, the effect of which may be likened to the threading of a needle through a ball of knitting wool. The immediate result is to sort the molecules into various classes on the basis of their topological relationship with the constraint. Some of them, for example, will pass on one side of the constraint and some on the other side, and there is an infinite set of categories for molecules which are looped around the constraining point for varying numbers of times. The essence of the problem in two dimensions is to calculate the probability that a flexible molecule of given length will fall into each of the various categories defined in this way. In practice, this is a random walk problem with restraints. It is no surprise that when the calculation has been carried out, the free energy of the assemblage turns out to be an additive function of the properties of the molecules in the several categories. One cheerful feature of the calculations is that the free energy comes out as an explicit function of the geometrical co-ordinates of the restraint, which means that elastic constants and the like can be calculated directly.

The three-dimensional problem is considerably more difficult, and in his latest article on the subject Professor Edwards has done little more than show how to sort long flexible molecules into categories on the basis of calculable topological invariants. The point restraints of the two-dimensional problem must be replaced by entanglements with other molecules, and for each degree of complexity of entanglement there is a separate invariant to be calculated. Professor

Edwards has calculated the first two of these, corresponding to the entanglement of molecules in twos and threes respectively Experience elsewhere, as in the evaluation of integrals representing multiple interactions in liquids, for example, would suggest that the value of calculating many more complicated members in this series will be limited More immediately, however, it is interesting that the expressions for the probability of molecular configurations defined by definite values of the invariant integrals turn out to be identical with the formalism of the quantum field theory of charged scalar mesons—problems of renormalization included One obvious possibility is that the construction of a series of Feynmann graphs of increasing complexity will be a convenient intuitive means of classifying molecular entanglements of increasing complexity Given the extra complexity of real assemblages of molecules, however, the chances are that the new theoretical calculation will help with the description of the properties of real assemblages of long molecules—pieces of rubber, for example—intuitively rather than by exact calculation It is therefore important that Professor Edwards has already described how existing theories of rubber-like materials should be modified to take account of molecular entanglements (*Proc Phys Soc*, **92**, 9, 1967)

New Plants from the Past

by Mary Lindley

DEVONIAN plants, two of them newly discovered, were the subject of a symposium held by the Palaeobotany Group of the Linnean Society on January 2

As Professor T M Harris said in his introduction, widespread botanical interest was only focused on the Devonian in 1917 when the famous deposit was found in the village of Rhynie in Aberdeenshire, when Kidston and Lang described the three vascular genera *Rhynia*, *Horneophyton* and *Asteroxylon* with their erect dichotomously branched stems, covered with leaves in the case of *Asteroxylon* and naked in the other two genera In the Rhynie Chert, Kidston and Lang also found specimens which they supposed to be the fertile part of *Asteroxylon mackiei*, the type species of the genus, but in 1964 Dr A G Lyon—a botanist who also happens to own the Rhynie Chert—described lateral sporangia of *A mackiei*, suggesting that the fertile parts found by Kidston and Lang were a separate plant, to be called *Nothia aphylla* At the meeting Dr W S Lacey described some work by a former student of his, Dr W El-Saadawy, who has examined new specimens and established that *Nothia aphylla* is a separate species with a slender dichotomously branching stem and terminal sporangia

Dr Lyon himself reported a new plant from the Rhynie Chert, the first for almost fifty years Two years ago he found some very localized remains which, by virtue of their xylem, could not be pieces of arachnid leg They turned out to be sections of a stem with epidermal hairs—unicellular outgrowths from epidermal cells—which clearly distinguish this plant from the others of the Rhynie Chert The stem has xylem—the conducting elements are tracheids with what appears to be annular thickening—surrounded by large phloem like cells and then cortex There were also many sporangia associated with these remains, but their mode of arrangement is uncertain An unusual feature is that the vascular tissue appears to pass into the sporangium and then fan out. Dr Lyon thinks that this plant is different enough to be put in a genus of its own, but he would not reveal the name that he is proposing for it, it remains the "hairy plant"

Another new plant was described by Mrs Dianne Edwards when she discussed the Lower Devonian genus *Cooksonia*, one of the earliest plants to be successful on land This genus, with terminal sporangia borne on a naked dichotomously branching stem, has been described from sedimentary rocks in England and also Czechoslovakia and Russia There is a great deal of variation within populations, and the relative maturity of specimens may add to the difficulty of the taxonomist, whose specimens may anyway be very poor Mrs Edwards was very dubious about the Russian species, *C rusanovii*, which is based on one very bad specimen She was also suspicious of the two species of *Cooksonia* described by Lang, who named the genus in 1937, and thinks that the two may grade into each other Mrs Edwards has also found better specimens of *C downtonensis* and thinks that this should perhaps be in a genus separate from Lang's *Cooksonia* Her own species of *Cooksonia* was found in Scotland, and is very like Lang's species In this case, however, the sporangia have thickening around them and each one has a definite and distinctive base Like Dr Lyon, Mrs Edwards would not divulge the name she has given to her new plant

A new find which has proved more mysterious was described by Mrs Margaret Mortimer She and Dr W G Chaloner have been studying specimens of a cellular membrane which first turned up in lower Devonian rocks in South Wales, and later in boreholes in Cambridgeshire and the Sahara The specimen shows a network of irregular cells, probably in more than one layer, containing regular circular pores This is quite unlike the cuticle of any Devonian vascular plant, and in this case there is no evidence of stomata On the assumption that it is not an animal membrane—it has not been recognized as belonging to any animal—the membrane compares best with the cuticle of some non-vascular Devonian plants, and best of all with *Spongiophyton* Although the specimens in question have nothing to compare with the larger pores in the cuticle of *Spongiophyton*, Mrs Mortimer and Dr Chaloner are inclined to think that this is the correct identification It clearly illustrates the sort of detective work in which the palaeobotanist has to indulge when identifying his specimens

Enzymes made Insoluble

from a Correspondent

THE attendance of 200 people at the Biochemical Society's colloquium on enzymes attached to solid matrices, held at the Medical College of St Bartholomew's Hospital last Friday, was no mean achievement for the organizers when it is considered that only a few years ago the subject was often regarded as obscure

Enzyme molecules have been made insoluble in four ways, by adsorption (ionic or otherwise), by immobilization in a gel, by cross-linking either immediately or after absorption on to a solid matrix and by covalently coupling them to a suitable insoluble polymer. Of these methods, the first is usually considered to be inadequate, although methods successfully employing such preparations have been reported and "irreversible" adsorption has been described. Cross-linking was not considered in detail at the meeting, but physical entrapment in a matrix was considered a serious rival of covalent attachment, on which most of the work has been carried out. It was suggested that if a gel were used in the preparation of the enzyme, for example, in electrophoresis, then the enzyme thus trapped could be treated as an "insolubilized" preparation. Some doubts were expressed about the stability of trapped enzymes. In general a covalently linked enzyme is more stable than the corresponding free enzyme. Inactivation by extremes of pH or temperature, oxidation or bacterial attack is reduced. There was little information about the stability of trapped enzymes except that in at least one case it was reported that there was no protection against oxidative inactivation.

Covalent attachment of enzymes was considered in some detail. About a dozen hydrolytic enzymes and half a dozen enzymes of other categories have been made insoluble by these methods. The amount of enzyme activity remaining after coupling varies from 30 per cent to 50 per cent and, in one or two cases, to 100 per cent of the free-solution activity. In some cases the supporting polymer may contain more than its own weight of enzyme.

Insoluble enzymes can be used as models for *in vivo* enzyme systems and as catalysts for analytical and preparative procedures. The possibility of using enzymes made insoluble also to reproduce the transport phenomena associated with natural membranes was discussed at the meeting, and the preparation of a system with potato-apyrase was reported. This work, however, is still in the early stages.

No striking process was described in the industrial and analytical uses of insoluble enzymes. Attention was drawn to the importance of diffusion as a limiting factor on the activity of enzyme preparations which have been made insoluble. Experiments were described in which the effective rate of catalysis by such a preparation can be doubled by increasing the stirring speed in the reaction vessel. Reasons were given for believing that a packed bed would be preferable to a continuous feed stirred tank when employing these catalysts. The preparation and use of enzymes attached to "porous sheets"—filter paper and filter cloth—were reported, as was the use of several such sheets in series.

Industrial Microbiology

THE Microbiology Group of the Society of Chemical Industry celebrated the New Year with a meeting on January 2 about the problems of maintaining pure cultures in industrial fermentations. This is an important economic question. Mr B. G. W. Hodds of Glaxo Ltd, Ulverston, provided a lucid introduction to the subject by describing how an industrial fermentation can frequently be ruined as a result of contamination by bacteria, yeasts, moulds, bacteriophages and actinophages. Sometimes the production of a micro-organism or one of its metabolites can be completely swamped by an intruder. In the production of streptomycin, for example, contamination by actinophage can cause complete lysis of the culture, which is why prudence suggests the addition of citrates to sequester calcium and magnesium ions necessary for the development of the actinophage. But how to know when a culture is contaminated? Mr Hodds emphasized the problem of distinguishing microscopically between contamination and abnormal development of the cultured organism. In brewing, to be sure, the low pH of fermentations and the antibacterial action of hops serve as a means of protection. Fermentations intended for the production of antibiotics also have a degree of self-protection except when contaminating micro-organisms are resistant to them. Bad luck or bad management here can mean the loss of a whole vat, the contents of which might be worth £10,000. How then to avoid contamination? This, said Mr Hodds, is not just microbiology but management. He pointed out that the training of recruits and the clear supervision of foremen are important factors in reducing the chances of contamination. Speedy identification and elimination are important if the contamination of a whole plant is to be avoided.

Much can also be done by good design, and Mr J. F. Thorley of Dista Products, Speke—the star turn at the symposium—urged that simplicity of design helps to keep out contamination. The more numerous the connexions to a vessel, the more difficult it is to sterilize. Smooth polished surfaces of stainless steel are a great help. So too is continuous welding.

But what is the cost of freedom from contamination? G. D. Wilkin of the Boots Pure Drug Company, Nottingham, said that any plant could be run under sterile conditions at a price. The more valuable the product and the larger the plant, the more it is worth paying for security.

The risk of contamination is most feared, however, in the production of vaccines, either viral or bacterial. Dr J. R. Hepple of Glaxo Laboratories Ltd explained how even a simple antigenic variation of an organism is enough to render a batch of vaccine useless. This means that the micro-organism must be kept in the pure form and in the correct physiological state, both in the seed and the batch cultures. But it is also necessary to ensure that the tissue on which a virus is being cultivated should be free from other viruses and bacteria.

More Light on Ribonuclease

from our Molecular Biology Correspondent

PANCREATIC ribonuclease is the enzyme about which most is known, in terms of both structure and function. The working out of the three-dimensional structure, supported by the accumulated mass of protein chemistry, is now beginning to pay impressive dividends: the current literature offers some especially interesting examples.

A second pancreatic ribonuclease—from the rat—has been fully sequenced by Beintema and Gruber (*Biochim. Biophys. Acta*, **147**, 612, 1967), and it is remarkable to discover that differences from the bovine enzyme occur in no fewer than one-third of the positions in the chain. Frequently there are substitutions of polar (for non-polar) residues, but it appears that in all cases the replacements fit without strain into the model obtained for bovine ribonuclease by Richards and his associates; this leads one to expect that the structure will be essentially the same, despite the large differences in sequence. The residues known to be parts of the active centre remain in place, and the overall charge is also essentially unchanged. Most of the substitutions are in fact at the surface of the molecule: in the closely packed hydrophobic interior, the steric restrictions are exacting. Among the substitutions are three new proline residues, none happily in α-helical sections of the chain. A partial sequence of horse pancreatic ribonuclease is also presented, and shows considerable differences from the other two.

The rat enzyme has three extra residues at the *N*-terminal end, and the *S*-peptide region is therefore enlarged to 23, as well as differing in no fewer than 10 of the other 20 residues from bovine *S*-peptide. (The *S*-peptide, it will be recalled, is the terminal segment which can be removed after proteolytic fission of one bond, with complete loss of activity: if it is returned to the truncated enzyme, it attaches non-covalently, with full restoration of activity.) This is interesting, in view of recent work on synthesis of *S*-peptide analogues. It is known that six residues can be excised from the *S*-peptide without detriment to its function. Finn and Hofmann (*J. Amer. Chem. Soc.*, **89**, 5298; 1967) have now shown that elimination of his-12—part of the active centre—from the *S*-peptide leads to loss of reactivity, but hardly at all of affinity for the residual *S*-protein. The sites which are vital for attachment to *S*-protein, rather than activity, are glu-2, asp-14 and met-13, and these may not be tampered with. All these, as expected, are unchanged in the rat enzyme, whereas all six inessential residues (15–20) are changed. Scoffone *et al.* (*ibid.*, 5450) have also synthesized *S*-peptide analogues: arg-10 has been replaced by ornithine, and phe-8 by tyrosine, isoleucine or alanine. In the last two cases binding is diminished, but tyrosine replaces phenylalanine with hardly any disadvantage. This is curious, for phe-8 is an internal residue, so the usual steric exclusivity is violated. Even in the other two cases, activity remains when a large excess of peptide is added to induce a measure of binding.

Yet another fascinating observation is that of Neumann *et al.* (*Europ. J. Biochem.*, **3**, 171; 1967), who have achieved partial reduction of pancreatic ribonuclease with a new reagent, phosphorothioate. In general, the four disulphide bonds are protected in the native state, but with this reagent two are cleaved in aqueous solution. The broken cystines have been identified by peptide mapping. As in one or two other cases recently reported, the partly cleaved ribonuclease retains its native conformation by all criteria. Thus it is immunologically identical with the native protein, and the three buried tyrosines remain non-titratable, and are exposed only on denaturation. In particular, the activity towards RNA is fully intact, and the activity towards cytidine cyclic phosphate is actually enhanced more than two-fold—it must be supposed by virtue of increased flexibility. The two surviving cystines are essentially "internal" residues. The susceptibility of the protein to attack by trypsin is much increased, which suggests that a general loosening of the structure can be tolerated, and that the steric limitations for activity are quite modest in large tracts of the sequence, as the wholesale substitutions in the rat enzyme also indicate.

Model Cell Membranes

from a Correspondent

Artificial cell membranes, less than 100 Å thick, can be prepared by allowing a film of lipid to form across a hole in a polythene cup separating two aqueous phases. Increasingly, these preparations are being used as model systems to test particular hypotheses in membrane transport. Thus, Tosteson and his colleagues (Andreoli *et al.*, *J. Gen. Physiol.*, **50**, 1729; 1967) have been trying for a number of years to define the molecular basis of the difference between the HK (high potassium) and LK (low potassium) strains of sheep, where potassium refers to the concentration of this ion in the red blood cells of these animals. Transport studies have shown that the two strains differ in the ionic permeabilities of their cell membranes; the HK sheep are sodium selective, and the LK sheep prefer potassium ions. In the present paper, the lipids have been isolated from the cell membranes, artificial membranes prepared from these lipids and the permeability of such membranes measured. It is disappointing that artificial membranes from the two strains of sheep differ in no significant way; the artificial membranes do not distinguish between sodium and potassium and are also more permeable to these ions than to chloride. In contrast, the intact cell, for both strains, has a chloride permeability some 10^6 times greater than that for cations. The difference between intact cells and model membranes seems to lie in the contribution of the membrane protein, and it is here that the gene distinguishing the HK and LK strains must act.

Another example of the impact of model membrane studies comes from Cass and Finkelstein (*J. Gen. Physiol.*, **50**, 1765; 1967), who have compared the water permeability of thin lipid films, when water movement occurs by diffusion (using isotopically labelled water) or by hydrodynamic flow (on setting up an osmotic gradient across the membrane). Studies of cell membranes have often shown that these two permeability coefficients differ. Because hydrodynamic flow across porous membranes, but not diffusion flow, is affected by the pore size, results such as these suggest that water moves through cell membranes along narrow channels. It was very surprising, therefore, that early studies on the artificial lipid membrane—presumably a continuous bilayer—showed a difference between the two permeability coefficients. Cass and Finkelstein now show that the early results were almost certainly caused by the presence of unstirred layers of water in the diffusion experiments and that for the lipid bilayers the diffusion and hydrodynamic permeabilities for water do not differ. Hence water movement most probably occurs after dissolution in the lipid. It is pertinent to recall, therefore, Dainty's suggestion that diffusion studies on intact cells may be in error because of the presence of unstirred layers. For the human erythrocyte, however, where the two permeability coefficients differ by a factor of 2·4, an elegant study (Sha'afi *et al.*, *J. Gen. Physiol.*, **50**, 1377; 1967) has shown that the unstirred layer is only 5·5μ thick, and that the effect of this layer is insignificant. Valuable as the studies on artificial lipid membranes may be, therefore, they strongly suggest that membrane proteins play at least as important a part as lipid in determining the permeability properties of cell membranes.

Reconstruction of Three Dimensional Structures from Electron Micrographs

by

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General principles are formulated for the objective reconstruction of a three dimensional object from a set of electron microscope images. These principles are applied to the calculation of a three dimensional density map of the tail of bacteriophage *T4*.

THE standard high resolution electron microscope has a depth of focus of several thousand Ångströms, making the image a two dimensional superposition of different levels in the three dimensional structure. The focus cannot be adjusted to different levels within the object, and so three dimensional structures are difficult to analyse. Stereo-electron micrographs do not overcome this difficulty satisfactorily, as will be shown.

Our method starts from the obvious premise that more than one view is generally needed to see an object in three dimensions. We determine first the number of views required for reconstructing an object to a given degree of resolution and find a systematic way of obtaining these views. The electron microscope images corresponding to these different views are then combined mathematically, by a procedure which is both quantitative and free from arbitrary assumptions, to give the three dimensional structure in a tangible and permanent form. The method is most powerful for objects containing symmetrically arranged subunits, for here a single image effectively contains many different views of the structure. The symmetry of such an object can be introduced into the process of reconstruction, allowing the three dimensional structure to be reconstructed from a single view, or a small number of views. In principle, however, the method is applicable to any kind of structure, including individual, unsymmetrical particles, or sections of biological specimens.

Summary of Procedure

Electron micrographs are selected in which the details of the structure show up best, as judged for example in the phage tail described later, by their optical diffraction patterns[1,2]. The optical density in each image is sampled at regular points on a grid by an automatic microdensitometer linked to a computer (unpublished work of U. W. Arndt, R. A. Crowther and J. F. W. Mallett), which converts the image into a set of numbers representing the density at each grid point. These numbers are now transformed by computation into a set of Fourier

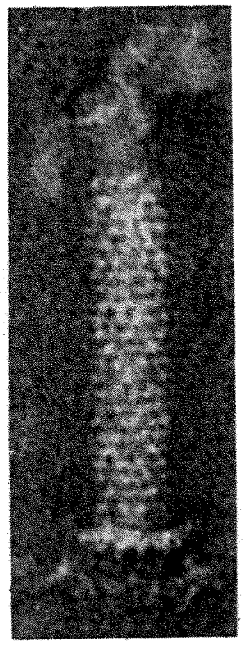

Fig. 1

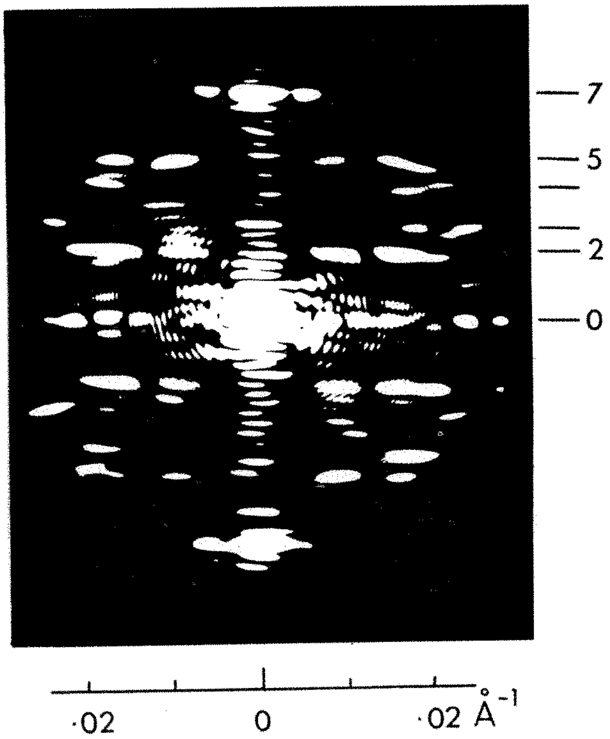

Fig. 2

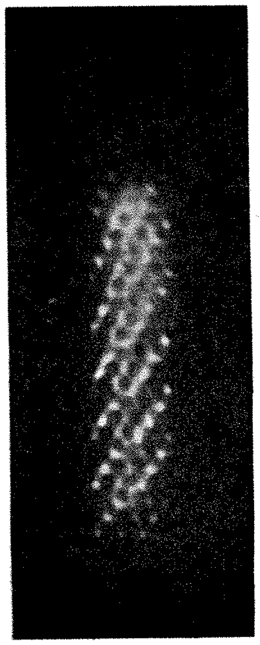

Fig. 3

Fig. 1. Electron micrograph of a tail of bacteriophage *T4*, negatively stained with uranyl formate. (× 500,000.)

Fig. 2. Optical diffraction pattern of the phage tail image in Fig. 1. Stronger exposures show that the order in the micrograph extends to spacings of about 25 Å. The strong meridional peak on the seventh layer line arises from the spacing of 38 Å between annuli. The layer lines are approximately equally spaced at the orders of an approximate repeat of 7 × 38 = 266 Å. The helical selection rule for the diffraction pattern is $l = 2n + 7m$, the only permitted values of n being those which are multiples of six.

Fig. 3. Optically filtered image of phage tail in Fig. 1, admitting only the diffracted rays corresponding to the far side of the particle. The dominant features are two distinct sets of oblique striations, which correspond to two distinct sets of helical lines running along two cylindrical surfaces of different diameter, and thus correspond to features at different depths in the particle.

amplitudes and phases*. A Fourier synthesis is then carried out which takes into account the symmetry of the object† and which builds up a three dimensional map of the structure. The density at each point on this map is made up by the summation of Fourier components contributed by all the different images of the object. This final step is similar to the method which is used by crystallographers for building up three dimensional maps of molecules from the amplitudes and phases of the X-rays diffracted by a crystal. The X-ray diffraction pattern lacks information about the phases, but these are readily calculated here from the density distribution in the original electron micrographs‡.

Application to the Tail of Bacteriophage *T4*

The tail of the *T*-even bacteriophages is made up of a core surrounded by a contractile sheath[8]. This contains protein subunits arranged in successive annuli which are rotated with respect to one another, so that the subunits also lie along oblique helical lines. On injection of the phage DNA into the host cell the sheath contracts, riding up the core. By comparing extended and contracted sheaths, we hope to discover the path of the contraction process.

Fig. 1 shows an electron micrograph of an extended phage tail which has been negatively stained. Its optical diffraction pattern, reproduced in Fig. 2, is essentially symmetrical about the meridian; because the tail is helical, this shows that the micrograph is made up by superposition from equally contributing substructures at the near and far sides of the particle[1]. The diffracted rays from the unwanted side of the particle can now be filtered out, and a good image of the other side obtained by optical recombination of the remaining diffracted rays[2]. Fig. 3 shows such an image from one side of the phage tail, but the picture is still complex, because it contains detail coming from different radii of the particle. This difficulty is overcome by our new reconstruction method which allows us to work in three dimensions and thus to separate the various radial contributions§.

Fig. 5 shows a model of the phage tail, consisting of the core together with its sheath, obtained by application of this method to the image in Fig. 1. It represents a Fourier synthesis of the particle at a resolution of 35 Å, in which the solid parts represent regions inaccessible to the negative stain. Comparison of such density maps from four different particles revealed only slight differences. The model contains two sets of black wires threaded through the two most prominent helical grooves; they form parallelograms which mark out the unit cells. There are six unit cells spanning the equator of the particle, corresponding to the number of subunits in the annulus[10,11].

We find a hole of about 30 Å diameter along the axis of the particle as we move outwards from the centre. This is formed probably by the packing of protein subunits in the tail core about a rotational symmetry axis. The resolution, however, is not sufficient to show up any fine periodic variations in the surface bounding this hole*. The model does show strong azimuthal fluctuations in density at two radii. Six helical tunnels of about 30 Å diameter lie at a radius of 65 Å, corresponding to the boundary between the sheath and the tail core (Fig. 5*c*). The matter which alternates with the tunnels at this radius presumably represents the connexions between tail and core. (These are to be expected, since the sheath is found in the extended state only when surrounding a core. Free sheaths are always contracted[12].)

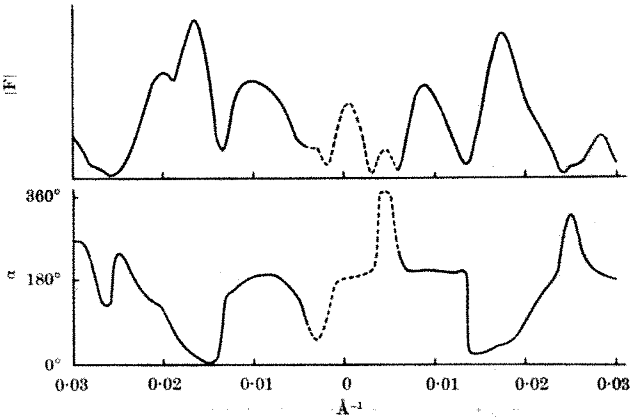

Fig. 4. Part of the computer-generated Fourier transform of the image of the tail in Fig. 1. The Fourier amplitude |F| along the second layer line is shown (compare Fig. 2), together with the accompanying phase α. The dotted parts of the curves near the middle of the diagram depend upon the exact region used to produce the Fourier transform; the regions used to produce this figure and Fig. 2 were not exactly the same.

A complex set of protuberances extends from a radius of about 90 Å to one of 120 Å at the outermost edge of the particle. They begin in the region of the model marked *L* (Fig. 5*a* and *b*) between the annuli, then sweep up to the right, as they pass through the region of an annulus, and finally join up by diagonal bridges (*S*) with the units on the upper left and lower right. The finer of the two sets of black wires near the surface passes under the bridges. The lower left-hand part of the protruding unit is almost in contact with the unit below and to the left. We cannot be sure, however, whether these units are really not in contact or whether we merely chose the contours marking the boundary between protein and stain at slightly too high a density.

Surprisingly, the cross-striations which give the appearance of annuli in the electron microscope are absent in the model. The cross-striations in the image are produced by the superposition of outer gaps and inner holes and do not correspond to deep serrations in the particle.

General Principles of Three Dimensional Reconstruction

Because of its high rotational symmetry, only a single two dimensional electron micrograph was needed to determine the three dimensional structure of the phage

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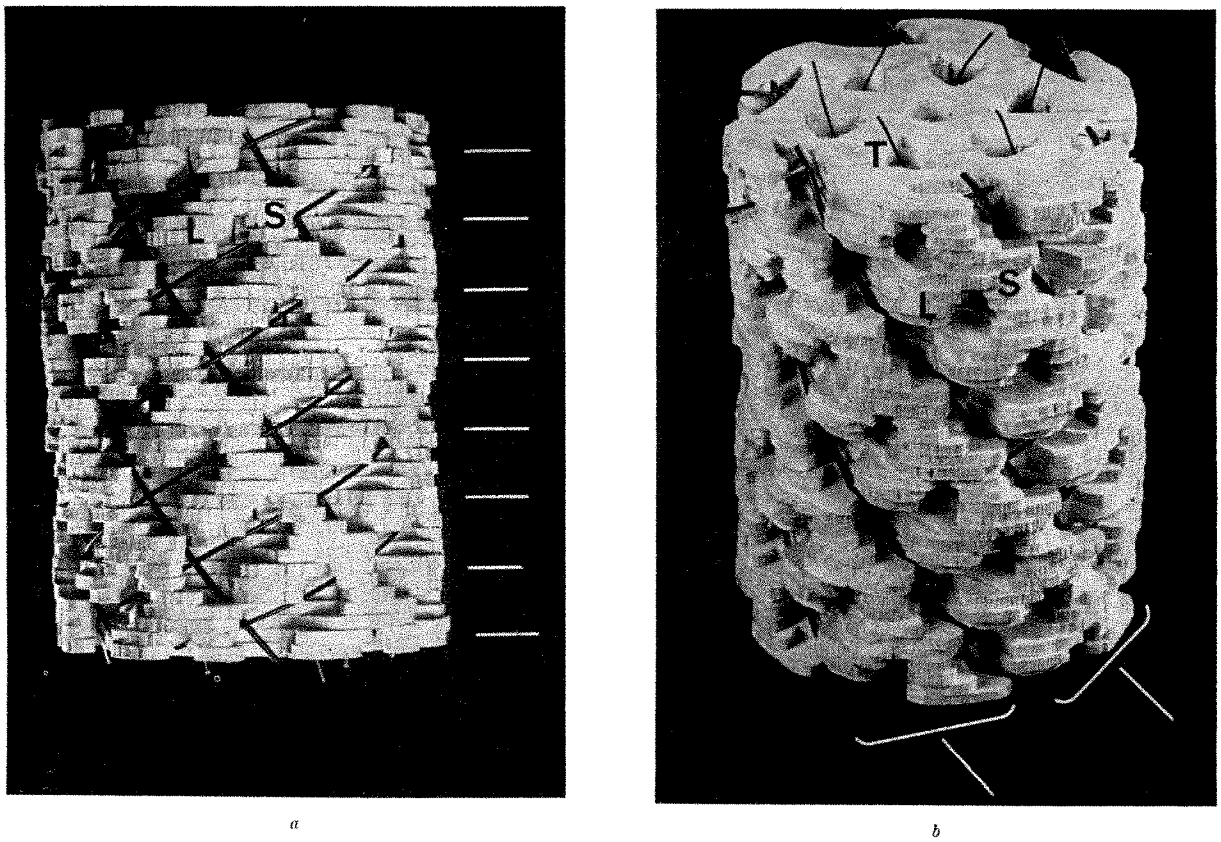
* Our method can be reformulated so as to carry out the reconstruction process without going through the intermediate stage of calculating Fourier coefficients, but this stage provides a useful point at which to check and if necessary correct the data. For example, in the case of a particle with helical symmetry like the phage tail, the relationship of the phases on the two sides of the meridian of a computer-generated Fourier transform provides a check that the axis of the particle has been correctly chosen (compare Fig. 4). If the axis were not quite correctly chosen, its position can be refined in Fourier space.

† For example, for a helical particle the three dimensional Fourier programme takes the form of a Fourier–Bessel synthesis into which the appropriate helical parameters can be inserted[3].

‡ The parallel with X-ray diffraction can be carried further, for it is possible to determine the Fourier amplitudes by optical diffraction from the electron microscope images. The determination of phases optically is more difficult, but we have tried out two optical techniques for phase determination. (1) The straightforward, but tedious, method in which diffracted rays are allowed to interfere pairwise to produce fringes, the positions of the fringe minima with respect to some reference point giving the phase. The procedure was carried out on our optical filtering system (compare ref. 2), but is very laborious and was less accurate than the densitometry method. (2) A holographic method in which the diffracted rays from the subject are allowed to interfere with a strong reference beam coming from a point in the same plane as the subject. The holographic technique using this particular geometry has been variously termed "Fraunhofer diffraction holography"[4] and "Fourier transform holography"[5]. This method has the same basis as the heavy-atom method in X-ray crystallography[6]. The method was not pursued intensively, because our first trials showed that, with subjects such as ours containing very fine detail, a proper interferometric system would have to be constructed[7].

§ Optical filtering is basically a two dimensional operation and gives direct results when applied to structures which are essentially two dimensional, such as overlapping sheets[9], thin-walled tubes (our work in preparation on the structure of the polyheads of bacteriophage *T4*; Kiselev, N. A., De Rosier, D. J., and Klug, A., work in preparation on the structure of tubes of catalase) or shallowly grooved solid objects (compare ref. 2).

* It should be stated that, in computing the map of the structure, it has been assumed that all the matter in the tail has the same symmetry. If the core had a different symmetry from the sheath, small modifications would be introduced into the model, but the main features described in this paper would be unchanged.



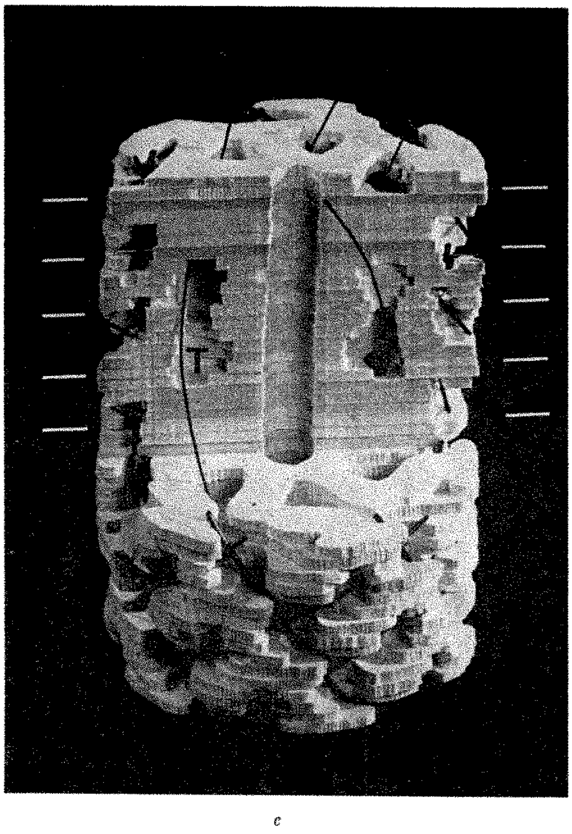


Fig. 5. Model of the phage tail in the extended state. The portion shown is about one-third of the actual length. The model was built from a series of sections in a three dimensional Fourier density map which includes data to spacings of 35 Å. *a*, Side view. The black wires lying in the prominent helical grooves on the surface serve to mark out the unit cells of the repeating structure. The horizontal lines at the side of the photograph mark the position of the annuli of subunits which can be seen in the electron micrograph in Fig. 1. *b*, Model tilted forward. The fine black wires *T* follow the path of the helical tunnels at an inner radius of about 60 Å. *c*, Same as *b*, but with part of the front half removed. The helical tunnels *T* lie between the tail core and the sheath.

The six pronounced helical ridges on the surface which run at an angle of about 30° to the particle axis (marked by brackets in *b*) can be identified with the helical "windings" which have been revealed at the surface by shadowing with metal[18]. This identification fixes the absolute hand of this family of helices. The same family of helices can be seen as the stronger of the two sets of oblique lines in the filtered image in Fig. 3, but their apparent hand is reversed there because it is the filtered image corresponding to the far side which is shown.

tail We now outline the conditions for the recovery of any type of three dimensional structure from transmission photographs and we formulate the part played by symmetry more precisely Large objects are examined usually by taking electron micrographs of thin sections, but this is not possible for small particles Instead of working with the particle directly, however, equivalent structural information in the form of its Fourier amplitudes and phases can be used If these could be measured in three dimensions, they could be used in a Fourier synthesis to compute the structure itself

The electron microscope image represents a projection* of the three dimensional density distribution in the object at all levels perpendicular to the direction of view According to a theorem familiar to crystallographers, the Fourier coefficients calculated from a projection of a three dimensional density distribution form a section through the three dimensional set of Fourier coefficients corresponding to that distribution By collecting many different projections of a structure in the form of electron microscope images, it should therefore be possible to collect, section by section, the full set of Fourier coefficients required to describe that structure (Fig 6) The number of projections needed to fill Fourier space roughly uniformly depends on the size of the particle and on the resolution, in much the same way as the number of zero-layer precession photographs in a three dimensional X-ray analysis depends on the unit cell size and the desired resolution Similarly, rotation or screw symmetry reduces the number of sections required, because different orientations of the particle present identical projections† The advantage of symmetry is illustrated in Table 1 which gives the number of independent views required to solve, to a resolution of about 30 Å, comparable structures of linear dimensions of about 250 Å

Table 1

Symmetry class	Example	No of views
Helical	*T*4 phage tail	1
Icosahedral	Tomato bushy stunt virus	~2
No symmetry	Ribosome	~30

It may be helpful to restate the role of symmetry differently The projection of an object with rotational symmetry is the sum of projections of its asymmetric unit in the various symmetry-related orientations (and positions) In the reconstruction process the three dimensional structure of the asymmetric unit is recovered from the various projections of it which are contained in a single image of the whole object Looked at in this way, an electron microscope image of the phage tail, which has forty-two subunits in its axial repeat, effectively presents projections of the subunit in twenty-one different orientations, more than enough to reconstruct the structure‡ The simplicity introduced by the symmetry of the phage tail made us choose it for our first attempt at three dimensional electron microscopy

At this point, it is worth discussing the reason why high-resolution stereo-electron microscopy is not, in general, an adequate technique for solving a structure Stereo-microscopy does not reproduce structures in a quantitative and tangible form, and has other drawbacks Information from pairs of corresponding points in a stereo-pair is combined visually Even if one had ideal pictures in which point-like features were clearly resolved, some points in a complex, deep structure would probably be obscured by others in front of them. To overcome this difficulty, many pairs of stereo-pictures would have to be taken This brings one back to the requirements discussed above, but the procedure does not tell one how these views might be combined, even subjectively. For these reasons, stereoscopy is only suitable for seeing details confined to the neighbourhood of a single surface or for particularly simple three dimensional objects

General Requirements for Implementation

If more than one projection of an object is needed to resolve its structure, such projections can be obtained in two ways The most obvious one is to systematically tilt

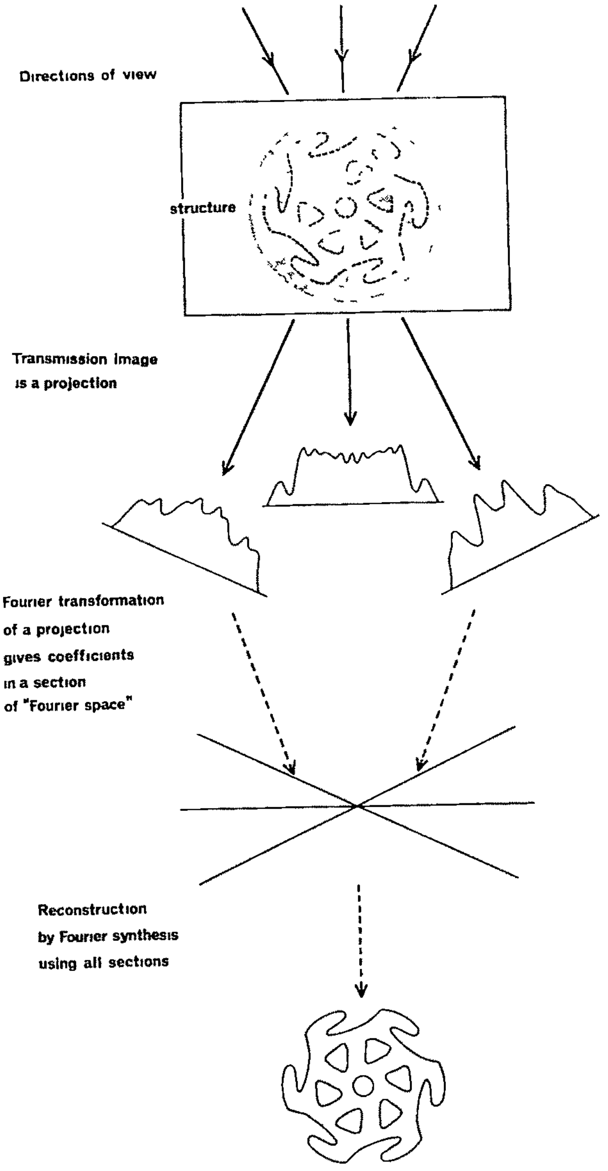

Directions of view
structure
Transmission image is a projection
Fourier transformation of a projection gives coefficients in a section of "Fourier space"
Reconstruction by Fourier synthesis using all sections

Fig 6. Scheme for the general process of reconstruction of a structure from its transmission images

and photograph a single particle in the electron microscope If all the necessary projections are collected regardless of particle symmetry, no assumptions are needed to calculate the three dimensional structure The synthesis will reproduce any symmetry that the particle possesses, but also any distortion it has suffered, or any irregularity in the stain distribution These defects, however, can be detected by comparing Fourier syntheses of different particles For a complete set of projections, the particles must be tilted accurately by small increments to a total tilt angle of 180° This, however, presents the technical problems both of covering the complete range of angles and of preserving the structure during successive exposures to the electron beam Symmetry lessens the difficulties by reducing both the number of different projections and the maximum tilt angle required So that symmetry can be used, the orientations of the symmetry axes with respect to the tilt axes must be found

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* In precise terms, it is the logarithm of the intensity of the transmitted electron beam in the image which represents the projection of the three dimensional distribution of absorbing (strictly scattering) material along the path of the beam For a distribution $\varrho(x, y, z)$, the transmitted intensity I is, in conditions of single scattering of electrons, related to the incident intensity I_0 by the expression $I(x, y) = I_0 \exp[-\mu \int \varrho(x, y, z)\, dz]$, where μ is the mass absorption coefficient and z is measured along the direction of the beam This interpretation on a mass-thickness basis is, of course only valid if the stain is amorphous[14], and if the spatial order in the liquid-like arrangement of scattering atoms is not shown up at the resolution with which one is concerned in the image[15] This condition is obeyed for the scale of structure normally investigated in biological applications of electron microscopy Likewise the condition of single scattering is obeyed for the thicknesses of stain involved

For our quantitative work a measure of the transmitted electron beam is given by the optical density on the electron microscope plate which is proportional to it[16] Thus in effecting the Fourier transformation of the electron microscope images, logarithms of the optical densities should be used to obtain the Fourier coefficients of the structure $\varrho(x, y, z)$ Taking the logarithm is easy in numerical processing, but presents a problem when optical methods are used to measure Fourier amplitudes and phases, for the logarithm of transmitted intensity in an optical system is directly proportional to the optical density and not to the logarithm of the optical density

† This statement assumes that the distribution of stain possesses the particle symmetry (for example, it could have the form of a cylinder for a helical particle) Departures from this must be taken into account, and the technical problem will be discussed elsewhere

‡ In terms of helical diffraction theory the amplitude of the Fourier transform of the phage tail has cylindrical symmetry to the working resolution because there is no overlap of Bessel functions of different order on the layer planes[17]

Alternatively, different images from a field of particles are, in principle, projections of the same structure (although in practice allowance must be made for variations in staining or perturbations in the different particles) The exact orientation of each particle in relation to the direction of view must be determined in order to relate correctly the Fourier space section obtained from it to those obtained from other particles This would mean determining the orientation of the particle for each image before its exact structure is known In practice, ancillary tilting experiments would help in the recognition of the views[18,19] Recognition and determination of the angle of view might be done more precisely by some form of refinement process, such as the simulation of particle images using a provisional model of the structure[18,19]. This would also help in assessing the preservation of detail in the different images It is important to integrate the information contained in the images of different particles so that the effects of noise coming from the sources mentioned above can be averaged out

Concluding Remarks

Our method of three dimensional reconstruction relates the analysis of electron microscope images to the theory that has been developed for X-ray diffraction analysis The difference is that the "phase", which together with the amplitudes of the Fourier components allows the reconstruction of a three dimensional map, is lost in recording the X-ray diffraction data It is preserved, however, by the focusing of the diffracted electron beam into an image At the same time, one can work with a single or a few particles and one is not confined to working with highly ordered crystals or fibres as in protein crystallography. While small numbers of particles can be used, more variability must be expected than is encountered in the average structure from, for example, a protein crystal containing 10^{15}–10^{17} particles*

Because the theory underlying this method is a very general one, the method can be used for all kinds of objects It may be necessary, however, to modify the various procedures used according to the particular problem. In the example given here we have used single particles, but the method could work equally well with ordered aggregates or arrays such as sections of muscle or small crystals

We thank Dr J. T Finch for the electron micrographs, R A Crowther for programming the densitometer and for help with the computer processing, Dr K C Holmes for making his Fourier–Bessel programme available and Miss Marion Holder for technical assistance We also thank Professor Gill and the manager of the Computer Centre, Imperial College of Science and Technology, for making facilities available We thank Drs H E Huxley and M F Perutz for helpful criticism of earlier versions of this article. One of us (D J De R) was aided by a grant from the American Cancer Society

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* Because the preservation of detail in electron micrographs is not usually perfect, many examples must be examined and assessed The method of optical diffraction[1] provides an objective assessment of the information contained in an image of a periodically repeating structure, for it displays an average of the genuine, repeating features We have found by this technique that the resolution in electron micrographs of negatively stained biological specimens can be as high as 15–20 Å (refs 1 and 2)

[1] Klug, A , and Berger, J E , *J Mol Biol* ,**10**, 565 (1964)
[2] Klug, A , and De Rosier, D J , *Nature*,**212**, 29 (1966)
[3] Klug, A , Crick, F H C , and Wyckoff, H W , *Acta Cryst* ,**11**, 199 (1958)
[4] Leith, E N , and Upatnieks, J , *J Opt Soc Amer* , **54**, 1295 (1964)
[5] Stroke, G W , *App Phys Lett* , **6**, 201 (1965)
[6] Bragg, W L , *Nature*,**166**, 399 (1950)
[7] Vander Lugt, A , *Trans IREE*, IT-10, 139 (1964)
[8] Brenner, S , Streisinger, G , Horne, R W , Champe S P , Barnett, L , Benzer, S , and Rees, M W , *J Mol Biol* ,**1**, 281 (1959)
[9] Finch, J T , Klug, A , and Nermut, M V , *J Cell Sci* , **2** (December, 1967)
[10] Moody, M F , *J Mol Biol* ,**25**, 201 (1967)
[11] Krimm, S , and Anderson, T F , *J Mol Biol* , **27**, 197 (1967)
[12] See, for example, Kellenberger, E , and Boy de la Tour, E , *J Ultrastruct Res* ,**11**, 545 (1964)
[13] Williams, R C , and Fraser, D , *Virology*, **2**, 289 (1956)
[14] Hall, C E , *Introduction to Electron Microscopy*, ch 9 (McGraw-Hill, New York, 1953)
[15] Heidenreich, R D , *Fundamentals of Transmission Electron Microscopy*, ch 1 (Interscience, London, 1964)
[16] Various contributions in *Symp on Quantitative Electron Microscopy* (edit by Bahr, G F , and Zeitler, E H) (The Williams and Wilkins Company, Baltimore, 1965)
[17] Franklin, R E , and Klug, A , *Acta Cryst* , **8**, 777 (1955)
[18] Finch, J T , and Klug, A , *J Mol Biol* ,**15**, 344 (1966)
[19] Klug, A , and Finch, J T , *J Mol Biol* (in the press, 1968)

Response of Pine Seedlings to Mechanical Stimulation

by
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When needles of pine seedlings are deflected downwards there is an electrical response which can be measured.

WHEN developing electrophysiological techniques for forest tree physiology research, I observed a hitherto unreported electrical "response" to leaf fascicle movement in young slash (*Pinus elliottii* variety *elliottii* Engelm) and loblolly (*P taeda* L) pine trees The effect can be elicited consistently by sharply deflecting a fascicle downward while recording the electrical potential between two or more points on the stem of the tree with non-polarizing electrodes The response is rapid and sharply defined and seems to be typical of tissue depolarization

A tracing of a single recorded response, obtained from a 2 yr old potted loblolly pine seedling is shown in Fig 1 Electrical potentials were led off through cotton-wick tapwater bridges to Ag–AgCl electrodes, amplified by a Keithley 603 d c. electrometer-amplifier (input resistance 10^{12} ohms), and displayed on a potentiometric recorder Conventional high impedance d c amplification and oscilloscope recordings were also made frequently In the case of Fig 1, the stimulated fascicle was located about midway between the apical and basal electrode contact points The input system was floating, with a resistance to earth of about 10^{10} ohms Single-ended inputs, and earthed seedlings with differential inputs, gave the same results

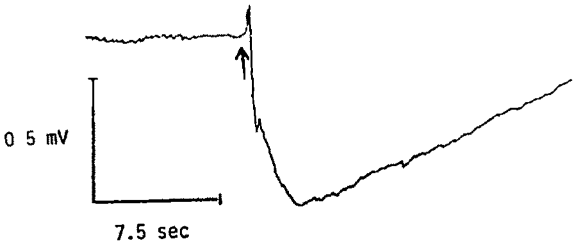

Fig 1 Response of *Pinus taeda* seedlings to needle fascicle deflexion Arrow indicates approximate time of stimulation Peak at right of arrow illustrates the initial fast component of the response A downward response indicates electrical negativity of the apex of the seedling with respect to the base

The magnitude of the response to maximal stimulation varied considerably with the condition of the tree and among trees, but an average of 406 measurements taken on eight seedlings was 0 72 ± 0 58 (standard deviation) mV, a typical response In a series of forty-five measurements on two trees, with a Keithley 603 d c electrometer-amplifier and a direct-coupled Tektronix *RM* 565 dual-beam oscilloscope the response lasted on average 22 0 ± 5 9 sec Most of the fascicle deflexions in this series were produced by means of a shielded pulse-driven d c solenoid with its armature attached to the needles by means of a string or light plastic rod No differences in the amplitude or duration of the response were attributable to tree species.

Effects of Deflexion

A brief description of a recent set of experiments employing two amplifying and recording channels (*A* and *B*) illustrates typical features of what has been observed so far Ag–AgCl electrodes were attached by cotton threads soaked in 0 001 molar KCl to three points on a 2 yr old potted loblolly pine seedling in the laboratory One electrode (apical) was placed on the terminal bud of the seedling and led to the "positive" input terminal of amplifier *A* A second electrode (middle), contacting the stem about midway, was connected in common with the "negative" input terminal of amplifier *A* and the "positive" side of amplifier *B*. The third (basal) electrode led from a point near the base of the seedling to the "negative" input terminal of amplifier *B* Outputs from the two amplifiers were connected separately to two adjacent channels of a four-channel Texas Instruments Servo-Riter recorder

Deflexion of a fascicle near the middle (common) electrode drove this point negatively (depolarization) Stimulation between this point and the apical contact resulted in depolarization of the tissue at the apical electrode, whereas stimulation between the middle and basal contacts gave the same result as stimulation at or near the middle electrode Stimulation in the immediate vicinity of an electrode contact usually produced a negative-going response at that point Stimulation between two electrodes produced a negative-going potential change at the upper or apical contact point Stimulation of fascicles on branches produced no detectable response at electrodes on the main stem or on other branches, but the usual responses were obtained from electrodes on the affected branches Responses appeared on lateral branches above the point of stimulation on the main stem, but not on those below this point

"Dead" seedlings did not ordinarily show a response. The difficulty of killing a seedling without affecting the elasticity of the tissue. and of determining precisely when a seedling is actually dead, renders a definite statement on this point impossible at present.

Girdling the stem with a hot wire above or below the point of stimulation resulted in altered response curves, ranging from disappearance of the initial fast component (Fig 1) to reversal of polarity. The most consistent effect noted was reversal (from negative-going to positive) on destruction of stem tissue between the point of stimulation and the basal electrode contact. Transverse knife cuts into the xylem immediately above and below the stimulated fascicle resulted in the disappearance of the initial rapid component of the response, but the slow or prolonged component persisted unaltered.

Application of electrolyte solutions (for example, 1–3 molar KCl) to the point of fascicle attachment to the stem usually led to a greatly reduced electrical response (Occasional absence of this effect in a few early records was apparently caused by failure of the solution to penetrate into the living tissue) Solutions of non-electrolytes, distilled water and mineral oil have not shown any effect on response amplitude Application of 0 1–1 normal KCN resulted in a marked reduction in amplitude, but this may have been principally a result of electrical shorting by the electrolyte rather than any metabolic effect In all cases, response amplitudes returned to pretreatment levels after drying or flushing of the point of application with distilled water

Characteristics of the Responses

General characteristics of the observed responses can be summarized as follows (*a*) Stimulation at intervals of about 20 sec produced maximal reproducible responses Increasing the stimulus rate produced responses arising from the point of recovery to the previous response, but with reduced amplitude Stimulus intervals of 5 sec or less led to a sustained depolarization with small transient "responses" that were probably stimulus artefacts

(*b*) No precise measurements of mechanical stimulus intensity or rate of stimulus onset were attempted, but actuation of the solenoid armature by square current pulses of varying amplitudes and by a voltage ramp of variable slope applied to the coil indicated an "all-or-none" type of response rather than a graded one Current pulses of about 4 m amp or less gave angular fascicle deflexions of less than 30 degrees and resulted in small transient and atypical voltage fluctuations, whereas pulses of 5 m amp or more moved the fascicles at least 45 degrees and led to the typical maximal response Similarly, response to a slow rate of stimulus onset (voltage ramp applied to the coil), if there was any, was maximal and showed the rapid initial potential change associated with sudden fascicle deflexions (*c*) Efforts to establish the magnitude of the delay between stimulus and response have so far been unsuccessful There was a slight delay, but it was difficult to measure because of the nature of the method of mechanical stimulation With very rapid deflexions, measurements from photographs of oscilloscope traces gave values of about 2 m/sec, which is far more rapid than any previously reported conduction times for plant action potentials Attempts to utilize electrical stimulation were made, but without success

(*d*) The magnitude of the recorded response did not seem to decrease appreciably with distance from the point of stimulation, although the response from a fascicle very near a recording point (within 1 cm or less) was usually greater than at more distant points. Spatial decrement could thus very well be exponential near the fascicle.

(*e*) The effect of temperature on the responses was not studied systematically, but casual observation indicated that the relationship, if any, was slight.

I have not succeeded in finding a fully plausible explanation for these observations. The possibility of shifting electrode contacts has been thoroughly tested and ruled out. Regardless of type of contact used (for example, cotton wick, chlorided silver or platinum wires inserted into the stem or clamped in place), the response persists unaltered. Furthermore, the consistent return of the potential to pre-stimulus levels within a few seconds argues against electrode contact shift as a significant contributing factor.

Similarly, electrostatic artefacts were eliminated by earthing the seedling, and puffs of air directed against the fascicles with sufficient force to deflect them produced the same response as mechanical contact. Electrical noise ("cable noise") resulting from mechanical tissue deformation does not seem to explain the phenomenon either, because of the relatively long recovery period.

Possible Explanations

There are at least three other possibilities, two of them essentially biological rather than physical. First, the response could be caused by depolarization of stem tissue in the immediate vicinity of the fascicle base. If the overall longitudinal seedling biopotential is the algebraic sum of polarized cells in a series-parallel equivalent circuit[1], the drop in potential difference resulting from slight injury in the area including the base of the deflected fascicle would account for the observed response. This would be true especially in those cases where the overall potential difference is in the direction of apical positivity with respect to the base of the seedling, and the response is negative, that is a reduction in potential difference. In those cases where the seedling apex is electronegative with respect to the base, however, the response should be recorded as "positive" (toward zero). Such a clearcut relationship has not been established, although positive responses are usually, but not always, associated with apical negativity.

Second, the response could be a rapidly propagated electrical impulse. All attempts to establish a reasonable transmission time have, however, proved fruitless. If a delay exists it is so slight as to cast serious doubt on its biological significance.

Finally, the mechanical deformation produced by flexure of the point of fascicle attachment could produce sudden changes in hydrostatic pressure within the tree, possibly altering cell membrane permeability at distant electrode contact points and thus producing the observed electrical changes. If this is indeed the case, the action must be strongly directed acropetally, posing intriguing anatomical and morphological problems.

Jacobson[2] has recently reported what he refers to as a "negative receptor potential" in response to mechanosensory hair stimulation in *Dionaea muscipula* Ellis (Venus fly-trap). Although his method of recording differed slightly from mine in that he recorded directly from the sensory hair, his negative receptor potential bears a marked resemblance to the potentials reported here both in waveform and duration (the *Dionaea* potentials were of greater magnitude, perhaps because of the recording method used).

A similar potential change after a series of stroking stimuli had been applied to the outer leaf blade of *Dionaea muscipula* is apparent in the recording contained in a report by DiPalma *et al*.[3] The authors suggest that small protruding structures (stellate trichomes) on the leaf surface serve as touch receptors to sensitize the trigger hairs to mechanical stimuli.

It is possible then that an electrical potential change is generally associated with mechanical displacement of such plant structures as hairs, needle fascicles, stellate trichomes and other similar organs.

I thank Dr Bruce I. H. Scott for his encouragement and advice.

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[1] Lund, E. J., *Bioelectric Fields and Growth* (University of Texas Press, Austin, 1947).

[2] Jacobson, S. L., *J. Gen. Physiol.*, **49**, 117 (1965).

[3] DiPalma, J. R., McMichael, R., and DiPalma, M., *Science*, **152**, 539 (1966).

Urinary Metabolites in Parkinson's Disease

by

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Although DMPEA does not seem to be a component of the pink spot in Parkinsonism, high urinary concentrations of tryptamine may be associated with some cases of the disease. This suggests that in such cases tryptamine hydroxylation is defective.

FRIEDHOFF AND VANWINKLE reported in 1962 that a compound characterized as 3,4-dimethoxyphenethylamine (DMPEA) by its paper chromatographic behaviour and a colour reaction (pink spot when treated with a sequence of reagents believed to be specific for phenethylamines), was unique to the urine of schizophrenic individuals[1]. Both the identification of the urinary component and its relationship to schizophrenia, however, have become controversial issues. Sen and McGeer found the amine in the urines of eleven out of twenty-two schizophrenics[2], and Bourdillon *et al* observed a substance like DMPEA in several urines from schizophrenics[3]. Others, however, have been unable to correlate DMPEA or the pink spot or both with this disease (for leading references see ref. 4).

We, too, could not obtain unequivocal evidence for or against the presence of 3,4-dimethoxyphenethylamine in the urine of schizophrenics using the Friedhoff technique because of the presence of other components in the same region of the paper chromatogram[5] An improved system of isolation[5] based on thin-layer chromatography has made it possible for us to effect a more selective separation of the urinary amines, indeed, the pink spot area of a paper chromatogram prepared by the Friedhoff technique invariably revealed the presence of several components when rerun in our thin-layer system[5] With this method, our studies of the urines of schizophrenics obtained from Dr Friedhoff tended to confirm his findings that DMPEA is unique to the urines of some schizophrenic patients More extensive investigations of a larger number of urines from other sources failed, however, to confirm these findings and we concluded that the positives in the first group were attributable to unique nutritional or environmental factors not related to schizophrenia[6].

To pursue these studies further, urines of normal and schizophrenic individuals were examined for 3,4-dimethoxyphenylacetic acid, the principal metabolic end product[7–9] of 3,4-dimethoxyphenethylamine The results of these studies showed low concentrations of the acid in urines of both normal and schizophrenic individuals, and no significant difference between the two groups On the basis of this evidence it was concluded that the excretion of DMPEA is not characteristic of schizophrenia[6] Friedhoff and Furiya recently reported finding the acid in the urine of both types of individuals, although the concentrations they found were considerably greater than we observed[10] Despite the final statement in their paper that they continue to find DMPEA only in urines of schizophrenics, our conclusion seems to be supported by a number of recent publications[11–15], and the suggestion that the pink spot is a "red herring" when applied to the schizophrenic state seems to represent an accurate appraisal of this situation[16] Most recently Perry *et al* have suggested that the pink spot may be monoacetyl or monopropionylcadaverine[17]

Although a relationship between DMPEA and schizophrenia now seems doubtful, recent evidence has suggested that this amine has some significance in Parkinson's disease Barbeau and his associates, using the Friedhoff technique, found that urines of 80 per cent of patients afflicted with this disease contained the pink spot component—tentatively identified as DMPEA[18] They were also able to show that administered DMPEA causes akinesia-like symptoms in animals[19] A programme to examine the pink spot components in urines of patients with Parkinson's disease with the technique employed in our earlier studies was thus undertaken with the co-operation of Dr Barbeau After this research was initiated, other workers confirmed Barbeau's findings concerning the incidence of a pink spot in urines of Parkinsonians, but could find no evidence supporting its identification as DMPEA[20].

The Parkinson urines, obtained from two sources and from patients who had been off drug treatment for 48 h before collection, gave the results shown in Table 1 Seven out of seventeen gave significant pink spots when examined by the Friedhoff technique An additional seven were positive in this respect, but here the colour was not judged to a greater intensity than observed for some normal urines None of these urines showed detectable amounts of DMPEA when examined by thin-layer chromatography Confirmation of the absence of this amine was obtained by gas chromatography of specimens selected on the basis of a high intensity pink spot The acid fractions of these urines were examined for 3,4-dimethoxyphenylacetic acid by the method described before[6], no differences between the normal and diseased groups were observed, which agrees with the absence of significant amounts of the precursor amine

Boulton *et al* have suggested that the pink spot in urines of schizophrenics and Parkinsonians may be *p*-tyramine, for they observed this compound to exhibit chromatographic and chromogenic properties in the Friedhoff system similar to those of DMPEA, and it was found in a single pooled specimen from four Parkinsonian patients in approximately twice the concentration found in that of a schizophrenic[21] Unfortunately, the current studies with Parkinson urines were not run in such a manner that *p*-tyramine could be detected On the other hand, our investigations revealed that tryptamine is both isographic with DMPEA in the Friedhoff system and characterized by a similar although not identical colour sequence when treated with the ninhydrin-modified Ehrlich reagents It would thus contribute to the overall colour of the spot, which on paper is diffuse and masked by neighbouring contaminants Accordingly, extracts of urines from a number of Parkinson patients were subjected to paper chromatography and the pink spot zone was eluted from paper with methanol and analysed by thin-layer chromatography Certain of the urinary extracts were also analysed directly by this method The results shown in Table 1 demonstrate that there is a reasonable correlation between the colour intensity of the pink spot on the paper chromatogram and the tryptamine content as measured by thin-layer chromatography (confirmation of the presence of this amine was obtained by gas chromatography), certainly enough to suggest that tryptamine is one of the principal components of the pink spot

Table 1

Urine	Pink spot by paper strip	Tryptamine estimate by TLC
11–W	5	3
9–W	4	3
10–W	3	1
876–W	3	5
939–B	2	5
12–W	2	1
5–W	2	2
5–N	2	3
3–W	1	0
1–W	1	0
2–W	1	1
4–N	1	2
2,009–B	1	3
915–B	1	1
7–W	1	0
877–B	1	0
6–W	0	0
4–W	0	0
8–W	0	2
1–N	0	3
2–N	0	0
3–N	0	0

The suffix W denotes a sample from Dr Waltz, B from Dr Barbeau and N a normal urine specimen An arbitrary value of 0 to 5 was used to estimate the intensity of the pink spot on paper and the tryptamine spot on the thin-layer chromatography (TLC)

In conclusion, our studies with urines of Parkinson patients have revealed that DMPEA is not a component of the pink spot, nor is there any consistent qualitative difference between the urines of normal and diseased individuals examined with respect to the composition of the pink spot On the other hand, the urines of patients which showed the more intensely coloured spots were also found to have correspondingly high concentrations of tryptamine Thus it seems possible that high urinary concentrations of tryptamine are associated with some but not all cases of Parkinson's disease, and that this amine is a principal component of the pink spot observed in these cases Furthermore, the finding by Barbeau *et al* that the urinary excretion of 5-hydroxyindoleacetic acid is diminished in patients with Parkinson's disease[22] is compatible with the concept of an increased excretion of tryptamine, and it is therefore reasonable to suggest that tryptophan hydroxylation may be defective in this diseased state Indeed, the administration of 5-hydroxytryptophan is reported to improve some symptoms of Parkinson's disease (personal communication from A Barbeau). Of further interest are the observations that brain concentrations[23] and the urinary excretion[24] of dopamine are decreased in Parkinson patients The fact that administered DOPA improves some symptoms led Cotzias *et al.* to suggest that tyrosine hydroxylation, the rate limiting step in dopamine formation, may be defective in this disease[25] Such a fault would be expected to result in an increased excretion of *p*-tyramine and, as noted here, Boulton *et al* have found that this amine is a component of the pink spot from Parkinson patients[21]. If both tyrosine and tryptophan hydroxylation prove to be deficient in Parkinson's disease, such a defect could be attributable to many factors including low concentrations of the individual hydroxylases Because, however, a reduced pteridine[26,27] is an obligatory requirement for both tyrosine and tryptophan hydroxylase it is possible that a deficiency of this cofactor may also be rate limiting and thus of significance in the aetiology of this disease

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[1] Friedhoff, A. J., and VanWinkle, E., *Nature*, **194**, 897 (1962).

[2] Sen, N P., and McGeer, P. L., *Biochem. Biophys. Res. Comm.*, **14**, 227 (1964)

[3] Bourdillon, R E , Clarke, C A , Ridges, A P , Sheppard, P. M., Harper, P , and Leslie, S A , *Nature*, **208**, 453 (1965)

[4] Closs, K , Wad, N and Ose, E , *Nature*, **214**, 483 (1967)

[5] Kuehl, F A , Hichens, M , Ormond R E , Meisinger M A P , Gale, P , Cirillo, V J , and Brink, N G , *Nature*, **203**, 154 (1964)

[6] Kuehl, F A , Ormond, R E , and VandenHeuvel, W J A , *Nature*, **211**, 606 (1966), and unpublished results

[7] Friedhoff A J , and Hollister, L E , *Biochem Pharmacol* , **15**, 264 (1966)

[8] Kuehl, F A , in *Amine and Schizophrenia* (edit by Himwich, H E , Kety, S S , and Smithies, J R), 23 (Alden Press, Ltd , Oxford, 1967)

[9] Charalampous, K D , and Tansey, L W , *J Pharmacol Exp. Therap* , **155**, 318 (1967)

[10] Friedhoff, A J , and Furiya, K *Nature*, **214**, 1127 (1967)

[11] Williams, C H , Gibson, J G , and McCormick, W O , *Nature*, **211**, 1195 (1966)

[12] Boulton, A A , and Felton, C H , *Nature*, **211**, 1404 (1966)

[13] Bell, C E , and Sumerville, A R , *Nature*, **211**, 1405 (1966)

[14] Faurbve, A , and Pind, K , *Acta Psychiat Scand* , **40**, 240 (1964), ibid , **42**, 136 (1966)

[15] Perry, T L , Hansen, S , MacDougall, L , and Schwartz, C J , *Nature*, **212**, 146 (1966)

[16] Annotations, *Lancet*, ii, 848 (1966)

[17] Perry, T L , Hansen, S , and MacDougall, L , *Nature*, **214**, 984 (1967)

[18] Barbeau, A , de Grot, J , Joly, J , Raymond-Trembley, D , and Donaldson, J , *Rev Canad Biol* , **22**, 469 (1963)

[19] Barbeau, A , Tetrealt, L , Oliva, L , Morazain, L , and Cardin, L , *Nature*, **209**, 719 (1966)

[20] Boulton, H A , and Felton, C A , *Lancet*, ii, 964 (1966)

[21] Boulton, D H , Pollitt, R J , and Majer, J R , *Nature*, **215**, 132 (1967)

[22] Barbeau, A , and Jasmin, G , *Rev Canad Biol* , **20**, 837 (1961)

[23] Bernheimer, H , and Hornykiewicz, O , *Klin Wschr* , **43**, 711 (1965)

[24] Barbeau, A , and Raymond-Trembley, D , *Parkinson's Disease Trends in Research and Treatment*, 79 (Grune, New York, 1965)

[25] Cotzias, G C , Van Woert, M U , and Schiffer, L. M , *New Eng J Med* , **276**, 374 (1967)

[26] Nagatsu, T , Levitt, M , and Udenfriend, S , *J Biol Chem* , **239**, 2910 (1964)

[27] Hosoda, S , and Glick, D , *J Biol Chem* , **241**, 192 (1966)

Late DNA Synthesis in Heterochromatin

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It seems to be a rule of chromosome replication that heterochromatin synthesizes its DNA at a later stage than the rest of the chromosome.

UNTIL 1959 heterochromatin was considered to be an elusive chromosome material difficult to approach experimentally at the cytological level[1]. Work with phosphorus-32 and tritium had not then produced any relevant information about this chromosomal material.

The first experimental demonstration that heterochromatin synthesizes its DNA later than the euchromatic regions of the chromosome complement was obtained in the grasshopper *Melanoplus differentialis*[2,3] In the male of this species there is a large heterochromatic *X* chromosome at prophase The autosomes are chiefly euchromatic The injection of tritiated thymidine into the body cavity revealed that in the spermatocytes, at zygotene and pachytene, the heterochromatic *X* and the autosomes were labelled at different times In the testicular tubules the spermatocytes are grouped in cysts which are synchronized at a given meiotic stage. The cysts move along the tubule as meiosis progresses, so that, from their relative position, it is possible to tell which have developed the farthest. In sections of this material, the spermatocytes of the cysts which were at a late stage in development were labelled only in the heterochromatic *X* chromosome, whereas cysts at an earlier stage of development were only labelled in the autosomes This result established that heterochromatin in this species, and in this tissue, replicated later than euchromatin

To find out whether this phenomenon occurred in other organisms, leaves from seedlings of rye (*Secale cereale*) were exposed to tritiated thymidine In this organism[4,5] all chromosomes are heterochromatic on both sides of the kinetochore and possess heterochromatic "knobs" at most ends The autoradiographic picture of interphase nuclei showed that in this species heterochromatin also replicated at a different time from euchromatin[16] Since then many people have studied the same phenomenon in plant, animal and human material by tritium autoradiography

Cytology of Heterochromatin

Heterochromatin as originally described by Heitz[7] represents a differential behaviour of the chromosome phenotype Heterochromatic regions are characterized, in interphase and early prophase nuclei, as "chromosome regions that form massive blocks of Feulgen positive material"[12].

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Heterochromatin is a state of the chromosome rather than a substance, as is sometimes assumed, because it does not occur at all stages of development In *Drosophila*, heterochromatin is absent in the early embryonic stages, but it is found at later stages in the three largest chromosomes[13] In the male of the grasshopper *Melanoplus* the X chromosome is not heterochromatic in the spermatogonia, but becomes so later in the spermatocytes

As Schultz[12] pointed out, "nucleic acid starvation"[14] or "negative heteropycnosis"[15] should not be included in heterochromatin If we do so, every differential state of the chromosome during the cycle of division could qualify as heterochromatin The original definition would break down, everything in the chromosome becoming heterochromatin depending on the appearance of the region used as a standard Recent results confirm the idea that negative heteropycnosis is a phenomenon distinct from heterochromatin

Spectrophotometric measurements have shown that the amount of DNA per unit area is two to three times greater in the heterochromatin than in the euchromatin of the same nucleus[16] This is not the case in negatively heteropycnotic regions in which there is no indication of increase or decrease in DNA content[17]. Thus this result contradicts the assumption that negative heteropycnosis is the reverse state of positive heteropycnosis, a term also used for heterochromatin. Furthermore, autoradiographic studies in *Trillium* disclose that cold-induced negative heteropycnotic regions can occur in chromosomes which had completed their DNA synthesis before the cold treatment began[18] This result, as well as the spectrophotometric measurements, shows that the designation sometimes given to such chromosomal segments of "nucleic acid starved regions" does not describe appropriately the actual macromolecular organization of these regions.

Negative heteropycnosis is thus not necessarily the opposite of heterochromatin, although it is a particular state of the chromosome, and probably an equally significant one Furthermore, it is not in agreement with the available evidence to call heterochromatin "positive heteropycnosis" as it has sometimes been described We should keep the original description of this well defined state of the chromosome which is characterized by the appearance of a massive block of Feulgen positive material at a given stage of development Other states of the chromosome should be considered as separate phenomena until we have a better knowledge of the macromolecular organization of the chromosome during these states

Autoradiography and Heterochromatin

Information about DNA synthesis obtained by different laboratories using tritium autoradiography has varied in the degree of accuracy with which the relation between the autoradiographic picture and the occurrence of heterochromatin has been established in a given tissue Accordingly, these results may be divided into three main groups (*a*) Autoradiographic studies were carried out in the same tissue and at the same stage at which heterochromatin has been well established (*b*) Tritiated thymidine was administered to tissues where heterochromatin was not recognizable but the chromosomes studied were heterochromatic in another tissue (*c*) Tritiated thymidine was added to a given tissue, but no exact information was available about the occurrence of heterochromatin in the tissue studied or in other tissues This group contains some of the results for sex chromosomes or presumed sex chromosomes

The experiments of groups (*b*) and (*c*), although relevant to this problem, cannot be considered to give reliable information about the relation between the occurrence of heterochromatin and the sequence of DNA synthesis To establish this relation with accuracy it is necessary (i) that there is clear-cut evidence that a given chromosome or chromosome segment is actually heterochromatic, and (ii) that the autoradiographic pictures are studied in the tissue where heterochromatin is displayed

A well known phenomenon, since Heitz's discovery of heterochromatin, is that a given chromosome is not necessarily heterochromatic in all the tissues of an organism, and that not all sex chromosomes are heterochromatic

Relation between Heterochromatin and Late Replication

In this article, we will deal chiefly with the evidence from the first group of results, where the two conditions just postulated are fulfilled. There are now many results in this category available from different laboratories These allow us to assess the general validity of the findings originally made in *Melanoplus* and *Secale*

Four species of plants (Table 1) have been found to exhibit this phenomenon *Secale cereale*[19,20], *Fritillaria lanceolata*[21], *Vicia faba*[22] and *Hordeum vulgare*[23] In these species heterochromatin is displayed, in root and leaf nuclei and other tissues It shows up at interphase in the form of chromocenters and at prophase as large chromomeres or deeply stained segments Tritiated thymidine was administered to these tissues and the autoradiographic picture was studied at interphase, prophase and metaphase In the four species, the chromocenters at interphase, the large chromomeres at prophase and the corresponding regions at metaphase were found to replicate later than the euchromatic regions (Table 1).

Four species of insects (Table 2) exhibit the same phenomenon *Melanoplus differentialis*, *Bombyx mori*, *Pseudococcus obscurus* and *Drosophila melanogaster* Three of them, *Drosophila*, *Melanoplus* and *Pseudococcus*, are classical examples of organisms with heterochromatin The behaviour of the X chromosome in *Melanoplus* was mentioned earlier in this article In *Drosophila*, the heterochromatin of which was initially studied by Heitz[7–11] at

Table 1 PLANTS IN WHICH INCORPORATION OF TRITIATED THYMIDINE WAS STUDIED IN THE SAME TISSUE AND AT THE SAME STAGE AND WHEN HETEROCHROMATIN HAS BEEN WELL ESTABLISHED

Species	Autoradiography		Heterochromatin		Replication period of heterochromatin	References, autoradiography	References, heterochromatin
	Tissue	Stage	Tissue	(1) Stage (2) Chromosomes			
Secale cereale	Leaves Roots	Interphase Prophase Metaphase	Leaves Roots	(1) Interphase Prophase (2) Proximal, and median regions of all chromosomes and "knobs"	Later than euchromatin	19, 20	6, 64, 65
Fritillaria lanceolata	Roots	Interphase	Roots	(1) Interphase (2) Chromocenters	Later than euchromatin	21	21
Vicia faba	Roots	Interphase	Roots	(1) Interphase Prophase (2) Near centromeres and other regions	Later than euchromatin	22	66
Hordeum vulgare	Roots	Metaphase	Roots	(1) Interphase Prophase (2) Proximal regions of all chromosomes	Later than euchromatin	23	65

prophase and metaphase in somatic cells, the whole *Y* chromosome, the proximal regions of the *X* and of chromosomes II and III are heterochromatic The incorporation of tritiated thymidine into this same tissue where heterochromatin is observed reveals the late replication of the heterochromatic regions relative to the euchromatic ones[24]. Particularly striking is the case of *Pseudococcus* where a whole set of chromosomes is heterochromatic[25]. This whole set undergoes DNA replication later than the euchromatic set in the same nucleus[26] In *Bombyx*[27], heterochromatin in the spinning glands is also found to be late replicating when tritiated thymidine is administered to this tissue In the four cases the autoradiographic picture was studied in the same tissue and at the same stage, when the heterochromatin was described

Mammals, including man, give most examples confirming the rule of late replication of heterochromatin We include in Table 3 only the cases where DNA replication has been studied in the chromocenters at interphase With the exception of a few cases, only such conditions allow an accurate study of the sequence of DNA replication in heterochromatin relative to euchromatin Only when the information from the chromocenters at interphase, or from heterochromatic chromosomes at prophase, can be directly related to specific chromosomes at metaphase can we state that the late DNA replication seen at metaphase is representative of the heterochromatin observed at interphase or prophase Metaphase chromosomes are seldom heterochromatic

Man is the species in which the relation between the metaphase and interphase pictures has been best established because of the occurrence of individuals with multiple *X* chromosomes The number of chromocenters (Barr bodies) seen at interphase in diploid cells is equal to the number of *X* chromosomes minus one (according to the rule of Harnden[2]) Thus in man the autoradiographic picture of the *X* chromosome at metaphase can be considered to furnish reliable information about the late DNA replication of heterochromatin[29–31] Studies of DNA replication in individuals with multiple sex chromosomes have confirmed the relation between the number of Barr bodies seen at interphase and the number of late replicating *X* chromosomes present at metaphase[32–38] Furthermore, there have been several studies of the tritium autoradiographic picture of Barr bodies at interphase which confirm the late replication of heterochromatin in man at the chromocenter stage[39–41] In addition to the sex chromatin (Barr bodies), autosomal heterochromatin has also been described at interphase in the human male and female This replicates at a different time from the euchromatin[42,43]

The Chinese hamster (*Cricetulus griseus*) has *X* and *Y* chromosomes which form distinct chromocenters at interphase (ref 43 and personal communication from W Schmid) These were found to replicate later than the euchromatic regions of the autosomes in interphase nuclei and at metaphase[44,45] A similar situation is found in the Syrian hamster (*Mesocricetus auratus*) and in the guinea-

Table 2 INSECT SPECIES IN WHICH INCORPORATION OF TRITIATED THYMIDINE WAS STUDIED IN THE SAME TISSUE AND AT THE SAME STAGE AND WHERE HETEROCHROMATIN HAS BEEN WELL ESTABLISHED

Species	Autoradiography		Heterochromatin		Replication period of heterochromatin	References, autoradiography	References, heterochromatin
	Tissue	Stage	Tissue	(1) Stage (2) Chromosomes			
Melanoplus differentialis	Testis	Prophase of meiosis	Testis	(1) Prophase of meiosis (2) *X* chromosome	*X* later than euchromatic autosomes	4, 57	4, 57
Bombyx mori	Spinning glands	Interphase	Spinning glands	(1) Interphase (2) Chromocenters	Later than euchromatin	27	27
Pseudococcus obscurus	Somatic cells	Interphase Metaphase	Somatic cells	(1) Interphase (2) Whole heterochromatic chromosome set	Later than euchromatic set	26	25, 67
Drosophila melanogaster	Embryonic cells	Prophase Metaphase	Embryonic cells	(1) Prophase Metaphase (2) Whole *Y*, prox reg of *X* and of chrom II and III	Later than euchromatin	24	68

Table 3 MAMMALIAN SPECIES WHERE THE INCORPORATION OF TRITIATED THYMIDINE WAS STUDIED IN THE SAME TISSUE AND AT THE SAME STAGE WHERE HETEROCHROMATIN HAS BEEN WELL ESTABLISHED

Species	Autoradiography		Heterochromatin		Replication period of heterochromatin	References, autoradiography	References, heterochromatin
	Tissue	Stage	Tissue	(1) Stage (2) Chromosomes			
Cricetulus griseus (Chinese hamster)	Embryonic tissues	Interphase Metaphase	Embryonic cells Kidney and nerve cells	(1) Interphase (2) Chromocenters representing *X* and *Y*	Later than euchromatin	44	69 and personal communication from W Schmid
Chinchilla lanigera (chinchilla)	Kidney cell cultures	Interphase Metaphase	Kidney cell cultures	(1) Interphase (2) Chromocenters representing *X* and *Y*	Later than euchromatin	48	48
Microtus agrestis (field vole)	Bone marrow cultures Fibroblast cells	Interphase Metaphase	Bone marrow cultures Fibroblast cells	(1) Interphase (2) Chromocenters whole *Y* and part of *X*	Later than euchromatin	46, 47	46, 47
Mesocricetus auratus (Syrian hamster)	Kidney cell cultures	Interphase Metaphase	Kidney cell cultures Nerve cells	(1) Interphase (2) Major and minor chromocenters	Later than euchromatin	46	46
Cavia cobaya (guinea-pig)	Kidney cell cultures	Interphase Metaphase	Kidney cell cultures Nerve cells	(1) Interphase (2) Large and small chromocenters	Later than euchromatin	46	46
Canis familiaris (dog)	Kidney cell cultures Bone marrow	Interphase Metaphase	Kidney cell cultures Buccal mucosa	(1) Interphase (2) Chromocenters One *X* chromosome	Later than euchromatin	49	49
Donkey–grevy zebra hybrid	Skin cultures	Interphase Metaphase	Skin cultures	(1) Interphase (2) Chromocenters One *X* chromosome	Later than euchromatin	50	50
Mus musculus (mouse)	Kidney epithelial cells	Interphase	Kidney epithelial cells	(1) Interphase (2) 4–20 chromocenters	Later than euchromatin	51	51
Homo sapiens (man)	Various somatic tissues (fibroblasts, blood, skin)	Interphase Metaphase	Various somatic tissues (fibroblasts, blood, skin, nerve cells)	(1) Interphase (2) Chromocenters One *X* chromosome (Barr body) and autosomes	Later than euchromatin	29–43	42, 43, 70–72

pig (*Cavia cobaya*), in which several major and minor chromocenters, present in the interphase nucleus, have been found to replicate late relative to the euchromatin[46].

The sex chromosomes of *Microtus agrestis*[47] are among the largest known in mammals and their heterochromatin can be seen at interphase in the form of very large chromocenters and at prophase. In the male chinchilla[48], the *Y* and the *X* chromosome are heterochromatic at interphase forming two large chromocenters. In these two species the pattern of uptake of tritiated thymidine into DNA was studied at metaphase, and also at interphase where the distinct chromocenters were found to replicate later than the euchromatin.

In the dog[49], the donkey–grevy zebra hybrid[50] and in the mouse[51] there are one or several chromocenters at interphase. These represent either one *X* chromosome (dog and donkey–grevy zebra hybrid) or cannot be directly related to sex chromosomes (mouse). In every mammalian species included in Table 3 the autoradiographic picture and the heterochromatin were studied in the same tissue at interphase, disclosing the late replication of the chromocentral heterochromatin.

Rule of Chromosome Replication

These results for the seventeen species of plants, insects and mammals (Tables 1–3) allow us to formulate the following rule of chromosome replication. When tritiated thymidine is incorporated into the cells of a given tissue in which there are massive Feulgen positive chromosome regions at interphase or early prophase, the heterochromatic regions replicate their DNA later than the euchromatic segments. This takes place when the autoradiographic picture is studied in the same tissue and at the same stage in which heterochromatin is displayed. This phenomenon occurs irrespective of the evolutionary position of the organism and of the tissue in which heterochromatin is observed.

If a chromosome is heterochromatic in a given tissue and replicates its DNA later than the euchromatin, it does not necessarily follow that it replicates its DNA later in other tissues where it may be euchromatic. This phenomenon was demonstrated in the grasshopper *Melanoplus*, in spermatocytes, the *X* chromosome is heterochromatic at prophase, and replicates its DNA later than the autosomes[2,52]. The same chromosome is, however, euchromatic or slightly negatively heteropycnotic in the spermatogonia, and in this tissue the incorporation of tritiated thymidine takes place at the same time as in the autosomes[53].

This result is most significant in demonstrating that it is invalid to study the autoradiographic picture of a given chromosome in a tissue where this chromosome is not known with accuracy to be heterochromatic, and from such a result we draw conclusions about the relation between heterochromatin and the sequence of DNA replication.

In *Microtus oregoni* Hsu *et al* [54] described results which they believed contradicted the rule of chromosome replication. In this species Ohno *et al* [55] had observed a large chromocenter in embryonic liver cells which they interpreted as representing two-thirds of the *X* chromosome seen at prophase. Hsu *et al* did not administer tritiated thymidine to this tissue but to lung fibroblasts grown *in vitro*, in which they then studied DNA replication at metaphase, when heterochromatin is invisible. They did not find late replication of the segments presumed to be heterochromatic at interphase in the liver, and they concluded that this case represented a departure from the relation between late replication and heterochromatin. Autoradiography was carried out in one tissue and heterochromatin was observed in another, and therefore such an experiment cannot be used as an example in establishing the relation between late replication and heterochromatin.

It is not a corollary of our DNA rule that any chromosome or chromosome region which is late replicating should be heterochromatic. Thus within the chromosomes of various species different regions replicate at different times showing no indication of being heterochromatic. When heterochromatin is not present, other factors, not yet apparent, determine the sequence of replication of various segments within and between chromosomes[57].

It can be stated at present that what determines the presence of heterochromatin also determines the late replication of a chromosome, or, in other words, what changes the condensation cycle of the chromosome at the same time shifts its timing of DNA replication. What determines the late replication of a chromosome or chromosome region, however, may not necessarily determine the onset of heterochromatin.

It should be noted that heterochromatin was originally discovered by its expression at the chromosome phenotype, and it is still primarily recognized by the changes which occur at this level. What happens at the gene level or at the molecular level of chromosome organization, when heterochromatin becomes apparent at the microscopic level—a jump from the Angstrom to the micron level—is unknown. The synthesis of *m*RNA in heterochromatin and in euchromatin, as well as the role of histones in the repression of this synthesis, is now being studied[60–63] and these results may give clues which will allow us to put the microscopic and molecular information into a coherent framework.

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[1] Schultz, J *Cold Spring Harbor Symp Quant Biol* , 12, 179 (1947)

[2] Caspersson, T O , *Cell Growth and Cell Function*, 185 (W W Norton, New York, 1950)

[3] Lima-de-Faria, A , *Hereditas*, 42, 85 (1956)

[4] Lima-de-Faria, A , *Science*, 130, 503 (1959)

[5] Lima-de-Faria, A , *J Biophys Biochem Cytol* , 6, 457 (1959)

[6] Lima-de-Faria A , *Chromosoma*, 5, 1 (1952)

[7] Heitz, E , *Jahrb Wiss Bot* , 69, 762 (1928)

[8] Heitz, E , *Planta*, 12, 775 (1931).

[9] Heitz, E , *Z Zellforsch* , 20, 237 (1933)

[10] Heitz, E , *Biol Zbl* , 54, 588 (1934).

[11] Heitz, E , *Z f ind Abst und Vererbgsl* , 70, 402 (1935).

[12] Schultz, J , *Cold Spring Harbor Symp Quant Biol* , 12, 179 (1947)

[13] Brown, S W , *Science*, 151, 417 (1966)

[14] Darlington, C D , and La Cour, L , *J Genet* , 40, 185 (1940).

[15] White, M J D , *Animal Cytology and Evolution*, 375 (London and New York, Cambridge University Press, 1945)

[16] Lima-de-Faria, A , *J Biophys Biochem. Cytol* , 6, 457 (1959).

[17] Woodard, J , and Swift, H , *Exp Cell Res* , 34, 131 (1964), Woodard, J , Gorovsky, M , and Swift, H , *Science*, 151, 215 (1966)

[18] Boothroyd, E R , and Lima-de-Faria, A , *Hereditas*, 52, 122 (1964).

[19] Lima-de-Faria, A , *J. Biophys Biochem Cytol* , 6, 457 (1959).

[20] Darlington, C D , and Hague, H , in *Chromosomes Today*, 1 (edit by Darlington, C D , and Lewis K R), Suppl to *Heredity*, 19, 102 (1966)

[21] Pelc, S R and La Cour, L F , in *The Cell Nucleus* (edit by Mitchell, J S), 232 (Butterworth and Co , London, 1960).

[22] Evans, H J , *Exp Cell Res* , 35 381 (1964).

[23] Kusanagi, A , *Chromosoma*, 20, 125 (1966).

[24] Barigozzi C Dolfini, S , Fraccaro, M , Jiepolo, L , and Raimondi, G R , *Exp Cell Res* , 43, 231 (1966)

[25] Hughes-Schrader, S , *Advances in Genetics* (edit by Demerec, M), 2, 127 (1948)

[26] Baer, D , *Genetics*, 52, 275 (1965)

[27] Karnkowska-Gorska, Z , *Bulletin de l'Academie Polonaise des Sciences*, 8, 353 (1960)

[28] Harnden, D G , *Lancet*, ii, 488 (1961)

[29] German, J L , *Trans NY Acad Sci* , 24, 395 (1962)

[30] Gilbert, C W , Muldal, S , Lajtha, L. G , and Rowley, J , *Nature*, 195, 869 (1962)

[31] Morishima, A , Grumbach, M M , and Taylor, J H , *Proc US Nat Acad Sci* , 48, 756 (1962)

[32] Morishima, A , Grumbach, M M , and Taylor, J H , *Proc US Nat Acad Sci* , 48, 756 (1962)

[33] Grumbach, M M , Morishima, A , and Taylor, J H , *Proc US Nat Acad. Sci* , 49, 581 (1963)

[34] Rowley, J , Muldal, S Gilbert, C W , Lajtha, L G , Lindsten, J , Fraccaro, M , and Kaijser, K , *Nature* 197, 251 (1963)

[35] Atkins L , Book, J A Gustavson, K -H , Hansson, O , and Hjelm, M , *Cytogenetics* 2 208 (1963)

[36] Giannelli, F , *Lancet*, i, 863 (1963)

[37] Mukherjee, B B , Miller, O J , Breg, W R , and Bader, S , *Exp Cell Res* , 34, 333 (1964)

[38] Hsu J C , and Lockhart L H , *Hereditas*, 52, 320 (1964) Atkins, L , and Gustavson, K H , *Hereditas*, 51, 135 (1964)

[39] Atkins L , Böök J A Gustavson, K.-H., Hansson, O , and Hjelm, M., *Cytogenetics*, 2, 208 (1963)

[40] Bishop, A , and Bishop, O N , *Nature*, **199**, 930 (1963)
[41] Comings, D E , *Cytogenetics*, **6**, 20 (1967)
[42] Lima-de-Faria, A , and Reitalu, J , *J. Cell Biol* , **16**, 315 (1963).
[43] Lima-de-Faria, A , Reitalu, J , and O'Sullivan, A M , *Chromosoma*, **16**, 152 (1965)
[44] Taylor, J H , *J Biophys Biochem Cytol* , **7**, 455 (1960).
[45] Hsu T C , *J Cell Biol* , **23** 53 (1964)
[46] Schmid, W , *Arch Klaus-Stiff Verer b Forsch* , **42**, 1 (1967)
[47] Wolf, U , Flinspach, G , Bohm, R , and Ohno, S , *Chromosoma*, **16**, 609 (1965)
[48] Galton, M Benirschke, K , and Ohno, S , *Chromosoma*, **16**, 668 (1965)
[49] Fraccaro, M , Gustavsson, I , Hulten, M , Lindsten, J , Mannini, A , and Tiepolo, L , *Hereditas*, **52**, 265 (1964)
[50] Benirschke, K , Low, R J , Brownhill, L E , Caday, L B , and De Venecia-Fernandez J , *Chromosoma*, **15**, 1 (1964)
[51] Tiepolo, L , Fraccaro, M , Hulten, M , Lindsten, J , Mannini, A , and Gen-Ming L Ming, *Cytogenetics*, **6**, 51 (1967)
[52] Lima-de-Faria, A , *Hereditas*, **47**, 674 (1961)
[53] Nicklas, R B , and Jaqua R A , *J Cell Biol* , **23**, 67*A* (1964)
[54] Hsu T C , Schmid, W and Stubblefield, E , in *Role of Chromosomes in Development*, 83 (Academic Press, New York, 1964)
[55] Ohno S Jainchill J , and Stenius, C , *Cytogenetics* **2**, 232 (1963)
[56] Ohno, S , Becak, W , and Becak, M L , *Chromosoma*, **15**, 14 (1964)
[57] Taylor, J H , *Exp Cell Res* , **15**, 350 (1958)
[58] Lima-de-Faria, A , *Hereditas*, **47**, 674 (1961)
[59] Lima-de-Faria, A , Bianchi, N O , and Nowell, P , *Hereditas* (in the press)
[60] Allfrey, V G , and Mirsky, A E , *Cold Spring Harbor Symp Quant Biol* , **28**, 247 (1963)
[61] Bonner, J , *The Molecular Biology of Development*, 155 (Clarendon Press, Oxford, 1965)
[62] Berlowitz, L , *Proc US Nat Acad Sci* , **53**, 68 (1965)
[63] Frenster, J H , in *The Cell Nucleus—Metabolism and Radiosensitivity*, 27 (1966)
[64] Lima-de-Faria, A , *Proc Seventh Intern Bot Cong* , 217 (1950)
[65] Tjio, J H , and Levan, A , *Anales de la Estacion Experimental de Aula Dei*, **2**, 21 (1950)
[66] McLeish, J , *Heredity*, Suppl 6, 125 (1953)
[67] Brown, S W , and Nur, U , *Science*, **145**, 130 (1964)
[68] Heitz, E , *Z f ind Abst und Vererbgsl* , **70**, 402 (1935)
[69] Taylor, J H , *J Biophys Biochem Cytol* , **7**, 455 (1960)
[70] Barr, M L , in *The Sex Chromatin* (edit by Moore, K L), 128 (Saunders Co , 1966)
[71] Klinger, H P , and Schwarzacher, H G , *J Biophys Biochem Cytol* , **8**, 345 (1960)
[72] Atkins, L , Book, J A , Gustavson, K -H , Hansson, O , and Hjelm, M , *Cytogenetics*, **2**, 208 (1963)

Structure and Distribution of Glycosyl Diglycerides in Bacteria

by

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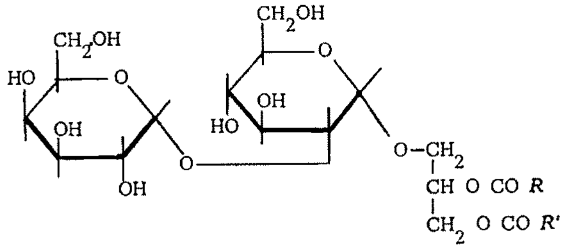
Glycosyl diglycerides are widely distributed in Gram-positive bacteria. Those examined so far contain only glucose, galactose or mannose, and they can be classified according to the nature of the disaccharide attached to their diglyceride component. This chemical classification can be related to general bacterial taxonomy.

THE recognition of the metabolic and structural importance of lipids has led to increased interest in them and in particular bacterial lipids. The wealth of information available on the phosphatide and fatty acid composition of numerous bacteria has enabled certain phylogenetic relationships to be established and this subject has been extensively reviewed[1,2]. New types of lipids have been discovered, and two of the most interesting recent developments have been the recognition of the widespread occurrence of the amino-acid esters of phosphatidyl glycerol[3] and the glycosyl diglycerides[4]. The structure, function and distribution of glycosyl diglycerides in bacteria have been studied in a number of laboratories, and this article reviews certain structural features and relationships that are emerging from this work.

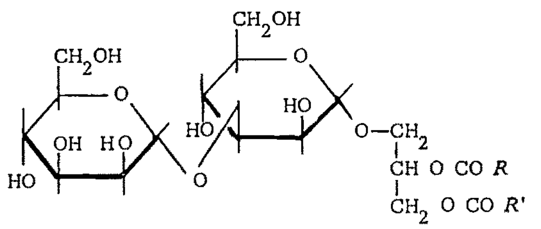
With the exception of extensive investigations by Lederer and his associates on the glycolipids of mycobacteria[5], no information was available on the structure and distribution of glycolipids in other bacteria until, in 1961, Macfarlane reported the presence of a mannosyl diglyceride in *Micrococcus lysodeikticus*[6]. The following year Polonovski, Wald and Paysant-Diament isolated a diglucosyl diglyceride from *Staphylococcus aureus*[7], although in neither case was the full structure determined. Our interest in this field developed from the observation that the lipids of a *Pneumococcus* Type I contained considerable quantities of 1-[*O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-glucopyranosyl]-2,3-diglyceride (Fig 1)[8]. Independently, Kaufman, Kundig, Distler and Roseman proposed an identical structure for the glycolipid from *Pneumococcus* Type XIV (ref 9). Several Gram-positive bacteria were then examined and the presence of similar compounds was established in all of them[4]. More recently, many other bacteria have been shown to possess this type of lipid, the widespread distribution of which is firmly established. From these studies, summarized in Table 1, certain tentative conclusions may be drawn concerning their structure and distribution.

The principal glycolipid in nearly all the organisms shown in Table 1 is a diglycosyl diglyceride. This type of lipid, first discovered in plants[10], is composed of a disaccharide residue glycosidically linked to the 1-position of an α β-diglyceride. Five groups have so far been recognized, depending on the nature of the disaccharide, these are the α-glucosylglucosyl, β-glucoslyglucosyl, α-galactosylglucosyl, β-galactosylgalactosyl and α-mannosylmannosyl diglycerides. Within each group the complete structure of at least one member has been determined and is shown in Fig 1. In four of the groups where structure determinations have been carried out on several members (indicated by an asterisk in Table 1), the diglycosylglycerols produced by hydrolysis of the glycolipids with alkali have identical structures within each group. Glucose, galactose and mannose are the only sugars found so far in these glycolipids and, of the five types of diglycosylglycerols, four contain disaccharides of a single hexose. The two anomeric centres in the glycosides have the same configuration and, in the four glycosides containing glucose or galactose or both, an α-linked disaccharide contains a 1→2 linkage between the two sugar residues whereas a β-linked disaccharide has a 1→6 linkage.

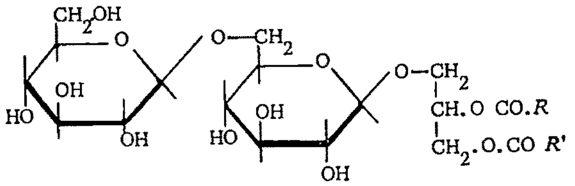
The glycolipids yield a single glycoside on hydrolysis with alkali, with the exception of those from *Arthrobacter globiformis*, where both a dimannosyl and a digalactosyl diglyceride occur together This organism is known to exist in two morphological forms[11] and it is possible that these two forms each contain a single but different diglycosyl diglyceride. Polonovski *et al* have reported[12] that the glycoside from *Staphylococcus aureus* is a mixture of two isomeric diglucosylglycerols containing respectively a 1→6 and a 1→3 linkage between the two sugars, but their evidence is inconclusive and has not been confirmed in our studies on *S. aureus*. Biosynthetic studies have shown that in *Micrococcus lysodeikticus*[13] and *Pneumococcus* Type XIV (ref 9), a monoglycosyl diglyceride is the immediate biosynthetic precursor of the diglycosyl diglyceride and it is probable that this mechanism of biosynthesis is usual In only three organisms, however, has a significant amount of a monoglycosyl diglyceride been detected Furthermore, in two of these organisms, *Arthrobacter globiformis* and *Mycoplasma laidlawii B*, a diglycosyl diglyceride is also present and the relative amounts of these two components will presumably depend on culture conditions and the growth phase at the time of isolation Small quantities of triglycosyl diglyceride have been detected in several lactobacilli and *Streptococcus faecalis*, and in *Lactobacillus plantarum* 17–5 it is the sole glycolipid component Gas–liquid chromatographic studies on the fatty acid composition of the glycolipids have shown a similar distribution to that in the other lipids



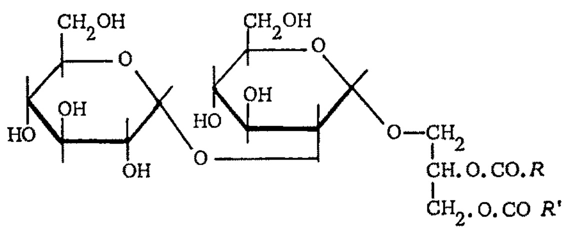
1-[*O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-glucopyranosyl]-2,3-diglyceride



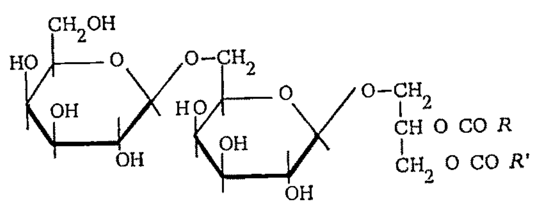
1-[*O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl]-2,3-diglyceride



1-[*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl]-2,3-diglyceride



1-[*O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl]-2,3-diglyceride



1-[*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl]-2,3-diglyceride

Fig 1 The structures of the five types of diglycosyl diglyceride isolated from Gram-positive bacteria

Table 1 DISTRIBUTION OF GLYCOSYL DIGLYCERIDES IN BACTERIA

Diglucosyl diglycerides	Reference
α-Glycoside	
* *Streptococcus faecalis NCIB* 8191	4 and N Shaw (unpublished work)
* ,, ,, *ATCC* 9790	22
,, ,, *St* 9	4
,, *pyogenes*	16
,, *pyogenes*, derived L-form	16
* *Mycoplasma laidlawii B*	23
β-Glycoside	
* *Staphylococcus lactis* I3	21
* ,, *saprophyticus* I2	4 D E Brundish (unpublished work)
* ,, *lactis NCTC* 7944	,,
* ,, *aureus*	,,
* *Bacillus subtilis*	,,
Galactosylglucosyl diglycerides	
* *Pneumococcus* Type I	24
* ,, Type XIV	9
* *Lactobacillus casei ATCC* 7469	25
* ,, *buchneri NCIB* 8007	4 N Shaw (unpublished work)
,, *plantarum NCIB* 7220	26
,, *helveticus NCIB* 8025	26
Dimannosyl diglycerides	
* *Micrococcus lysodeikticus*	13
* *Microbacterium lacticum*	N Shaw (unpublished work)
* *Arthrobacter globiformis*	27
Digalactosyl diglycerides	
* *Arthrobacter globiformis*	27
Monoglycosyl diglycerides †	
* *Arthrobacter globiformis*—galactopyranosyl diglyceride	27
* *Mycoplasma mycoides*—galactofuranosyl ,,	28
* ,, *laidlawii B*—glucopyranosyl ,,	23
Triglycosyl diglycerides †	
Lactobacillus plantarum 17–5–glucosylgalactosylglucosyl diglyceride	4

Gram-negative bacteria examined for glycosyldiglycerides[20] *Aerobacter aerogenes Bacillus polymyxa Escherichia coli K*12 *E coli B Serratia marcescens, Pseudomonas fluorescens, P aeruginosa, Vibrio succinogenes*

* Complete structure has been determined.

† The organisms listed contain these glycolipids as principal components Small quantities have been observed in other organisms

Two conclusions may be drawn from a study of the distribution of these glycolipids. First, they have only been isolated from Gram-positive bacteria and, second, significant relationships concerning the nature of the glycolipid present and the taxonomy of the organisms are being revealed Although an extensive survey of Gram-negative bacteria has not been carried out, none of those examined in detail (Table 1) contained glycosyl diglycerides It would be premature to claim their universal occurrence in Gram-positive bacteria, but the presence of a glycosyl diglyceride has been shown in all such organisms examined in these laboratories. Their presence in the mycoplasma, a group of organisms lacking the normal rigid bacterial cell wall, is especially interesting and adds further support to the similarities which have already been established between the mycoplasma and the L-forms of Gram-positive bacteria[14]

The distribution of the five groups of diglycosyl diglycerides shows that the galactosylglucosyl diglycerides are found in the lactobacilli and two strains of pneumococcus, the α-glucosylglucosyl diglycerides are found in streptococci and *Mycoplasma laidlawii B*, the β-glucosylglucosyl diglycerides are found in staphylococci and *Bacillus subtilis* and the dimannosyl diglycerides are found in *Micrococcus lysodeikticus* and the closely related organisms *Arthrobacter globiformis* and *Microbacterium lacticum* Thus while the same glycolipid can be found in organisms belonging to different families, members of the same genus or closely related species contain identical glycolipids

Quantitative studies have shown that the amount of glycolipid is variable, ranging from a few per cent of the total lipid in the lactobacilli and staphylococci to more than 40 per cent in *Microbacterium lacticum* and *Mycoplasma laidlawii B* Until more evidence is obtained concerning the function of these compounds and their rate of accumulation and turnover in the organism, it is difficult to draw any useful conclusions from these figures In common with the phosphatides of Gram-positive bacteria, their location in the membrane has been firmly established in several instances[15–17]

There is still no evidence to support earlier suggestions[18] that these glycolipids might be concerned in the transfer of sugar residues from nucleoside diphosphate sugars to polysaccharide chains, although the occurrence of the larger compounds would be consistent with such a function More recent events, however, have made this less likely because it has been shown that in the biosynthesis of lipopolysaccharides[19] and peptidoglycans[20] sugar residues are attached to an intermediate lipid pyrophosphate in which the lipid is a polyisoprenoid alcohol, a phospholipid intermediate has also been demonstrated in the biosynthesis of a teichoic acid (L J Douglas, N. L. Blumsom and J Baddiley, unpublished work) An alternative function has been suggested by Brundish, Shaw and Baddiley[21] from an examination of the shape of diglycosyl diglycerides These glycolipids can adopt a conformation in which all the hydroxyl groups lie on one side of the molecule and the lipophilic components (fatty acids, ring oxygen of sugars and glycosidic oxygens) lie on the other side The hydrophilic regions of a number of molecules could come together to form a "pore" in the membrane through which small ions and water-soluble metabolites could pass

The conclusions presented here are based on a study of relatively few organisms and consequently may be invalidated by further work in this field In our view, however, the results obtained so far are sufficiently encouraging to suggest that this approach will complement the results of phosphatide and fatty acid analysis in the study of phylogenetic relationships in bacteria

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[1] Kates, M *Adv Lipid Res*, 2, 17 (1964)
[2] Ikawa, M, *Bact Rev* 31, 54 (1967)
[3] Macfarlane, M G, *Adv Lipid Res*, 2, 91 (1964)
[4] Brundish, D E, Shaw, N, and Baddiley, J, *Biochem J*, **99**, 546 (1966)
[5] Asselineau, J, in *Les Lipides Bacteriens* (Hermann et Cie, Paris, 1965)
[6] Macfarlane, M G, *Biochem J*, **80**, 45P (1961)
[7] Polonovski, J, Wald, R, and Paysant-Diament, M, *Ann Inst Pasteur*, **103**, 32 (1962)
[8] Brundish, D E, Shaw, N, and Baddiley, J, *Biochem Biophys Res Comm*, 18, 308 (1965)
[9] Kaufman, B, Kundig, F D, Distler, J, and Roseman, S, *Biochem Biophys Res Comm*, **18**, 312 (1965)
[10] Carter, H E McCluer, R H, and Slifer, E D, *J Amer Chem Soc*, **78**, 3735 (1965)
[11] Stevenson, I L, *Canad J Microbiol*, 9, 509 (1963)
[12] Polonovski, J, Wald, R, and Petek, F, *Bull Soc Chim Biol*, Paris, **47**, 409 (1965)
[13] Lennarz, W J, and Talamo, B, *J Biol Chem*, **241**, 2707 (1966)
[14] "Biology of Mycoplasmas", *Ann NY Acad Sci*, **143** (1967)
[15] Vorbeck, M L, and Marinetti, G V, *Biochemistry*, 4, 296 (1965)
[16] Cohen, M, and Panos, C, *Biochemistry*, 5, 2385 (1966)
[17] Bishop, D G, Rutberg, L, and Samuelsson, B, *Europ J Biochem*, **2**, 448 (1967)
[18] Distler, J, and Roseman, S, *Proc US Nat Acad Sci*, **51** 897 (1964)
[19] Wright, A, Dankert, M, Fennessey, P, and Robbins, P W, *Proc US Nat Acad Sci*, **57** 1798 (1967)
[20] Higashi Y, Strominger, J L, and Sweeley, C C, *Proc US Nat Acad. Sci*, **57**, 1878 (1967)
[21] Brundish, D E, Shaw N, and Baddiley, J, *Biochem J*, **105**, 885 (1967)
[22] Vorbeck, M L, in *Abst Ann Meet Amer Chem Soc* (1966)
[23] Shaw, N, and Smith, P F, *Bact Proc* 108 (1967)
[24] Brundish, D E, Shaw, N, and Baddiley J, *Biochem J*, **97** 158 (1965)
[25] Shaw N, Heatherington, K, and Baddiley J, *Biochem J* (in the press).
[26] Le Roux, N, dissertation, Univ Newcastle upon Tyne (1967)
[27] Walker, R W, and Bastl, C P, *Carbohyd Res*, 4, 49 (1967)
[28] Plackett P, *Biochemistry*, 6, 2746 (1967)
[29] Heatherington, K, dissert, Univ. Newcastle upon Tyne (1966).

Measurement of an Antigen–Antibody Reaction by a Static Method utilizing a Porous Gel

by

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Using radioactively labelled markers of high and low molecular weight, as well as radioactive ligand, it has been shown that binding of antibody to hen egg lysozyme is nearly complete.

THE availability of cross-linked dextrans or polyacrylamide gels of varying pore size ('Sephadex', 'Bio-Gel') has led to their use in the quantitative estimation of interactions between a protein and a small molecule, between protein molecules or protein subunits Such interactions have usually been measured by using columns of the gel[1–5] A static method, which is less time consuming and which more readily permits measurements in a steady state, was described by Fasella *et al*[6] In applying the method to an antigen–antibody interaction we have encountered difficulties resulting from non-specific adsorption to the gel of protein which is present at low concentrations By using radioactively labelled markers of high and low molecular weight, as well as radioactive ligand, we have been able to detect small amounts of non-specific adsorption and to evaluate unlabelled carriers for their ability to reduce non-specific adsorption by displacement of the adsorbed ligand Also, such markers provide reference points which allow direct calculation of the extent of binding The system which has been investi-

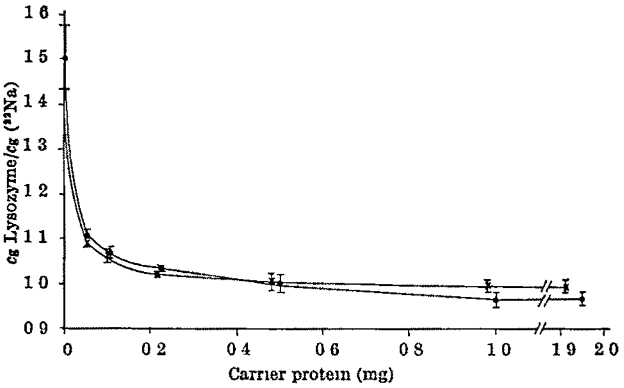

Fig 1 Effect of unlabelled carrier protein on the ratio, c_g (iodine-125-lysozyme)/c_g (sodium-22) ● Unlabelled horse heart cytochrome *c* added as the carrier ×, Unlabelled lysozyme as carrier Total volume, 4 6 ml Other details are in the text Experiments in triplicate with standard deviations indicated by the bars

gated is the combination of hen egg lysozyme (molecular weight, 14,600) with its rabbit antibody (molecular weight, 150,000) The method should, however, have general applicability

'Bio-Gel *P*-60', 100–200 mesh, was obtained from the Bio-Rad Company and was allowed to swell in a buffer containing 0 15 molar sodium chloride and 0 003 molar sodium borate, *p*H 8 Crystallized lysozyme was obtained from the Mann Research Laboratories The extinction coefficient, $E_{1\text{ cm}}^{1\text{ per cent}}$ at 280 mμ, was taken as 26 (ref 7) Lysozyme and non-specific rabbit γG-immunoglobulin (IgG) were labelled with iodine-125 and iodine-131, respectively (0 5 to 2 0 atoms of iodine/molecule of protein[8]) Unreacted iodine was removed by gel filtration or by dialysis More than 99 per cent of the radioactivity in each preparation could be precipitated by 5 per cent trichloroacetic acid, with unlabelled bovine IgG present as a carrier Sodium-22 chloride was obtained from the Nuclear Chicago Company

Equilibration of protein solution with the gel was carried out in a 5 ml vial with a screw cap and a polyvinyl chloride–polyvinyl acetate gasket In most experiments, 2 5 ml of solution and approximately 2 g of a suspension of swollen beads were placed in the vial which was then cooled in an ice-bath The system was equilibrated by inverting the vial ten times and allowing the beads to settle after each inversion Control experiments showed that five or six inversions were sufficient for equilibration The mixture was transferred to a weighed test tube which was used for gamma counting The supernatant was completely removed with a very fine pipette, transferred to another tube and weighed Radioactivities of the beads and supernatant were measured in a 3-channel 'Packard' gamma scintillation counter The radioactivity which was determined for each isotope was corrected for the contributions, at the window settings used, by the other isotopes present in the mixture A minimum of five thousand counts above background was recorded in each channel

An experimental parameter which is useful in discussing these data is the concentration of an isotope within the gel suspension after equilibration, relative to its concentration in the supernatant fluid This value, which we designate c_g, depends on the volume within the pores of the gel which is accessible to the isotope, on the spaces between the beads (void volume) and on the amount of material, if any, which is adsorbed to the gel Experimentally, c_g is evaluated by determining the net radioactivity/g of supernatant solution (A) and the radioactivity/g of the suspension of beads (B), c_g is the ratio B/A

In preliminary experiments, c_g was determined for sodium-22 in solutions of sodium-22 chloride having approximately 1,000 counts/min/ml, but in which total concentrations of sodium chloride varied (0 01, 0 05, 0 1, 0 15, 0 2 and 0 3 molar); the corresponding values of c_g were 0 96, 0 97, 0 96, 0 95, 0 94 and 0 96. The constancy of c_g with increasing concentration suggests that the fraction of sodium which is non-specifically bound to the beads is not significant at the concentrations which were tested Further evidence comes from a comparison of c_g (sodium-22) with the weight of buffer in 1 g of a suspension of 'Bio-Gel *P*-60' This weight was found to be 0 946 g, in close agreement with the average value of c_g

A mixture was prepared in triplicate at *p*H 8 containing 0 15 molar sodium-22 chloride, 0 003 molar sodium borate and iodine-125-lysozyme at a final concentration of 0 022 μg/ml The average ratio, c_g (lysozyme)/c_g (sodium), was 1 50 and quite variable (Fig 1, ordinate intercept) suggesting non-specific adsorption of lysozyme to the gel When unlabelled lysozyme was added as carrier in increasing amounts, this ratio decreased and approached a constant value close to unity (Fig. 1) This constancy and the close agreement between the values for sodium and lysozyme suggest that the fraction of lysozyme which is non-specifically adsorbed becomes negligible as the total concentration of carrier increases above 0 1 mg/ml

For studying interactions with rabbit antilysozyme, however, such concentrations are not very practical because, at equivalence, the weight ratio of antibody to lysozyme is about 14 1, so that large amounts of antibody would be needed Also, it is essential to use very low concentrations of lysozyme to avoid formation of precipitates during the half-hour period before removal of supernatant from the gel When the concentration of lysozyme used is less than 1 μg/ml, no turbidity is observed in control experiments during this time interval

Another basic protein, horse heart cytochrome c (Sigma Chemical Co), was found to be equally as good as unlabelled lysozyme in reducing non-specific adsorption (Fig 1) Precipitin data for lysozyme and its rabbit antibody in the presence and absence of added cytochrome c are shown in Fig 2 The data show that the

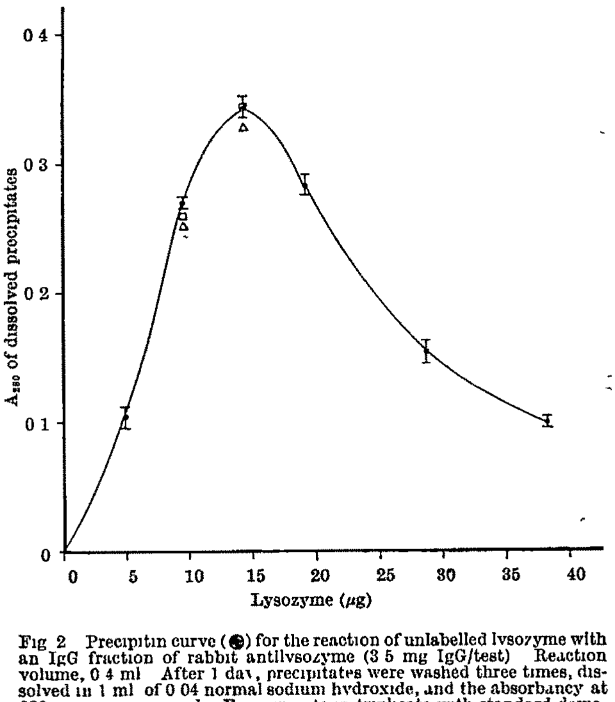

Fig 2 Precipitin curve (●) for the reaction of unlabelled lysozyme with an IgG fraction of rabbit antilysozyme (3 5 mg IgG/test) Reaction volume, 0 4 ml After 1 day, precipitates were washed three times, dissolved in 1 ml of 0 04 normal sodium hydroxide, and the absorbancy at 280 mμ was measured Experiments in triplicate with standard deviations indicated by the bars Two experiments (△) were repeated in duplicate in the presence of 1 mg of horse heart cytochrome *c*, and two (□) with iodine-125-lysozyme in place of the unlabelled protein The average mean deviation for the experiments with labelled iodine-125-lysozyme or with cytochrome *c* present was 0 010 units of absorbance

cytochrome *c* does not compete with lysozyme for antibody combining sites, it was therefore used as carrier in subsequent experiments.

Rabbit iodine-131-IgG (molecular weight, 150,000) was used as an indicator which is excluded from 'Bio-Gel *P*-60'. The value of c_g (IgG) was found to be 0·48 ± 0·015 and was essentially independent of concentration in the range which was investigated (0·02 to 3·5 mg/ml).

Fig. 3 shows the effect of addition of antilysozyme (IgG) to a mixture at *p*H 8 containing 0·15 molar sodium-22 chloride, 0·003 molar borate, non-specific iodine-131-IgG (0·46 mg), iodine-125-lysozyme (0·1 μg) and cytochrome *c* (1 mg). The total volume, including 2·6 g of bead suspension, was approximately 4·6 ml. With increasing concentrations of antilysozyme the ratio, c_g (lysozyme)/c_g (sodium-22), decreased from 0·95 to 0·55. At the same time the ratio, c_g (lysozyme)/c_g (iodine-131-IgG), decreased from 1·94 to 1·05 (Fig. 3). The close agreement between c_g of non-specific IgG and that of the lysozyme–antibody complex is consistent with the interpretation that both are excluded from the pores of 'Bio-Gel *P*-60' and that essentially all of the lysozyme is bound when an excess of antibody is present. That the binding is nearly complete is supported by other data, described later.

It can be shown that in the absence of non-specific adsorption a linear relationship should exist between the fraction of lysozyme which is bound to antibody and c_g (lysozyme). On this basis, the amount of antibody (IgG fraction) required to bind 50 per cent of the 0·1 μg of lysozyme present is estimated, from the data of Fig. 3, to be 10·2 μg. On the assumption that all of the antibody and antigen are precipitated at equivalence (Fig. 2), 10·2 μg of antibody globulin contains 0·60 μg of antibody. On this basis the ratio, moles of antibody present/moles of lysozyme bound, was 1·4 when 50 per cent was bound.

To check the results by an independent method, complexes of lysozyme and antibody were precipitated with sheep antiserum. Varying amounts of antilysozyme (IgG) and non-specific IgG were mixed to give a total of 0·2 mg of protein in a volume of 1·6 ml. of borate buffer, *p*H 8. Iodine-125-lysozyme (0·05 μg in 0·05 ml.) was added, followed after 10 min by 0·4 ml. of sheep antiserum of high titre which was prepared against the heavy chains of rabbit IgG. In preliminary experiments with iodine-131–rabbit IgG, 92 per cent of the iodine-131 was precipitated in these conditions. In two experiments, 1 mg of horse cytochrome *c* was present. The concentration of lysozyme was chosen so as to be very similar to the concentration outside the gel in the experiments of Fig. 3, at the point corresponding to 50 per cent binding.

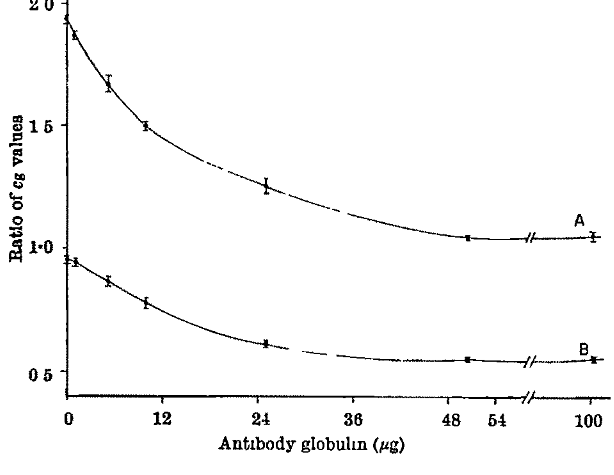

Fig. 3. Effect of added antibody (IgG fraction) on the ratio, c_g (iodine-125-lysozyme)/c_g (iodine-131-IgG) (curve *A*), or c_g (iodine-125-lysozyme)/c_g (sodium-22) (curve *B*). Details are in the text. Experiments in triplicate with standard deviations indicated by bars.

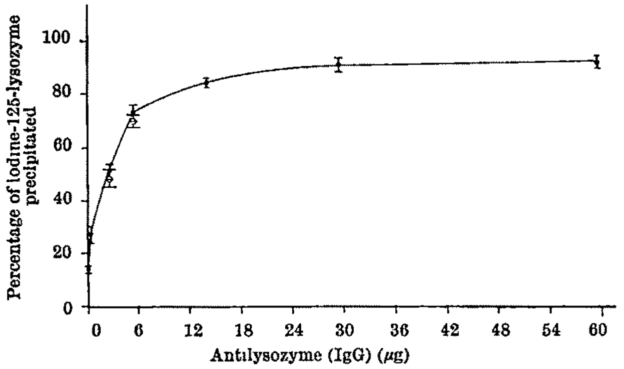

Fig. 4. Indirect precipitation of complexes of iodine-125-lysozyme and rabbit antilysozyme (IgG fraction) by sheep antiserum to H chains of rabbit IgG. Mixtures contained 0·05 μg iodine-125-lysozyme, an amount of the antilysozyme shown on the graph, and 0·4 ml. of the sheep antiserum. In two experiments (open circles), 1 mg cytochrome *c* was also present. Reaction volume, 2 ml. Experiments in triplicate with standard deviations indicated by bars.

After standing for 1 day in the refrigerator, each precipitate was separated by centrifugation, washed three times and the total radioactivities in the combined supernatant fluids and in the dissolved precipitate were measured.

The results (Fig. 4) show that the amount of antibody globulin which is needed to react with half of the antigen (0·025 μg) is 3·1 μg. In making this computation the amount of antigen which is non-specifically precipitated in the absence of antibody (0·006 μg, ordinate intercept of Fig. 4) was subtracted from each value on the curve. The amount of antibody globulin required to bind 0·05 μg is therefore 6·2 μg, as compared with the value 10·2 μg which was derived from experiments using the gel (Fig. 3). The reason for the disparity is not apparent, but in view of the difference in conditions and reagents in the two sets of experiments the agreement seems reasonably close.

The difficulties which were encountered should be mentioned. One is the binding of lysozyme to non-specific IgG, which becomes appreciable at globulin concentrations above 2 mg/ml. Also, the binding of lysozyme to glass is significant at low concentrations in the absence of carrier protein. In experiments with another batch of 'Bio-Gel *P*-60', lysozyme was not completely included within the pores of the gel, which suggests that the pore size of 'Bio-Gel *P*-60' does not greatly exceed the size of lysozyme molecules. This, however, may be attributable to partial dimerization of the protein[7]. The precision of the method is poor when a small percentage of ligand is bound, because the magnitude of the experimental variation of c_g then approaches the differences between c_g of the ligand and c_g of the internal control (sodium-22). The experimental variation, expressed as fraction of ligand bound, is about 5 per cent when half is bound.

The method is useful for showing the binding capacity of an antibody or other protein and, if components of sufficiently high specific radioactivity are used, should permit determination of dilutions at which dissociation of two reacting proteins occurs, and thus their association constant.

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[1] Hummel, J. P., and Dreyer, W. J., *Biochim. Biophys. Acta*, **63**, 530 (1962).
[2] Winzor, D. J., and Scheraga, H. A., *Biochemistry*, **2**, 1263 (1963).
[3] Winzor, D. J., and Scheraga, J. A., *J. Phys. Chem.*, **68**, 338 (1964).
[4] Fairclough, jun., G. F., and Fruton, J. S., *Biochemistry*, **5**, 673 (1963).
[5] Gilbert, G. A., *Nature*, **212**, 296 (1966).
[6] Fasella, P., Hammes, G. G., and Schimmel, P. R., *Biochim. Biophys. Acta*, **103**, 708 (1965).
[7] Sophianopoulos, A. J., and Van Holde, K. E., *J. Biol. Chem.*, **239**, 2516 (1964).
[8] McFarlane, A. S., *Nature*, **182**, 53 (1958).

LETTERS TO THE EDITOR

ASTRONOMY

Spiral Structure and Galactic Magnetic Field

THE amount of data available on the magnetic field of the Galaxy has increased considerably since the discovery of linear polarization in the galactic background radio emission[1] There now exist three different methods for determining the structure of the field, these being (1) optical observations of interstellar polarization, (2) Faraday rotation measures of extra-galactic radio sources, and (3) the polarization of the galactic radio emission

The chief conclusions drawn from the data are summarized in Table 1 The distances given in the penultimate column are in most cases estimated from the dimensions of the region in which the particular effect is supposed to take place In some cases, the distances have been estimated by those doing the analysis or the observations

Table 1

Method	Direction of the field	Region of the Galaxy	Distance from the Sun	Ref
Interstellar polarization	$l=50°$	In plane of the Galaxy	~ 600 parsec	12
" "	$l=45°$	Along spiral arm	0–1,000 parsec	10
" "	$l=40°$–$45°$	In plane of the Galaxy	60–260 parsec	2
Radio background polarization	$l=50°$	In local spiral arm	~ 150 parsec	4
" " "	$l=70°$	" " "	~ 150 parsec	13
" " "	$l=50°$	" " "	> 110 parsec	2
" " "	$l\sim70°$	Beyond spiral arm	> 500 parsec	2
Faraday rotation measures of extra-galactic radio sources	$l=95°$	In plane of the Galaxy	~ 600 parsec	6
" " "	$l=95°$	Below galactic plane	~ 600 parsec	6
" " "	$l=275°$	Above galactic plane	~ 600 parsec	6
" " "	$l=80°$	In plane of the Galaxy	~ 600 parsec	14
" " "	$l=80°$	Below galactic plane	~ 600 parsec	14
" " "	$l=260°$	Above galactic plane	~ 600 parsec	14
" " "	$l=91°$	In plane of the Galaxy	> 1,100 parsec	2
" " "	$l=82°$	Below galactic plane	> 1,100 parsec	2
" " "	$l=260°$	Above galactic plane	> 1,100 parsec	2

Using the method I described earlier[2], I carried out a spherical harmonic analysis of the interstellar polarization data of Behr[3], and I carried out a similar analysis on the radio background polarization data of Berkhuijsen *et al*[4] and the data of Bingham[5] I also used the data of Gardner and Davies[6] for a spherical harmonic analysis of rotation measures From these analyses I deduced the direction of the field in different regions of the Galaxy

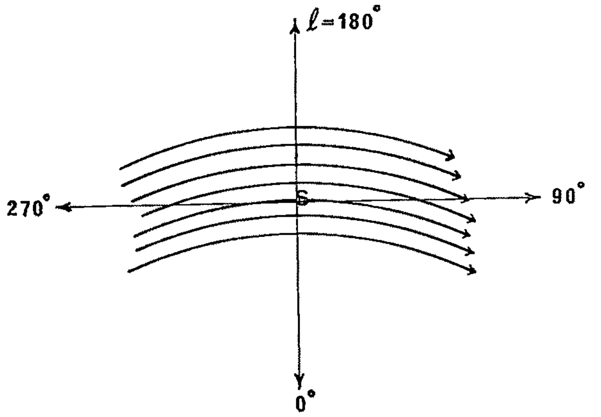

Fig 1 The large scale structure of the magnetic field deduced from Faraday rotation observations The angles give the galactic longitude, and *S* represents the position of the Sun

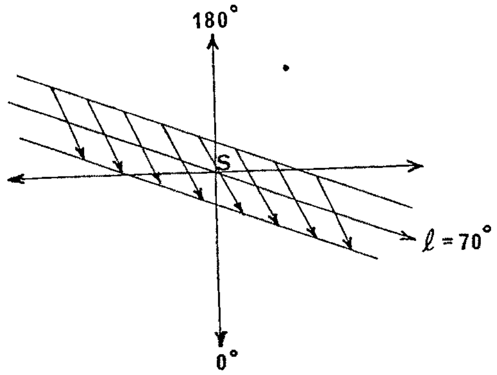

Fig 2 The lines parallel to $l=70°$ represent the local spiral arm The lines across the arm represent the field deduced from optical and radio polarization measurements

Recently, two models have been proposed which attempt to account for all the available observations In the first model, Hornby[7] suggested that the magnetic field in the local spiral arm has a form very similar to that of a tightly wound helix Stepien[8] had already shown that some forms of helices were consistent with the interstellar polarization data Hornby showed that his model made possible an explanation of the field reversal seen at intermediate latitudes He also showed that the model was able to explain the principal features of the background polarization data His model, however, is unable to explain the direction of the field in the plane of the Galaxy

Bingham and Shakeshaft[9] considered a model in which the magnetic field in the plane of the Galaxy converged towards the direction $l=70°$ Their model was able to explain some aspects of the interstellar polarization data It is not, however, consistent with the data in the direction $l=75°$ (see ref 10) Their model also included a magnetic loop above the plane With this loop, they were able to explain the field reversal above the plane Like Hornby, they ignored certain low latitude sources on the grounds that the Faraday rotation for these sources takes place beyond the local spiral arm As a result, their model is unable to explain the direction of the field in the plane of the Galaxy They show that their model is consistent with the background polarization data

I have shown[2] that a toroidal field in the plane of the Galaxy was able to explain the Faraday rotation data, if one assumed that the Faraday rotation was not confined to the local spiral arm I also showed that a simple radial component was not consistent with the data. Faraday rotation thus gives a field of the form shown in Fig 1 Optical polarization together with the polarization of the radio background emission, however, gives us a field of the form shown in Fig 2

Lin and Shu[11] suggested that the spiral arms are regions of high density in a wavelike pattern which covers the disk of the Galaxy. This pattern is formed by periodic displacements of stars and gas parallel to the galactic plane. These displacements are caused by motions caused by gravitational effects In such a theory the spiral arms, as well as any segment of a line parallel to them, are lines of equal phase These periodic motions would distort a simple toroidal field because the field is probably frozen into the interstellar matter If the field lines are not to cross each other, if they are to remain connected and if there are no large scale twists about axes parallel to the galactic plane, then there are only a few possible configurations for the field lines to take Let us represent the local spiral arm by the curve *AB* (see Fig 3) and a section of the local magnetic field by the curve *ab* Consider two points *c* and *d* chosen in such a way that *c* lies on a line of equal phase that lags, say, π behind *AB*, and *d* lies on a similar line that

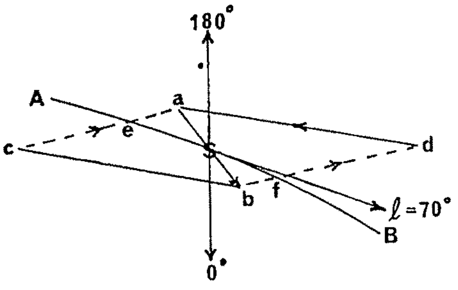

Fig 3. A schematic representation of possible ways of joining the local field to the large scale field (see text)

leads AB by π Thus c and d are 2π out of phase There are two ways of joining a and b to c and d Suppose we join d to a and b to c If we now follow the curve $dabc$ we move in a direction of steadily increasing phase all along the curve Thus this curve represents a configuration which could be obtained from the periodic disturbances. The curve, however, gives us a field that points from d to a and from b to c This is not consistent with the Faraday rotation measures of extra-galactic radio sources The other possibility is to join c to a and b to d In this case, the curve would cut the local spiral arm in points e and f as well as S (the position of the Sun) The field at e and f is in a direction differing from that at S. The field direction is therefore not uniquely defined by the phase, and this is impossible on our assumptions Thus such a situation could not arise from the type of motion we described earlier

A general toroidal field cannot be distorted by periodic disturbances, confined to the plane of the Galaxy, to give rise to the local spiral arm field, if the field lines are to remain connected and are not to cross each other This leads us to suppose that there may also be systematic motions perpendicular to the galactic plane or that the field lines in the spiral arms are not connected to the general galactic field

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[1] Westerhout G , Seeger, C L , Brouw, W N , and Tinbergen, J A , *Bull Astro Insts Neth* 16, 187 (1962)
[2] Seymour, P A H , *Mon Not Roy Astro Soc* ,134, 389 (1966)
[3] Behr, A , *Veroff Univ Sternw Gottingen*, 126 (1959)
[4] Berkhuijsen, E M , Brouw W N , Muller, C A , and Tinbergen, J A , *Bull Astro Insts Neth* ,17, 465 (1964)
[5] Bingham, R G , *Mon Not Roy Astro Soc* ,134, 327 (1966)
[6] Gardner, F F , and Davies, R D , *Austral J Phys* ,19 129 (1966)
[7] Hornbv, J M , *Mon Not Roy Astro Soc* ,133, 213 (1966)
[8] Stepien, K , *Acta Astro* ,14, 81 (1964)
[9] Bingham, R G , and Shakeshaft, J R , *Mon Not Roy Astro Soc* ,136, 347 (1967)
[10] Appenzeller, I , *Zeit Astrophys* , 64, 296 (1966)
[11] Lin, C C , and Shu, F H , *Ap J* ,140, 646 (1964)
[12] Serkowski, K , *Adv Astro Astrophys* ,1, 289 (1962)
[13] Mathewson, D S , and Milne, D K , *Austral J Phys* ,18, 635 (1965)
[14] Berge, G L , and Seielstad, G A , *Ap J* ,148, 367 (1967)

New Restriction on Models for Quasi-stellar Objects

THE inverse Compton effect has recently received attention as a possible source of the continuous radiation of quasi-stellar objects[1-3] Hoyle *et al* [4] concluded that quasi-stellar objects cannot be at cosmological distances because otherwise the inverse Compton effect would force the electrons to dump their relativistic energies in times so short that no known acceleration mechanism could be responsible for their original appearance Modifications of this argument have been discussed by several authors[5-9], in particular by Woltjer[10], who considered a radiation (and electron) flux restricted to a narrow cone about the radial direction

In all these discussions, only the spontaneous component of the Compton effect was considered There is also, however, a stimulated term which is a straightforward consequence of quantum mechanics and which was recognized as such as early as 1933 by Kapitza and Dirac[11] It is the purpose of this communication to show that the—necessary—inclusion of the stimulated processes makes an understanding of the radiation pattern of quasi-stellar objects in terms of currently proposed models extremely difficult

We assume that there are relativistic electrons moving through a radiation field Let their energy be such that, in familiar notation, $\gamma=[1-\beta^2]^{-1/2}\gg 1$, where $v=\beta c$ is the electron speed in the observer's frame Furthermore, let the energy ε of the photons in the observer's frame be such that $\varepsilon'\sim\gamma\varepsilon\ll\mu$, where ε' is the photon energy in the rest frame of the electron, $\mu=m_0c^2$ the rest energy of the electrons Finally, let θ be the angle between the photon and electron paths in the observer's frame, so that a photon travelling parallel to the direction of motion of the electron from behind corresponds to $\theta=0$ In the rest frame of the electron, however, all but the few photons arriving with $0\leqq\gamma^{-1}$ will seem to arrive with $\theta'\sim\pi$, so that $\cos(\chi'+\theta_i')\sim-\cos\chi'$, where χ' is the scattering angle in the electron frame

The probability of a Compton process is now proportional to $[1+n(\varepsilon)]$, where

$$n(\varepsilon)=[(\pi\hbar c)^3/\pi\varepsilon^2]\,N(\varepsilon) \qquad (1)$$

and $N(\varepsilon)$ is the number of photons of energy ε per cm³, $2\pi\hbar$ being Planck's constant and c the speed of light $n(\varepsilon)$ is an invariant, so that $n'(\varepsilon',\theta')=n(\varepsilon,\theta)$ The power generated through Compton scattering can then be written as[12]

$$\begin{aligned}4\pi P_c=2\pi^2r_0{}^2\int\sin\chi'\,d\chi'\int\sin\theta_i\,d\theta_i\,(1+\cos^2\chi')\,(1-\beta\cos\theta_i)\\ \times\{\gamma^2(1-\beta\cos\theta_i)\,[1-\beta\cos\chi']-1\}\\ \times\int d\varepsilon_i\,c\,\varepsilon_i\,N(\varepsilon_i)\,[1+n(\varepsilon_f)]\end{aligned} \qquad (2)$$

In equation (2) we have taken the Thomson approximation to the Klein–Nishina cross-section θ_i and θ_i', in the respective reference frames, are the angles of the incoming photon (energy ε_i in the observer's frame); ε_f is the energy of the outgoing photon

It may suffice to derive a lower bound of the energy transferred from the electrons to the radiation field This we can obtain by rather simple considerations, a detailed analysis will appear elsewhere To arrive at such an estimate, we first consider scattering angles $\chi'>1/\alpha$, where $\alpha<\gamma$ Almost all events in this domain lead to an increase in the photon energy, whereas scatterings with $\chi'<1/\alpha$ will be essentially symmetric in energy gain or loss Thus, if we extend the integration over χ' from $\cos\chi'=-1$ to $\cos\chi'=1-1/2\alpha^2$ only, we are certain to collect energy gains The detailed calculation shows that $4\alpha^2\sim\ln\gamma^2$ Similarly, we restrict the integration over θ_i to the $\cos\theta_i$ range between -1 and $1-1/2\gamma^2$ With the total flux $F=\int cN(\varepsilon)\varepsilon d\varepsilon$ we find for the spontaneous component

$$4\pi P_c^{sp}=4\pi\,(32\pi/9)\,\gamma^2\,r_0{}^2\,F \qquad (3)$$

Writing $\cos\chi'=x$, $\cos\theta_i=y$, the stimulated component reads

$$\begin{aligned}4\pi P_c^{st}=2\pi^2r_0{}^2\int dx\int dy\,(1+x^2)\,(1-\beta y)\,\{\gamma^2(1-\beta y)\\ \times\,(1-\beta x)-1\}\int d\varepsilon_i\,F(\varepsilon_i)\,\pi^2h^3c^2\varepsilon_f{}^{-3}\,F(\varepsilon_f)\end{aligned} \qquad (4)$$

Initial and final energies are related by $\varepsilon_f=\varepsilon_i\,\gamma^2\,(1-\beta y)\times(1-\beta x)$

In order to proceed, we have to choose a specific flux spectrum. We first consider the case of constant flux, introducing boundaries $\varepsilon_1=h\nu_1$ and $\varepsilon_2=h\nu_2$ at which we cut off the spectrum In addition, for the stimulated term, we must ascertain that both initial and final photon energies lie within the flux boundaries This amounts to limiting the ε integration between $\varepsilon_i = \varepsilon_1$ and $\varepsilon_f = \varepsilon_2$, or between $\varepsilon_i = \varepsilon_1$ and $\varepsilon_i = \varepsilon_2/\gamma^2\,(1-\beta y)\,(1-\beta x)$ The integrations lead to the result

$$4\pi P_c^{st} = 4\pi\,\frac{[\ln(2\gamma^2)]^2}{32\gamma^4}\,r_0^2\,(c^2/h)\,\frac{F^2}{\nu_1^2\nu_2^2} \qquad (5)$$

The total flux F is about $F(\varepsilon)$ ε_2 Taking $\gamma^2 \sim 10^2$ in the logarithm, we find the ratio

$$R_1 = P_c^{st}/P_c^{sp} = 3\ 7\times10^{46}\,\frac{F_0Q^2}{\gamma^6\nu_1^2\nu_2^2} \qquad (6)$$

Here we introduced the flux F_0 at Earth distance, Q being the ratio of the distance between Earth and quasi-stellar object and the radius of the quasi-stellar object

Similarly, the power spectrum for a monochromatic flux with index −1 (ref 13, Table 5 1) yields

$$4\pi P_c^{st} = 4\pi\,(3/64)\,\gamma^{-4}\,r_0^2\,(c^2/h)\,\frac{F_0Q^2}{[\ln(\nu_2/\nu_1)]^2\nu_1^4} \qquad (7)$$

The expression corresponding to equation (6) now reads

$$R_2 = P_c^{st}/P_c^{sp} = 6\ 1\times10^{44}\,\frac{F_0Q^2}{\gamma^6[\ln(\nu_2/\nu_1)]^2\nu_1^4} \qquad (8)$$

Finally, the calculation for Woltjer's model[10] shows that, although the spontaneous component is significantly reduced, the stimulated component is very nearly the same as in the isotropic case, provided that photons and electrons are not aligned to within an angle of order $1/\gamma$

It is worth emphasizing that equations (6) and (8) refer to monochromatic electrons But because the γ dependence is very strong and with a negative exponent, the implication is that in a continuous energy distribution the slowest electrons lose their energy first, so that in effect we have a lower limit for the electron energies in a stationary situation

If we insert the values (compatible with *3C* 273) $F_0 = 10^{-8}$, $\gamma = 10^2$, $\nu_1 = 10^7$, $\nu_2 = 10^{13}$, r (quasi-stellar object) $= 10^{17}$, d (quasi-stellar object) $= 10^{27}$ (cosmological distance) so that $Q^2 = 10^{20}$ (all units in CGS), we find $R_1 = 3\ 7\times10^6$ If we adopt the local hypothesis for quasi-stellar objects the numerical value of R_1 is reduced by roughly a factor 10^4 As already mentioned, Hoyle *et al*[4], from considering the spontaneous component, concluded that the electrons in quasi-stellar objects could survive, if the sources were not at cosmological distances Adding the stimulated component seems to preclude even this possibility, at least for electrons with $\gamma \sim 10^2$ to 10^3 The reduction in lifetime is even more drastic for a flux spectrum $F(\varepsilon) \sim \varepsilon^{-1}$, namely, $R_2 = 2\ 7\times10^{14}$ for the same values of F_0

Thus, and regardless as to whether we adopt the local or the cosmological hypothesis, we are left with two choices

(1) The radiation field of quasi-stellar objects is caused by electrons supplied *in situ* or, at any rate, not accelerated anywhere near the locus of the radiation field The electrons at the low energy end of the spectrum are then decelerated "instantaneously", feeding the radiation field by means of stimulated Compton effect It is interesting to speculate that if this mechanism were operating, the radiation field would be made to align itself more and more with any preferred direction of particle motion If the particle motion is highly anisotropic, for example, purely radial, and if the radiation field has reached a state of confinement to a cone about the direction of particle motion of half-angle γ^{-1}, the stimulated scattering mechanism ceases to be efficient At this time, the low energy portion of the photon spectrum may have been seriously depleted This effect could obviously be cited as an explanation for the observed curvature of the spectrum of many quasi-stellar objects

(2) The alternative is for the radiation field to be produced by a mechanism that does not involve relativistic electrons

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[1] Greenstein, J L, and Schmidt, M, *Astrophys J*, **140**, 1 (1964)
[2] Ginzburg, V L, Ozernoi, L M, and Syrovatsky, S I, *Soviet Phys Dokl*, **9**, 683 (1964)
[3] Shklovskii, I S, *Sov Astro*, **9**, 683 (1966)
[4] Hoyle, F, Burbidge, G R, and Sargent, W L W, *Nature*, **209**, 751 (1966)
[5] Rees, M J, *Nature*, **211**, 805 (1966)
[6] Rees, M J, and Sciama D W, *Nature*, **213**, 374 (1967)
[7] Hoyle, F, and Burbidge, G R, *Nature*, **212**, 1223 (1966)
[8] Hillier, R R, *Nature*, **212**, 1334 (1966)
[9] Pfleiderer, J, and Grewing, M, *Science*, **154**, 1452 (1966)
[10] Woltjer, L, *Astrophys J*, **146**, 597 (1966)
[11] Kapitza, P L, and Dirac, P A M, *Proc Cambridge Phil Soc*, **29**, 297 (1933)
[12] Felten, J E, and Morrison, P, *Astrophys J*, **146**, 686 (1966)
[13] Burbidge, G R, and Burbidge, E M, in *Quasistellar Objects* (Freeman and Co, San Francisco, 1967)

PLANETARY SCIENCE

Incoming Solar Radiation over the Tropical Pacific

THERE seems to be a much larger amount of total incoming solar radiation (direct and diffuse radiation on a horizontal surface as recorded by the Eppley pyranometer) at the ocean surface over the equatorial Pacific—and probably over much of the tropical Pacific as a whole—than has usually been thought It also seems that the incoming solar radiation over the central and western parts of the equatorial Pacific dry zone would closely approach the extremely large amounts received over the tropical and subtropical desert areas of the continents These statements are based on a study of recorded incoming solar radiation data for Wake and Canton Islands in relation to associated cloud distributions and also on comparisons of the long-term Canton Island cloud distribution with that of several other small islands in the central and western equatorial Pacific (Both Wake and Canton seem to be reasonably representative marine sites by virtue of their small land areas, lack of relief and complete isolation from continental influence)

The recent global illustrations by Budyko[1] show upward revisions of the incoming solar radiation over the tropical Pacific when compared with his[2,3] earlier depictions and the works of Kimball[4] and Black[5], but they are still not indicative of the recorded incoming solar radiation values at Wake and Canton Islands For example, Budyko's 1963[1] representation of the average annual solar radiation reception at both these sites would indicate values slightly in excess of 160 kLangleys* Wake Island, however, shows an average annual reception of 194 kLangleys[6], based on a 9 year period of record, and the average annual figure for Canton Island is 218 kLangleys[7], based on a 12 year period of record Why do such large differences occur ?

There is an extreme lack of solar radiation recording stations over the tropical Pacific, and it is apparent that computed values for incoming solar radiation, based on cloud cover data from the much more numerous weather observing sites, have had a controlling influence on the tropical Pacific representation Computations based on meteorological data usually consider only mean total sky cover, and most of the formulae in use were developed originally for middle latitude conditions where cloud distributions and solar altitude ranges are quite different from those of the tropics Even during the highly unusual meteorological period July 1957 to June 1958, when Canton received 82 22 in of rainfall (usual annual precipitation

* One Langley is 1 cal cm⁻² min⁻¹

Table 1 AVERAGE TRANSMISSION PERCENTAGES FOR DESIGNATED SOLAR ALTITUDE RANGES IN STIPULATED SKY COVER CONDITIONS

Coverage (in tenths)	Solar altitude ranges 0°–14°	15°–29°	30°–44°	45°–59°	60°–74°	75°–90°	Average
<2 0 TC	46 9 (5)	65 6 (10)	73 2 (10)	76 6 (12)	77 8 (17)	77 6 (5)	69 6 (Pertains essentially to clear sky)
10 0 TC and <2 0 TOC	45 3 (36)	63 9 (24)	72 3 (24)	76 1 (26)	76 8 (26)	77 4 (6)	68 6 (Pertains essentially to cirriform overcast)

TC, total sky cover, TOC, total opaque sky cover. Figures in parentheses refer to the number of cases in the particular category

29 43 in) and had 9 0 tenths average total sky cover (mean annual sky cover 6 3 tenths), it received approximately 195 kLangleys

It seems quite significant that a 43 per cent increase in mean annual sky cover only caused an 11 per cent reduction from the average annual incoming solar radiation for Canton (The 11 per cent figure is based on approximately eleven months of data, because all radiation data for February 1958 were missing If, however, the missing data were available, it is difficult to see how the annual reduction would be increased beyond 12 per cent) The explanation of this situation seems to lie in the cloud distribution by type and amount During the period July 1957 to June 1958, Canton experienced 10 0 tenths thin cirriform clouds with scattered or no low clouds about 40 per cent of the time This percentage is very high but would, of course, be much higher if specifications were changed so as to include the same low cloud criteria (scattered or none) and all cirriform ceilings (that is, 6 to 10 tenths cirrus or cirrostratus) This thin cirriform cloud cover, which was the principal contributor to total sky cover during fair weather, apparently had very little effect on the incoming solar radiation Even during this year of relatively high precipitation and cloud cover, the percentage of time with disturbed weather and its associated heavy opaque cloud cover and precipitation was relatively small when compared with the percentage of time during which fair weather prevailed

To ascertain the reduction in radiation caused by thin cirriform clouds alone, it was first necessary to determine the incoming solar radiation during clear sky conditions at Canton There were so few clear hours from July 1957 to June 1958 that it was necessary to consider all observations with less than 2 0 tenths total sky cover, after eliminating those situations when there were obviously low cloud patches directly between the pyranometer and the Sun (This was accomplished by a study of the Canton Island radiation traces for each hour involved Large, short period downward displacements in the radiation trace were indicative of the periods when low cloud patches were above the pyranometer) To obtain incoming solar radiation when just the cirriform overcast was present, those hours with 10 0 tenths cirrostratus or cirrus and less than 2 0 tenths total opaque sky cover were used, following the same procedure to eliminate cases where a low cloud patch was directly between the pyranometer and the Sun (The opaque sky cover in these fair weather situations ordinarily pertains to low cloud patches) Table 1 contains average transmission percentages for the incoming solar radiation within the various solar altitude ranges (The individual transmission percentages, which were used to arrive at the average values of Table 1, were obtained by dividing the recorded hourly incoming solar radiation at the surface by the computed hourly incoming solar radiation at the top of the atmosphere, and then multiplying the result by 100) Figures in this table indicate that the thin cirriform overcast did not cause a significant reduction of the incoming solar radiation regardless of solar altitude The general decrease in transmission percentage with decrease in solar altitude, which affected both categories similarly, was caused by the increase in air mass penetrated at the lower solar altitudes For the long period record (a 209 month US Air Force Air Weather Service uniform summary of surface weather observations for Canton Island), although the mean cloud amounts show a considerable reduction from the amounts recorded for the July 1957 to June 1958 period, a similar cloud distribution is shown with high clouds as the important contributor to total sky cover

The incoming solar radiation pattern over the tropical Pacific is likely to resemble that shown by Wyrtki[8], in that a tropical minimum reception would be expected along the zone most frequently occupied by the intertropical convergence zone of the northern hemisphere, and a maximum would be expected in the equatorial dry zone The incoming solar radiation amounts, however, would seem to be considerably higher At Wake Island's location (19° 17′ N, 166° 39′ E) Wyrtki's chart gives about 165 kLangleys/yr (recorded average about 194 kLangleys/yr) and at Canton's location (2° 46′ S, 171° 43′ W) his chart also indicates about 165 kLangleys/yr (recorded average about 218 kLangleys) Wyrtki's incoming solar radiation figures exclude that part of the solar radiation reflected from the ocean surface, but, because this only amounts to about 6 to 7 per cent of the total incoming solar radiation, it cannot account for the large differences between the observed and computed values

If Canton Island, during this year of unusually large average total sky cover (9 0 tenths) and heavy rainfall (82 22 in), recorded about 195 kLangleys of incoming solar radiation, there must be a very large portion of the tropical Pacific receiving annually in excess of 180 kLangleys and a sizable portion receiving in excess of 200 kLangleys It is expected that the maximum annual reception over the Pacific would occur in the central and western portions of the equatorial dry zone where values may reach 200 kLangleys or more The large cirriform contribution to total sky cover seems to have prevented earlier recognition of this situation In those cases where a balance has been achieved, using the computed solar radiation figures, compensating errors most probably occur in one or more of the remaining heat budget terms

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[1] *Atlas Teplovago Balansa Zemnogo Shara* (edit by Budyko, M I) (Glavnaia Geofizicheskaia Observatoriia, Moscow, 1963)

[2] *Atlas Teplovago Balansa* (edit by Budyko, M I) (Gidrometeorologischeskoe Izdatel'stovo, Leningrad, 1955)

[3] Budyko, M I , in *The Heat Balance of the Earth's Surface* (1956) (translated Off Tech Ser , US Dept Commerce Washington, DC, 1958)

[4] Kimball, H H , *Mon Weather Rev* , 56, 393 (1928)

[5] Black, J N , *Arch Meteorol Geophys Bioclimatol* , Ser B, 7, 165 (1956)

[6] *Local Climatological Data Annual Summary with Comparative Data, Wake Island, North Pacific* Environ Sci Ser Admin (Washington, DC, 1966)

[7] *Local Climatological Data, Annual Summary with Comparative Data, Canton Island, South Pacific*, Environ Sci Ser Admin (Washington, DC 1966)

[8] Wyrtki, K , *J Geophys Res* , 70, 4547 (1965)

Semi-annual Variation in Thermospheric Density

KING-HELE[1] has reported a semi-annual variation in thermospheric density at about 190 km with a maximum to minimum ratio of 1 45 Cook's hypothesis[2] that the semi-annual variation is caused by the modulation of the helium content of the lower thermosphere, originally coined to account for the variation at 1,100 km, clearly cannot be applied to explain changes at 190 km. The reconciliation between theory and observation is in essentially the same state as it was a year ago There is the hypothesis of Anderson[3] that the semi-annual variation results from a migration of the perigee points of the satellites studied with latitude in such a manner that the drag at perigee is small when over the winter pole which is supposed to be cold (and of lower density than the equatorial regions) Debate is continuing about Anderson's hypothesis[4,5] and no doubt the issue can be resolved when sufficient values of density for a wide range of perigee latitudes and over all seasons are available, such as the simultaneous changes at 190 and 1,100 km Anderson quotes as support my colleagues' computations[6], which show a cold winter pole The influence of motions, however, was neglected in these computations and it has recently been shown that the influence of vertical motions is quite substantial (ref 7 and unpublished work of R E Dickinson, C P Lagos and R E Newell) Satellite drag observations by Jacchia[8] and Keating and Prior[9] show higher densities over the winter pole than over the summer pole Jacchia and Slowey[10] could reduce —but not eliminate—the effect by manipulating parameters in their model, indeed, the effect was predicted before observation[11] and seems to increase with altitude as expected from theory

Another suggestion[12] is that the modulation of extreme ultraviolet radiation is responsible for the semi-annual change, just as it is responsible for the changes in the densities during the 27 day and solar cycles Unfortunately, while there may have been an association between extreme ultraviolet and density in 1962–63, there seems to have been density changes[13] without changes in the extreme ultraviolet in several other years

Density changes are observed at 190 km and may be caused by pressure change at some lower level or by temperature changes within the 100–190 km layer (if the influence of changes in composition is ignored at these altitudes) At 80 km there is an annual density and pressure variation with a maxima in summer in the region[14] 30°–60° N. The question is what other mechanisms are available to produce temperature changes in the 100–200 km layer? Johnson (see Jacchia[13]) has proposed large-scale meridional motions which subtract more heat from the region during the solstice than during the equinoxes, thereby producing a semi-annual density variation Another possibility would seem to be that Joule heating associated with ionospheric currents has a semi-annual variation Such heating has been considered by many authors (see Cole[15]) Both disturbance currents, caused by magnetic storms and usually measured by K_p, and steady currents denoted as S_q, have maxima at the equinoxes King-Hele has attempted to eliminate large variations in K_p from his results by omitting nineteen large storms in the period His elimination criterion is not stated but seems to be about $K_p \leqslant 5_0$ from inspection of the data There are very few days with very low K_p, 74 days out of 386 have $K_p \leqslant 2^-$ Radio aurora can sometimes be observed when $K_p = 2$, and by implication Joule heating will be associated with the ionospheric currents, at least in polar regions It is clearly difficult to assess the heating effect of many small disturbances, but it is well known that K_p shows equinoctal peaks It has been suggested that the peaks result from leakage of energy from the large scale waves in the lower atmosphere into the thermosphere[16] The transmission between tropopause and mesopause is a maximum near the equinoxes when the winds are changing from westerly to easterly or vice versa.

The quiet day currents are also at a maximum near the equinoxes[17] and it is possible that vertical fluxes associated with tidal motions can penetrate into the lower thermosphere at these times when the prevailing zonal winds at 100 km are also close to zero in middle latitudes[18] There is some (slight) experimental evidence that the diurnal tide does seem to pepetrate more easily at these times[19]. The amplitudes of the S_q currents are largest over the equator and if a typical equinoctal current is taken as 4×10^5 amp (I) per 60° of latitude spanning the equator, and an average column conductivity (σ) between 100 and 120 km is taken as 10 mho (ignoring the complications introduced by the tensor nature of conductivity), then the Joule heating (I^2/σ) is 0 36 erg cm^{-2} sec^{-1} For a typical winter current of 3×10^5 amp the corresponding heating is 0 21 erg cm^{-2} sec^{-1}

What is the temperature change in the 100 to 120 km region necessary to account for an observed density change of about 1 4 at 180 km, if all other parameters including the temperature between 120 and 180 km are kept constant? An estimate from the hydrostatic equation and the equation of state using 10 km increments in height gives a value of about 40° K for the mean increase in temperature necessary in the 100–120 km layer In turn an energy flux of about 0 1 erg cm^{-2} sec^{-1} acting for 10 days is implied, the danger of such simple estimates is obvious because composition, temperature gradient and downward heat flux will all change concomitantly, but nevertheless the fact is that the small Joule heating inferred from the currents can be significant in altering the density of the 180 km level Small changes in K_p, when $K_p < 5$ are also associated with substantial current changes as measured in terms of the numbers already mentioned, although these changes are largest at high latitudes Similarly, the larger change in Joule heating associated with K_p storm events may account for the observed density changes associated with K_p (ref 20) and the differences between day and night currents may also be important in the observed diurnal changes[21]. In so far as the S_q currents are thought to be driven by atmospheric motions and some K_p changes may also be driven from below, King-Hele's report may be treated as additional experimental evidence for a leakage hypothesis A test of all these items, including Johnson's proposal of meridional motion, could be made by comparing the results from a global network of meteor wind stations, as proposed elsewhere[19], with thermospheric densities

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[1] King-Hele, D G , *Nature*, 216, 880 (1967)
[2] Cook, G E , *Planet Space Sci* , 15, 627 (1967)
[3] Anderson, A D , *Planet Space Sci* , 14, 849 (1966)
[4] King-Hele, D G , *Planet Space Sci* , 14, 863 (1966)
[5] Anderson, A D , LMSC–6–77–67–40 (Lockheed Missiles and Space Company, Palo Alto, California, 1967)
[6] Lagos, C P , and Mahoney, J R , *J Atmos Sci* , 24, 88 (1967)
[7] Lagos, C P , *MIT, Dept of Meteorology*, Rep No 20, 134 (1967)
[8] Jacchia, L G , *Smithsonian Astrophys Obs Spec Rep* , No 184 (1965)
[9] Keating, G M , and Prior, E J , *Space Res* , VII, 1119 (North-Holland Publishing Co , Amsterdam, 1967)
[10] Jacchia, L G , and Slowey, J , *Space Res* , VII, 1077 (North-Holland Publishing Co , Amsterdam, 1967)
[11] Newell R E , *Problems of Atmospheric Circulation* (edit by Garcia, R V , and Malone, T F), 106 (Spartan Books, Washington, 1966)
[12] Chandra, S , and Krishnamarthy, B V , *Planet Space Sci* (in the press)
[13] Jacchia, L G , *Space Res* , V, 1152 (North-Holland Publishing Co , Amsterdam, 1965)
[14] Kantor, A J , and Cole, A E , *J App Meteor* , 4, 228 (1965)
[15] Cole, K. D , *Space Sci Rev* , 5, 699 (1966)
[16] Newell, R E , *Nature*, 211, 700 (1966)
[17] Matsushita, S , and Maeda H , *J Geophys Res* , 70, 2535 (1965)
[18] Greenhow, J S , and Neufeld, E L , *Quart J Roy Meteor Soc* , 87, 472 (1961)
[19] Newell, R E , and Dickinson, R E , *Pure and Applied Geophysics* (in the press)
[20] Jacchia, L G , Slowey, L , and Verani, F , *J Geophys Res* , 72, 1423 (1967)
[21] King-Hele, D G , and Quinn, E , *Planet Space Sci* , 14, 1023 (1966)

Stable Lead in Fossil Ice and Bones

DURING the past hundred years about 1×10^{14} g of lead has been dispersed by man in the biosphere[1], and the question arises as to whether this has increased the exposure of modern societies to chronic lead intoxication The chief sources of lead in the atmosphere are the combustion of petrol and coal and the contributions from both these sources are now of the same order of magnitude although in the past the greater contribution was from coal[2] In effect, the existing concentrations of lead in different components of the biosphere are now much higher than the natural concentrations which have been calculated[3] To investigate the temporal variations in the lead content of the environment and in the human body I have collected samples of glacial ice representing the precipitations of the past hundred years, and also samples of human bones covering the period from the third to the twentieth centuries In these samples lead was determined by dithizone colorimetry according to the method described earlier[4]

Ice samples were collected from a miniature corrie glacier in the Great Mieguszowiecki Cirque and from a glacier in the cave Grota Lodowa in the Tatra Mountains Both localities have already been described[5] As Fig 1 shows, the mean concentration of lead in the ice has increased steadily during the past hundred years The mean concentration which has been found in contemporary samples (1960–65) of 75 µg/l. was about sixteen times greater than that in the oldest samples from about 1861–66, in which the mean concentration was 5 0 µg/l These results are similar to the data of Murozumi *et al* [6], who found that the concentration of lead in the Greenland snow dating from 1905 was sixteen times lower than in the snow of 1964 The low concentrations of lead during 1861–1966 are probably not the natural concentrations, because in that time the precipitations could have been polluted by industrial activity in the neighbouring Silesian centres It is surprising that the increasing concentrations of lead in the ice are similar to the increasing annual use of coal as a source of energy[7] This correlation is not necessarily exclusively caused by the volatilization of the lead in fuel combustion, but it is probably connected with the increase in general industrial activity, related to the rate of coal burning

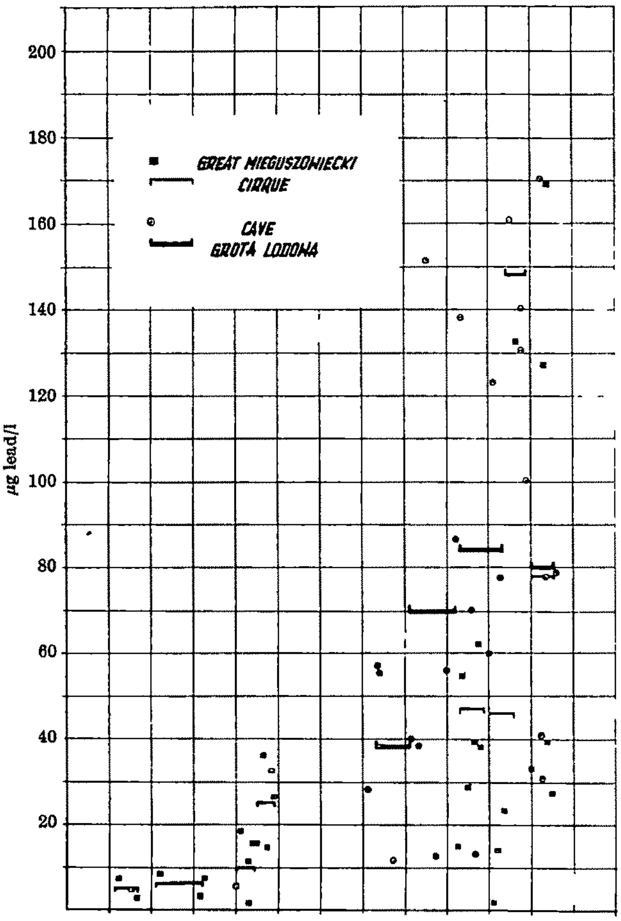

Fig. 1 Concentrations of stable lead in the glacier ice Lines represent the mean concentrations for a period

The samples of human bones were collected in four churches in Cracow and its region Most of them were preserved in wooden or stone coffins which were stored in dry underground crypts, although some bones from the Tyniec Monastery (samples 7–12) were buried directly in the loess beneath the central part of the church The third century bones were collected in a limestone cave near Kroczyce in the province of Katowice, where the skeletons were found lying on the dry stony floor Samples 1 and 2 from this cave were collected in 1936, immediately after the cave was discovered, and samples 3, 4 and 5 were collected in 1967 The latter samples could have been contaminated by visitors

Table 1 CONCENTRATION OF STABLE LEAD IN HUMAN FOSSIL BONES

No of skeleton	Age	Locality	Lead concentration µg/g of dried bone	Type of bone
1	3rd century	Kroczyce	1 81	Pubis
2	3rd century	Kroczyce	1 84	Scapula
3	3rd century	Kroczyce	2 13	Ulna
4	3rd century	Kroczyce	2 55	Humerus
5	3rd century	Kroczyce	3 40	Tibia
6	11th century	Tyniec Monast	92 5	Temporal + frontal
7	13th century	Tyniec Monast	16 0	Vertebra
7			26 07	Temporal + frontal
7			35 6	Tibia
8	13th century	Tyniec Monast	87 5	Phalanx
8			185 9	Talus
8			318 6	Vertebra
9	13th century	Tyniec Monast	19 6	Mandibula
9			24 8	Temporal + frontal
10	13th century	Tyniec Monast	8 52	Tibia
11	14th century	Tyniec Monast	5 6	Temporal + frontal
11			7 8	Ulna
12	17th century	Tyniec Monast	31 1	Ulna
13	1650	Bielany Monast	199 3	Femur
14	1710	Reformatów Ch	33 8	Femur (cortical)
14			58 6	Femur (trabecular)
15	1750	Bielany Monast	22 7	Femur
16	1792	Bielany Monast	15 4	Temporal + frontal
17	1797	Bielany Monast	58 7	Femur
18	18th century	Mariacki Church	80 8	Ulna
19	18th century	Mariacki Church	46 1	Phalanx
20	18th century	Mariacki Church	64 4	Ulna
21	About 1800	Bielany Monast	38 8	Tibia
22	Early 19th century	Mariacki Church	116 5	Femur
23	Early 19th century	Mariacki Church	25 9	Vertebra
23			31 6	Femur
24	1835	Reformatów Ch	55 1	Phalanx
25	1838	Reformatów Ch	59 2	Talus
25			91 9	Phalanx
Recent (mean of 20 samples)		Poland	3 9	Vertebrae

As can be seen from Table 1, the low concentrations of lead in the third century bones contrast with the high lead content of the bones from the eleventh to the nineteenth centuries The unusually high concentration of lead in the bones of an individual from the thirteenth century (skeleton No 8) was in all probability acquired during life, because relatively low concentrations of lead were found in other skeletons from the same locality which were buried 1 to 2 metres away from skeleton 8 The high concentrations of lead in the bones of humans living in Poland between the eleventh and nineteenth centuries are similar to the concentrations which were observed in contemporary bones in the United Kingdom. Germany and the United States[8-12] On the other hand the lead contents of the bones of contemporary residents of Poland are lower than they were in the past, although the contamination of the environment with lead, as reflected by the concentration of lead in the fossil precipitations, has increased during the past hundred years This suggests that in Poland the contribution from the atmosphere to the level of lead in the population is now smaller than the contribution from other sources in the past These sources were probably tin kitchen utensils which were widely used, and which contained large proportions of lead[13,14] It can also be supposed that the high head content in the bones of contemporary residents of western countries is probably caused not by pollution of the atmosphere resulting from industrial activity, but rather by dietary habits and contamination of food

The low lead concentrations which were found in the third century bones of 1 81 and 1 84 µg/g of dried bone are remarkably near the value of 0 85 µg/g, which was theoretically estimated to be the natural concentration[3] According to T Dabrowska (private communication) the third century inhabitants of Poland probably had no contact with metallic lead, and it can be supposed that their bones show the natural concentration of lead, which is about 1–2 µg/g of dry bone

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[1] Chow, T J , and Patterson, C C , *Geochim Cosmochim Acta*, 26, 262 (1962)

[2] Jaworowski, Z , *Stable and Radioactive Lead in Environment and Human Body* (Nuclear Energy Information Centre, Warsaw, 1967)

[3] Patterson, C C , *Arch Env Health*, 11, 344 (1965)

[4] Jaworowski, Z , *Nukleonika*, 8, 333 (1963)

[5] Jaworowski, Z , *Nature*, 212, 886 (1966)

[6] Murozumi, M , Chow, T J , and Patterson, C C , NYO–3450–1, 213 (1960)

[7] Putnam, P C , *Energy in the Future* (D Van Nostrand Co , Inc , New York 1953)

Thompsett, S L , *Biochem J* 30, 345 (1936)

[9] Kehoe, R A , *Occupat Med* , 3, 156 (1963)

[10] Lynch, G R , Slater, R S , and Osler, T G , *Analyst*, 59, 787 (1934)

[11] Weinig, E , and Borner, B , *Arch Toxic* ,19, 34 (1961)

[12] Hill, C R , and Jaworowski, Z , *Nature* 190, 353 (1961)

[13] Friend, J N , *Man and the Chemical Elements* (Charles Griffin and Co Ltd , London, 1951)

[14] Hensel, W , *Slowianszczyzna Wczesnosredniowieczna* (Panstwowe Wydawnictowo Nauowe, Warszawa, 1965)

Variations in *Pc* 1 Frequency with Latitude

Recordings of the magnetic micropulsations called *Pc* 1 from two auroral zone stations, College, Alaska, and Sodankyla, Finland (64 6° N and 63 8° N. geomagnetic, respectively), and one subauroral station, Nurmijaarvi, Finland (57 8° N), show a marked latitude variation in mid-frequency of structured *Pc* 1 events Analysis of data for a 3 month period (Table 1) shows that the average frequency of structured *Pc* 1 at Nurmijaarvi, the lower latitude station, is significantly higher than that for the auroral zone stations Fig. 1 shows a 6 Hz *Pc* 1 event which was recorded at Nurmijaarvi

Table 1 AVERAGE *Pc* 1 FREQUENCIES (HZ) OF THE HALF-HOURLY OCCURRENCES IN 1967

	January	May	June
Nurmijaarvi	1 32	2 39	1 95
Sodankyla		0 79	0 96
College	0 72	0 74	0 57

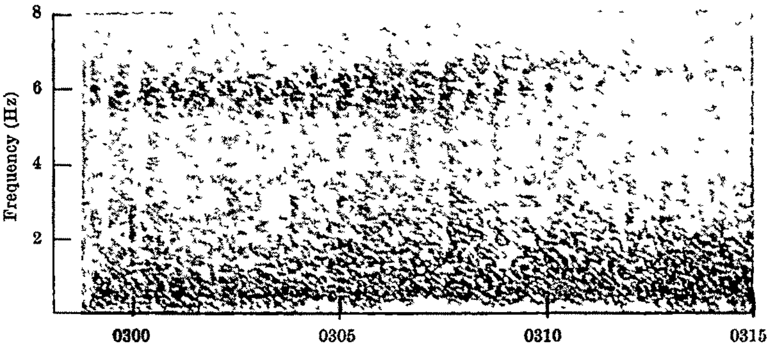

Fig 1 Sonagram showing very high frequency structured *Pc* 1 event, Nurmijaarvi, Finland June 7, 1967

There is considerable evidence that structured *Pc* 1 originate within the magnetosphere on "closed" field lines[1-5] Satellite observations have shown that the closed field lines extend to approximately 75° geomagnetic latitude on the day side and to 69° on the night side during magnetically quiet times, with the boundaries tending to move towards the equator with increasing *Kp* (ref 6) The location of the sources of *Pc* 1 within this region remains to be determined Furthermore, the existence of a latitude-frequency relationship within this *Pc* 1 source region is important, because some theoretical mechanisms involve the ion gyroresonance instabilities[7-9], and the ion gyrofrequency increases inwards in the magnetosphere's equatorial plane Some other mechanisms, for example, "hydromagnetic whistlers"[9], do not require this latitude variation in source frequency if a simple broad-band initiating impulse is postulated

The mid-frequencies for each of the three stations for June are presented in a diurnal variation display (Fig 2) The "occurrences" are half-hour intervals which contain structured *Pc* 1 activity Note that during the midday hours all of the Nurmijaarvi occurrences were above 1 1 Hz, whereas at Sodankyla and College all occurrences were $\leqslant$1 1 Hz

A complication which arises here from the fact that it is possible for Sodankyla to be outside the "closed" field line region on some occasions of high *Kp*, while Nurmijaarvi is inside Thus if *Pc* 1 do tend to occur in the outer part of the "closed" line region, then events would tend to be recorded at Nurmijaarvi at times of higher *Kp* than at Sodankyla, and the apparent latitude variation in *Pc* 1 frequency could be caused instead by the changing level of magnetic activity We averaged the *Kp* indices for times of the *Pc* 1 occurrences for May and for June For the College occurrences *Kp* averaged 1 95 and 1 91, and for Sodankyla 1 77 and 1 74, but for Nurmijaarvi the averages were 2 65 and 2 40 Thus in these data the more southerly station tended to experience structured *Pc* 1 at times of higher *Kp* To remove the effect of variations in *Kp* we deleted Nurmijaarvi *Pc* 1 occurrences from the May data for times when $Kp > 2^+$ The remaining *Kp* then averaged 1 63 and the average *Pc* 1 mid-frequency was 2 03 Hz Similarly, for June, the restriction $Kp \leqslant 3-$ brought the Nurmijaarvi average *Pc* 1 frequency down to 1 77 Hz, still well above the Sodankyla average frequency From these and other results it seems that variations in *Kp* cannot fully account for the observed *Pc* 1 frequency-latitude variations

Structured *Pc* 1 events recorded at one of the Finland stations in the summer months were usually not received at the other, in spite of a separation in latitude of only 6° For example, of the forty-six and sixty-five occurrences in May 1967 at Nurmijaarvi and Sodankyla, respectively

(Fig. 3), only three were simultaneous, with appearance of similar frequency-time on both records.

In Fig 2 there is a wide spread in frequencies for night-time occurrences. This suggests that propagation across field lines, north and south from the source lines, may be best at night, and thus that a station receives signals from a wider range of latitudes at night This idea is consistent with the fact that California receives *Pc* 1 signals essentially only at night[10] This further suggests that propagation across field lines should be best in winter months To check this, we analysed the data for January 1967 There was a remarkable number of occurrences at Nurmijaarvi and at College—343 and 124, respectively. In contrast, in May and June the occurrences/month/station averaged approximately seventy The average *Pc* 1 frequencies for January (Table 1) were more nearly equal at the two stations as compared with the summer data Also, the average *Kp* values for *Pc* 1 occurrences were nearly equal in January These results are seen to be consistent with the idea that the *Pc* 1 signals propagate best across field lines at night and in winter Thus it seems that the latitude spread in *Pc* 1 frequencies is most reliably studied by using daytime and summer data We note again from Fig 3 that at midday all Nurmijaarvi occurrences were >1·1 Hz and all College and Sodankyla occurrences were <1·1 Hz

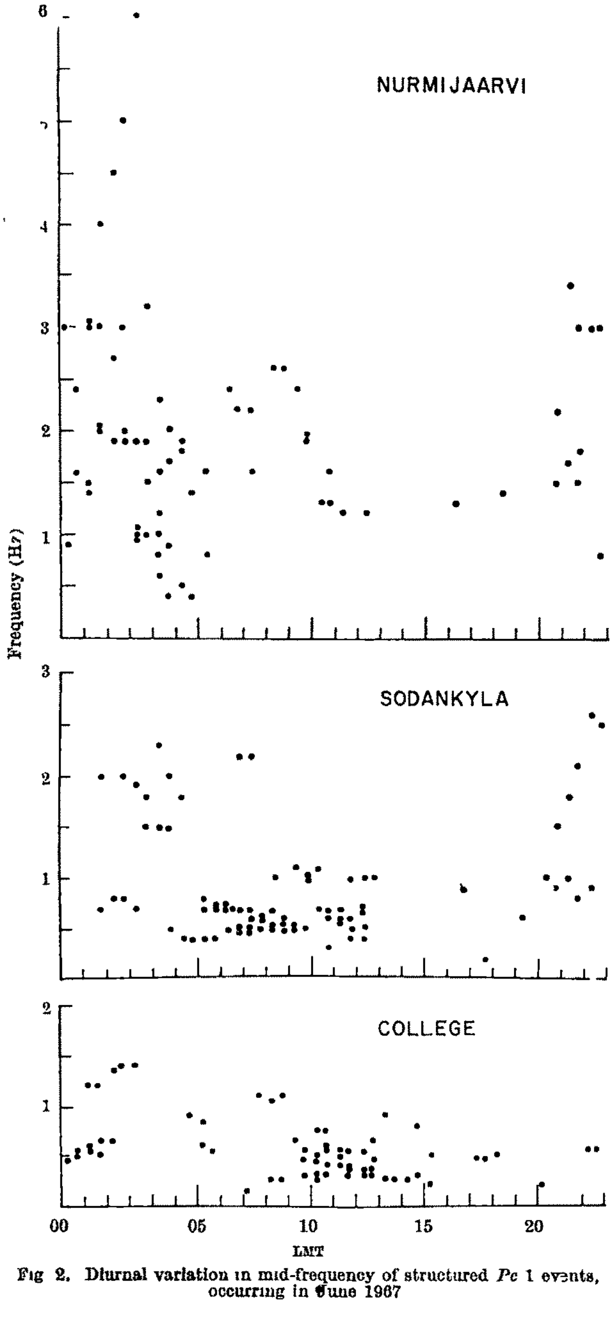

Fig 2. Diurnal variation in mid-frequency of structured *Pc* 1 events, occurring in June 1967

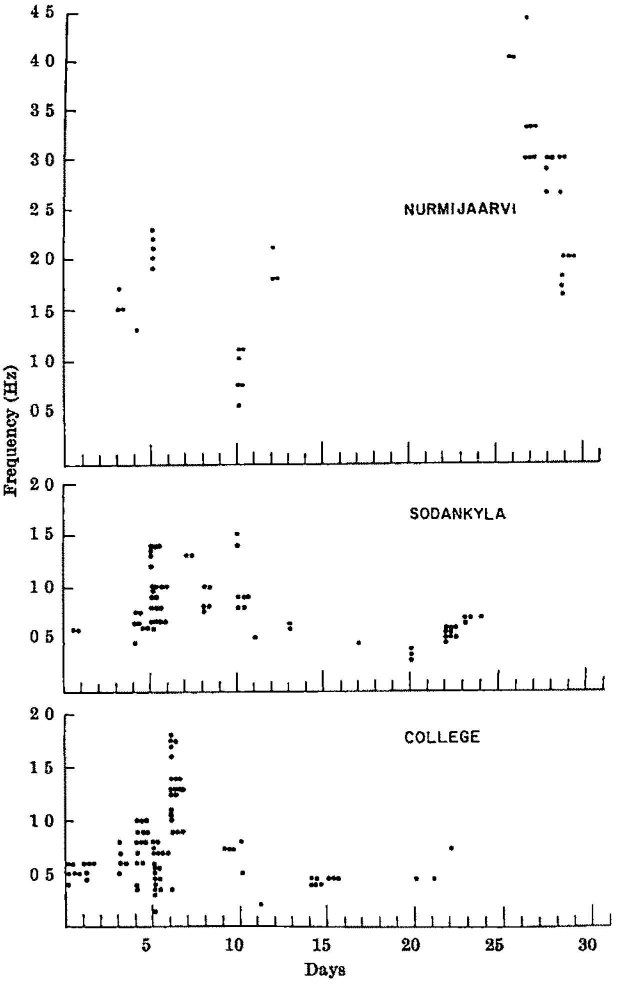

Fig 3 Mid-frequency of all structured *Pc* 1 events recorded at Nurmijaarvi, Sodankyla and College, May 1967

Theoretical works show that the higher frequencies are damped more heavily in the ionosphere[11] We therefore expect that if Nurmijaarvi is located near the source field lines for the higher *Pc* 1 frequencies, then stations at lower latitudes may record lower average *Pc* 1 frequencies than Nurmijaarvi, because of the attenuation of the highs in an ionospheric duct during southward propagation of the signal Tepley and Wentworth[12] recorded *Pc* 1 with average frequency near 1 Hz at Palo Alto, at about 40° N

There are certainly possible explanations for the *Pc* 1 frequency variation other than the latitude variation in equatorial proton gyrofrequency For example, there could be latitude variations in the magnitudes of certain wave damping mechanisms in the ionosphere and exosphere, and there could be more than one type of ion involved with a latitude spread in the distribution of the types. Dowden[13] has suggested that helium atoms as well as protons are involved in *Pc* 1 generation At present, however, there is no evidence for the existence of either of those latitude variations

The results presented here do seem to favour the *Pc* 1 theories which depend on the ion gyroresonance mechanism[7–9], and present difficulties for certain other mechanisms

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[1] Lokken, J E , Shand, J A , and Wright, C S , *J Geophys Res* , 68, 789 (1963)
[2] Tepley, L R , *J Geophys Res* , 69, 2273 (1964)
[3] Gendrin, R E , and Troitskaya, V A , *Radio Science*, 69D 1107 (1965)
[4] Wentworth, R C , Tepley, Lee, Amundsen, K D , and Heacock, R R , *J Geophys Res* , 71, 1492 (1966)
[5] Wescott, E M , Hessler, V P , and Kenny, J F , *Nature*, 212, 170 (1966)
[6] Armstrong, T , *J Geophys Res* , 70, 2077 (1965)
[7] Tepley, L R , and Wentworth, R C , Final Report Contract No *NAS5*-3656, Lockheed Research Laboratory, Palo Alto, Calif (1964)
[8] Cornwall, J M , *J Geophys Res* , 70, 61 (1965)
[9] Jacobs, J A , and Watanabe, T , *Planet Space Sci* , 15, 799 (1967)
[10] Benioff, H , *J Geophys Res* , 65, 1413 (1960)
[11] Akasofu, Syun-Ichi, *Radio Science*, 69D, 361 (1965)
[12] Tepley, L R , and Wentworth, R C , *J Geophys Res* , 67, 3317 (1962)
[13] Dowden, R L , *J Geophys Res* , 72, 2020 (1967)

Origin of the Newgale Shingle Embankment, St. Bride's Bay, Pembrokeshire

MANY of the shingle accumulations round the coastline of Britain pose problems of origin because there is no apparent source of supply for them One such accumulation is that at Newgale in Pembrokeshire (Fig 1), a study of which has provided evidence relating both to the source of supply of the shingle and to the reasons for its concentration This evidence may be of general interest with regard to the problem of explaining how such banks originate

St Bride's Bay is highly irregular in plan, the bay-head consisting of several small beaches separated by headlands The only substantial accumulation of shingle is at Newgale, where an embankment 17 ft high extends for 1 25 miles Steers[1] has suggested that "Along the northern shore of St Bride's Bay beach material travels eastward and the storm beach at Newgale thus accumulates, at least in part" This view attempts to explain both the origin and concentration of the shingle By his further assertion that "It (the embankment) is formed of stones of local origin"[1], Steers seems to imply that cliff erosion and the alongshore transport of the debris are principally responsible for the embankment The difficulties with this suggestion are first that there are erratics present, and second, it is doubtful whether shingle could move along the northern shore because of the minor headlands, which would obstruct movement, even with a lower sea level

To explain the presence of flint shingle on the coast of southern England far from possible sources, Hardy[2] developed an idea of Steers[3] in which a rising post-glacial sea level is envisaged "sweeping up" shingle in front of it This hypothesis would seem applicable at Newgale The presence of erratics restricts the provenance of the shingle, at least in part, to drift Such deposits do not exist on land near Newgale, and so it is suggested that the source of the erratics is drift on the bay floor As a rising sea level "sweeping up" erratics from the drift would also remove stones of local origin, the drift could be the direct source of most of the embankment's shingle. It is therefore unnecessary to infer movement along the northern shore

Steers's hypothesis of littoral movement attempted to explain the origin and concentration of the shingle The rising sea level theory solves only the former problem With reference to the latter, Hardy has suggested that in southern England[2] and Norfolk[4] shingle structures are associated with areas of concentrated constructive wave energy An attempt has been made to test this idea at Newgale

The principal directions of wave approach to St Bride's Bay lie in the south western quadrant Wave refraction diagrams of the Arthur, Munk and Isaacs type[5] drawn for various directions within this quadrant for periods between 3 and 10 sec show that energy levels increase generally northwards along the bay-head, which is to be expected because of the sheltering effect of the southern headland Maximum energy is therefore associated with the shingle accumulation, although the association is not confined to long constructive waves Hardy's view is that this association of shingle with concentrations of constructive energy can be explained in that the sorting of shingle to produce a beach is achieved largely by constructive waves, so that any concentration of these will lead to a shingle concentration Hardy's work is concerned with beaches built from loose shingle on the sea bed and not from chalk *in situ* The removal of stones from drift would require a concentration of shorter, steeper, destructive waves before the building of an embankment by longer, less steep, constructive waves It is considered significant that waves of several lengths show maximum energy levels at Newgale

There is thus at Newgale an association of high energy values with a shingle accumulation For a causal relationship to be inferred it must be shown that a similar concentration of energy existed during the post-glacial rise in sea level The isobaths (Fig. 1) demonstrate that the bay form would have been in existence throughout this rise, so that the shelter effect of the southern headland would be a dominant factor controlling the energy distribution patterns at successive coastlines Maximum energy levels throughout the rise would therefore be in

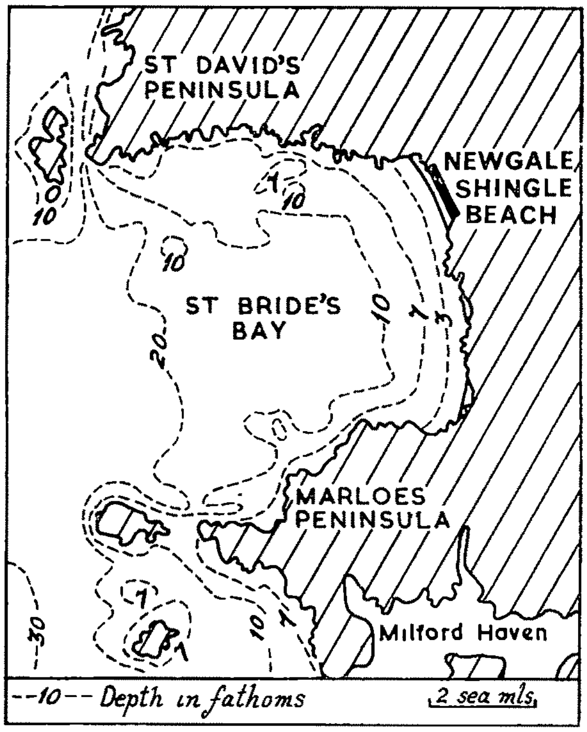

Fig 1 St Bride's Bay

the northern part of the bay. In addition, each successive bay shape would be relatively more inset, resulting in a progressive intensification of the shelter effect, which would in turn result in a tendency for the alongshore energy differential to become more pronounced with time.

I think that the Newgale embankment is composed largely of drift derived shingle from the bay floor and that the reason for its location is that wave energy has been continuously concentrated in the northern part of the bay. The postulated causal relationship between concentrated wave energy and shingle accumulation gains support from this investigation.

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Received November 22, revised December 18, 1967.

[1] Steers, J. A., in *The Coastline of England and Wales*, 158 (Cambridge, 1946).

[2] Hardy, J. R., *Twentieth Intern. Geograph. Cong. London, 1964*.

[3] Steers, J. A., in *The Sea Coast*, 160 (London, 1953).

[4] Hardy, J. R., *Trans. Inst. Brit. Geog.*, **34**, 53 (1964).

[5] Arthur, R. S., Munk, W. H., and Isaacs, J. D., *Trans. Amer. Geophys. Un.*, **33**, 855 (1952).

Theoretical Model for the Chandler Wobble*

EULER is usually credited with having been the first to show that an axially symmetric rigid body, with a fractional difference between the equatorial and polar moments of inertia equal to that of the Earth, could undergo a free nutation with a period of about 300 days. That is, in a body-fixed co-ordinate system, the instantaneous axis of rotation would describe a cone about the polar axis with a 300 day period. It could have been expected that such a motion, even if present primordially, would have been damped almost completely by natural dissipative processes within the Earth. Such a motion would show itself in a periodic variation in astronomic latitude of a given site on the Earth's surface, because the rotation axis moves only slightly with respect to an inertial frame[1]. Despite the expectance of almost complete damping, repeated attempts were made in the nineteenth century to uncover indications of a variation in latitude with a 10 month period. None was definitely established, but in 1891 Chandler[2] announced a variation with a period of 428 days, about 40 per cent larger than predicted. Newcomb soon realized that the period of free nutation for the Earth would be greater than the rigid-body value, because of the fluid nature of the oceans and elastic yielding of the solid earth, and he proposed that Chandler's observations were indeed of the free nutation[3]. Systematic observations of latitude variations have been made since the turn of the century and clearly indicate the presence of an oscillation with this 14 month period (see Fig. 1). The amplitude of this oscillation has a maximum of about 0.3 sec of arc, that is the inclination of the instantaneous axis of rotation to the figure axis does not seem ever to exceed about 0.3 sec of arc.

What prevented this free nutation from being completely damped? Many suggestions of mechanisms to excite the Chandler wobble have been put forward during the past seventy-five years, but none has proved satisfactory. The question is still considered by most geophysicists to be largely unanswered. The purpose of this communication is to describe a new, and admittedly speculative, model that we conceived independently. The idea is based on Fig. 1 which, although extending over too short a time interval to be definitive, appears to have an envelope characteristic of "beat" phenomena. We therefore propose that these latitude variations do indeed exhibit beats, attributable to a mechanical interaction between the upper layer with the remainder of the Earth. We at first contemplated a core–mantle separation, but seismological and magnetic field evidence seems to indicate that the core has insufficient rigidity to possess a free nutation frequency nearly equal to the corresponding frequency of the mantle alone. We were thus led to the supposition, which must be regarded as speculative, that the important interaction effectively separating the Earth into two parts occurs in the upper mantle, perhaps somewhere between the depths of about 400 and 1,000 km in a region where the shear velocities of seismic waves change rather sharply. The separate free nutation frequencies of the two parts, modified by the coupling, can be expected to be present in the Chandler motion. The data (Fig. 1) suggest a beat with a half-period between 40 and 50 years, corresponding to a difference in period of the two component frequencies of between 12 and 10 days, respectively. Results of theoretical calculations, although sensitive to the assumed values for the relevant Love numbers and the differences in equatorial and polar moments of inertia of the two parts, indicate that such a beat period is reasonable for our proposed model. (Of course, if the model proves adequate, the observed period provides a further condition on these properties.)

We suppose that this resonant oscillation is excited asynchronously, through a nonlinear interaction, by the differential luni-solar torques exerted on the two layers. Using a suitably parameterized model, we are investigating numerically the possible motions of this complicated, three degrees of freedom, dynamical system. Apart from the well-known expressions for the luni-solar gravitational torques, the model includes a restoring torque (inertial coupling term), which is proportional to the separation of the figure axes of the two layers, and a viscous torque which is a non-linear function of the difference in angular velocity of the coupled parts. In first approximation both layers are considered rigid. The transfer of the required energy between the two layers seems possible on the basis of preliminary calculations. Quantitative results, to be reported in a subsequent communication, will include the relations between the model parameters and the amplitudes and frequencies of the Chandler motion.

How may this model be checked experimentally? The supposition that a beat frequency is present in the Chandler motion can, of course, be tested conclusively by awaiting future measurements. In fact, data have accumulated for 12 years since Fig. 1 was compiled and would be expected to exhibit a decreasing amplitude in the oscillation. Although the nineteenth century observations may not be very reliable, we intend to re-examine the best of these and, after separating the annual term[1], seek evidence bearing on the predicted amplitude modula-

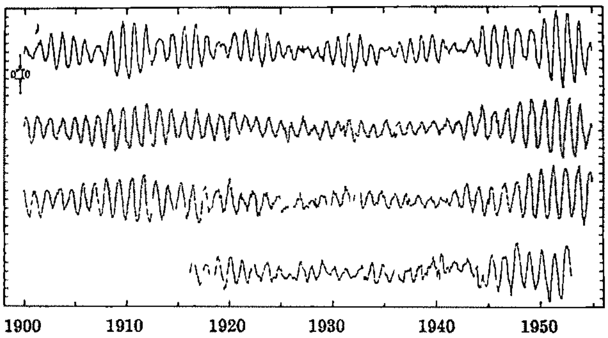

Fig. 1. Variations of the two orthogonal components of the position of the Earth's rotation axis with respect to the axis of the figure (from ref. 1). The top two curves show the unsmoothed observations for one component, before (first curve) and after (second) removal of the seasonal variation. The last two curves, in reversed order, show similar variations for the second component. The first three curves were obtained by the International Latitude Service, the last with the Photographic Zenith Tube at Washington.

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* Because of a misunderstanding and a delay in the post, an uncorrected version of this note appeared (*Nature*, **216**, 669, 1967).

tion of the Chandler term The presence of a beat would not definitely establish the correctness of our model, but would provide strong evidence in support of the basic idea Even if the beat were absent, and the apparent change in amplitude illusory, it is still quite conceivable that the excitation of the Chandler motion could take place through the differential luni-solar gravitational torques acting, say, on the core and mantle which may interact through a non-linear coupling

The presence of two important frequency components in the Chandler motion is, of course, also consistent with the apparent breadth of the power spectrum of the polar motion in the vicinity of 0 8 cycles/yr[1] In the past, the corresponding decrease in the amplitude of the auto correlation of the Chandler data—approximately 10 per cent for a 14 month lag—was interpreted as indicating a damping time of approximately a decade and led to suggestions that the free nutation was being excited by random impulses[1] In our model, the amplitude of the auto correlation will also decrease, as observed, but after longer intervals will rise again, the period being equal to that of the beat frequency Furthermore, the problem of the anomalously small Q value for the Earth, inferred from the "damping" of the Chandler motion, is automatically disposed of

If this model of the Chandler wobble proves fruitful, it might be interesting to consider the more complicated case of a non-axially symmetric mantle which may be appropriate for Venus or the Moon

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[1] See, for example, Munk, W H , and MacDonald, G J F , in *The Rotation of the Earth* (Cambridge Univ Press, London, 1960)

[2] Chandler, S C , *Astron J* ,11, 65 (1891)

[3] Newcomb S *Astron J* , 11, 81 (1891)

PHYSICS

Dimensional Problem of the Power Law in Rheology

The power law equation, between shear stress, τ, and strain rate, γ, may be written $\dot{\gamma}=k\tau^{\alpha}$ it has often been shown[1,2] to be in accordance with experimental data obtained for many non-Newtonian fluids The equation is open to the logical objection that it is dimensionally inconsistent unless the constant of proportionality, k, is not a true constant at all but has variable dimensions which depend on the properties of the sample under observation Nedonchelle and Schutz[3] have shown experimentally that for one particular non-Newtonian system there is a relationship between the constant of proportionality, k, and the exponent, α, such that the equation may be reduced to a dimensionless form containing three dimensionally stable constants which are independent properties characterizing the material in its environment.

Scott Blair[4] attempted to provide a theoretical basis for the existence of the power law, and in one form[5] of his derivation he introduced four constants, two of which were particular values of a function, which he designated f in his paper In his treatment, this function was subsequently eliminated from the argument, but it can easily be shown that the constants of Nedonchelle and Schutz and of the particular values of Scott Blair for f are closely related

Writing the dimensionless equation of Nedonchelle and Schutz in the form

$$\frac{\dot{\gamma}}{\gamma_0} = \left(\frac{\tau}{\tau_0}\right)^{\alpha} \tag{1}$$

to correspond with the form given here, by replacing their D by γ and their a by $1/\alpha$, gives, on taking logarithms

$$\log \dot{\gamma} - \log \dot{\gamma}_0 = \alpha \log \tau - \alpha \log \tau_0 \tag{2}$$

From the theoretical derivation of Scott Blair, α is the ratio of two constants, which he denotes by a and b Equation (2) may then be rewritten

$$b \log \dot{\gamma} - b \log \dot{\gamma}_0 = a \log \tau - a \log \tau_0$$

or

$$a \log \tau - b \log \gamma = a \log \tau_0 - b \log \dot{\gamma}_0 \tag{3}$$

Scott Blair gives (his equations (2) and (4))

$$a \log \tau = f_1 - f \qquad b \log \dot{\gamma} = f_2 - f$$

so that

$$a \log \tau - b \log \dot{\gamma} = f_1 - f_2 \tag{4}$$

Combining equations (3) and (4) gives

$$\alpha \log \tau_0 - b \log \gamma_0 = f_1 - f_2$$

This becomes an identity if

$$f_1 \equiv a \log \tau_0 \quad f_2 \equiv b \log \dot{\gamma}_0 \tag{5}$$

These expressions (5) give the relation between the experimentally determinable constants τ_0 and $\dot{\gamma}_0$ of Nedonchelle and Schutz, and the constants of integration f_1 and f_2 of Scott Blair

Thus from the results of Nedonchelle and Schutz, it can be deduced that a minimum of three independent characteristic constants are necessary and sufficient for the power law to be dimensionally acceptable, and these three constants are compatible with the theoretical derivation of Scott Blair.

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[1] Philippoff, W , in *Viskositat der Kolloide* (Dresden, 1942)

[2] Spriggs, T W , Huppler, J D., and Bird, R. B , *Trans Soc. Rheol* , 10, 191 (1966)

[3] Nedonchelle, Y , and Schutz, R A , *CR Acad Sci* , 265 C, 16 (1967)

[4] Scott Blair, G W , *Rheologica Acta*, 4, 53 (1965)

[5] Scott Blair, G W , and Prentice, J H , *Cah du Gpe Fr de Rhéol*, 2, 75 (1966)

Luminescent Properties of Rocks, Meteorites and Natural Glasses under Proton Bombardment

Suggestions that some transient lunar events[1] are luminescent phenomena, excited by solar protons, have aroused interest in the luminescent properties of putative lunar surface materials We have studied the luminescence produced in various minerals and meteorites by a beam of 146 MeV protons from the Atomic Energy Research Establishment synchrocyclotron Both our proton flux, about 10^8 protons sec^{-1} cm^{-2}, and the ionization density are many orders of magnitude lower than those used by other workers[2-4], thus we avoid their problems of radiation damage and simulate more closely the conditions on the Moon's surface

Our specimens, powdered to a grain size of 10–100μ, were mounted on stainless steel disks using silicone grease, and viewed through one of a set of seven broad-band interference filters by a single EMI 9558 phototube Provision was made for evacuating the sample enclosure, for cooling the samples to liquid nitrogen temperature and for heating them electrically We thus obtained spectra between 3800 Å and 7400 Å at −180° C, +20° C and +100° C We also studied the thermoluminescence produced by irradiation at −180° C followed by heating, at about 12° per minute, to +180° C

Individual samples will be discussed in detail elsewhere, here we note that they may be assigned according to their brightness, spectra and thermoluminescence into one of several distinct groups, corresponding in the case of geological specimens roughly to their petrological type, and in the case of meteorites to their classes according to the Prior–Hey catalogue[5] These groups and their members are listed in Table 1, which also shows their average integrated light output at −180°, +20° and +100° C, their characteristic thermoluminescence (if any), and the ratio of light output at −180° to that at +100° C (the "cold enhancement factor") In Figs 1 and 2 we show representative spectra, at −180° and +100° C, of members of some of these groups The data are normalized to an intensity of $8\ 7 \times 10^{7}$ protons/sec The absolute magnitude of the brightness was found by calculation from the known parameters of the apparatus.

In geological samples, we note that the luminescent intensities are in all cases similar at +20° C and +100° C, but greatly enhanced at −180° C In similar studies, Nash[3] found that the response of a basalt sample was unaffected by a temperature change from +120° C to −80° C, we also find that a reduction to −80° C does not sensibly alter the response of basalts or granites, but that a further reduction to −180° C has a dramatic effect We show in Fig 3*a* the blue response of a granite specimen as a function of temperature and conclude that between −80° and −180° C the relative probability of radiative transitions increases sharply for these common minerals The granites show this low temperature enhancement most strikingly, their brightness at −180° C is of the same order as that of 0 004″ of *NE* 102*A* scintillator The granites also show an intense red thermoluminescence, with peaks near −140° and −30° C, a typical glow curve is shown in Fig 3*b* Energy efficiencies at +20° C varied between about 10^{-5} for the ultrabasic rocks, and about 2×10^{-4} for the granites, assuming an optical depth of 40μ This represents excellent agreement with Nash[3] (energy efficiencies typically 10^{-4}–10^{-5})

Of meteorites, we studied eleven chondrites and three achondrites Our results at +20° C agree well with those of Geake and Walker[2] The enstatite achondrite Khor Temiki was by far the most intensely luminescent Our spectrum for this, together with that of Geake and Walker for comparison, is shown in Fig 2*a* The effects of temperature variation are interesting Some classes of meteorite are brighter at +100° C than at −180° C, unlike any terrestrial rocks we studied This behaviour, typified by a cold enhancement factor less than unity, is accompanied by thermoluminescence near to or above room temperature We show in Fig 3*d* the red thermoluminescence of Khor Temiki, with, for comparison, the glow curve obtained by Sun and Gonzalez[4] for Cumberland Falls, another enstatite achondrite The recent fall at Barwell seems anomalous In all respects, it resembles the bronzites more than the hypersthenes with which it is classified[6] on a mineralogical basis Figs 2*b*, *c* and *d* show the spectra at −180° C and +100° C of Ogi (a typical bronzite), Barwell and Sinai (a hypersthene), respectively This observation seems significant and (in view of the clear mineralogical distinctions[7] between the classes of chondrites, particularly falls) somewhat surprising

Finally, we studied five specimens of tektites and three terrestrial glasses Four of the tektites are very similar, they have featureless spectra of low intensity and exhibit only a small low temperature enhancement and no thermoluminescence The Moldavite, on the other hand, has a strong orange-red peak and a total light output about five times that of the others Fig 2*e* compares its spectrum at +20° C with that of the Australite, and with the low temperature spectrum of Ries impactite glass The latter's resemblance to the Moldavite is intriguing, but the difference in cold enhancement factors argues against any genetic relationship between these two geographically associated glasses

Our observations of the intense thermoluminescence of granites suggest an explanation of the transient red glow

Table 1

	Average light output*			Cold enhancement factor†	Thermoluminescence
	+20° C	−180° C	+100° C		
Geological Samples					
I Basic ferromagnesian rocks and minerals (dunite, enstatite, augite, olivine)	13	30	11	2 7	Nil
II Extrusive rocks and breccia (five specimens of ash pumice and basaltic lava)	25	68	27	2 5	Nil
III Ancient volcanic breccia (Ordovician ash and tuff)	57	460	63	7 3	Weak blue peaks near −140° C
IV Potash felspar (orthoclase)	266	449	320	1 4	Intense blue peak near +20° C
V Granites (seven specimens)	238	3,352	266	12 6	Intense red peaks near −30° C and −140° C
Meteoric Samples					
VI Hypersthene chondrites (Sinai, Tenham)	12	24	14	1 7	Nil
(VIa Barwell	30	28	38	0 7	Strong green-blue peak above +180° C)
VII Bronzite chondrites (Cobija, Mornans, Ogi, Sindhri)	18	19	28	0 7	Strong blue peak above +180° C
VIII Pigeonite chondrite (Karoonda)	8	25	13	1 9	Nil
IX Enstatite chondrites (Daniel's Kuil, Khairpur)	92	97	119	0 8	Very strong red peak near 0° C
X Carbonaceous chondrites (Cold Bokkeveld, Orgueil)	8	10	5	2 0	Nil
XI Hypersthene achondrite (Johnstown)	11	36	10	3 6	Nil
XII Enstatite achondrite (Khor Temiki)	1,778	1,730	1,997	0 9	Intense red peak near 0° C
XIII Plagioclase achondrite (Juvinas)	115	132	89	1 5	Weak red peak near 0° C
Glasses					
XIV Tektites (Australite, Bediasite, Indochinite, Rizalite)	11	14	10	1 4	Nil
(XIVa Moldavite	48	85	54	1 6	Nil)
XV Natural glasses (Darwin glass, Ries impactite)	15	158	12	13 2	Nil
(XVa Libyan Desert glass	471	1,345	350	3 8	Nil)

* These figures are the integrated light output from 3800 Å–7400 Å In this table and on the figures, one unit corresponds to the emission of 2×10^{4} photons/sec, which for our proton flux is equivalent to an energy efficiency of about 7×10^{-7} (assuming an optical depth of 40μ)

† Ratio of light output at −180° C to that at +100° C

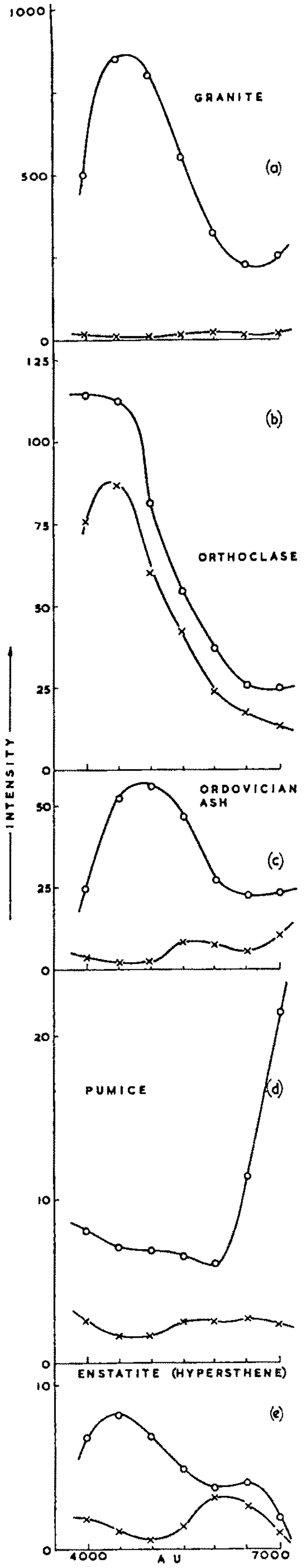

Fig 1 Luminescent spectra of various geological samples. Open circles represent data at −180° C, crosses are data at +100° C

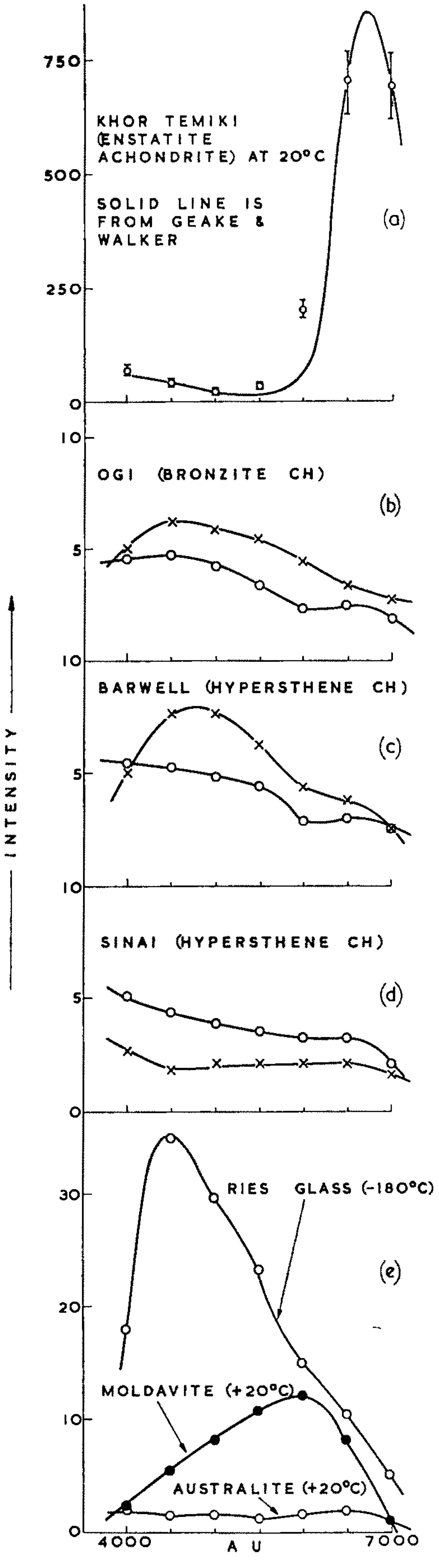

Fig 2 Luminescent spectra of various meteoritic and vitreous samples. Open circles represent data at −180° C, crosses are data at +100° C (except where stated otherwise)

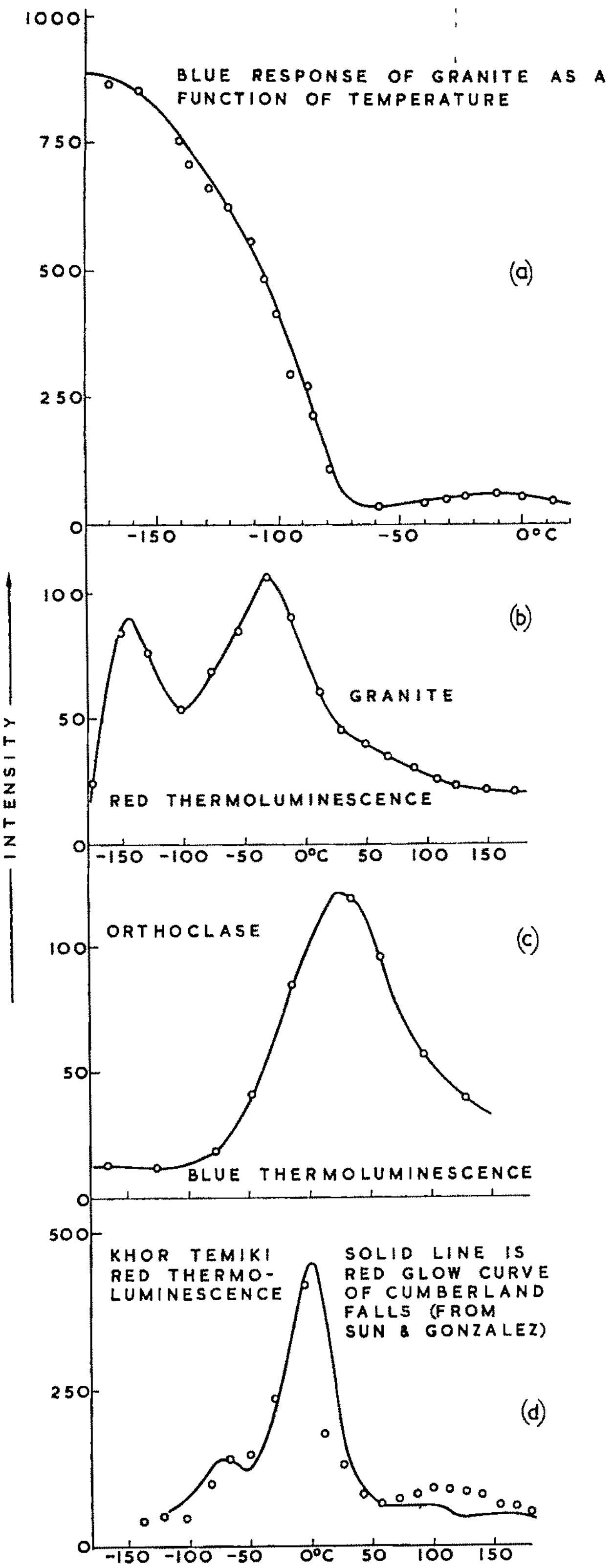

Fig 3 Luminescent response as a function of temperature (*a*), and thermoluminescent effects (*b, c, d*), for various samples

seen repeatedly in regions of the moon which, from their distribution, are believed[8] to be prone to tectonic disturbances In such active regions it may sometimes happen that cold sub-surface material, possibly granitic, is suddenly disturbed and exposed to surface insolation, a previous history of low-temperature irradiation may then give rise to visible thermoluminescence Using values for proton fluxes cited by More and Tiffany[9], we calculate, for example, that lunar material at a depth of about 10 g cm^{-2} will absorb about 10^{10} MeV g^{-1} cm^{-2} y^{-1} Such irradiation at[10] − 70° C for about half a million years might then yield, on sudden heating, light outputs of the same order as that of reflected sunlight

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[1] Middlehurst, B M, *Observatory*, **86**, 239 (1966)
[2] Geake, J E, and Walker, G, *Geochim Cosmochim Acta*, **30**, 929 (1966)
[3] Nash, D B, *J. Geophys Res*, **71**, 2517 (1966)
[4] Sun, K H, and Gonzalez, J L, *Nature*, **212**, 23 (1966)
[5] Hey, M H, *Catalogue of Meteorites*, 77 (third ed) (British Museum, 1966)
[6] Jobbins, E A, Dimes, F G, Binns, R A, Hey, M H, and Reed, S J. B, *Mineral Mag*, **35**, 881 (1966)
[7] Mason, B, in *Meteorites*, 78 (Wiley, 1962)
[8] Middlehurst, M B, and Moore, P A, *Science*, **155**, 449 (1966)
[9] More, K A, and Tiffany, O L, *Proc Symp Radiation Hazards in Space 1962 USAEC*, TID-7652, 682 (1963)
[10] Troitsky, V S, *Radio Sci*, **69D**, N12, 1585 (1965)

THE SOLID STATE

Annealing of Single Phase Polycrystalline Sulphide Ores

In two earlier communications[1,2] it was demonstrated that the grain boundary shapes in many sulphide ores could be ascribed to interfacial free energy requirements, and thus that their grain structures were analogous to those of metals and ceramics It was also suggested that many ores had been naturally annealed

Subsequent work has confirmed the importance of interfacial free energies in the development of grain shape, but it has also shown that the fully annealed state is far less common than was originally thought In fact it now seems that the fully annealed state is rare among granular ores of "massive" type—a conclusion reached as a result of the development of a new, though very simple, method for expressing degree of annealing

Following the earlier work, we began investigating the effects of temperature on the grain structure of single phase sulphide aggregates, and on dihedral angles in two phase and polyphase aggregates It soon became apparent that heating led to a decrease in the spread of angle values as measured on a random polished section, and that this decrease was in some way related to both time and temperature Unknown to us at the time, Ikeuye and Smith[3] had observed an analogous phenomenon in the

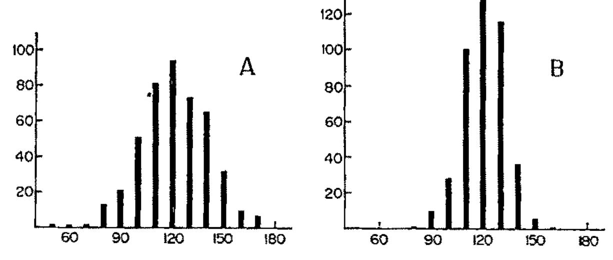

Fig 1 Frequency distribution of angles as measured on a random plane for polycrystalline chalcopyrite ($CuFeS_2$) *A*, Distribution for 450 random measurements before heating, *B*, distribution for 450 random measurements after heating at 300° C for 47 days

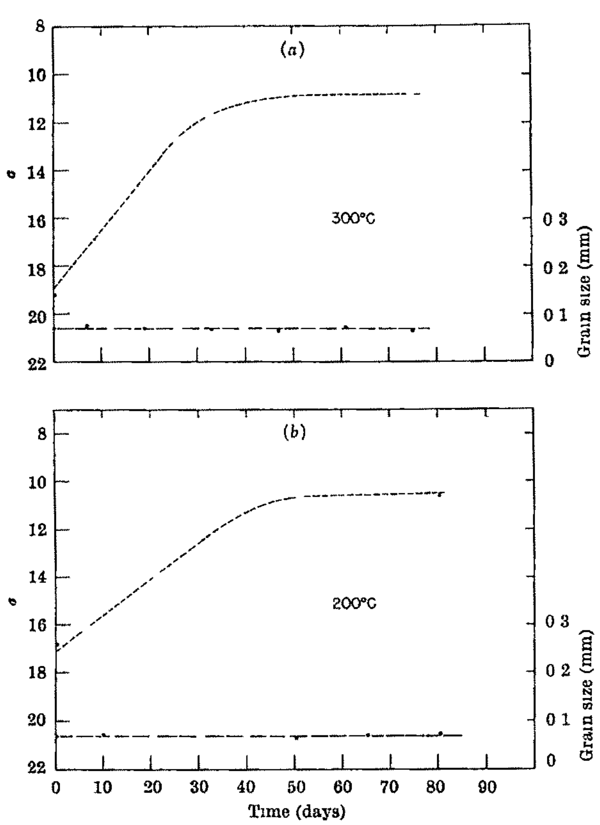

Fig. 2. Plots of changes in spread with time at (a) 300° C, and (b) 200° C for chalcopyrite.

change of the dihedral angle of aluminium–tin alloys (95 per cent to 5 per cent) when cold-rolled and then progressively annealed at 350° and 450° C. By plotting frequency distributions of a standard 400 random angles measured after cold-rolling and then at successive stages of the anneal, Ikeuye and Smith showed a clear change in mode (from 20° to approximately the true dihedral angle of about 60°) and also a decrease in spread of values "as measured". This, of course, involved two phases and thus "impurity diffusion", whereas the present work has been concerned with single-phase grain boundary migration, but the general analogy is clear.

In Fig. 1 are shown frequency distributions of angles, as measured on a random plane, observed by us in polycrystalline chalcopyrite ($CuFeS_2$) from one of the copper orebodies at Mount Isa, Queensland. Fig. 1*A* depicts 450 random measurements on a polished slug of this chalcopyrite prior to heating and Fig. 1*B* depicts a similar 450 measurements on the same slug after heating at 300° C for 47 days. Decrease in spread is clear.

Changes of this kind can be investigated quantitatively by introducing a measure of spread, and then plotting this against time for any chosen temperature. Where a fixed number of angles is measured throughout an experiment, the standard deviation of the frequency of angles as measured is an adequate and convenient index.

In Fig. 2, changes in spread exhibited by two specimens of Mount Isa copper ore heated at 200° and 300° C, respectively, are shown. Apart from occasional deformation twins and the moderate incidence of subgrains, there was no gross microscopical evidence of deformation prior to heat treatment. For each experiment, a single slug (about 2 × 1 × 1 cm) was used. This was etched initially with ammonium hydroxide–hydrogen peroxide and a random 450 angles measured. The etch was then removed, the slug placed in glass tubing which was evacuated and sealed off, and the whole was then heated at the appropriate temperature for the appropriate time. The slug was then removed, repolished, etched and measured again, and so on. It is clear from Fig. 2 that the initial grain boundary arrangement in both cases was immature (that is, not fully annealed). On heating and on the development of atomic mobility, the boundaries began to move towards equilibrium positions, such movement being more or less linearly related to time. On attaining equilibrium (that is, completion of the anneal) the standard deviation, σ, stopped decreasing and the curve flattened as shown. The ultimate spread is rather greater than that which might have been expected from random sectioning itself[4], but this is undoubtedly caused by the slight anisotropy of the chalcopyrite, and by the small impurity content inevitable in any natural ore. Fig. 2 shows that rate of annealing is, as might be expected, related to temperature.

A statistical study of this kind is valuable because it depicts the overall rate of annealing of a polycrystalline aggregate. It is also of interest, however, to observe rates of grain boundary adjustment at individual triple junctions, an experiment first carried out on a metal (a tri-crystal of tin) by Aust and Chalmers[5]. Such an experiment has been carried out by us on a single triple junction in a specimen of galena (PbS) from Coeur d'Alene, Idaho. Unlike the Mount Isa chalcopyrite, this galena showed a high degree of deformation, obvious even to the naked eye. Grains were elongated and often bent, the whole having the appearance of a sulphide schist. The behaviour of the chosen triple junction (that is, the relative movement of the three grain boundaries) at 300° C is shown in Fig. 3. Position 0 indicates the initial positions of the grain boundaries, and positions 1 to 9 show their arrangements as observed at intervals during the 21 days of the experiment. Considering that the temperature was a moderate one from a geological point of view, the amount of movement achieved in this short time is quite remarkable.

We have also obtained results similar to those of Fig. 2 for galena and sphalerite. Rates of annealing for the three minerals are generally comparable and increase fairly systematically with temperature. Current experiments are concerned with the low temperature regions in an attempt to estimate activation temperatures for grain boundary movement in the more common sulphides.

In view of the low temperatures at which grain boundary movement can be induced in sulphides, and the rapidity with which this progresses, it is perhaps surprising

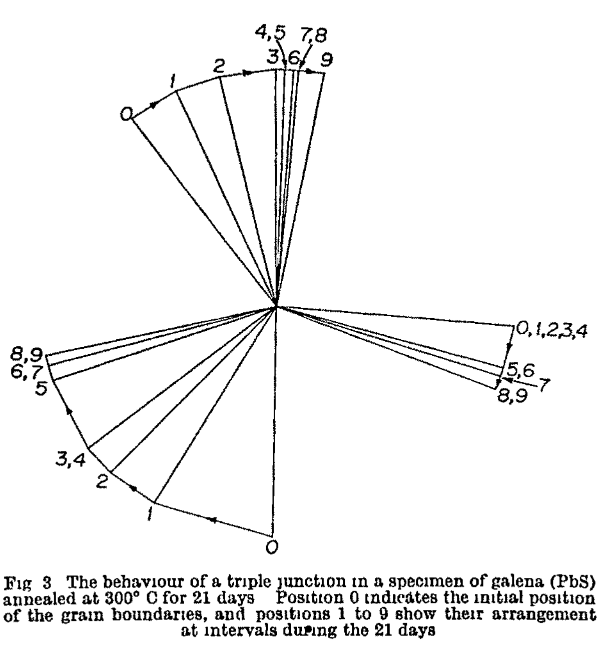

Fig. 3. The behaviour of a triple junction in a specimen of galena (PbS) annealed at 300° C for 21 days. Position 0 indicates the initial position of the grain boundaries, and positions 1 to 9 show their arrangement at intervals during the 21 days.

that any sulphide ores remain unannealed in nature It leads us to suspect that they may be a good deal less static than has been generally thought, and, in fact, that they are being more or less continuously deformed and annealed It follows from this, at least for "massive" ores, that present textures are unlikely to indicate the original physical nature of a deposit or the processes by which it was formed

This work has been carried out under the auspices of the Committee on Experimental Geology and Geophysics at Harvard University, and the Broken Hill Mining Managers Association, and we wish to acknowledge the generous support of both of these We also wish to acknowledge the important part played by N Gow in the initial stages of the investigation, and the helpful advice of Professor Cyril Stanley Smith as it progressed

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[1] Stanton, R L, *Nature*, 202, 173 (1964)
[2] Stanton, R L, *Inst Min Met*, 74, 33 (1964)
[3] Ikeuve, K K, and Smith, C S, *Met Trans*, 185, 762 (1949)
[4] Smith, C S, in *Imperfections in Nearly Perfect Crystals* (edit by Shockley) (Wiley, New York, Chapman and Hall, London, 1950)
[5] Aust, K T and Chalmers, B, *Proc Roy Soc*, A, 201, 210 (1950)

MOLECULAR STRUCTURE

Interaction between Model Compounds of Actinomycin *D* and DNA: DNA Dependent RNA Synthesis

DIMETHYL-actinocinyl-*bis*-(carboxylate) (AMD 1) and dimethyl-actinocinyl-*bis*-(L-threonate) (AMD 2) bind to the double stranded DNA (see following article) by a mechanism similar to that suggested for aromatic hydrocarbons[1] The binding sites may be related to conformational defects the concentration of which can be drastically reduced by addition of spermidine This is very likely the result of a re-ordering of the Watson and Crick model in solution caused by a stereospecific complex between the polyamine and DNA (refs 2 and 3)

This conclusion strongly suggests that in contrast to actinomycin *D* (ACT *D*), no specific sites are involved in the binding of AMD 1 and AMD 2 to DNA Some correlation has been found to exist for actinomycin between interaction in specific binding sites and inhibition of DNA-dependent RNA synthesis in the presence of RNA polymerase[4], and so we decided to study this reaction in the presence of AMD 1 and AMD 2.

For this purpose, DNA–AMD 1 and DNA–AMD 2 complexes to be used as templates in the RNA synthesis were prepared by equilibration of fine crystals of the two compounds with DNA (Sigma, Type 1) solutions in the cold (4° C) for 4 days and centrifugation, as described in the following article A sample of DNA solution was treated in the same way and used as control RNA polymerase (Miles) from *Micrococcus lysodeikticus* with an enzyme activity of 700 U/mg (ref 5) was used Reactions were performed at 30° C, 10 min in a final volume of 0 3 ml containing 0 1 molar *tris* *p*H 7 5, 2 5 mmolar $MnCl_2$, 1 6 mmolar spermidine hydrochloride (Fluka), 1 mmolar CTP, GTP, UTP and ^{14}C-ATP (2,100 c p m / mμmole), and 200 mμmoles of DNA control or DNA-AMD 1 or DNA-AMD 2 and 4 μg of the enzyme The inhibition of RNA synthesis by actinomycin was studied in the same experimental conditions, by adding to the DNA control increasing amounts of actinomycin *D* (a gift from Merck, Sharp and Dohme Research Laboratories)

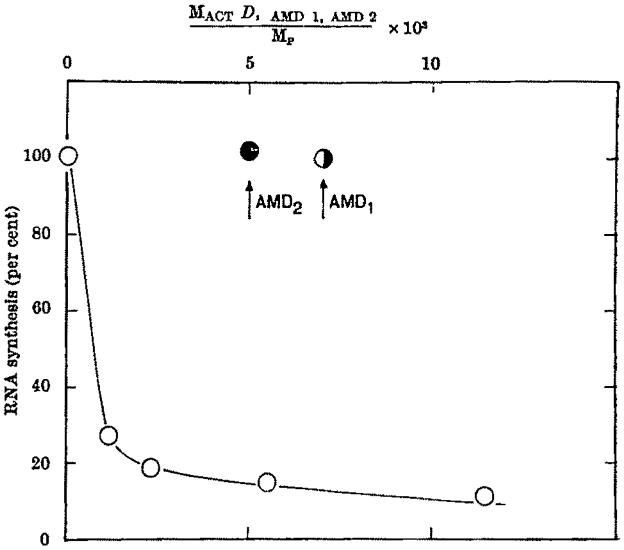

Fig 1. Percentage of RNA synthesis in presence of ○, actinomycin *D*, ◑, AMD 1, and ●, AMD 2 100 per cent corresponds to the incorporation of 2 mμmoles of ^{14}C-AMP For experimental details see text

The reaction was terminated by chilling in ice and adding an equal volume of cold 10 per cent trichloroacetic acid, the precipitate was collected on a 'Millipore' filter and washed extensively with cold 5 per cent trichloroacetic acid. The filters were dried and counted in a liquid scintillation counter (Nuclear Chicago). The results of these experiments are shown in Fig 1 Compounds AMD 1 and AMD 2 do not inhibit the DNA-dependent RNA synthesis and give the same results as the DNA control at concentrations comparable with those of actinomycin *D* which inhibits the reaction more than 80 per cent. In absence of spermidine the extent of the RNA synthesis is 25 per cent lower for both DNA control and DNA-AMD 1 or DNA-AMD 2

These results indicate that specific binding of actinomycin *D* to DNA which causes inhibition of RNA synthesis must necessarily involve the peptide lactone, although the isolated aromatic moiety of actinomycin can also interact with DNA

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[1] Liquori, A. M, De Lerma, B, Ascoli, F, Botrè, C, and Trasciatti, M, *J Mol Biol*, 5, 521 (1962)
[2] Liquori, A M, Costantino, L, Crescenzi, V, Elia, V, Giglio, E, Puliti, R, De Santis Savino, M, and Vitagliano, V, *J Mol Biol*, 24, 113 (1967)
[3] Liquori, A M, Ascoli, F, and De Santis Savino, M., *J. Mol Biol*, 24, 123 (1967)
[4] Reich, E, and Goldberg, I H, in *Progress in Nucleic Acid Research and Molecular Biology* (edit by Davidson, J N, and Cohn, W E), 3, 184 (Academic Press, 1964)
[5] Nakamoto, T., Fox, C F, and, Weiss, J B, *J Biol Chem*, 239, 167 (1964).

Interaction between Model Compounds of Actinomycin *D* and DNA: Physicochemical Studies

THE remarkable inhibitory effect of actinomycin *D* on DNA dependent RNA synthesis has been correlated with its ability to interact in a specific way with the DNA template[1]. The nature of the sites of interaction has

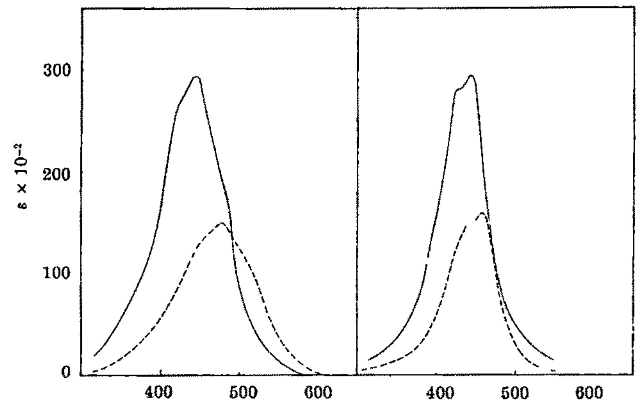
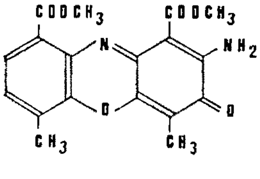
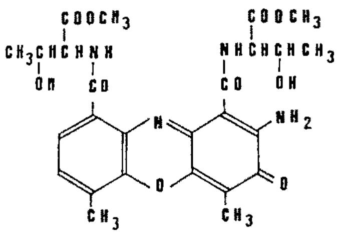


Fig 1 Absorption spectra of AMD 1 (left) and AMD 2 (right) in an aqueous solution of DNA (- - - -) and in water (———)

been investigated by physicochemical and biochemical methods[2-4] For this purpose several chemical modifications of the actinomycin have been tried Studies of simple chemical analogues of actinomycin which lack the peptide lactone moiety have, however, probably been discouraged by the great decrease in water solubility which is produced by the removal of the non-phenoxazone region of the molecule Previous experiments in our laboratory have provided useful information on the interaction between DNA and polycyclic aromatic hydrocarbons[5,6], which are sparingly soluble in water, through the introduction of a technique based on solubilization phenomena This method has been used in the present work in order to study the interaction of DNA with two model compounds containing the chromophore group of actinomycin



AMD 1
Dimethyl-actinocinyl-bis-(carboxylate)



AMD 2
Dimethyl-actinocinyl-bis-(L-threonate)

These compounds were prepared from 2-methoxy-toluic acid (Aldrich) through either five or six steps, respectively[7-9] Their solubilities in DNA solutions and in water were determined according to the technique described earlier[5,6] Fine crystals of AMD 1 and AMD 2 were equilibrated with solutions of DNA (Sigma, type 1) or with doubly distilled water for 3 days at 25° C by gentle shaking in the dark

After centrifugation at 60,000*g* for 1 h in the Spinco LHV ultracentrifuge, or filtration through sintered glass G4, the solutions were extracted with chloroform and the concentration in the organic layer was determined from the absorption at 437 mμ for AMD 1 ($\varepsilon = 27{,}000$) and at 444 mμ for AMD 2 ($\varepsilon = 27{,}800$) In Table 1 the solubilities in water and in DNA solutions are reported

Table 1

Compound	Solubilities at 25° C in Water	 DNA solution* (after deduction of the solubilities in water)
AMD 1	$1\ 6 \times 10^{-6}$ moles/l	$3\ 2 \times 10^{-5}$ moles/l
AMD 2	$1\ 0 \times 10^{-6}$ moles/l	$1\ 2 \times 10^{-5}$ moles/l

* DNA concentration 4 m equiv /l phosphate

The solubility in water of both compounds is extremely low compared with that of actinomycin[10] In the presence of DNA, however, there is a marked increase In Fig 1, the absorption spectra of AMD 1 and AMD 2 in water and in DNA solution are shown As in the case of actinomycin[1], a marked red-shift, 31 mμ for AMD 1 and 13 mμ for AMD 2, and a hypochromic effect (AMD 1, $\varepsilon_{475} = 15{,}000$, AMD 2, $\varepsilon_{457} = 16{,}000$) are observed in DNA solution with respect to the aqueous solutions

Both the solubilization of AMD 1 and AMD 2 in water, induced by DNA, and the red-shift of the absorption spectra, are very reminiscent of similar effects which were observed in the case of aromatic hydrocarbons[5] For the latter compounds it has been proposed that their molecules are inserted into defective regions of double stranded DNA and establish perpendicular interactions with the bases The crystal and molecular structures of complexes between aromatic hydrocarbons and a fully methylated purine strongly support this model[11-13]

A similar explanation could apply to the interaction between DNA and AMD 1 and AMD 2 The average number of phosphorous atoms per molecule of AMD 1 and AMD 2 bound can be calculated from the data in the table after making allowance for the solubilities in water The values so obtained are 1 44 and 1 125 which are of the same order of magnitude as those obtained for 3,4-benzpyrene.

As a further control of the hypothesis of a common mechanism for the binding of AMD 1 and AMD 2 and aromatic hydrocarbons to DNA, the influence of spermidine in the solubilization of AMD 1 and AMD 2 in the presence of DNA was studied

The curves of Fig 2 compare very closely with a similar curve showing the effect of spermidine in the binding of 3,4-benzpyrene which was studied with the same technique[6] As in the case of aromatic hydrocarbons the decrease of binding sites for AMD 1 and AMD 2 which was brought about by addition of spermidine can be interpreted as being caused by the elimination of the conformational defects as a result of a stereo-specific complexing of the polyamine to DNA[14] In conclusion, whereas actinomycin presents a strong binding to specific sites followed by a weak extensive binding, according to the mechanism proposed for the insertion of AMD 1 and

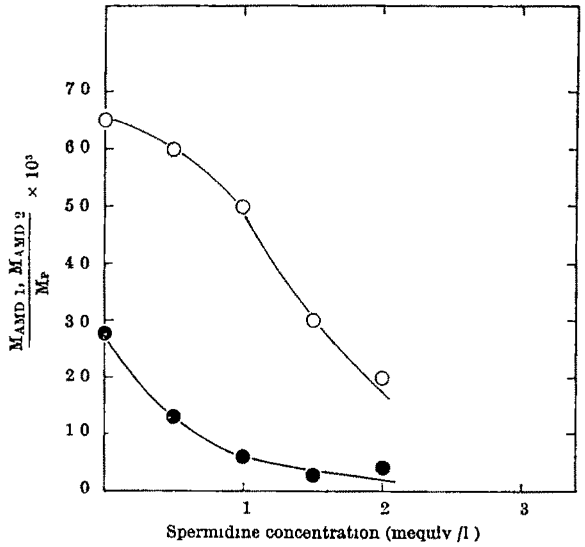


Fig 2 Ratio $\frac{M_{AMD\,1}, M_{AMD\,2}}{M_P}$ corresponding to the number of (○) AMD 1 moles $M_{AMD\,1}$ or (●) AMD 2 moles $M_{AMD\,2}$ solubilized/mole of DNA monomer unit M_P, expressed as phosphorus equivalents, as a function of spermidine concentration DNA concentration 2 mequiv /l phosphate Buffer 10^{-2} molar sodium chloride – 10^{-3} molar sodium citrate, pH 7 1

AMD 2 in conformational defects of DNA, no specificity should be expected in this latter case This is regardless of the similar optical changes which were observed when actinomycin and AMD 1 or AMD 2 interact with DNA

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[1] Reich, E , and Goldberg, I H , in *Progress in Nucleic Acid Research and Molecular Biology* (edit by Davidson, J N , and Cohn, W E), 184 (Academic Press, 1964)

[2] Goldberg, I H , Rabinowitz, M , and Reich, E , *Proc US Nat Acad Sci* , 48, 2094 (1962)

[3] Kahan, E , Kahan, F M , and Hurwitz, J , *J Biol Chem* ,238, 2491 (1963)

[4] Cerami, A , Reich, E , Ward, D C , and Goldberg, I H , *Proc US Nat Acad Sci* , 57, 1036 (1967)

[5] Liquori, A M , De Lerma, B , Ascoli, F , Botrè, C , and Trasciatti, M , *J Mol Biol* , 5, 521 (1962)

[6] Liquori, A M , Ascoli, F , and De Santis Savino, M , *J Mol Biol* , 24, 123 (1967)

[7] Brockmann, H , and Muxfeldt, H , *Chem Ber* , 91, 1242 (1958)

[8] Mauger, A B , and Wade, R *J Chem Soc* , 3126 (1965)

[9] Weinstein, B , Crews, O P , Leaffer, M A , Baker, B R , and Goodmann, L , *J Org Chem* ,27 1389 (1962)

[10] Gellert, M , Smith, C E , Neville, D , and Felsenfeld, G , *J Mol Biol* ,11, 445 (1965)

[11] De Santis, P , Giglio, E , Liquori, A M , and Ripamonti, A , *Nature*,191, 900 (1961)

[12] Damiani, A , De Santis, P , Giglio, E , Liquori, A M , Puliti, R , and Ripamonti, A , *Acta Cryst* ,19, 340 (1965)

[13] Damiani, A , Giglio, E , Liquori, A M , Puliti, R , and Ripamonti, A , *J Mol Biol* ,20, 211 (1966)

[14] Liquori, A M , Costantino, L , Crescenzi, V , Elia, V , Giglio, E , Puliti, R , De Santis Savino, M , and Vitagliano, V *J Mol Biol* ,24, 113 (1967)

Molecular Structures of Propargyl Alcohol and Propargyl Amine from Microwave Spectra

PROPARGYL alcohol and propargyl amine are of particular structural interest, because they belong to the rare class of substituted derivatives of methanol and methylamine in which the only possibilities of rotational isomerism are those associated with the COH or CNH_2 groups We have assigned microwave spectra for six isotopic forms of propargyl alcohol and for the normal form of propargyl amine The moments of inertia show that the rotamers concerned are those in which the OH and both NH bonds occupy gauche positions, that is, subtend dihedral angles in the region of 60° with respect to the acetylenic group. In the alcohol, the properties of the potential function for internal rotation are reflected in the occurrence of spectra of molecules in a very low-lying excited state and in the occurrence of series of spectral lines with first-order Stark effects

Table 1 ROTATIONAL CONSTANTS

Molecule and state		*A* (MHz)	*B* (±0 1) MHz	*C* (±0 1) MHz
HCC CH_2OH	state 1	33,000	4,700 23	4,232 25
	state 2	—	4,684 19	4,227 99
HCC CH_2OD	state 1	—	4,612 73	4,130 68
	state 2	—	4,600 74	4,127 71
DCC CH_2OH	state 1	—	4,360 65	3,944 66
	state 2	—	4,345 77	3,940 89
HCC CD_2OH	state 1	—	4,578 71	4,087 81
	state 2	—	4,564 15	4,084 25
DCC CH_2OD	state 1	—	4,282 04	3,852 89
	state 2	—	4,271 32	3,850 08
H CC CD_2OD	state 1	—	4,500 08	3,992 13
	state 2	—	4,489 53	3,989 81
HCC CH_2NH_2		29,619 46 ±0 1	4,595 12	4,160 62

The spectroscopic constants are shown in Table 1 For the alcohol, observed transitions are all caused by the component of the dipole moment in the *A*-axis of inertia, a total of some hundred lines were assigned on a rigid-rotor basis For the amine, lines caused by components of the dipole in both the *A*- and *B*- axes were assigned

The spectrum of the alcohol consists of two chief series of lines, the intensities of one series being only slightly greater than those of the corresponding members of the second For convenience, we have distinguished the constants for the stronger series as those of the ground state (state 1) and those for the other series as belonging to a very low-lying excited state (state 2) A number of lines caused by other states of the molecules are also present With any reasonable set of structure parameters the constants of all forms correspond convincingly only with those expected for the gauche rotamer In particular, the changes in *B* and *C* accompanying deuteration can be accounted for only with this geometry, which is that expected by analogy with the conformation found for allyl alcohol[1] Since beginning this work, we have learnt of similar measurements on propargyl alcohol by Dr E. Hirota[2], at the University of Tokyo, and we are grateful to him for allowing a comparison of the data obtained by our two groups Agreement of the rotational constants is very good, and Dr Hirota has made a considerably deeper study of the internal rotation problem, which we shall accordingly not discuss in detail here The suggestion that the low-lying excited states arise from a rather easy tunnelling of the OH proton from one of the two equivalent gauche positions to the other fits well with Dr Hirota's theoretical analysis of the spectra, including the series of lines with first-order Stark effects

The rotational constants for propargyl amine, all of which can be evaluated with high accuracy, show that four hydrogen atoms are outside the HCCCN plane, and thus the spectra are those of the form in which the NH_2 group straddles the acetylenic bond In accordance with this we have not found lines at the accurately predicted frequencies where absorption caused by any *C*-component of the dipole moment would lie Support is also found in the nuclear quadrupole coupling constants for nitrogen, which have the values

$$eqQ_a = -3\ 00\ \text{MHz},\quad eqQ_b = 1\ 34\ \text{MHz},\quad eqQ_c = 1\ 66\ \text{MHz}$$

The coupling tensor is thus roughly symmetrical about the *A*-axis of inertia and the constants are approximately consistent with the lone-pair electrons on the nitrogen being directed roughly along the *A*-axis This would be expected for the proposed rotamer, but not for the other rotamer in which the lone pair is adjacent to the carbon–carbon triple bond No intense satellite spectra have been observed for the amine, in contrast with the alcohol, and this, too, conforms with the proposed geometry

We are continuing to seek spectra of the alternative rotamers of both these substances, and it is hoped that evidence of the importance, or otherwise, of these will be obtained from high resolution infrared studies of both molecules, over a range of temperatures, which are in progress Work on the microwave spectra is also being extended in order to obtain more detailed structural information

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[1] Murty, A N , and Curl, R F , *J Chem Phys* , 46, 4176 (1967)

[2] Hirota, E , *J Mol Spectrosc* (in the press)

Empirical Correlation between the Molecular Electric Field Gradients and the Average Molecular Polarizabilities of some Halogen Compounds

A NUCLEAR quadrupole resonance signal[1] is split into two components by electrostatic fields[2] with up to maximum field strengths of 50 kV/cm The separation between the two components is a linear function[2] of the polarizability of the molecular species under investigation The aim of this communication is to demonstrate the general validity of this particular relationship[2] which is in fact independent of any restricting experimental conditions, for example application of an electrostatic field (Stark effect[2]) Although there are relatively few compounds for which both the nuclear quadrupole resonance and molecular polarizability data are available, the general validity of the correlation between the molecular polarizability and nuclear quadrupole resonance parameters, such as the molecular electric field gradients q_{zz}, can be demonstrated conclusively in the series of the homologous compounds CH_3Cl, CH_2Cl_2 and $CHCl_3$ The relevant experimental data[3-7] are shown in Table 1 and illustrated in Fig 1

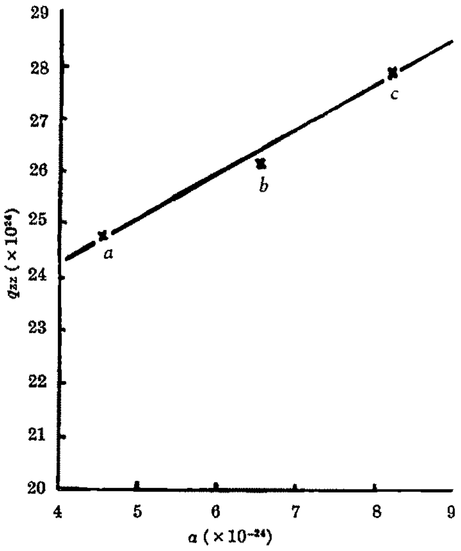

Fig 1 Linear correlation between the molecular electric field gradient q_{zz}, and the average molecular polarizability $\bar{\alpha}$ of the non-spherical molecules CH_3Cl (*a*), Ch_2Cl_2 (*b*) and $CHCl_3$ (*c*)

The molecular electric field gradients, q_{zz}*, used for Fig 1 were calculated from the nuclear quadrupole resonance frequencies of chlorine-35 in the molecules under investigation It should also be mentioned that the electron polarizability[8,9], $P_E + P_A$, is linearly related to the molecular electric field gradient

The existence of the general correlation implies that for the compounds considered a similar relationship might hold for the nuclear quadrupole resonance and Raman data because the appearance of Raman vibrations depends upon the molecular polarizability, which in turn is related to the molecular electric field gradient It is therefore interesting to note that if the Raman active $\nu_{C-Cl}(\pi)$ A_1 frequencies[10] (expressed in wave numbers) are plotted against the nuclear quadrupole resonance frequencies, the points representing the various chloromethanes fall on a smooth curve (see Fig 2)

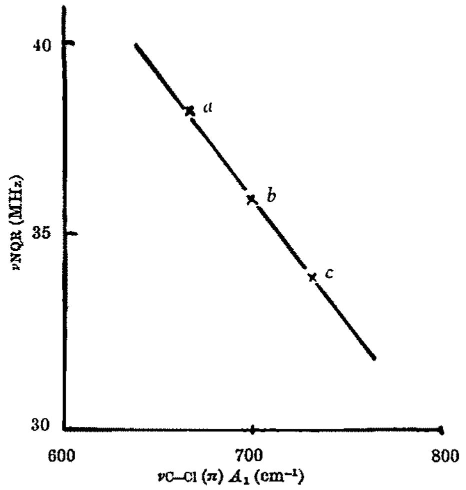

Fig 2 Linear correlation between the nuclear quadrupole resonance (NQR) frequency and the ν_{C-Cl} (π) A_1 Raman vibration of the non-spherical molecules CH_3Cl (*a*), CH_2Cl_2 (*b*) and $CHCl_3$ (*c*)

The Raman frequencies used in Fig 2 are summarized in Table 1

In the series of the chlorine derivatives of methane carbon tetrachloride seems to behave anomalously with respect to the rule developed This suggests that in the case of spherical molecules other rules are necessary to describe the dependence of the molecular electric field gradient on the molecular polarizability The new correlation (equation (3)) can be deduced in the following way

According to Clausius[11] and Mosotti[12], the molecular polarizability of sphere-like molecules is, to a good approximation, a function of the third power of the molecular radius R,

$$\alpha = R^3 \tag{1}$$

On the other hand, the molecular electric field gradient at the chlorine nucleus is a function[13] of the inverse of the third power of the average distance, r, between the quadrupolar nucleus and the $3p_z$ valence electron of chlorine,

$$q_{zz} = (-2)\frac{2l}{2l+3}\left\langle\frac{1}{r^3}\right\rangle_{3p} \tag{2}$$

Combining equation (1) with equation (2) gives

$$q_{zz} \sim (-2)\frac{2l}{2l+3} \times \left(\frac{1}{\alpha}\right) \tag{3}$$

From equation (3) it would be expected that in the case of sphere-like molecules the molecular electric field gradient is proportional to the inverse of the molecular polarizability Equation (3) takes into account the fact that the quotient $1/\alpha$ concerns the sphere-like molecule as a whole while $<1/r^3>_{3p}$ characterizes one of the four chlorine atoms of the tetrachloride

The validity of equation (3) can be checked with the series of compounds CCl_4, $SiCl_4$, $GeCl_4$ and $SnCl_4$ For the graphic representation of equation (3), the electron polarization[8,9] values P_{E+A} of the tetrachlorides are used, for the molecular polarizability is not available in all four cases This procedure is justified because according to the equation

$$P_E + P_A = \frac{n_\infty^2 - 1}{n_\infty^2 + 2} \times \frac{M}{\rho} = \frac{4\pi}{3} \times N \times \alpha$$

the electron polarization is proportional to the molecular polarizability Fig 3 shows a plot of the molecular electric field gradient of sphere-like molecules against the inverse of the electron polarization •

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* The molecular electric field gradient was calculated with the aid of the following formula[21]

$$\nu = \frac{e^2 Q q_{zz}}{2h}$$

where e is the electronic elementary charge, Q is the electric nuclear quadrupole moment of ^{35}Cl, q_{zz} is the molecular electric field gradient at the ^{35}Cl nucleus h is Planck's constant and ν is the pure nuclear quadrupole resonance frequency

Table 1

Substance	Nuclear quadrupole resonance frequency of ^{35}Cl (at 77° K)	Molecular electric field gradient q_{ZZ}	Average molecular polarizability $\bar{a}$	Electron-polarization (P_E+P_A)	$\nu_{C-Cl}(\pi)$ A_1 Raman frequency (cm^{-1})
CH_3Cl	34 029 MHz (ref 3)	24 75 × 10^{24} cm^{-3}	4 56 × 10^{-24} cm^3 (ref 7)	13 6 cm^3 (ref 8)	732 cm^{-1} (ref 10)
CH_2Cl_2	35 991 MHz (refs 3–5)	26 17 × 10^{24} cm^{-3}	6 48 × 10^{-24} cm^3 (ref 7)	20 cm^3 (ref 8)	700 cm^{-1} (ref 10)
$CHCl_3$	38 254 MHz (refs 3, 4, 6)	27 81 × 10^{24} cm^{-3}	8 23 × 10^{-24} cm^3 (ref 7)	26 6 cm^3 (ref 8)	667 4 cm^{-1} (ref 10)

As indicated by Fig 3, equation (3) describes in a fairly satisfactory manner the linear dependence of the molecular electric field gradient upon the inverse of the electron polarization, and therefore also upon the inverse of the molecular polarizability in all cases except that of $SiCl_4$ The anomalous behaviour of $SiCl_4$ with respect to equation (3) is a consequence of the particular double bond character which causes both the silicon–chlorine bond distance and the chlorine nuclear quadrupole resonance frequency to deviate irregularly from those of the homologous tetrachlorides A further reason is a possible strong deviation of solidified $SiCl_4$ from the spherical symmetry which is characteristic of this molecule in the gaseous state In this regard it should be noted that the nuclear quadrupole resonance spectra of the tetrachlorides of the elements of the fourth chief periodic group can only be recorded in the solid state (that is, at 77° K) The experimental findings relevant to the particular problem of $SiCl_4$ are summarized in refs 14 and 15

Table 2

Substance	Nuclear quadrupole resonance frequency of ^{35}Cl (at 77° K)	Molecular electric field gradient q_{ZZ}	Electron-polarization (P_E+P_A)
CCl_4	40 6 MHz (refs 3, 4, 6) (average value)	29 52 × 10^{24} cm^{-3}	28 2 cm^3 (ref 8)
$SiCl_4$	20 464 MHz (ref 3)	14 88 × 10^{24} cm^{-3}	28 7 cm^3 (ref 9)
$GeCl_4$	25 746 MHz (ref 3)	18 72 × 10^{24} cm^{-3}	38 cm^3 (ref 8)
$SnCl_4$	24 294 MHz (ref 3)	17 66 × 10^{24} cm^{-3}	45 5 cm^3 (ref 8)

Although the data considered are rather limited in number, it is conclusively shown that the molecular symmetry determines the nature of the dependence of the molecular electric field gradient upon the molecular polarizability The results presented thus supplement various empirical correlations put forward For example, Gerdil[16] showed that a linear correlation exists between the nuclear quadrupole resonance and infrared absorption frequencies (thus continuing earlier investigations by Lucken and Whitehead[17]) Further, Iredale[18] related nuclear quadrupole resonance frequencies to polarographic half-wave potentials, and Meal[19] and Bray and Barnes[20] demonstrated a linear correlation between nuclear quadrupole resonance coupling constants of substituted chlorobenzenes and the Hammett σ values of the substituents

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[1] Dehmelt, H G , and Kruger, H , *Naturwissenschaften* , **37**, 111 (1950)
[2] Duchesne, J , Read, M , and Cornil, P , *J Phys Chem Solids*, **24**, 1338 (1963)
[3] Livingston, R , *J Phys Chem* , **57**, 496 (1953)
[4] Gutowsky, H , and McCall, D , *J Chem Phys* , **32**, 549 (1960)
[5] Pound, R , and Watkins, G , *Prog Nucl Phys* , **2**, 21 (1952)
[6] Livingston, R , *Phys Rev* , **82**, 289 (1951)
[7] Stuart, H A , in *Die Struktur des Freien Moleküls*, 440 (Springer-Verlag, 1952)
[8] Landolt-Bornstein, in *Zahlenwerte und Funktionen*, 1, Part 3, 515 (Springer-Verlag, 1951)
[9] *Gmelin's Handbuch der Anorganischen Chemie, Silicium*, eighth ed
[10] Landolt-Börnstein, *Zahlenwerte und Funktionen*, |1, Part 2, 260 121, 267 (Springer-Verlag, 1951)
[11] Clausius, R , *Mechanische Warmetheorie*, 2, 94 (1874)
[12] Mosotti, O F , *Mem Math Fis Modena*, **24**, Part II, 49 (1850)
[13] Townes, C H , and Dailey, B P , *J Chem Phys* , **17**, 783 (1949)
[14] Das, T P , and Hahn, E L , *Solid State Phys* , suppl 1, 101 (1958)
[15] Huckel, W , in *Structural Chemistry of Inorganic Compounds*, 471 (Elsevier, 1951)
[16] Gerdil, R , *Nature*, **212**, 922 (1966)
[17] Lucken, E A C , and Whitehead, M A *J Chem Soc* , 2459 (1961)
[18] Iredale, T , *Nature*, **177**, 36 (1956)
[19] Meal, H C , *J Amer Chem Soc* , **74**, 6121 (1952)
[20] Bray, P J , and Barnes, R G , *J Chem Phys* , **22**, 1787 (1954), ibid , **27**, 551 (1957)
[21] Das, T P , and Hahn, E L , *Solid State Phys* , suppl 1, 18 (1958)

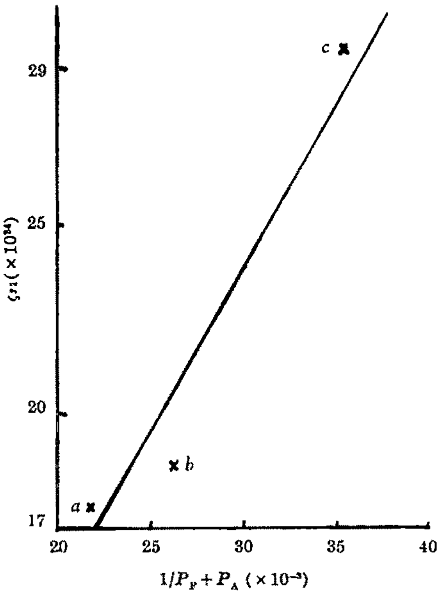

Fig 3 Linear correlation between the molecular electric field gradients q_{ZZ}, and the inverse of the electron polarization, $1/P_E + P_A$ of sphere-like molecules a, $SnCl_4$, b, $GeCl_4$, c, CCl_4

BIOCHEMISTRY

DNA Dynamics in Rat Granulopoiesis studied by Cytophotometry

THE theory that the DNA content of cells remains constant has been widely accepted, but some workers[1–3] presume that DNA content decreases either during the mitosis of the precursor cell (hypoploidy[4], haploidy[5]) or during the interphase maturation of the myelocyte stage into the granulocyte stage Another open question is which myeloid cells are capable of DNA synthesis and mitosis ? Most authors express the belief that these are the myeloblasts, promyelocytes and myelocytes[6,7] Recent evidence, however, indicates that some metamyelocytes can synthesize DNA This has been shown in the bone marrow of the guinea-pig after haemorrhage, by labelling with ^{3}H-thymidine and by cytophotometry[8], in irradiated rats as well as normal rats, by autoradiographic methods[9]; and in humans, by cinematomicrography[10]

These data and the fact that we had previously[11] observed division in the more differentiated myeloid cells of rats justified an investigation to find out, by different methods, whether DNA synthesis and mitosis could be seen in rat metamyelocytes in homoeostatic conditions

Myeloid cells were obtained from twenty Wistar rats of either sex weighing 80–120 g The animals were decapitated, the bone marrow plug was removed and

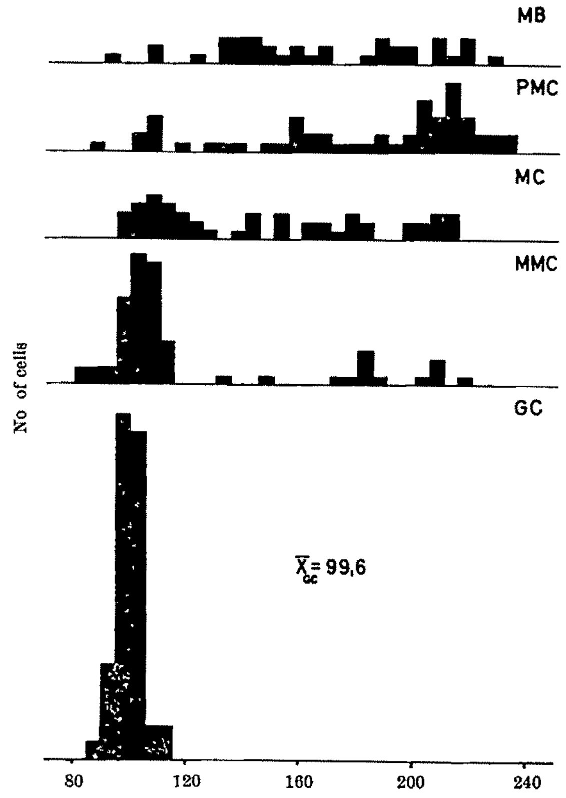

Fig. 1. Histograms showing DNA content in myeloblasts (MB), promyelocytes (PMC), myelocytes (MC), metamyelocytes (MMC), and granulocytes (GC) of myeloid cells.

thoroughly mixed with Tyrode solution by careful shaking. The cells were sedimented on quartz plates, and fixed in a 9 : 1 mixture of methanol and formalin, stained by the Feulgen reaction, and mounted with 'De Pe *X*' medium. DNA content was measured with an Opton *Umsp*-I in monochromatic light of wavelength 560 mμ, and expressed in arbitrary units. Differential diagnoses were made in a phase contrast microscope fitted with an ocular micrometer (×1,250). The stages of maturation were identified by the criteria described earlier[11]. The DNA content in 306 myeloid cells was recorded in five compartments: myeloblasts, promyelocytes, myelocytes, metamyelocytes and granulocytes. Histograms are shown in Fig. 1.

The diploids fell within the range of 85–115 arbitrary units. These were the granulocytes and most of the metamyelocytes. The rest of the metamyelocytes were hyperdiploids and tetraploids. Measurements showed them to amount to 21 per cent, but because we had selected, rather arbitrarily, the more intensely Feulgen-reactive metamyelocytes, it is safe to say that they amounted only to a small percentage. Most, but not all, the metamyelocytes with a higher DNA content were larger than average.

Myelocytes showed the frequency distribution of a highly proliferative population. The slight shift to the right of the peak of the diploid DNA values was probably caused by cells in the early *S* phase.

The DNA content of a large proportion of the promyelocytes fell within the range 190–233 arbitrary units, that is, they were tetraploids. The diploid cells were conspicuous for their small number. The ratio between 2*n*, hyperdiploid, and 4*n* promyelocytes was 1 : 3 : 4, indicating the presence among them of a number of cells with a comparatively long G_2. To establish the most reliable differential diagnoses possible with the phase contrast microscope, only cells with perforated ring shaped nuclei were selected for the DNA measurements, except in the myeloblasts.

The frequency distribution of DNA contents in myeloblasts showed a small heap of diploid cells. They were too few in number to establish the ratio between them and the hyperdiploid and tetraploid cells.

Not one of the 306 myeloid cells measured was of higher than tetraploid values ($AU_{max}=233$, a single promyelocyte).

To test our cytophotometric data, some preparations were stained not with Feulgen but according to the Pappenheim method for autoradiography (unpublished work of G. Rappay and A. Balazs). These studies showed that between 10 and 12 per cent of the metamyelocytes took up ^{3}H-thymidine and that they incorporated approximately as much labelled thymidine as did the promyelocytes with perforated nuclei.

Our findings suggest the following principal conclusions. We found that 50 per cent of the promyelocytes were diploids and hyperploids and that in none of the promyelocytes was the DNA content more than double that in the diploid myelocytes. This seems to contradict the earlier assumption that the division of promyelocytes leads to hypoploid[4] or even haploid[5] amounts of DNA. This assumption is also in marked contrast with evidence produced by Lennartz *et al.*[12] that the time of DNA synthesis (t_S) is twice as long in tetraploid as in diploid cells, whereas in promyelocytes and myelocytes it is about the same length[6,13]. Concerning the earlier assumption that DNA content decreases during interphase maturation[1-3,7,14], we found a shift to the left in the heap of metamyelocytes in relation to the myelocytes. Nevertheless, on cytophotometric evidence alone it is difficult to decide conclusively whether the shift was caused by actual loss of DNA or by the additive effect of cells in the early *S* phase, or to both. The transition between metamyelocyte and granulocyte is easier to interpret adequately. In the present work, the mean DNA value for all metamyelocytes and granulocytes was 121·5 and 99·6, respectively, but if we disregard the hyperdiploid and tetraploid metamyelocytes we find that the difference between the two diploid cell heaps is not statistically significant.

It is now generally accepted[6,7] that the myelocyte compartment is the last in which there is DNA synthesis during granulocytosis. Yet some metamyelocytes, chiefly megametamyelocytes with a nuclear diameter of 13–15μ, had been reported to be hyperdiploids or tetraploids[15]. We made the same observation with metamyelocytes of smaller diameter (10–12·5μ) (ref. 11). We have never encountered greater than tetraploid DNA values (compare reported findings in rabbit metamyelocytes[15]).

The large heap of promyelocytes can be interpreted in either of two ways. (1) These cells are members of a homogeneous population with a long G_2 period. Because t_S has been shown[12] to be independent of $t_{G_1}+t_M$, a shortened G_1 phase looks obvious. No doubt a uniform cell compartment in which the ratio of t_S to t_{G_2} is 1 : 1·3 does strike one as quite unusual. The other, and perhaps more likely, explanation is that there is, at least in the rat, a heterogeneous population of promyelocytes. Because t_S is the same throughout[6,12], while t_G gets longer as myeloid differentiation progresses[6,9,10], it seems justified to postulate, in addition to the incessantly maturing and dividing population, the existence of another promyelocyte population which has a comparatively long G_2. These cells might serve as a rapidly mobilizable reserve in rat bone marrow, as was suggested in some other respects by Gelfant[15] as well as by Lord[9].

We have heard of Morrison's paper (*Trans. Amer. Microsc. Soc.*, 84, 17, 1965) only after completion of our manuscript. His findings are in good agreement with our results and support in some aspects our conclusions.

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[1] Marinone, G , *Rev d'Hématol* , 9, 341 (1954)
[2] Hale, A J , in *VIII Kongress d Europ Ges f Haematologie*, Vienna (1961)
[3] Muller, D , *Klin Wschr* , 42, 224 (1964)
[4] Kinosita, R , and Ohno, S , *Naturwissenschaften*, 41, 381 (1954)
[5] Miyoshi, K , and Kono, H , *Tokushima J Exp Med* , 7, 196 (1960)
[6] Patt, H M , and Maloney, M A , *Ann. NY Acad Sci* , 113, 515 (1964)
[7] Schumacher, H R , and Erslev, A J , in *Nuclear Haematology* (edit by Szirmai, E), 89 (Academic Press, New York–London, 1965)
[8] Harris, P F , and Kugler, J H , *Exp Cell Res* , 42, 196 (1966)
[9] Lord, B I , *Brit J Haematol* , 11, 130 (1965)
[10] Boll, I , *Granulocytopoese unter physiologischen und pathologischen Bedingungen* (Springer, Berlin–Heidelberg–New York, 1966)
[11] Balázs, A , and Rappay, G , *Acta Biol Hung* , 17, 256 (1967)
[12] Lennartz, K J , Schummelfeder, N , and Maurer, W , *Naturwissenschaften*, 53, 21 (1966)
[13] Strickmans, P , Cronkite, E P , Fliedner, T M , and Ramos, J , *Nature*, 211, 717 (1966)
[14] Garcia, A M , *Acta Histochem* , 17, 246 (1964)
[15] Gelfant, S , *Exp Cell Res* , 26, 395 (1962)

Intermolecular Relationship between Neutral-salt-soluble and Acid-soluble Collagen

Soluble collagen can be extracted from collagenous material with neutral salt solutions and weak acids These extracts display many similar physical and chemical characteristics, but differ significantly with respect to their solubility Neutral-salt-soluble collagen has been shown to be the precursor collagen[1,2], with acid-soluble collagen being the more mature fibre-forming protein[3] The relationship between these soluble collagens which is usually accepted relies on the presence or absence of intramolecular cross links Apart from hydrogen bonds no intramolecular bonds are considered to occur in neutral-salt-soluble collagen On the other hand, acid-soluble collagen is formed as intramolecular bonds are introduced into neutral-salt-soluble collagen Such a relationship alone is insufficient, however, to explain the very different solubility properties of these two forms of collagen It is therefore proposed that neutral-salt-soluble and acid-soluble collagen are related on an intermolecular basis in their native environment, but that their subsequent extraction in mildly acid and basic conditions results in the degradation of terminally located telopeptides, which play a vital part in the properties of these collagen preparations The evidence for this theory is now outlined

A topic which has been the subject of some dispute is the relative proportions of α and β-sub-units in denatured soluble collagens Some workers have shown that neutral-salt-soluble collagen contains only α-sub-units[4,5], while others have shown the presence of β-sub-units as well[2,4,7,8] Some authors[9-11] have reported no differences in the sub-unit composition of neutral-salt-soluble and acid-soluble collagen prepared from the same source A more detailed study of the monomeric nature of neutral-salt-soluble collagen, and its preparation, would seem to explain many of the contradictory findings about the relationship between neutral-salt-soluble and acid-soluble collagen The term monomeric tropocollagen refers here to the native monomer with intact terminally located telopeptide side chains It is postulated[12-14] that side chains rich in acidic residues are present at the one end of the molecule, inducing a small net negative charge, while side chains rich in basic residues are present at the other extremity of the molecule, inducing a small net positive charge A number of workers[15-17] have isolated telopeptides rich in both acidic and aromatic amino-acids, while Cooper and Davidson[18] have reported the presence of three relatively large basic telopeptides While the acidic telopeptides have been shown to be labile in alkaline conditions, the basic telopeptides may be labile in acid conditions The failure to report the presence of such basic telopeptides in many acid-soluble collagen preparations would suggest that they are lost during preparation in weakly acid conditions

In the case of neutral-salt-soluble collagen the initial extract yields a large collagen fraction which when precipitated by lowering the *p*H with acetic acid becomes largely insoluble in 10 per cent NaCl even after very exhaustive extraction The initial ability of such neutral-salt soluble collagen to form insoluble polymeric material as well as to gel at body heat[19,20] would strongly suggest an intermolecular polymerization mechanism involving telopeptide linkages Furthermore, the extraction of the initial collagen precipitate with dilute acetic acid results in a soluble collagen fraction incapable of undergoing vigorous polymerization or gelating at body heat The progressive depolymerization of the initial collagen precipitate in weakly acid conditions resulting in the loss of telopeptide side chains would explain such findings

If native neutral-salt-soluble collagen is the tropocollagen monomer, then the possible dimeric character of acid-soluble collagen must be considered Acid-soluble collagen prepared by repeated phosphate precipitation in alkaline conditions has been fractionated on CM-cellulose yielding the α_3, α_1, β_{11}, β_{12} and α_2 sub-units[21,22] It must be noted, however, that acid-soluble collagen prepared using no phosphate precipitation has been shown to yield major unknown components[23-27] Davidson and Cooper[26] have related four such components to the $\alpha_1\alpha_1$, $\alpha_2\alpha_2$, $\beta_{11}\beta_{11}$ and $\beta_{12}\beta_{12}$ dimeric sub-units, respectively, and attribute their presence to the dimeric nature of the parent material Such a dimeric parent material would explain the β_{22}-sub-unit reported by Bornstein *et al* [28], as well as the presence of only β-sub-units in an acid-soluble extract reported by Bakerman and Hersh[29]

The presence of dimeric sub-units in solutions of denatured collagens immediately stresses the need for extreme caution in the interpretation of sedimentation data Davidson and Cooper[26] have shown that the major $\alpha_1\alpha_1$ and $\alpha_2\alpha_2$ components sediment with the β-sub-units, and suggest that the minor $\beta_{11}\beta_{11}$ and $\beta_{12}\beta_{12}$ components (molecular weights approximately 350,000) may be incorrectly characterized as gamma components A molecular weight of 260,000 for the collagen monomer[30], as well as the wide range of molecular weights and $\alpha \cdot \beta$ ratios reported in the literature, would support such findings Such evidence immediately casts doubt as to the labile nature of the β-sub-unit in certain prescribed conditions when using evidence from sedimentation[10,17,31-33] Mazurov and Orekhovitch[2] found no degradation of the β-sub-unit at *p*H 9 The depolymerization of the $\alpha_1\alpha_1$ and $\alpha_2\alpha_2$ components may in some cases explain such findings

While numerous values for the intrinsic viscosity of collagen appear in the literature (see summary by Kahn and Witnauer[34]), the absence of values extrapolated to zero rates of shear makes any comparison difficult Davison and Drake[30] quote a value of 10 dl /g for pronase treated monomeric tropocollagen at zero rate of shear, and attribute their higher values for untreated acid-soluble collagen (17–20 dl /g) as resulting from the presence of linear polymers Kahn and Witnauer[34] quote a value of 27 dl /g for acid-soluble collagen at low rates of shear, which would support the theoretical supposition that the collagen dimer is terminally linked with a 10 per cent overlap[35], and would display an intrinsic viscosity of approximately 30 dl /g This evidence would also suggest that acid-soluble collagen is dimeric

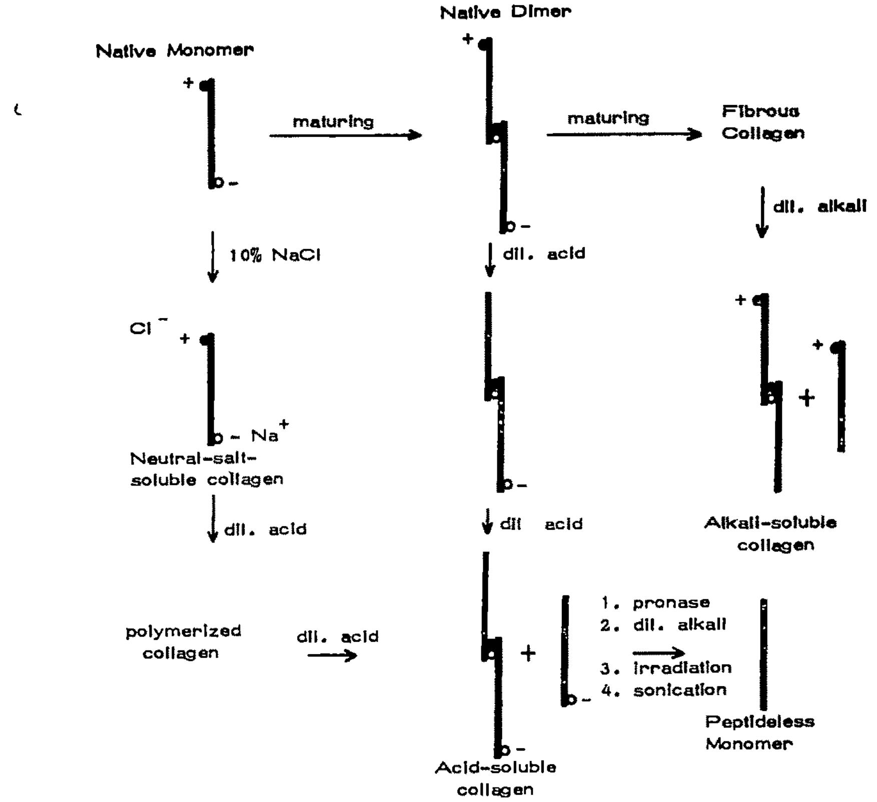

Fig 1 Intermolecular relationships between soluble collagens

When related to the current methods of extracting soluble collagens, much can be explained using this hypothesis First, the preparation of neutral-salt-soluble collagen would initially yield an active monomer which when acidified would yield an insoluble polymer (Fig 1) Subsequent extraction with dilute acetic acid would yield a mixture of soluble inactive monomer and inactive dimer, which on further purification would result in the progressive enrichment of inactive monomeric collagen Second, the preparation of acid-soluble collagen after previous removal of neutral-salt-soluble collagen would result in an initial extract of inactive dimeric collagen which on further purification would be progressively enriched with inactive monomeric collagen In both cases the resultant monomer-dimer content will depend on the number of purification stages carried out, as well as on the *p*H conditions used These conclusions would indicate that native monomeric tropocollagen can only be prepared in a neutral medium of high ionic strength Such a medium would effectively smother any electrostatic interaction of the high active collagen monomer, and so inhibit polymerization (Fig 1)

The term linear polymerization seems to explain the well ordered mechanism of fibre formation from native tropocollagen monomers, while the term aggregation can be applied to those preparations in which the partial or complete destruction of telopeptide side chains has taken place, resulting in an impaired ability of such preparations to form insoluble native fibres

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[1] Harkness R D, Marko, A M, Muir, H M, and Neuberger, A, *Biochem J*, **56**, 558 (1954)
[2] Mazurov, V I, and Orekhovitch V N, *Biochemistry*, **25**, 630 (1960)
[3] Jackson, D S, and Bentley, J P, *J Biophys Biochem Cytol*, **7**, 37 (1960)
[4] Piez, K A, Lewis, M S, Martin, G R, and Gross, J, *Biochim Biophys Acta*, **53**, 596 (1961)
[5] Jackson D S, *Abstract 141st Meeting of the American Chem Soc*, 83 (1962)
[6] Peiz, K A Weiss, E, and Lewis, M S, *J Biol Chem*, **235**, 1987 (1960)
[7] Orekhovitch, V N, Shpikiter, V O, Mazurov, V I, and Kounina, O V, *Bull Soc Chim Biol*, **42**, 505 (1960)
[8] Heidrich, H G, and Wynston, L K, *Hoppe-Seyl Z*, **342**, 166 (1965)
[9] Nanto, V, Maatela, J, and Kulonen, E, *Acta Chem Scand*, **17**, 1604 (1963)
[10] Davidson, R J, and Cooper, D R, *Biochem J* (in the press)
[11] Davidson, R J, and Cooper, D R, *Biochem J* (in the press, 1968)
[12] Schmitt, F O, Levine, L, Drake, M P, Rubin, A L, Pfahl, D, and Davison, P F, *Proc US Nat Acad Sci*, **51**, 493 (1964)
[13] Hodge, A J and Schmitt, F O, *Proc US Nat Acad Sci*, **44**, 418 (1958)
[14] Schmitt, F O, *Proc Amer Phil Soc* **100**, 476 (1956)
[15] Bornstein, P, Kang, A H, and Piez, K A, *Proc US Nat Acad Sci*, **55**, 417 (1966)
[16] Rubin, A L Pfahl D Speakman, P T, Davison, P F, and Schmitt, F O, *Science*, **139**, 37 (1963)
[17] Drake, M P, Davison, P F, Bump, S, and Schmitt, F O, *Biochemistry*, **5**, 301 (1966)
[18] Cooper, D R, and Davidson R J (in the press)
[19] Fessler, J H, *Biochem J*, **76**, 452 (1960)
[20] Fessler, J H, *Biochem J*, **76**, 463 (1960)
[21] Piez, K A, Eigner, E A, and Lewis, M S, *Biochemistry*, **2**, 58 (1963)
[22] Piez, K A, *J Biol Chem*, **239**, C 4315 (1964)
[23] Schleyer, M, *Hoppe-Seyl Z*, **329**, 97 (1962)
[24] Hollmen, T, and Kulonen, E, *Biochim Biophys Acta*, **93**, 655 (1964)
[25] Francois, C, and Glimcher, M J, *Biochim Biophys Acta*, **97**, 366 (1965)
[26] Davidson, R J, and Cooper, D R, *J S African Chem Inst*, **20**, 69 (1967),
[27] Tristram, G R, Worrall, J, and Steer, D C, *Biochem J*, **95**, 350 (1965)
[28] Bornstein, P, Martin, G R, and Piez, K A, *Science*, **144**, 1220 (1964)
[29] Bakerman, S, and Hersh, R T, *Nature*, **201**, 190 (1964)
[30] Davison, P F, and Drake, M P, *Biochemistry*, **5**, 313 (1966)
[31] Chun, E H L, and Doty, P, in *Recent Advances in Gelatin and Glue Research* (edit by Stainsby, G), 261 (Pergamon Press, 1958)
[32] Astrup, H N, Marko, A M, and Young E G in *Recent Advances in Gelatin and Glue Research* (edit by Stainsby, G), 76 (1958)
[33] Doty, P, and Nishihara, T, in *Recent Advances in Gelatin and Glue Research* (edit by Stainsby, G), 92 (1958)
[34] Kahn L D, and Witnauer, L P, *J Biol Chem*, **241**, 1784 (1966)
[35] Hodge, A J, and Petruska, J A, in *Aspects of Protein Structure* (edit by Ramachandran, G N), 289 (Academic Press, New York, 1963)

Proposed Procedure for the Allocation of Trivial Names to the Gibberellins

THE symbols A_1, A_2, A_3 and A_4 were originally used by Takahashi *et al.*[1,2] to denote the first four gibberellins, isolated from the fungus *Gibberella fujikuroi* This convenient trivial nomenclature was continued by MacMillan *et al* [3-5], Cross *et al* [6-8], Hanson[9,10] and Galt[11,12] to include gibberellins A_5 to A_{17} Continuity of the sequence gibberellins A_1 to A_{17} has been maintained, and confusion avoided, for the following two reasons First, gibberellins A_1 to A_4 were named by one group at the University of Tokyo and gibberellins A_5 to A_{17} by a second group, who kept in personal contact when the Akers Research Laboratories, ICI, Ltd, where they worked was closed down. Fortuitously, there was no overlap in time between the numbering of these two groups of gibberellins by these two groups of workers Second, Tamura and his group and Mitsui and his colleagues have avoided possible confusion while waiting for an agreed procedure for the allocation of gibberellin A numbers, by using provisional names for their recently discovered plant gibberellins based on their source, namely, Bamboo[13,14], Pharbitis[15], Canavalia-I and -II[16,17], Lupinus-I[18] and Lupinus-II (personal communication from K Koshimizu and T Mitsui) gibberellins

Several plant physiologists have suggested to us that the present circumstances provide an' opportunity to devise an entirely new system of trivial names, based on structural type We believe that this would result in complete confusion Names based on the original source of isolation have obvious disadvantages, for example, Bamboo gibberellin has been found[19] in seed of *Phaseolus multiflorus* We therefore propose that the gibberellin A_1 A_n system of trivial names be extended to include the Bamboo, Pharbitis, Canavalia and Lupinus gibberellins (see Fig 1) We further propose the adoption of the following procedure for the allocation of gibberellin A numbers to future gibberellins One of us (J MacMillan) will allocate consecutive numbers gibberellin A_{24} A_n on request His task will simply be one of book-keeping and the release of a number will imply no judgement on the correct application of gibberellin nomenclature; the naming of a compound by this gibberellin number would

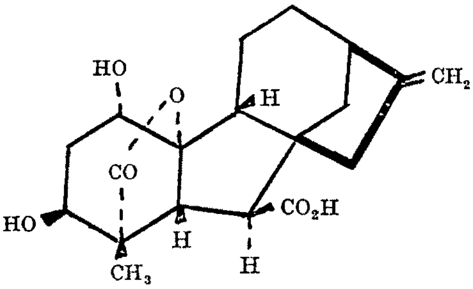
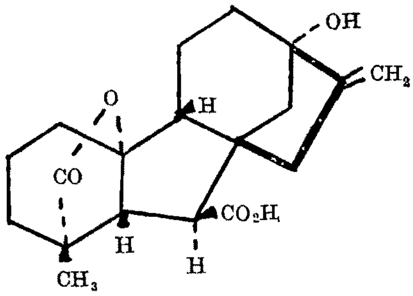
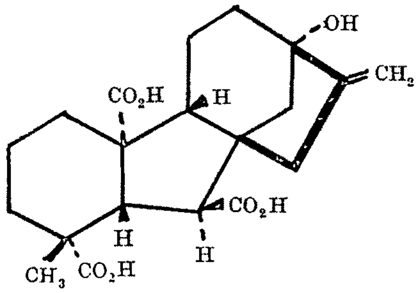
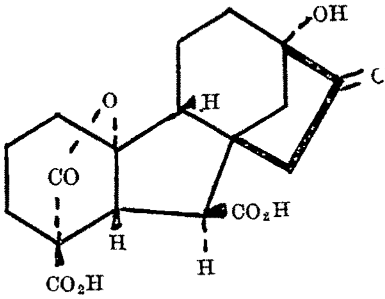
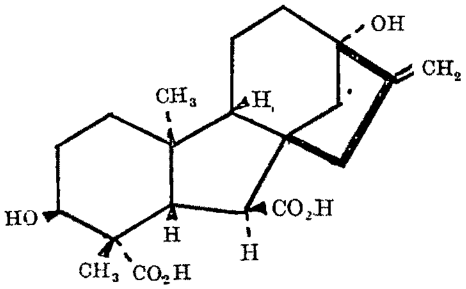
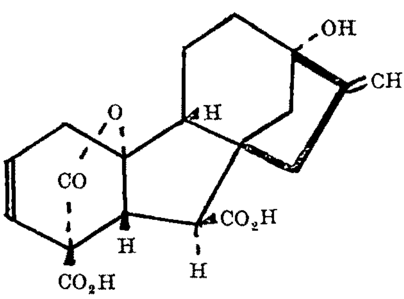
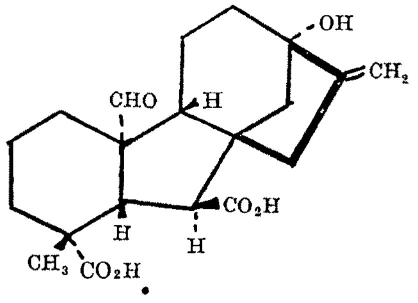
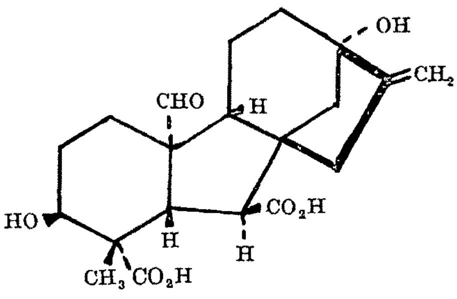
Provisional name	Structure*	Proposed A number	Provisional name	Structure*	Proposed A number
	 HO O CH$_2$ H CO HO CO$_2$H H H CH$_3$	A_{16}†	Pharbitis		A_{20}
	 OH CH$_2$ CO$_2$H H CO$_2$H H H CH$_3$ CO$_2$H	A_{17}	Canavalia-I	 OH CH$_2$ O H CO CO$_2$H H H CO$_2$H	A_{21}
Lupinus-I	 OH CH$_2$ CH$_3$ H HO CO$_2$H H H CH$_3$ CO$_2$H	A_{18}	Canavalia-II	 OH CH$_2$ O H CO CO$_2$H H H CO$_2$H	A_{22}
Bamboo	 OH CH$_2$ CHO H CO$_2$H H H CH$_3$ CO$_2$H	A_{19}	Lupinus-II	 OH CH$_2$ CHO H HO CO$_2$H H H CH$_3$ CO$_2$H	A_{23}

Fig 1 * The depicted absolute stereochemistry is mainly by analogy, except for A_{20} † Isolated as the methyl ester only remain the sole responsibility of the investigator(s). Following precedent, these *A* numbers should be used only for naturally occurring, fully characterized compounds which possess the gibbane skeleton and the appropriate biological properties; *A* numbers need not be used for naturally occurring derivatives of previously described gibberellins, for example, 2-O-acetyl gibberellin A_3 (ref. 20). To help determine whether a suspected new gibberellin is distinct from those previously described, as complete a collection as possible of infrared and mass spectra of the known gibberellins will be held by us both.

These proposals have been discussed with, and approved by, other workers in the field. We hope that they will be generally adopted.

It should be noted that the gibberellin *A* numbers do not necessarily follow the order of discovery. For example, gibberellin A_3 was the first characterized gibberellin and, indeed, the name gibberellic acid takes precedence over gibberellin A_3 although the latter name is now more convenient. Also, gibberellin A_{17} was isolated after gibberellins A_{18} to A_{22}.

A complete list of the gibberellins known at present and their structures is provided by our Fig. 1 and Fig. 1 of Cavell *et al.*[21].

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[1] Takahashi, N., Kitamura, H., Karawada, A., Seta, Y., Takai, M., Tamura, S., and Sumiki, Y., *Bull. Agric. Chem. Soc., Japan*, **19**, 267 (1955).

[2] Takahashi, N., Seta, Y., Kitamura, H., and Sumiki, Y., *Bull. Agric. Chem. Soc. Japan*, **21**, 396 (1957).

[3] MacMillan, J., Seaton, J. C., and Suter, P. J., *Tetrahedron*, **11**, 60 (1960).

[4] MacMillan, J., Seaton, J. C., and Suter, P. J., *Tetrahedron*, **18**, 349 (1962).

[5] Pryce, R. J., and MacMillan, J., *Tetrahedron Lett.*, 4173 (1967).

[6] Cross, B. E., Galt, R. H. B., and Hanson, J. R., *Tetrahedron*, **18**, 451 (1962).

[7] Cross, B. E., and Norton, K., *J. Chem. Soc.*, 1570 (1965).

[8] Cross, B. E., *J. Chem. Soc. (C)*, 501 (1966).

[9] Hanson, J. R., *Tetrahedron*, **22**, 701 (1966).

[10] Hanson, J. R., *Tetrahedron*, **23**, 733 (1967).

[11] Galt, R. H. B., *J. Chem. Soc.*, 3143 (1965)

[12] Galt, R. H. B., *Tetrahedron* (in the press).

[13] Tamura, S., Takahashi, N., Murofushi, N., Iriuchijima, S., Kato, J., Wada, Y., Watanabe, E., and Aoyama, T., *Tetrahedron Lett.*, **75**, 279 (1966).

[14] Murofushi, N., Iriuchijima, S., Takahashi, N., Tamura, S., Kato, J., Wada, Y., Watanabe, E., and Aoyama, T., *Agric. Biol. Chem.*, **30**, 917 (1966).

[15] Takahashi, N., Murofushi, N., Yokota, T., and Tamura, S., *Tetrahedron Lett.*, 1065 (1967).

[16] Tamura, S., Takahashi, N., Murofushi, N., Yokota, T., Kato, J., and Shiotani, Y., *Planta*, **75**, 279 (1967).

[17] Takahashi, N., Murofushi, N., Yokota, T., Tamura, S., Kato, J., and Shiotani, Y., *Tetrahedron Lett.*, 4861 (1967).

[18] Koshimizu, K., Fukui, H., Kusaki, T., Mitsui, T., and Ogawa, Y., *Tetrahedron Lett.*, 2459 (1966).

[19] Pryce, R. J., MacMillan, J., and McCormick, A., *Tetrahedron Lett.*, 5009 (1967).

[20] Schreiber, K., Schneider, G., Sembdner, G., and Focke, I., *Phytochemistry*, **6**, 867 (1967).

[21] Cavell, B. D., MacMillan, J., Pryce, R. J., and Sheppard, A. C., *Phytochemistry*, **6**, 867 (1967).

PATHOLOGY

Segmental Demyelination of Peripheral Nerves in Krabbe's Disease

The current literature contains many references to peripheral neuropathies in a variety of conditions[1-4]. This communication describes a peripheral neuropathy associated with Krabbe's globoid cell leucodystrophy which is a progressive heredofamilial neurological disorder of infancy, and is usually diagnosed by either brain biopsy or at necropsy by the demonstration of characteristic "globoid" cells in the white matter. Bachhawat *et al.*[5] have described a deficiency of cerebroside sulphotransferase in the brain of two cases. Clinical descriptions and details of investigations of a series of patients suffering from this rare condition are in preparation.

Peripheral nerves were obtained at biopsy or necropsy in four cases of Krabbe's leucodystrophy aged between 7 months and 21 months. Portions were "snap" frozen or fixed in buffered formalin. Part of one biopsy was processed for electron microscopy. Paraffin sections were stained with haematoxylin and eosin, periodic acid–Schiff (PAS), haematoxylin and van Gieson and Luxol fast blue. Acid phosphatase was demonstrated in cryostat sections of unfixed nerve[6]. Cryostat sections were also stained with toluidine blue, cresyl fast violet, Sudan black, PAS and Luxol fast blue. Nerves from control cases were processed similarly. Nerves fixed in formalin were partially teased and stained with Sudan black in propylene glycol before final teasing from 30 per cent glycerol.

In the paraffin sections, the nerves showed considerably less myelin than the controls, and there was evidence of myelin breakdown. Cryostat sections showed excessive acid phosphatase activity in the Schwann cell cytoplasm (Fig. 1), but no metachromasia could be demonstrated. The acid phosphatase activity was present in fine granular form and at the periphery of vacuoles. Teased nerves showed segmental demyelination (Fig. 2). Some remyelination was also evident.

Electron microscopy provided evidence of an active process in the nerve. Many fibres had large vacuoles containing myelin debris within the Schwann cell cyto-

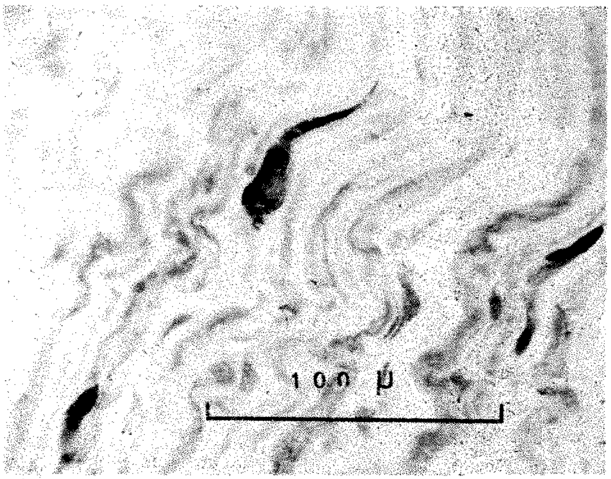

Fig. 1. Sural nerve cryostat section. Acid phosphatase activity in the cytoplasm of Schwann cells.

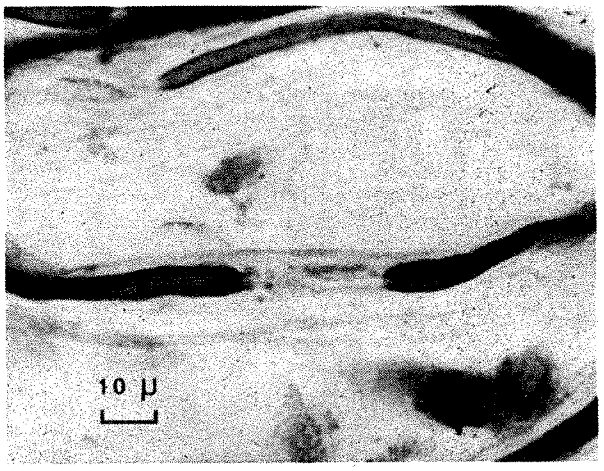

Fig. 2. Teased nerve stained with Sudan black showing a demyelinated segment.

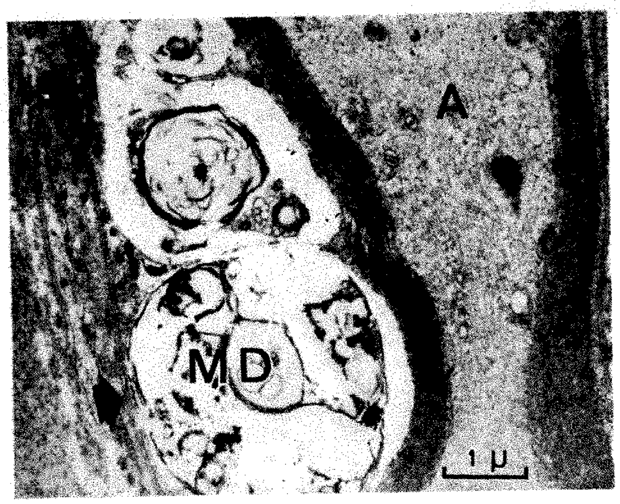

Fig. 3. Sural nerve. Large vacuoles containing myelin debris (*MD*) adjacent to myelin sheath. Axon (*A*) appears normal. Arrows indicate boundary of Schwann cell.

plasm (Fig. 3). Other fibres appeared to be remyelinating and occasional fibres showed the changes of Wallerian degeneration.

The pathological changes associated with Krabbe's globoid cell leucodystrophy have hitherto been thought to be confined to the brain, and previous workers have found no abnormalities in peripheral nerves[7,8]. Allen and de Veyra[9], however, have recently shown in one case "minimal peripheral nerve abnormalities" which they considered to be a late expression of a maximally developed disease. The results obtained in each of our cases show that the nerves of patients with Krabbe's disease are poorly myelinated and that there is segmental demyelination. A deficiency of cerebroside sulphotransferase in Krabbe's disease could explain the findings in the peripheral nerves. The lack of this enzyme would prevent the formation of cerebroside sulphate (sulphatide), an essential component of myelin, and thus the myelin formed would be unstable. It is suggested that in suspected cases of Krabbe's leucodystrophy nerve biopsy may be helpful in establishing the diagnosis, particularly if this is done in conjunction with studies of nerve conduction time, which in each of our cases was found to be markedly retarded.

Metachromatic leucodystrophy from which this disorder must be distinguished can be recognized by deposits of sulphatide staining metachromatically with cresyl fast violet in peripheral nerve and by the demonstration of metachromatically staining cells in the urine[10]. Urinary aryl sulphatase activity is also reduced in this condition[11].

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[1] Dayan, A. D., and Williams, R., *Lancet*, ii, 133 (1967).

[2] Kocen, R. S., Lloyd, J. K., Lascelles, P. T., Fosbrooke, A. S., and Williams, D., *Lancet*, i, 1341 (1967).

[3] Kasef, R., and Das Gupta, T. K., *Brit. J. Cancer*, **21**, 411 (1967).

[4] Dayan, A. D., *J. Neurol. Neurosurg. Psychiat.*, **30**, 311 (1967).

[5] Bachhawat, B. K., Austin, J., and Armstrong, D., *Biochem. J.*, **104**, 15c (1967).

[6] Lake, B. D., *J. Roy. Microsc. Soc.*, **85**, 73 (1966).

[7] Hagberg, B., Sourander, P., and Svennerholm, L., *J. Neurol. Neurosurg. Psychiat.*, **26**, 195 (1963).

[8] D'Agostino, A. N., Sayre, G. P., and Hayles, A. B., *Arch. Neurol.*, **8**, 82 (1963).

[9] Allen, N., and de Veyra, E., *J. Neuropath. Exp. Neurol.*, **26**, 456 (1967).

[10] Lake, B. D., *Arch. Dis. Child.*, **40**, 284 (1965).

[11] Austin, J., McAfee, D., Armstrong, D., O'Rourke, M., Shearer, L., and Bachhawat, B. K., *Biochem. J.*, **93**, 15c (1964).

IMMUNOLOGY

Ability of Leucocytes to recognize some Foreign Gamma Globulins during Pinocytosis

We have previously demonstrated that pinocytosis of autologous immunoglobulins (Igs) altered by physical (heating at 80° C, X, γ or β-rays) or chemical agents ($(NO_3)_2Hg$; $(NO_3)_2Pb$) is significantly increased as compared with unaltered Igs[1,2]. We have now extended our investigations by studying the pinocytosis of unaltered Igs from various species. It is already known that Igs markedly increase pinocytosis by various types of cells[4-8].

Pinocytosis of Igs was investigated by fluorescence microscopy of a mixture of 10^6 guinea-pig peritoneal exudate cells and 6·6 mg of Igs/ml., incubated for 30 min at 37° C. The guinea-pig peritoneal exudate was obtained after two intraperitoneal injections of polyvinylpyrrolidone and contained about 90 per cent of polymorphonuclear leucocytes. Autologous, homologous, human, chicken, tortoise and carp Igs were prepared by chromatography on DEAE 'Sephadex' and labelled with fluorescein isothiocyanate[9]. The pinocytosis index was expressed as the percentage of leucocytes showing fluorescent pinocytic vacuoles[10] (Table 1).

Table 1. INDEX OF PINOCYTOSIS OF VARIOUS GAMMA GLOBULINS BY GUINEA-PIG PERITONEAL EXUDATE LEUCOCYTES

Gamma globulin species	No. of experiments performed	$\bar{x}_e \pm \sigma$	$\bar{x}_p$ *
Autologous	24	15·5 ± 1·1	$14{\cdot}66 < \bar{x}_p < 16{\cdot}34$
Homologous	24	31·9 ± 3·1	$30{\cdot}22 < \bar{x}_p < 35{\cdot}58$
Human Igs	30	45·4 ± 2·7	$44{\cdot}03 < \bar{x}_p < 46{\cdot}77$
Chicken	7	40·51 ± 4·95	—
Tortoise	5	44·42 ± 8·2	—
Carp	7	55 ± 6·03	—

The significance of the results was estimated by the χ^2 test and Student's *t* test. The index of pinocytosis differed for each kind of Igs (autologous, human, reptile, fowl), although differences were not significant from one species to another ($P > 0{\cdot}05$). Fish Igs show a maximum index, significantly different from the autologous and homologous ($P < 0{\cdot}01$), but only doubtfully significant against tortoise Igs ($0{\cdot}01 < P < 0{\cdot}05$).

* The mean population was calculated using the Student parameter (*t*), for a probability of 0·005.

As shown in Table 1, the pinocytosis index for autologous Igs was significantly less than that observed for homologous Igs ($P < 0{\cdot}01$) and this latter was significantly less than the index observed for heterologous Igs ($P < 0{\cdot}01$).

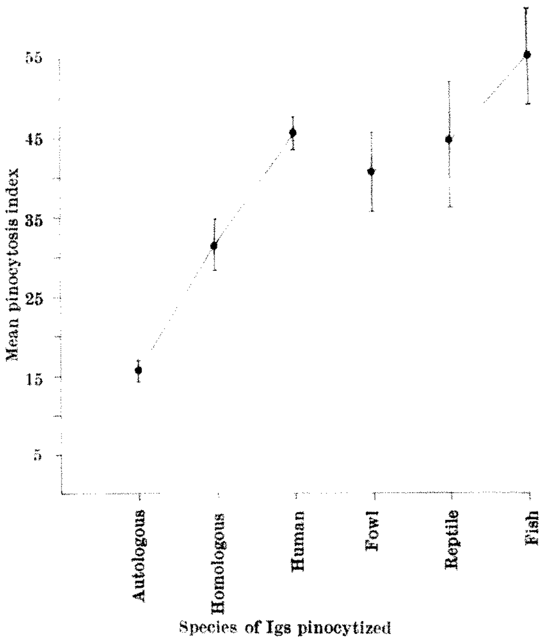

Fig. 1

Our experiments show that polymorphonuclear leucocytes can discriminate between autologous and foreign or autologous immunoglobulins denatured in various ways[1,2].

The mechanism by which discrimination occurs is unknown. Many authors considered that discrimination of foreign or altered matter at the leucocytic level is mediated by opsonins or natural antibodies[11-17]. It seems very likely that the immunological mechanisms of recognition implied in the immune response were evolved from the cruder recognition process which also seems to be involved in our experiments.

As shown in Fig. 1, the pinocytosis index for the heterologous Igs is not obviously related to the phylogenetic distance.

The absence of correlation between the kind of radiations (X, γ, β) and the levels of the pinocytosis index (of irradiated autologous Igs) reported previously[2] also implies a more limited ability of polymorphonuclears to discriminate subtle differences.

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Received December 4, 1967.

[1] Bona, C., Sulică, A., Dumitrescu, M., and Vranialici, D., *Nature*, **213**, 824 (1967).

Gavrilescu, N., Ghyka, G., Sulică, A., and Bona, C., *First Nat. Cong. Genetics*, Bucharest (1967).

[3] Perkins, E. H., and Leonard, M. R., *J. Immunol.*, **90**, 238 (1963).

[4] Holter, H., and Marshal, jun., J. M., *C.R. Trav. Lab. Carlsberg*, **23**, 7 (1954).

[5] Chapman-Andresen, Cecily, *Exp. Cell Res.*, **12**, 397 (1957).

[6] Holter, H., and Holtzer, H., *Exp. Cell Res.*, **18**, 421 (1959).

[7] Bona, C., and Macovschi, O., *Arch. Roum. Pathol. Exp. Microbiol.*, **26**, 730 (1967).

[8] Cohn, Z., and Parks, A., *J. Exp. Med.*, **125**, 1091 (1967).

[9] Mesrobeanu, I., Bona, C., and Mesrobeanu, L., *Exp. Cell Res.*, **36**, 434 (1964).

[10] Mesrobeanu, I., Bona, C., Vranialici, D., Ioanid, L., and Mesrobeanu, L., *Z. für Immunitforsch.*, **133**, 108 (1967).

[11] Ada, G. L., Nossal, G. J. V., and Austin, C. M., *J. Exp. Biol. Med. Sci.*, **42**, 331 (1964).

[12] Nossal, G. J. V., and Ada, G. L., *Nature*, **201**, 580 (1964).

[13] Nossal, G. J. V., *Ann. NY Acad. Sci.*, **124**, 37 (1965).

[14] Boyden, S. V., *J. Theor. Biol.*, **3**, 123 (1962).

[15] Boyden, S. V., in *Advances in Immunology* (edit. by Dixon, P. J., and Humphrey, J. H.). **6**, 1 (Academic Press, New York, London, 1966).

[16] Humphrey, J. H., and Frank, M. M., *Immunology*, **13**, 87 (1967).

[17] Humphrey, J. H., Askonas. B. A., Auzins, Schechter, I., and Sela, M., *Immunology*, **13**, 71 (1967).

Location of Gamma Globulin in the Endocardium in Rheumatic Heart Disease by the Ferritin-labelled Antibody Technique

Gamma globulin can be demonstrated by the fluorescent antibody technique in some biopsy specimens of the left atrial appendage from patients with rheumatic heart disease and also *post mortem* in the heart in cases of acute rheumatic fever[1-5]. The distribution is focal and usually bears no direct relation to cellular rheumatic lesions, although gamma globulin can occasionally be demonstrated in the latter. We have used antibody labelled with ferritin to study the location of gamma globulin in relation to individual components of the endocardium and subendocardium.

Cryostat sections of fresh specimens of left atrial appendage from cases of mitral valvotomy were stained for gamma globulin by the fluorescein-labelled antibody technique (rabbit anti-human gamma globulin antiserum followed by goat anti-rabbit gamma globulin antiserum labelled with fluorescein).

For electron microscopy goat anti-rabbit and rabbit anti-human gamma globulin antisera were labelled with ferritin by the method of Singer[6], with various modifications. These preparations were kindly provided by Professor P. H. Gell. Cryostat sections (60μ thick) of four specimens positive for gamma globulin and four negative specimens were treated with antibody labelled with ferritin either by the double layering or the direct technique similar to the fluorescein-labelled antibody method. After washing for at least 30 min, sections were fixed in Caulfield's buffered osmium tetroxide, and the endocardium was embedded in 'Araldite' and methacrylate. Some sections were treated with phosphotungstic acid during dehydration.

As further controls, one specimen from a case of calcific aortic stenosis negative for gamma globulin by the fluorescein technique was similarly treated, and sections from all specimens were treated with ferritin solution diluted to a colour resembling that of the labelled antisera.

With fluorescent microscopy irregular areas of the endocardium and subendocardium showed specific fluorescence. Only one specimen contained small numbers of cellular lesions in the subendocardium and in some of these there was focal fluorescence. In most areas, however, the gamma globulin bore no relation to cellular lesions and was present in specimens without such lesions.

The tissue had suffered considerable distortion by freezing and autolysis, but most structures were readily identifiable on the electron microscope.

In the control sections, stained with diluted ferritin solution, a small amount of ferritin was seen chiefly in relation to collagen and sometimes in the cement substance between collagen fibrils. A few groups of particles were present in the interstitial spaces, and occasionally were found attached to fibroblasts, macrophages and smooth muscle cells. A similar appearance was noted in the negative specimens treated with labelled antisera and in the control specimens of the positive cases.

In contrast to this, sections containing gamma globulin showed large amounts of ferritin in the endocardium and subendocardium, but in some zones it was almost entirely absent. The ferritin was heavily concentrated in relation to collagen fibrils and when these occurred separately particles were closely applied to the fibrils. With large collagen fibres ferritin was located on and between fibrils and in cross-section could be seen in the cement substance, either closely applied to the fibril or separated from it by a narrow zone. Sometimes small groups of ferritin were seen in the interstitial spaces between collagen fibres. In some of the fibrils with ferritin attached no periodicity could be recognized. Small areas of granular amorphous material were heavily studded with ferritin particles.

The elastic tissue showed only occasional ferritin particles on the surface and the cells of the endocardium showed no more ferritin than in the controls. Only one specimen contained cellular lesions and we failed to locate any in the embedded sections.

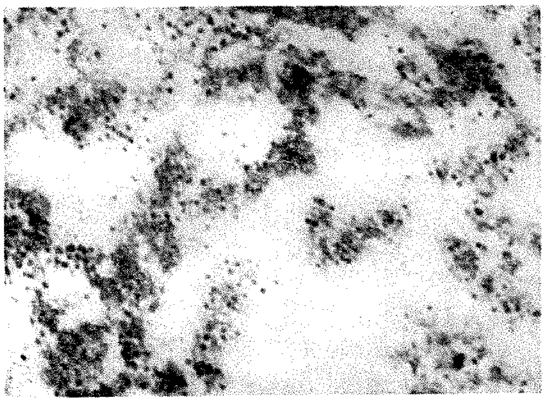

Fig. 1. Collagen fibrils and granular material with ferritin attached. (× 180,000.)

The specificity of the ferritin labelling technique is not very high because of the tendency of ferritin to stick to tissues, and attempts to improve it—for example, by removal of free ferritin—have been disappointing. In our material, the ferritin-labelled antibody was obviously not entirely specific, but the difference between the controls and the positive specimens was so marked that certain tentative conclusions are possible. The chief difference was quantitative and the effect could be recognized with the naked eye after staining by the faint yellowish colour of the sections positive for gamma globulin. The heaviest concentrations of ferritin were in relation to collagen fibrils, some of which showed loss of periodicity, granular material and the cement substance of collagen fibres. We have previously described granular material and fibrils without periodicity within and around cellular rheumatic lesions[7,8]. The possibility that ferritin was in the same situation as acid mucopolysaccharide was considered, but this was not thought to be the case because the distribution of acid mucopolysaccharide, as demonstrated by the colloidal iron method, is principally around fibres and very little is seen within a fibre between the fibrils. In addition, when the dialysed iron method was applied after staining with labelled antibody the distribution of both could be seen on the electron microscope, and although the two sometimes occurred in the same area, the heavy concentration of colloidal iron on the surfaces of fibres contrasted with the heavy concentration of ferritin in relation to collagen fibrils.

In spite of the difficulties of sampling and of non-specific "staining" we believe the differences are great enough between the controls and the test specimens to allow us to conclude that gamma globulin in rheumatic tissue is closely related to collagen fibrils or to the cement substance rather than to the ground substance between fibres.

In previous studies[7,9] we have shown that collagen itself and probably the cement substance take part in the formation of "fibrinoid" in rheumatic lesions. In this connexion the observation of Goldstein *et al.*[10] that a glycoprotein in heart valves cross reacts with streptococcus *A* polysaccharide may be important.

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[1] Vasquey, J. J., and Dixon, F. J., *Lab. Invest.*, **6**, 205 (1957).
[2] Vasquey, J. J., and Dixon, F. J., *Arch. Path.*, **66**, 504 (1958).
[3] Kaplan, M. H., *Ann. NY Acad. Sci.*, **86**, 974 (1960).
[4] Kaplan, M. H., and Dallenbach, F. D., *J. Exp. Med.*, **113**, 1 (1961).
[5] Wagner, B. M., *Ann. NY Acad. Sci.*, **86**, 992 (1960).
[6] Singer, S. J., *Nature*, **183**, 1523 (1959).
[7] Lannigan, R., and Zaki, S. A., *J. Path. Bact.*, **93**, 449 (1967).
[8] Lannigan, R., and Zaki, S. A., *Nature*, **198**, 898 (1963).
[9] Lannigan, R., and Zaki, S. A., *Nature*, **206**, 106 (1965).
[10] Goldstein, I., Halpern, B., and Robert, L., *Nature*, **213**, 44 (1967).

Gamma G4-Globulin Antibody causing Inhibition of Clotting Factor VIII

HAEMOPHILIA results from the absence of clotting factor VIII. The presence of a circulating inhibitor to factor VIII causes a disease which resembles haemophilia. Five patients with inhibitors shown to be γG-antibodies have been reported[1-3].

γG-antibodies can be separated into four subclasses (γG1, γG2, γG3 and γG4) and two types (κ and λ) based on antigenic differences in the heavy and light polypeptide chains, respectively[4]. In this report, the antibody to factor VIII found in one patient is of the γG4 sub-class with κ-type light chains. This is the first demonstration of antibody activity in the γG4 sub-class and the first determination of heavy chain sub-class characteristics of factor VIII inhibitors.

Previous studies of patient G. K.[2] had shown that decreased factor VIII activity was the only clotting deficiency and was caused by a γG-antibody which inhibits factor VIII. In this study identification of heavy chain subclass and light chain type was accomplished by immunoglobulin depletion. Antisera were directed against κ or λ light chains and either γG1, γG2 and γG3, referred to as anti-γG123, or γG4. Anti-γG123 was prepared by injecting Rhesus monkeys with pooled normal γG in complete Freund's adjuvant. The antiserum was absorbed with isolated γG4-myeloma protein and light polypeptide chains from pooled normal human γG. Immunoelectrophoretic and Ouchterlony tests showed that it precipitated only γG1, γG2 and γG3 sub-classes. Anti-γG4 was prepared by immunizing Rhesus monkeys with γG4-myeloma protein. The antiserum was absorbed with light chains, γG2-myeloma protein and hypogammaglobulinemic serum. The resulting antiserum precipitated only γG4-globulins.

Antisera to κ and λ-chains were prepared by immunizing rabbits with κ or λ-Bence Jones proteins in complete Freund's adjuvant. The antisera were absorbed to render them specific for either κ or λ-chains. Difficulties encountered in the clotting assay when whole rabbit antiserum was used were circumvented by precipitating the antiserum with 40 per cent saturated $(NH_4)_2SO_4$ and fractionating precipitates on DEAE-cellulose. Fractions showing antibody activity were used. All antisera were centrifuged to remove lipid and adsorbed with $BaSO_4$ to minimize any direct effect on the clotting time.

Immunoglobulin depletion studies were carried out with inhibitor-rich fractions eluted from a starch block electrophoresis of serum G. K. Aliquots of antiserum were added to the inhibitor fraction, incubated for 1 h at 37° C, 16 h at 4° C, and centrifuged to remove precipitates. Antiserum was added until there was no further precipitation. Preer and Ouchterlony methods confirmed the completeness of absorption.

The inhibitor was assayed by mixing the test sample with normal plasma diluted 1 : 7·5, incubating for 30 min at 37° C, and performing a one stage factor VIII assay[2,5]. Prolongation of clotting time beyond the normal plasma control indicated loss of factor VIII activity caused by the inhibitor. Normal plasma gave an average clotting time of 78 sec (Table 1). When the inhibitor fraction of serum G. K. was added to normal plasma, the time increased to 112 sec. The inhibitor fraction was depleted of either γG1, γG2, and γG3 or γG4 and re-evaluated for inhibitory activity. Complete loss of inhibitory activity followed depletion of γG4-globulins (Table 1). The clotting time of normal plasma plus inhibitor fraction depleted of γG1, γG2 and γG3 remained at 112 sec, indicating no loss of inhibitory activity (Table 1). The clotting time of normal plasma was unaffected by anti-γG4 serum but it was shortened with anti-γG123. This

Table 1. EFFECTS OF IMMUNOGLOBULIN ABSORPTION ON INHIBITORY ACTIVITY

	One stage factor VIII assay: clotting time (sec)		No. of determinations
	Average	Range	
Normal plasma	78	77–80	8
Normal plasma + inhibitor fraction	112	108–115	8
Normal plasma + inhibitor fraction absorbed with anti-γG123	112	110–114	6
Normal plasma + inhibitor fraction absorbed with anti-γG4	80	79–82	12
Normal plasma + anti-γG123	68	68–69	2
Normal plasma + anti-γG4	77	76–78	2
Haemophilic plasma	115	—	1
Haemophilic plasma + anti-γG123	115	—	1
Haemophilic plasma + anti-γG4	116	—	1

Normal or haemophilic plasma was incubated with the test sample for 30 min at 37° C. A one stage factor VIII assay was performed. In solutions containing normal plasma plus test sample, clotting times longer than that for normal plasma alone indicate the presence of a factor VIII inhibitor in the test sample. In solutions containing haemophilic plasma plus test sample, clotting times shorter than haemophilic plasma alone indicate the presence of factor VIII in the test sample.

effect was not caused by factor VIII in the antiserum, for it did not decrease the clotting time of haemophilic (factor VIII deficient) plasma (Table 1). The shortening of clotting time caused by anti-γG123 is unexplained.

To evaluate further the possibility that the inhibition of factor VIII is caused by a non-specific effect of elevated γG4-globulins, an inhibitor assay was performed on serum from another patient containing more than 30 mg/ml. of γG4-myeloma globulin. No factor VIII inhibition was found. Other evidence that inhibition was the result of a true antigen–antibody reaction was obtained from a previous experiment[2] which showed that pepsin digestion, known to destroy the Fc piece of γG-globulins and leave intact the antibody combining site, failed to reduce inhibitory activity.

Similar depletion experiments were carried out using rabbit antisera to κ and λ determinants. Anti-κ serum removed some of the anti-factor VIII activity, while anti-λ had no effect (Table 2). It was impossible to remove all anti-factor VIII activity with anti-κ antiserum. This was probably not caused by λ-type anti-factor VIII, for absorption with anti-λ did not remove any activity. Anti-κ serum may have produced soluble complexes with κ-type anti-factor VIII which retained inhibitory activity.

Table 2. EFFECTS OF ABSORPTION WITH ANTI-κ AND ANTI-λ SERUM

	One stage factor VIII assay: clotting time (sec) Average	Range	No. of determinations
Normal plasma	75	74–75	4
Normal plasma + inhibitor fraction	96	94–99	4
Normal plasma + inhibitor fraction absorbed with anti-κ	84	83–84	4
Normal plasma + inhibitor fraction absorbed with anti-λ	95	93–97	4
Normal plasma + anti-κ	75	74–76	4
Normal plasma + anti-λ	70	—	4

The factor VIII inhibitor in serum G. K. is therefore an antibody of the γG4 sub-class with κ-type light chains. This is consistent with previous observations of physical and chemical characteristics of the inhibitor[2]. A 7*S* sedimentation coefficient, resistance to reduction and alkylation, and decrease in sedimentation coefficient to 3·9*S* with pepsin–cysteine treatment are characteristics of γG-antibodies. Electrophoretic mobility of the inhibitor was faster than the bulk of γG-globulins. γG4-globulins from normal serum and γG4-myeloma are known to have electrophoretic mobilities similar to this inhibitor[6].

Previous investigations have demonstrated antibody activity in the γG1, γG2 and γG3 sub-classes[7]. γG4 sub-class is present in a low concentration in normal serum and has not previously been shown to have antibody activity. The present studies clearly show that γG4 can also possess antibody activity.

Earlier studies[2] failed to demonstrate precipitin reactions, complement fixation and passive cutaneous anaphylaxis. This may have been because there were insufficient concentrations of antigen (factor VIII) or antibody (inhibitor). It is also possible that γG4-antibodies are unable to participate in some or all of these reactions. Studies using γG4-myeloma proteins show that they can sensitize guinea-pig skin for reverse passive cutaneous anaphylaxis[8], but aggregated γG4 in contrast to the other sub-classes does not fix complement[9]. Presumably, γG4-antibodies lack the capacity to fix complement. More observations will be necessary to clarify the biological activities of γG4-antibodies.

Four reported cases have had only γG class inhibitors[1,2] and a fifth was restricted to γA-globulin[10]. Only Poulik's patient[3] had factor VIII inhibitors of mixed classes, γG and γM. In the four cases studied[1,3] only inhibitors with κ-type light chains could be found. In the present study the antibody was not only of one immunoglobulin class, γG, but contained only γG4-heavy chains and κ-type light chains. A similar tendency toward antigenic homogeneity has been observed in cold agglutinating antibodies[11] and erythrocyte auto-antibodies[12]. It has yet to be determined whether the antigen or the nature of the antibody-producing mechanism is responsible for the relative homogeneity of antibodies in these presumably auto-immune processes.

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[1] Shapiro, S., *J. Clin. Invest.*, **46**, 147 (1967).

[2] Andersen, B. R., and Troup, S. B., *J. Immunol.* (in the press, 1968).

[3] Poulik, M. D., and Lusher, J., *Fed. Proc.*, **26**, 312 (1967).

[4] *Bull. WHO*, **35**, 953 (1966).

[5] Margolis, J., *J. Clin. Pathol.*, **11**, 406 (1958).

[6] Grey, H., and Kunkel, H., *J. Exp. Med.*, **120**, 253 (1964).

[7] Terry, W. D., and Fahey, J. L., *Science*, **146**, 400 (1964).

[8] Terry, W. D., *J. Immunol.*, **95**, 1041 (1965).

[9] Ishizaka, T., Ishizaka, K., Salmon, S., and Fudenberg, H., *J. Immunol.*, **99**, 82 (1967).

[10] Glueck, H., and Hong, R., *J. Clin. Invest.*, **44**, 1866 (1965).

[11] Harboe, H., van Furth, R., Schubothe, H., Lind, K., and Evans, S., *Scand. J. Haematol.*, **2**, 259 (1965).

[12] Leddy, J., and Bakemeier, R., *J. Exp. Med.*, **121**, 1 (1965).

Enhancement of Skin Homografts and Dissociation of Immune Responses in Mice immunized with Lyophilized Tissues in Freund's Adjuvant

ATTEMPTS to achieve enhancement of skin homografts in actively or passively immunized animals have so far been unsuccessful, in contrast to the results of experiments with tumour homografts[1-7]. On the other hand, delayed sensitivity can be relatively easily inhibited in adult animals immunized with organ-specific tissue or some defined antigens in saline, or incomplete adjuvant, before sensitization with the same antigens in complete Freund's adjuvant[8-12]. The mechanism of this specific inhibition, which has been called immune deviation[11], and its relation to enhancement or to partial tolerance[12-15], have not been fully elucidated.

Using various schemes of pre-immunization, I have attempted to determine whether transplantation immunity was also effectively inhibited, as judged by the survival of skin homografts exchanged between mice of inbred strains differing at the *H*-2 locus. The results of experiments on *CBA/Jax* mice, immunized with lyophilized spleen cells from *A* strain mice, showed that different degrees of skin homograft enhancement could be achieved according to the dose of lyophilized tissue and the mode and time of its administration. The mode of immunization seemed to be most important in this respect, because a comparison of several experiments (one is illustrated in Fig. 1) revealed that the injection of antigen in incomplete or complete Freund's adjuvant resulted in a longer survival of homografts than immunization with the same or a larger dose of the same antigen in saline. In my experiments, immunization with antigen in incomplete adjuvant was most frequently used because repeated immunization

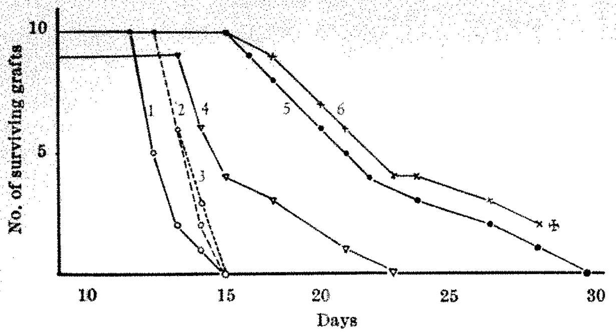

Fig. 1. Dependence on the mode of immunization. All injections were given intraperitoneally. Skin was grafted 6 days after the second injection. 1, Grafts only; $M = 11 \cdot 5$ (11–13). (M . . . Median survival time and the confidence limits with the confidence coefficient greater than 95 per cent.) 2, Incomplete adjuvant three times at intervals of 8 days; $M = 13$ (12–14). 3, Complete adjuvant three times at intervals of 8 days; $M = 13$ (12–14). 4, 6 × 10 mg of lyophilized tissue in saline at intervals of 4 days; $M = 14$ (13–19). 5, 3 × 10 mg of lyophilized tissue in incomplete adjuvant at intervals of 8 days; $M = 19 \cdot 5$ (16–27). 6, 3 × 10 mg of lyophilized tissue in complete adjuvant at intervals of 8 days; $M = 19 \cdot 5$ (16–27).

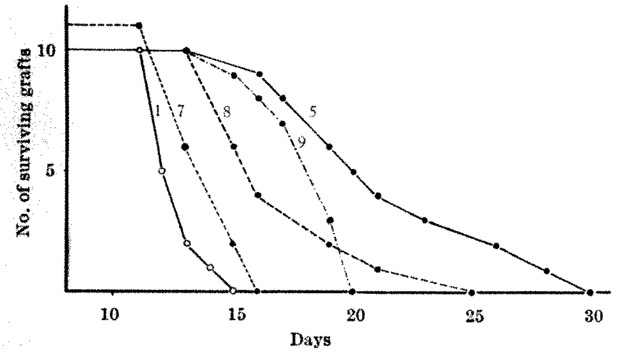

Fig. 2. Dependence on the time of skin grafting. All experimental groups were injected with 3 × 10 mg of lyophilized tissue in incomplete adjuvant at intervals of 8 days. All injections were given intraperitoneally. Skin grafted 6 days after the second injection. 7, Skin grafting 24 h after the first dose; $M = 14$ (12–15). 8, Skin grafting 6 days after the first dose; $M = 15$ (13–20). 9, Skin grafting 6 days after the third dose; $M = 17 \cdot 5$ (15–19).

in complete Freund's adjuvant was accompanied by relatively high mortality.

Most of the *A* strain skin grafts which survived for a prolonged period in experimental mice were completely or partially covered with hair, and their delayed rejection was of a chronic nature, so that they survived for some time with an increasing number of ulcerations. Such grafts were evaluated as positive as long as their epithelium could be distinguished. The ability to reject these grafts was not accompanied by the acquisition of transplantation immunity, because in all nineteen cases where second grafts were applied 14 days after complete rejection of the first grafts, the grafts survived as long as normal first set ones.

Figs. 2 and 3 show that apart from the mode of immunization two additional factors—the time schedule of immunization and dose of antigen—are of decisive importance for the prolongation of skin graft survival. When *CBA/Jax* mice were immunized repeatedly with three doses of lyophilizate from *A* strain spleens (10 mg each) at 8 day intervals before grafting, the prolongation of homograft survival was less pronounced than when skin grafts from *A* strain mice were applied after a second immunization and the third immunizing dose was injected after grafting (Fig. 3). Immunization with only one dose before grafting had a less pronounced effect, and, if the first immunizing dose was given as late as 24 h before grafting, the result was almost undetectable; the survival times of skin grafts in this group did not differ from the values observed in the control group injected with incomplete adjuvant only (Fig. 2). The effect of the dose of antigen on the prolongation of the survival of the skin graft is illustrated in Fig. 3. A two-fold increase of the dose in each individual injection, or the addition of a fourth immunization, prolonged the survival time of the skin graft.

These results agree with the finding that transplantation immunity could not be transferred by regional lymph node cells taken from treated mice. The inhibition of cellular immunity is also accompanied by inhibition of the formation of cytotoxic antibody, as is the case in the prevention of experimental autoimmune aspermatogenesis by injection of antigen in saline before sensitization with complete Freund's adjuvant[16]. No cytotoxic antibodies were detected in sera taken from experimental mice by the seventh day after grafting and as late as the fourteenth day; they were present only in low titres in comparison with control sera (Table 1). On the other hand, the titre of haemagglutinins was higher in the group pre-immunized with lyophilized tissues; this was especially pronounced in sera taken 14 days after grafting (Table 1).

Table 1. TITRES OF HAEMAGGLUTININS AND CYTOTOXIC ANTIBODIES IN EXPERIMENTAL AND CONTROL GROUPS

4 × 10 mg of lyophilized spleen tissue in incomplete adjuvant

7 days after grafting			14 days after grafting		
Grafts	Haemaggl.	Cytotox.	Grafts	Haemaggl.	Cytotox.
Experimental groups. Grafts from *A* strain mice 6 days after second injection					
+	64	0	+	250	0
+	64	0	+	250	8
+	64	0	+	120	8
+	32	0	+	250	4
+	120	0	+	32	4
+	64	0	+	64	4
Control groups. Grafts from *A* strain mice only					
+	8	8	–	0	16
+	16	8	–	16	32
+	64	16	–	32	64
+	120	16	–	8	64
+	32	4	–	16	64
+	32	0	–	64	8

The prolonged survival of skin grafts which occurred in actively immunized mice could be transferred only in part by antisera taken 6 days after a second immunization with lyophilized tissues in incomplete Freund's adjuvant (Fig. 4). A dose of serum, corresponding to 3·3 ml. given in eleven injections within 14 days, resulted in a short but significant prolongation of survival ($P < 0 \cdot 01$). The optimum conditions for passive transfer of enhancement in skin grafts and its dependence on haemagglutinin titre will be reported later.

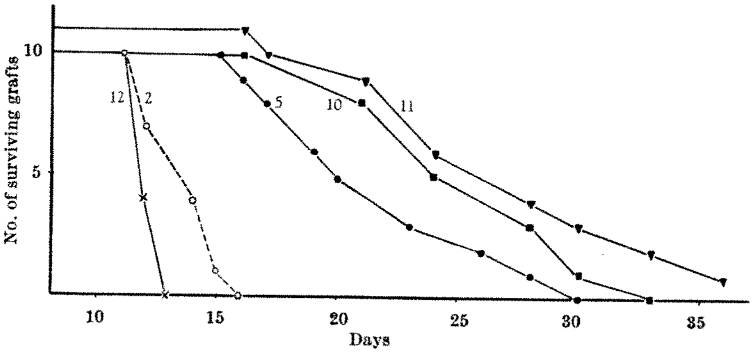

Fig. 3. Dependence on the dose of antigen. All injections were given intraperitoneally. Skin was grafted 6 days after the second injection. 10, 4 × 10 mg of lyophilized tissue in incomplete adjuvant at intervals of 8 days; $M = 25 \cdot 0$ (20–29). 11, 3 × 20 mg of lyophilized tissue in incomplete adjuvant at intervals of 11 days; $M = 24 \cdot 0$ (20–35). 12, Control grafts from *B*10.*D*2 strain; $M = 11 \cdot 0$ (10–12).

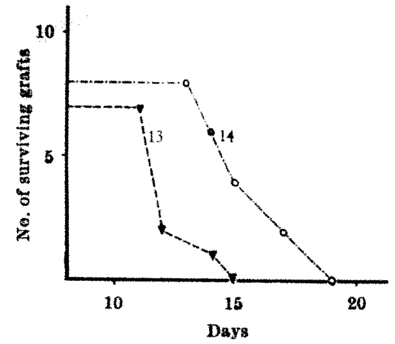

Fig. 4. Passive transfer. Eleven injections of sera, 0·3 ml., each daily (except Sunday). Skin grafting 24 h after the first injection. 13, Control sera from non-immunized mice; $M = 11·0$ (11–12). 14, Immune sera; $M = 14·5$ (13–18).

Prolonged survival of skin grafts after immunization with incomplete and even complete Freund's adjuvant is interesting and agrees with the results of experiments with tumour homografts[17]. In the analysis of the mechanism of this phenomenon, our model has the advantage that the survival of the animals is not endangered by the growth of the graft as is the case with tumour homotransplants.

On the basis of the results obtained in actively and passively immunized mice, it seems that there may be, in addition to antibodies which may be responsible for an afferent or central inhibition, another mechanism which plays a part in the inhibition of transplantation immunity in actively acquired enhancement of skin homografts. If various cell populations are responsible for different types of immune response[18,19], the most probable explanation of this mechanism seems to be a temporarily predominating proliferation of the cell population activated by pre-immunization with lyophilized tissues, and reacting by a harmless or beneficial type of immune response characterized by haemagglutinin formation. On the other hand, a harmful type of immune response leading, in normal conditions, to a rapid destruction of the graft shortly after grafting seems to be temporarily inhibited, perhaps as a result of the competition between immune responses.

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[1] Billingham, R. E., Brent, L., and Medawar, P. B., *Transplant. Bull.*, **3**, 84 (1956).
[2] Nelson, D. S., *Brit. J. Exp. Pathol.*, **42**, 1 (1961).
[3] Möller, G., *Transplantation*, **2**, 281 (1964).
[4] Moore, D. B., and Pareira, M. D., *Transplantation*, **3**, 627 (1965).
[5] Simmons, R. L., and Russell, P. S., *Transplantation*, **5**, 551 (1967).
[6] Kaliss, N., *Cancer Res.*, **18**, 992 (1958).
[7] Kaliss, N., *Ann. NY Acad. Sci.*, **129**, 155 (1966).
[8] Boyden, S. V., *Brit. J. Exp. Pathol.*, **38**, 611 (1957).
[9] Brown, P. C., Glynn, L. E., and Holborow, E. J., *J. Pathol. Bacteriol.*, **86**, 505 (1963).
[10] Chutna, J., and Rychlíková, M., *Folia. Biol.*, **10**, 177 (1964).
[11] Asherson, G. L., and Stone, S. H., *Immunology*, **9**, 205 (1965).
[12] Loewi, G., Holborow, E. J., and Temple, A., *Immunology*, **10**, 339 (1966).
[13] Asherson, G. L., *Immunology*, **10**, 179 (1966).
[14] Humphrey, J. H., *Brit. Med. Bull.*, **23**, 93 (1967).
[15] Borel, Y., Fauconnet, M., and Miescher, P., *J. Immunol.*, **98**, 881 (1967).
[16] Chutná, J., and Rychlíková, M., *Folia. Biol.*, **10**, 188 (1964).
[17] Cruse, J. M., and Michelson, I. D., *Z. Immun. For. Klin. Immun.*, **130**, 433 (1966).
[18] Oort, J., and Turk, J. L., *Brit. J. Exp. Pathol.*, **46**, 147 (1965).
[19] Parrott, D. M. V., and de Sousa, M. A. B., *Nature*, **212**, 1316 (1966).

Immunosuppressive Effects of Lymphoid Cell Proliferation in Mice receiving Anti-lymphocyte Globulin

ANTILYMPHOCYTE serum (ALS) and globulin (ALG) are potent suppressors of certain forms of immune response, particularly homograft rejection[1,2] and the primary antibody response[3]. Their mode of action has, however, been disputed. Although lymphopenia has been considered the essential cause of the immunosuppression[4] it has not always accompanied homograft acceptance which is induced by these agents[2,5]. "Blind-folding" and "sterile blast activation" of lymphocytes by ALG have been proposed as alternative mechanisms[2]. It has also been observed that renal homografts which have escaped rejection can nevertheless be infiltrated by lymphocytes, suggesting that the progeny of lymphoid cells exposed to ALG function inadequately[6].

We have already obtained evidence[7] that ALG ablates circulating blood lymphocytes. We now report that these cells need not necessarily have been functionally replenished even when the absolute blood lymphocyte count had returned to normal after the cessation of ALG injections. Moreover, ALG induces proliferative changes in the lymphoid tissues and indeed this could itself account for the prolonged delay in the return of normal immunological competence. This hypothesis was tested as follows.

Rabbit anti-mouse lymphocyte IgG (ALG) was prepared as previously reported[8]. C_3H mice were given daily intraperitoneal injections (10 mg) for 7 days. Twenty-four hours after the conclusion of these injections, one group of ALG-treated mice was given intravenously 150×10^6 lymphoid cells which were obtained by mixing equal numbers of cells from spleen, lymph nodes and bone marrow from normal C_3H donors. A second ALG-treated group received 10×10^6 peripheral blood lymphocytes which were obtained by the method of Hildemann[9] from the same strain. A third ALG-treated group received no cells, and a control group received 10 mg daily of normal rabbit globulin. All groups were injected intraperitoneally with 1 ml. of 20 per cent sheep erythrocytes on the day of transfer. Haemagglutination titres were determined 6 days later. Table 1 shows that blood lymphocytes restored the capacity of the mice which had been treated with ALG to produce an antibody response, but that cells obtained from lymphoid tissues were ineffective when given immediately after ALG. These findings support the contention that ALG acts primarily on circulating lymphocytes. Indeed the importance of blood borne lymphocytes in the initiation of antibody production by the spleen has recently been emphasized[10].

In order to determine whether the absolute blood lymphocyte count was any guide to the return of immunological competence after a course of ALG injections, we gave groups of twelve C_3H and C57 *Bl* mice daily injections of ALG for 10 days. At the end of this course, their mean absolute blood lymphocyte count was reduced to 500 per cmm but 14 days later the counts had returned to control levels (mean 6,400 per cu.mm). Only feeble haemagglutination titres (mean $\log_2$ titre 1·2) were obtained 6 days after challenge with sheep erythrocytes at this time, however, compared with a mean $\log_2$ titre of 6·0 in control mice.

Table 1. ANTIBODY TITRES TO SHEEP RED CELLS IN C_3H MICE AFTER RECEIVING ANTILYMPHOCYTE GLOBULIN (ALG) AND RECONSTITUTED WITH LYMPHOID CELLS

Group (No. of mice)	$\log_2$ haemagglutination titres at 6 days	Arithmetic mean $\log_2$ titre
ALG only (7)*	0. 0. 0. 0. 0. 0. 0.	0
ALG, then mixed lymphoid cells (7)†	0. 0. 0. 0. 0. 0. 0.	0
ALG, then peripheral blood lymphocytes (7)‡	4. 5. 6. 3. 5. 3. 0.	3·7
Controls (6)	5. 8. 8. 3. 5. 3.	5·3

* ALG in each group given daily for 6 days.

† 150×10^6 mixed lymph node, spleen and bone marrow cells injected intravenously in equal numbers.

‡ 10×10^6 cells injected intravenously.

Cells were given 24 h after ALG and all groups received 1 ml. 20 per cent sheep red blood cells intraperitoneally 24 h after last injection of ALG.

Similar results were obtained in a situation involving a red cell autoantibody. Twenty 2-month old *NZB* mice were injected twice weekly with 10 mg of ALG for 2 months, with resulting lymphopenia (mean 550 lymphocytes/cmm.). Twenty control mice received 10 mg of normal rabbit IgG. Although the absolute blood lymphocyte counts at 10 months of age were identical in the two groups (mean 4,400/cu.mm) all the ALG treated mice had negative antiglobulin tests compared with 80 per cent positive in the controls.

In contrast, although X-irradiation produced lymphocyte depletion of equivalent severity, its immunosuppressive effects were short lived. Thus C_3H mice which were challenged with sheep erythrocytes 14 days after 700 rads produced unimpaired haemagglutinin responses. Similarly, in experiments which are not reported here we have found that the incidence of positive anti-globulin tests at 10 months of age in *NZB* mice which had received 700 rads X-irradiation at 2 months of age does not differ significantly from that in non-irradiated mice.

The more prolonged immunosuppressive action of ALG compared with X-irradiation can be explained by considering the changes in the lymphoid organs which are induced by the former agent. We intravenously injected tritiated thymidine (1·0 μc./g body weight; specific activity 17·8 c./mM) into *C57Bl*, *NZB* and $(NZB \times NZW)\ F_1$ hybrid mice which had received ALG continuously in the same dosage as above for periods of up to 6 months. At 1, 24, 72 and 100 h, autoradiographs were obtained of imprints and sections from the peripheral blood, thymus, spleen, lymph nodes and bone marrow and analysed as described previously[7,8]. The specific activity of the DNA which was isolated from these tissues was measured by liquid scintillation counting[8]. Confirming our observations in rats[7,8], the specific activity of the DNA which was isolated from the lymphoid tissues of mice receiving ALG was significantly higher than that of the DNA obtained from control animals which had received normal rabbit IgG. The results of a typical experiment are shown in Table 2. The values in ALG-treated mice were higher than in the control mice and even exceeded those in the mice which had received X-irradiation, which is known to be followed by a phase of compensatory increase in DNA synthesis[11].

Table 2. INCORPORATION OF TRITIATED THYMIDINE (H^3Tdr) INTO THE LYMPHOID TISSUE DNA OF *NZB* MICE RECEIVING ANTILYMPHOCYTE IMMUNOGLOBULIN G (ALG), OR TOTAL BODY IRRADIATION, AND IN CONTROLS

Group (No. of mice)	Specific activity*		
	Thymus	Spleen	Lymph nodes
ALG treated (4)	34·8 (7·1–96·0)†	28·2 (12·5–40·3)	100·5 (12·8–322·0)
X-irradiated (3)	15·5 (9·8–20·0)	19·7 (18·8–20·2)	4·3 (2·6–6·5)
Controls (5)	2·7 (1·9–4·7)	14·7 (12·2–21·9)	4·8 (3·8–10·3)

ALG group received 10 mg of ALG twice weekly for 4 weeks.

X-irradiated group received 500 rads 3 days before labelling, delivered at 72·0 rads/min (250 kV, 1·2 mm Cu shield).

Controls received normal rabbit gamma globulin twice weekly for 4 weeks.

* C.p.m. per μg of DNA.

† Mean value and range.

The autoradiographs showed that the high specific activity of the DNA in the thymuses and spleens of the ALG-treated mice was attributable, as in similarly treated rats[7], to a striking increase in the numbers of highly labelled reticulum cells and large lymphocytes; these were randomly distributed throughout the cortex and medulla of the thymus and the white pulp of the spleen which was depleted of small lymphocytes. Reticulum cells (reticular lymphoblasts) and large lymphocytes were identified by the criteria of Bessis[12] and Frenkel *et al.*[11].

Furthermore, the high grain counts over these cells in the thymus and spleen fell sharply during the 100 h following labelling, and this fall was accompanied by the appearance after 24 h of labelled small lymphocytes in the thymic cortex and in the white pulp of the spleen. Because small labelled lymphocytes were not seen earlier, they must have arisen from division of labelled precursor cells. It is unlikely that they migrated from the blood, for these mice were still profoundly lymphopenic (< 500 lymphocytes/cu.mm.) and less than 1 per cent of the lymphocytes in their blood was labelled.

The high specific activity of the lymph-node DNA is clearly accounted for by the massive proliferation of plasma cells in the lymph nodes which is known to accompany ALG administration[13].

Levey and Medawar have suggested that an effect of ALG treatment is to produce "sterile activation" of lymphoid cells[14]. The results reported here support this contention, because they show that ALG provokes an intense proliferation of precursor cells leading to the appearance of immunologically incompetent lymphocytes in the circulating blood. Moreover, after ALG treatment, the capacity to make primary antibody responses can only be restored by transfer of peripheral blood lymphocytes from a normal isogeneic donor, and not by non-circulating lymphoid cells from spleen, lymph nodes or marrow. It is certain, however, that lymphocytes are not simply transformed into blast cells by the direct action of ALG as we have induced a blood lymphocytosis of 100,000/cu.mm. in mice by intravenous injection of pertussis vaccine and have then injected ALG. Despite a fall in the blood lymphocyte count to 300 per cu.mm. over a 24 h period, blast cells were not detected in the peripheral blood either morphologically or by tritiated thymidine labelling.

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[1] Woodruff, M. F. A., and Anderson, N. F., *Ann. NY Acad. Sci.*, **120**, 119 (1964).

[2] Levey, R. H., and Medawar, P. B., *Ann. NY Acad. Sci.*, **129**, 164 (1966).

[3] James, K., and Anderson, N. F., *Nature*, **213**, 1195 (1967).

[4] Kinne, D. W., and Simmons, R. L., *J. Immunol.*, **98**, 251 (1967).

[5] Anderson, N. F., James, K., and Woodruff, M. F. A., *Lancet*, i, 1126 (1967).

[6] Starzl, T. E., Marchioro, T. L., Porter, K. A., Iwasaki, Y., and Cerilli, G. L., *Surgery Gynec. Obstet.*, **124**, 301 (1967).

[7] Denman, A. M., and Frenkel, E. P., *Immunology*, **14**, 107, 115 (1968).

[8] Denman, A. M., Frenkel, E. P., and Ziff, M., *Proc. Soc. Exp. Biol. Med.*, **125**, 1127 (1967).

[9] Hildemann, W. H., Linscott, W. D., and Morlina, M. J., in *Ciba Foundation Symposium on Transplantation*, 236 (Churchill, London, 1962).

[10] Ford, W. L., and Gowans, J. L., *Proc. Roy. Soc.*, B, **168**, 244 (1967).

[11] Frenkel, E. P., Sugino, Y., Bishop, R., and Potter, R. L., *Rad. Res.*, **19**, 701 (1963).

[12] Bessis, M., *Cytology of the Blood and Blood Forming Organs* (Grune and Stratton, New York, 1956).

[13] Denman, A. M., and Frenkel, E. P., *J. Immunol.*, **99**, 498 (1967).

[14] Levey, R. H., and Medawar, P. B., *Proc. US Nat. Acad. Sci.*, **58**, 470 (1967).

Effect of Cyclophosphamide on the Production of Interferon

CYTOTOXIC drugs are widely used for the treatment of malignant disease. Many of these agents are also powerful immunosuppressants, affecting both humoral and cellular immunity. Other antiviral defence mechanisms can also be affected by cytotoxic drygs; for example, the production of interferon can be depressed by actinomycin[1,2] and puromycin[3]. Steroids have also been shown to suppress the production of interferon[4].

We describe here experiments which show that cyclophosphamide depresses interferon in the intact animal but is without activity in tissue culture systems.

Preliminary experiments indicated that 1 mg/ml. of cyclophosphamide was not toxic for cultured monkey kidney cells. It was also shown that this concentration of the drug had no effect on the growth of the Sendai strain of parainfluenza and the Kunz strain of influenza *A* virus in the same cells. These findings suggested that cyclophosphamide was having no effect on normal metabolic processes of the cell, the processes concerned

Table 1. EFFECT OF CYCLOPHOSPHAMIDE (1 MG/ML.) ON INTERFERON PRODUCTION INDUCED BY NEWCASTLE DISEASE VIRUS IN TISSUE CULTURES OF MOUSE *L* CELLS

Experiment No.	Interferon titres Without drug	Interferon titres With drug
1	33	37
2	13	11
3	32	35

Table 2. EFFECT OF CYCLOPHOSPHAMIDE ON INTERFERON PRODUCTION IN MOUSE LUNG INDUCED BY SENDAI VIRUS

Experiment No.	Dose (mg/kg)	Interferon titres Without drug	Interferon titres With drug
1	100	210	36
2	100	512	48
3	200	218	28
4	200	42	< 16
5	400	44	< 8
6	200	39	< 8
7	200	96	26

Assays were carried out on lungs removed on either the third or fifth day after infection.

with viral replication, and probably no effect on the processes concerned with the production of interferon.

The effect of cyclophosphamide on the production of interferon in tissue culture was determined directly, using the method of Paucker[5] in which interferon is induced in mouse *L* cells by Newcastle Disease Virus. Cyclophosphamide was incorporated in the tissue culture medium of the experimental cultures in a concentration of 1 mg/ml. and interferon was assayed by the quantitative haemadsorption method[6]. The results of three experiments are shown in Table 1, which demonstrates that cyclophosphamide had no effect on the yields of interferon.

In contrast, cyclophosphamide treatment consistently depressed the production of interferon in the intact animal. Swiss white mice were inoculated intranasally with $10^{5.5}EID_{50}$ of Sendai virus. The mice were killed and exsanguinated on either the third or fifth day after infection and their lungs were processed and assayed for interferon. Animals were inoculated subcutaneously with cyclophosphamide as indicated in Table 2, which shows the results of seven experiments with different batches of mice. Although there was considerable variation in the amounts of interferon produced in the different experiments, the titres were always considerably lower in the cyclophosphamide treated animals.

The difference between the *in vitro* and *in vivo* effects of cyclophosphamide on the production of interferon can be explained if certain metabolic processes of the intact animal are required to convert the drug into its active form[7]. This lack of activity in tissue culture has also been observed by Hampel *et al.*[8], who have shown that cyclophosphamide (in contrast to most cytotoxic drugs) does not damage the chromosomes of lymphocytes cultured *in vitro*, but chromosome damage can be demonstrated if these cells are treated with the serum from rats treated with cyclophosphamide.

These findings show that cyclophosphamide, like many other cytotoxic compounds, may adversely affect not only immune processes but also certain non-specific reactions concerned with defence against viral infections.

We thank Messrs Ward, Blenkinsop and Co., Ltd., for the cyclophosphamide, and Miss G. V. Martin for technical assistance.

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[1] Heller, E., *Virology*, **21**, 652 (1963).

[2] Wagner, R. R., *Trans. Assoc. Amer. Physic.*, **76**, 92 (1963).

[3] Buchan, A., and Burke, D. C., *Abst. Sixth Intern. Cong. Biochem.*, **1**, 46 (1964).

[4] Kilbourne, E. D., Smart, K. M., and Pokorny, B. A., *Nature*, **190**, 650 (1961).

[5] Paucker, K., *J. Immunol.*, **94**, 371 (1965).

[6] Finter, N. B., *Virology*, **24**, 589 (1964).

[7] Brock, N., and Hohrst, H. J., *Arzneimittel-forsch*, **13**, 1021 (1963).

[8] Hampel, K. E., Kober, B., Rösch, D., Gerhartz, H., and Meinig, K. H., *Blood*, **27**, 816 (1966).

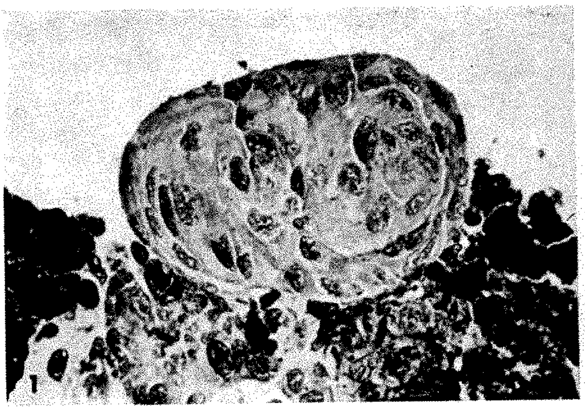
CYTOLOGY

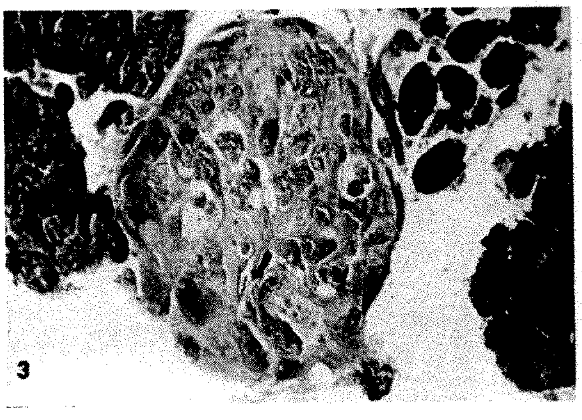
In vitro Culture of the Regenerating Lens

LENS regeneration by the dorsal iris in the lentectomized newt is a phenomenon which has been studied from the viewpoints of experimental embryology[1,2] as well as cell biology[3-7]. Most of the studies so far reported, however, have been performed *in vivo*. The potential advantages of performing such studies in controlled conditions led us to develop an *in vitro* method for the culture of the regenerating lens of the newt *Triturus viridescens*.

The technique involved placing the lens regenerates on agar cubes into which culture medium had been incorporated. The two media used were medium 199 with 15 per cent foetal bovine serum or Puck's *N*-16 (Microbiological Associates, Bethesda, Maryland). Penicillin was added at a concentration of 200 U/ml. The agar blocks were prepared by mixing either of these media with 4 per cent aqueous agar (noble Difco agar) solution 1 : 1 by volume and pouring into a 5 cm Petri dish to a depth of about 3 mm. After solidification, cubes of approximately 5 × 5 × 3 mm were cut out. These were placed into 3 cm tissue culture Petri dishes which had previously been filled with culture medium without agar up to a depth of 1 to 2 mm. The agar substrate was used to inhibit the outgrowth of cells from the regenerate, and the additional liquid medium was used to saturate the culture with nutrient solution.

The dorsal iris with the regenerating lens was dissected from the eye of the lentectomized newt in aseptic conditions, washed with Earle's balanced salt solution and placed on the surface of the agar block. The cultures were incubated at 25° C in 5 per cent carbon dioxide and





Figs. 1 and 3. 20 day old regenerates cultured for 5 days in Puck's medium *N*-16. Lens fibres are conspicuous and squamous cells are present at the periphery of the regenerates.

90 per cent relative humidity. Maintenance of the *p*H of the culture between 7 and 7·4 with a carbon dioxide atmosphere was essential for optimal development of the regenerates. Cultures, in which alkalinity developed, died or survived in a poor condition.

Regenerates at day 9, 15 and 20 after lens removal were cultured for 5, 7 and 10 days. Mitoses were seen in pigmented and depigmented cells and the differentiation of cells into fibres continued (Figs. 1, 3, 4, 5 and 6) as compared with controls (Fig. 2). Both culture media were suitable for developing the tissue; however, a better differentiation was observed when the medium with serum supplement was used.

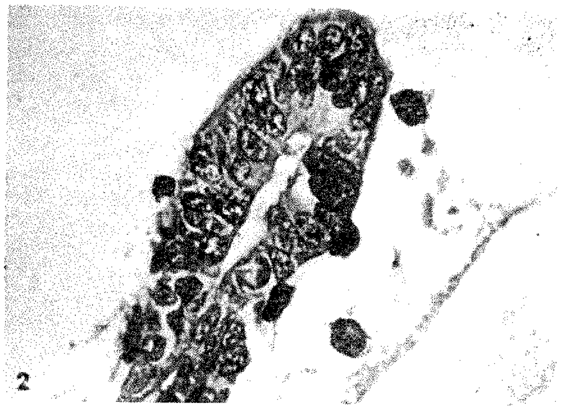


Fig. 2. Control for regenerate in Fig. 1. Both eyes were lentectomized in the same animal. This regenerate was fixed at the same time that regenerate in Fig. 1 was started in culture.

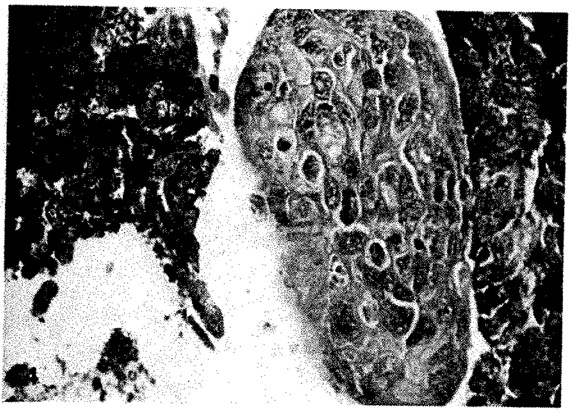


Fig. 4. 20 day old regenerate cultured for 10 days in medium 199 plus 15 per cent foetal bovine serum.

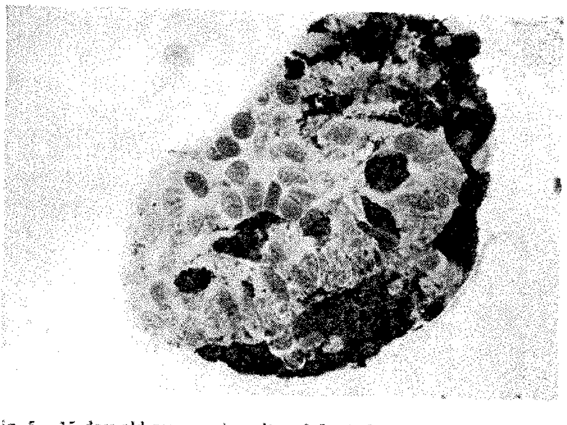


Fig. 5. 15 day old regenerate cultured for 7 days in medium 199 plus 15 per cent foetal bovine serum. Observe elongation of cells into fibres.

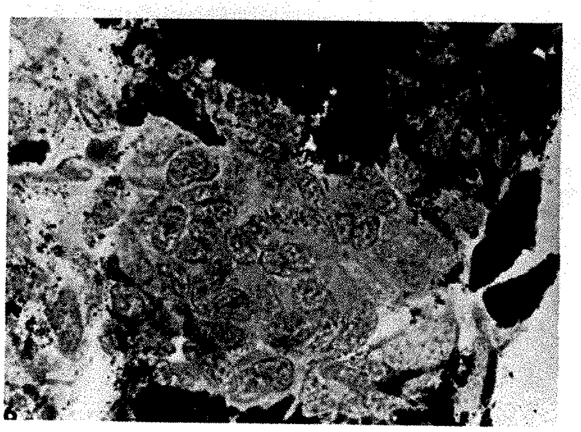


Fig. 6. 9 day old regenerate cultured for 7 days in medium 199 plus 15 per cent foetal bovine serum.

An interesting feature in the cultured regenerates was the disappearance of the presumptive lens epithelium. It seems that cells in this region of the lens regenerate elongated and form lens fibres (Figs. 3, 4 and 5) or become transformed to squamous type cells surrounding the developing lens (Figs. 1 and 3).

Our experiments suggest that as early as 9 days after lentectomy, the lens regenerate, can survive *in vitro* conditions and continue with further differentiation. The results on the further progress of differentiation *in vitro* from day 9 of regeneration onwards agree with the preliminary results of Reese (reported by Yamada[8]). In other studies, we have found that in these conditions the integrity of the cultured regenerates was maintained and the cells remained healthy for periods of up to 30 days. A more detailed study in the differentiative capacities of developing lenses at successive stages of regeneration is being performed.

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Received October 9; revised December 12, 1967.

[1] Stone, L. S., *Invest. Ophtal.*, **4**, 420 (1965).
[2] Reyer, R. W., in *Regeneration* (edit. by Rudnick, D), 211 (1962).
[3] Yamada, T., and Karasaki, S., *Develop. Biol.*, **7**, 595 (1963).
[4] Yamada, T., and Takata, Ch., *Develop. Biol.*, **8**, 358 (1963).
[5] Takata, Ch., Albright, J. F., and Yamada, T., *Develop. Biol.*, **9**, 385 (1964).
[6] Eisenberg, S., and Yamada, T., *J. Exp. Zool.*, **162**, 353 (1966).
[7] Eisenberg, Zalik S., and Yamada, T., *J. Exp. Zool.* (in the press).
[8] Yamada, T., in *Current Topics in Developmental Biology* (edit. by Moscona and Monroy) (in the press).

Effect of Cell Cycle on Recovery from Radiation Damage in the Mouse Liver

I HAVE studied the chromosomal aberrations which are induced in mouse hepatocytes by single and fractionated doses of X-rays given at various times before or after surgical partial hepatectomy. The data presented here show that the different phases of the cell cycle possibly differ not only in their sensitivity to chromosomal damage by radiation but also in their ability to recover from such damage.

Female *SAS*/4 mice 12 weeks old which had been anaesthetized with sodium pentobarbitone ('Nembutal'), 60 mg/kg body weight, were partially hepatectomized to remove two-thirds of the liver[1]. The surgery was performed so that subsequent killing, 66 h later, by cervical dislocation was always between 9.00–10.00 a.m., thus offsetting possible diurnal mitotic rhythm which has been reported to occur[2]. The remaining regenerating liver was excised and sections 5μ thick were stained with haematoxylin and eosin for microscopic examination. Similar anatomical areas of the anterior right lateral lobe were scanned in each mouse by one investigator using coded slides to score chromosomal abnormalities. The aberrations, bridges and fragments were scored in 100 anaphase/telophase figures in each liver, using approximately ten mice in each group. Irradiation was carried out with a 250 kVp, 15 mamp X-ray machine, filtered so as to give a dose rate of 105 rad/min at 30 cm focal spot distance. The filtration was 1 mm Al inherent and a 'Thoraeus II' filter which gave uniform beam over the field was used. Mice, which were unanaesthetized, breathing air, and which were constrained in a 'Perspex' tube with walls 0.5 cm thick, were irradiated, two at a time.

The upper curve in Fig. 1 is a plot of the logarithm of the fraction of normal mitoses against dose for cells exposed 24 h before partial hepatectomy, that is, while in the G_0 phase. The values which are plotted are relative to those in non-irradiated mice for which 94.5±0.7 per cent of normal mitoses was observed. There is a close fit to an exponential function with a fitted line drawn through all points having a $D_0 = 1{,}228 \pm 31$ rads and an extrapolation number of 1.00±0.02. The lower curve in Fig. 1 shows the same effect for irradiation which was given 24 h after partial hepatectomy when the regenerating cells were, as has been shown by autoradiography in this laboratory, to be in G_1 or early *S*. This line has a definite curvature and a fitted line on the last four points gives a $D_0 = 300$ rads and an extrapolation number of 6.7. There is thus a very large difference in radiation sensitivity, as far as chromosomal damage is concerned, between cells irradiated in the G_0 phase or early *S* phase. Furthermore, in the latter case, the curve has a pronounced shoulder which is indicative of repair of damage, while the upper curve has no shoulder.

In order to test whether a difference in ability to recover was detectable between G_0 cells and cells in G_1/S, fractionation experiments were carried out. The lower curve of Fig. 2 shows the relative proportion of normal mitoses which were observed after splitting a dose of 950 rads into two equal fractions, the first fraction being given at 24 h before partial hepatectomy and the second being given at the times shown—that is, 3, 9, 16, 21, 24 h later—right up to the time of the operation. There seems to be no significant difference in chromosomal damage between the single and the split dose over the 24 h period which was studied. Such a result suggests that no recovery of chromosome damage occurs in G_0 cells during 24 h. In contrast, the upper curve shows the increased proportion of normal mitoses, interpreted as "recovery", which takes place by splitting the same dose and giving the first half 24 h after partial hepatectomy and the second half at the times shown. Further experiments in this laboratory have shown that the reduced yield of aberrations which was observed was not caused by the second fraction of the dose being given to cells in a phase of increased radioresistance.

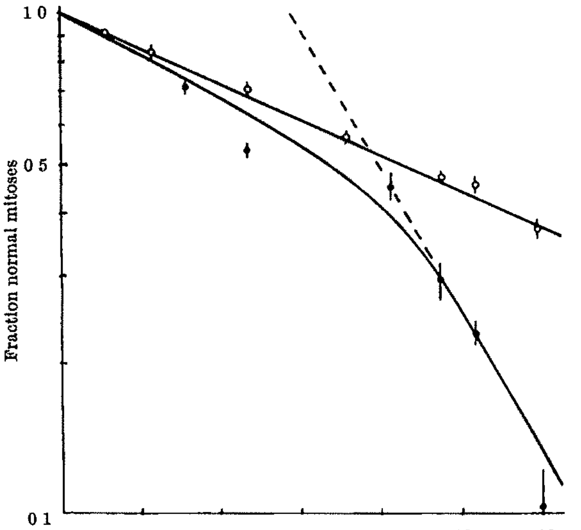

Fig. 1. A plot of the logarithm of fraction normal mitotic figures against doses of X-rays. ○, Doses given 24 h before partial hepatectomy; ●, Doses given 24 h after partial hepatectomy. More points would be desirable for the latter fitted line; this is, however, technically very difficult.

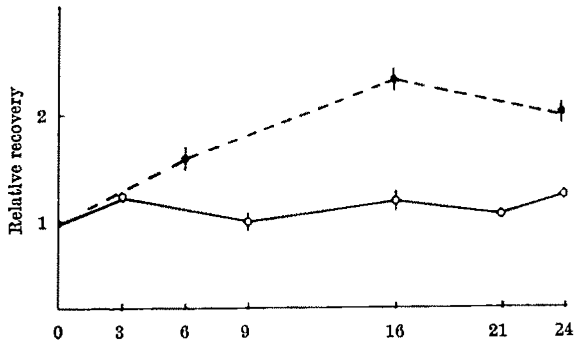

Fig. 2. Relative recovery factor for chromosomal damage induced by radiation against time between two fractions of 475 rads each. ●, Doses given after partial hepatectomy; ○, doses given before partial hepatectomy.

The percentage yields of chromosomal damage for G_0 cells (Fig. 1) are in accord with published values[3-7] although these authors used only one or two doses. The greater sensitivity of cells in actively dividing liver is in agreement with Albert[3]. The reasons for the difference in sensitivity remain obscure, but an oxygen effect could be involved because in regenerating liver an inflammatory reaction exists and increased oxygenation possibly results in increased sensitivity. Cater (see Holmes[8]), however, reported a 40 per cent increase in the consumption of oxygen in regenerating liver slices relative to that in normal liver; the tissues are therefore unlikely to be highly oxygenated unless there is a correspondingly greater oxygen supply, for which there is no evidence. Alternatively, increased sensitivity could be a result of the difference between time of irradiation and the time of assay of the damage, because in each case the latter was 66 h postoperatively and there was therefore a difference of 48 h between irradiation and assay times of cells irradiated 24 h before (G_0) and those irradiated 24 h after (G_1/S cells). Greater damage might be expected in the latter because they had less time available for any repair. The slight increase in damage which was observed for the 16 h split dose compared with that at 24 h, in the upper curve of Fig. 1, however, argues against this interpretation.

The lack of recovery in G_0 cells which were exposed to split doses is in agreement with Shapiro[5], who reported no sparing effect when two doses were split by a 7 day period. Several papers[3-7] report little or no decrease in the number of abnormalities when partial hepatectomies were performed many weeks or months after a single dose of radiation, and only one[7] noticed a decay in the proportion of "bridges" but not in overall percentage abnormalities. In contrast, two workers[9,10] showed chronic irradiation to be less effective than acute exposures; this is suggestive of repair, as one of the workers[9] excludes cell death at cell division as a factor lessening observed damage. The persistence of chromosomal abnormalities which were found in peripheral blood cultures[11] could represent another example of the lack of recovery in cells which were not actively in cell cycle.

There does seem to be a recovery, however, when split doses are given after partial hepatectomy. The upper curve in Fig. 2 shows a smooth early recovery with no evidence of fluctuations in recovery pattern as have recently been reported[12], although split doses separated by less than 6 h might reveal such patterns.

It might be suggested that the population feedback mechanism which keeps cells in G_0 (ref. 13) also inhibits the metabolic pathway necessary for repair of damage, as seen in cells which are in cycle. This might constitute a definitive characteristic of the G_0 phase.

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[1] Wilson, M. E., Tsuboi, K. K., and Stowell, R. E., *Cancer Res.*, 13, 80 (1953).

[2] Jackson, J., *Anat. Res.*, 134, 365 (1959).

[3] Albert, M. D., *J. Nat. Cancer Inst.*, 20, 309 (1958).

[4] Stevenson, K. G., and Curtis, H. J., *Rad. Res.*, 15, 774 (1961).

[5] Shapiro, I. M., *Intern. J. Rad. Biol.*, 3, 293 (1963).

[6] Albert, M. D., and Bucher, N. L. R., *Cancer Res.*, 20, 1514 (1960).

[7] Weinbren, K., and Cohen, M., *Brit. J. Radiol.*, 33, 419 (1960).

[8] Holmes, B. E., *Ionising Radiation and Cell Metabolism*, Ciba Foundation Symposium (edit. by Wolstenholme, G. E. W.), 225 (1956).

[9] Shapiro, I. M., Yarmonenko, S. P., and Paliga, G. F., *Nature*, 203, 315 (1963).

[10] Curtis, H. J., and Crowley, C., *Rad. Res.*, 19, 331 (1963).

[11] Doida, Y., Sugahara, T., and Horikawa, M., *Rad. Res.*, 26, 69 (1965).

[12] Evans, H. J., *Radiation Research* (edit. by Silini, G.), 482 (North-Holland Publ. Co., Amsterdam, 1967).

[13] Lajtha, L. G., and Gilbert, C. W., *Adv. in Biol. and Medical Physics*, 1 (Acad. Press, 1967).

PHYSIOLOGY

Iodine Compounds in Plasma of Rats: Effect of Exposure to High Environmental Temperature

THE rate of release of iodine-131 from the thyroid has been widely used as an index for thyroid activity and has been considered a reliable index for thyroid function[1]. It is also used as a basis for the rate of secretion of thyroxine (TSR) by the replacement method[2]. Recently, this laboratory[3] reported a higher rate of release of iodine-131 from the thyroid in rats following long-term exposure to a high environmental temperature (34° C). These findings prompted the question whether the composition of the substance(s) released might be constant in different physiological conditions, that is, would long-term exposure to environmental heat alter the composition of the iodinated compounds which were released by the thyroid gland? This experiment was undertaken in order to determine the nature of the iodinated compounds in plasma of rats during exposure to heat (34° C).

A group of fifty-six male rats of the Holtzmann strain (3 weeks old) was maintained in a climatic chamber at 28° C and 50 per cent relative humidity (RH). At the age of 6 weeks, twenty-four rats were exposed to a constant temperature of 34° C and 50 per cent RH and were maintained in these conditions for more than 20 weeks. Carrier-free iodine-131 was diluted in distilled water to contain 20 μc./ml. and 0.5 ml. was administered intraperitoneally to the rats. Twenty-four hours after the injection of iodine-131 blood samples (2–3 ml.) were collected by heart puncture from each rat. Blood samples were collected after 1, 3, 6, 12 and 20 weeks' exposure to heat (34° C). Rats which were maintained at 28° C were bled at the beginning and at the end of the experiment.

The iodine-131 compounds in plasma were separated using a descending chromatographic technique described by Heninger *et al.*[4]. The iodine-131 activity was assayed in a well type scintillation crystal detector.

Fig. 1 illustrates the percentage of iodinated compounds in the plasma of rats which were raised at 28° C and of others which were exposed to high temperature (34° C) for 1, 3, 6, 12 and 20 weeks. The triiodothyronine (T_3) and diiodothyronine (T_2) fractions were not adequately separated and the radioactivity of both fractions was therefore combined. The results at 28° C and 34° C indicate that thyroxine (T_4) contains most of the iodine-131 compounds in plasma. At 28° C, the rat plasma contains an average of 93.5 per cent T_4, 4.6 per cent $T_2 + T_3$, 0.6 per cent monoiodotyrosine (MIT) and 1.6 per cent inorganic iodine (I^-).

After exposure to heat at 34° C the iodine-131 compounds in plasma were not significantly different from the values which were obtained at 28° C after exposure for 1 and 3 weeks. After 6 weeks of exposure to 34° C the composition of the iodine-131 compounds was significantly ($P < 0.05$) different from the values which were obtained for these compounds at 28° C. The T_4 fraction was decreased and the I^-, MIT and $T_2 + T_3$ fractions were increased. After exposure for 12 and 20 weeks at 34° C the T_4 fraction was significantly lower ($P < 0.01$) than the values at 28° C. These data suggested a significant effect of long-term (at least 6–12 weeks) exposure to heat on the composition of iodine-131 compounds in plasma (Fig. 1).

The results which are presented confirmed that T_4 is the principal iodine-131 compound in the plasma of rat. Pitt-Rivers and Rall[5] reported 92–94 per cent T_4, 4.2–4.4 per cent T_3 and 1.3–3.4 per cent iodinated thyronines (MIT + DIT) in rat plasma. Laidlaw[6], however, found that in a few rats (three animals), plasma contained 60 per cent T_4, 48 h after the injection of iodine-131. The uniqueness of the data shown in Fig. 1 is the change which occurred in the iodine-131 compounds in the

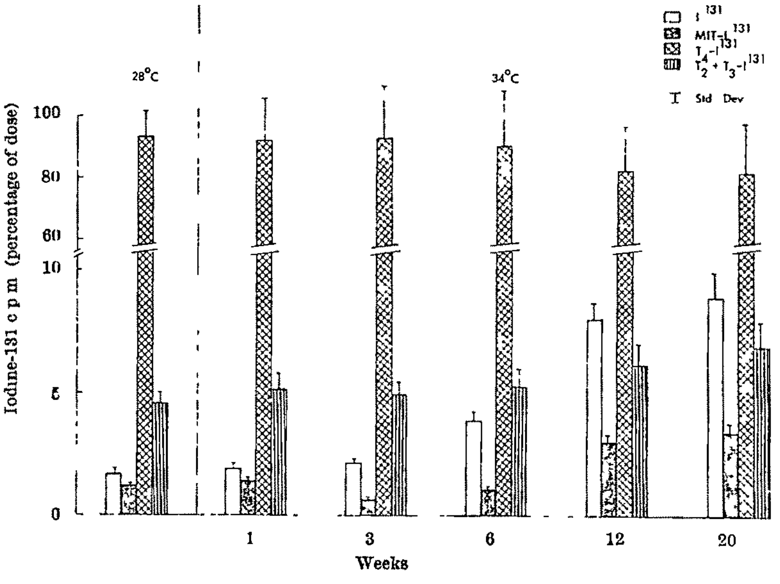

Fig. 1. Changes in iodinated compounds in plasma during long term exposure to heat (34° C).

plasma of rats which were exposed to 34° C. The data suggested that the composition of the thyroid secretion changes during acclimatization to heat (34° C) For example, the ratio of I^- T_4 changes from 1 55 at 28° C to 1 23, 1 10 and 1 . 9 at 34° C after exposure for 6, 12 and 20 weeks, respectively. The ratio of MIT $T_2 + T_3$ changed from 13 100 to 21 100, 51 100 and 49 100 at 34° C for 6, 12, and 20 weeks, respectively

To explain the changes which occurred in the composition of the iodinated compounds during heat acclimatization, there are three possibilities (*a*) perhaps the heat affects the permeability of thyroid cells resulting in the cellular release of more I^-, MIT and less T_4; (*b*) the heat could affect the acinar cellular processes which limit the biosynthesis of T_4, (*c*) changes in the percentage of I^- in plasma of rats which were exposed to heat could reflect either a reduction in renal clearance of I^- or a reduced uptake of iodine-131 by the thyroid, or both Details of body weight and other physiological functions at 34° C are reported elsewhere[7]

Knowing that the release rate technique measures the rate of all the iodine that comes out of the gland/unit of time, any changes in the composition of the iodine which is released from the gland are ignored by this technique and may mask the true secretory action of the thyroid gland Results obtained by the release rate technique should be explained with great caution when the animal is deviated from its thermoneutral environment and exposed to high or other ambient temperature conditions

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[1] Brown-Grant, K , Von Euler, C , Harris, B W , and Reichlin, S , *J Physiol* , 126, 1 (1954)

[2] Reineke, E P , and Singh, O N , *Proc Soc Exp Biol and Med* , 88, 203 (1955)

[3] Johnson, H D , Ward, M W , and Kibler, H H , *J Appl Physiol* , 21, 689 (1966)

[4] Heninger, R W , Larson, F C , and Albright, E C , *J Clin Invest* , 42, 1761 (1963)

[5] Pitt-Rivers, R , and Rall, J E , *Endocrinology*, 68, 309 (1961)

[6] Laidlaw, J C , *Nature*, 164, 927 (1949)

[7] Johnson, H D , *Growth* (in the press)

Regeneration of a Peripheral Nerve Implant into Goldfish Telencephalic Parenchyma

THE nerve fibres of a peripheral nerve implanted in the cortex of the rat[1] or cat[2,3] do not regenerate into the brain parenchyma The glial scar which lines the wound made in the parenchyma during nerve implantation seems to be a mechanical barrier to successful regeneration After nerve implantation and administration of the drug piromen, however, the peripheral axons which formerly had not grown through the glial scar now grow through the scar[2,3]

Several chemical agents have induced the growth of axons in the central nervous system, which demonstrated that the effect of the glial scar which seems to be a barrier in the normal course of regeneration can be negated[2-4]. In the goldfish, a glial-ependymal scar was observed after central nervous system insult This scar does not act as a mechanical barrier to regenerating axons of the central nervous system[5,6] The present experiments were undertaken to ascertain the tissue reaction of the telencephalon of the goldfish to an implanted peripheral nerve

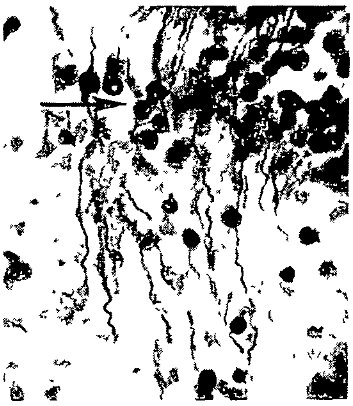

Fig 1 The peripheral nerve fibres regenerated through a dense glial-ependymal scar (arrow) into the telencephalic parenchyma. Silver stain (protargol, × 850)

Seven goldfish (*Carrasius auratus*) 3–6 yr old and approximately 17 cm long were used in these experiments The goldfish were anaesthetized with Tricaine methanesulphonate ('MS-222', Sandoz, 1 4,000) The cranium was opened with a trephine and the fatty substance surrounding the brain aspirated On the floor of the cranium a branch of the seventh nerve within the cranial cavity was exposed This nerve was dissected free, turned and implanted into a stab wound within the telencephalon A cap was used to close the cranium Animals were killed 90 days later and the degree of regeneration was assessed

Five animals were perfused with 10 per cent isotonic formol-saline, the brain removed, dehydrated, embedded in paraplast and sectioned at 10μ Alternate sections were stained with silver ('Protargol'), or with periodic acid–Schiff's reagent and haematoxylin Two animals were perfused with 2 5 per cent gluteraldehyde in a phosphate buffer (*p*H 7 4). The telencephalon with the implant was removed, washed in buffer and placed in buffered 2 per cent osmic acid for 2 h The tissue was then dehydrated and embedded in 'Araldite' Sections 1μ thick were stained with methylene blue, Azure II or toluidine blue

Although the stab wound in the telencephalon resulted in the formation of a glial-ependymal scar, the peripheral nerve fibres regenerated into the brain parenchyma (Fig 1) The glial-ependymal scar was from two to seven cells thick and was characterized by long interdigitating cytoplasmic processes of the cells constituting the scar The peripheral nerve fibres seemed to grow between these cellular processes and splay out into the brain parenchyma The nerve fibres did not grow as compact bundles of nerve fibres, but were discrete axons that grew between the glial, ependymal and neuronal cell processes The peripheral nerve fibres seemed normal when compared with cells of the contralateral unoperated telencephalic hemisphere

After administration of the drug piromen peripheral nerve fibres which usually do not grow into the brain parenchyma will successfully regenerate into the cortex of cats This phenomenon has been interpreted as the loosening of the glial scar allowing for the penetration of the nerve fibres[2,3] Recent evidence has demonstrated that except for the synaptic bed of Mauthner's cell[7] the extracellular space of the fish brain does not seem to differ from that of the mammalian brain[8] These data seem to demonstrate that the glial-ependymal scar of the goldfish, through which the peripheral nerves regenerated, is not morphologically more loose than the mammalian glial scar through which peripheral nerves do not regenerate The regeneration of axons in the mammalian nervous system seems to depend on the establishment of proper milieu for nerve regrowth This has been demonstrated by the fact that axons of the mammalian brain stem and spinal cord will resume growth (sprout) after partial denervation of the neurones in the surrounding parenchyma[8] The action of piromen could therefore be considered to induce a change in the permeability of the cellular constitution of the glial scar, thus creating a proper environment for the growth of the peripheral nerve fibres through the scar The chemical constituents of the telencephalon of the goldfish are such that a proper milieu is established within the brain parenchyma allowing for the successful regeneration of peripheral axons, whereas drugs or special operative procedures must be utilized in the mammalian brain to induce the proper neurotrophic environment

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[1] LeGros Clark, W E , *J Anat* , 77, 251 (1943)
[2] Clemente, C D , in *Regeneration in the Central Nervous System*, 147 (Thomas, Springfield, Illinois, 1955)
[3] Windle, W F , Clemente, C D , and Chambers, W W , *J Comp Neurol* , 96, 359 (1952)
[4] Scott, D , and Liu, C N , *Prog Brain Res* , 13, 127 (1964)
[5] Bernstein, J J , *Exp Neurol* , 17, 44 (1967)
[6] Bernstein, J J , and Bernstein, M E , *Exp Neurol* , 19, 25 (1967)
[7] Robertson, J D , Bodenheimer, T S , and Stage, D E , *J Cell Biol* , 19, 159 (1963)
[8] Palay, S L , McGee-Russell, S , Gordon, S , and Grillo, M A , *J Cell Biol* , 12, 385 (1962)
[9] Goodman, D C , and Horel, J A , *J Comp Neurol* , 126, 71 (1965)

Regional Distribution of Tritiated Acetylcholine in Rat Brain

THE almost universal occurrence of acetylcholine, other choline derivatives and cholinesterase in the vertebrate nervous system, and effects of cholinesterase inhibitors on the central nervous system have suggested that cholinergic systems play an important part in brain function The experimental evidence for such a role ranges from effects on the rate of discharge of single brain neurones to complex animal behaviour[1] Whittaker[2] has demonstrated that acetylcholine is located primarily in the specific tissue fraction consisting chiefly of nerve ending particles De Robertis[3] has located acetylcholine in the synaptic vesicles recovered from brain homogenates

Recent studies have demonstrated that ^{3}H-norepinephrine introduced into the brain by intraventricular injection is taken up by neurones which contain catecholamine, where it mixes with the endogenous norepinephrine stores[4] We therefore thought it of interest to determine whether ^{3}H-acetylcholine can also be accumulated in the brain in analogous fashion Because acetylcholine is reported to occur unequally in various regions of the brain[1], regional distribution of injected ^{3}H-acetylcholine was determined with the hope that differences in distribution among various brain regions may reflect the relative importance of this compound in these regions. Because intravenously injected acetylcholine does not readily penetrate the blood–brain barrier, ^{3}H-acetylcholine was administered into the lateral ventricle of the brain. (The acetylcholine was purchased from New England Nuclear Corporation, who state that the labelling is in the methyl group of the acetyl The correct designation should therefore be acetyl-^{3}H choline According to New England Nuclear Corporation and Nuclear-Chicago, tritiated acetylcholine, labelled either in the acetyl or *N*-methyl groups, has the biological and chemical properties of "cold" acetylcholine)

Female Sprague–Dawley rats weighing 180–200 g were anaesthetized with ether and fixed in a stereotaxic device A hole just large enough to accommodate a 27 gauge needle was drilled through the skull on the coronal suture 1 5 mm lateral to the bregma A polyethylene sleeve was fitted to penetrate 4 mm below the surface of the skull Penetration to this depth placed the tip of the needle in the lateral ventricle This was verified by dissection of fresh brains after the injection of indian ink Physostigmine hydrochloride (1 mg/kg) was injected subcutaneously 5–10 min before the injection of acetyl-^{3}H choline into the ventricle in order to inhibit hydrolysis of the administered acetylcholine Acetyl-^{3}H choline (5 μc 4 2 mg/mc) was drawn into a 50 μl Hamilton syringe fitted with the 27 gauge needle described here and injected into the lateral ventricle After 5 sec, the needle was withdrawn and the hole covered with the index finger to prevent leakage The animals were decapitated 30 min after injection of the acetyl-^{3}H choline. The brains were removed immediately and rinsed in cold 0 9 per cent sodium chloride They were then dissected in cold saline into nine regions Each brain portion was blotted, weighed and homogenized in 4–10 ml of cold 0 4 normal perchloric acid, according to the method of Hebb *et al* [5] After centrifugation, a 1 ml aliquot of the supernatant fluid was transferred to 10 ml of Bray's solution Total radioactivity was measured by means of a Nuclear Chicago liquid scintillation counter

Our results indicate that acetyl-^{3}H choline accumulated in the brain tissue The rate of disappearance of radioactivity from the whole brain was relatively rapid with reduction within 30 min to half the activity that was present at 2 min (Fig 1) If true uptake of the acetyl-^{3}H choline into the physiological pool of endogenous acetylcholine is assumed, our results indicate a rather rapid turnover of acetylcholine in the rat brain Analysis of various regions of the rat brain indicates (Table 1) that acetyl-^{3}H choline was not taken up equally The differences in uptake among some regions were highly significant We have included data on the occurrence of endogenous acetylcholine in Table 1 in order to enable comparison with the distribution of acetyl-^{3}H choline No data on the occurrence of endogenous acetylcholine in the hippocampus and diencephalon are available for comparison with our finding that the greatest accumulation of acetyl-^{3}H choline occurred in these regions The basal ganglia, where Votava[1] found considerable concen-

Table 1 ACCUMULATION OF ^{3}H-ACh IN VARIOUS REGIONS OF THE BRAIN

Region*	Acetyl-^{3}H choline (c p m /mg)						Endogenous† acetylcholine (μg/mg)
	1	2	3	4	5	Mean	
(1) Hippocampus	33 6	40 0	40 8	55 6	56 4	45 3	
(2) Diencephalon	34 4	30 0	23 6	39 2	21 2	29 7	
(3) Basal ganglia	17 2	16 8	19 2	—	—	17 7	4 073 ± 0 653
(4) Cerebral hem * exclusive of forebrain regions listed	9 2	15 6	18 8	—	—	14 5	2 195 ± 0 598 ‡
(5) Medulla	17 6	5 2	6 0	7 2	4 2	8 2	2 471 ± 0 573
(6) Mesencephalon	10 8	6 4	3 6	12 0	6 0	7 8	3 864 ± 0 550
(7) Cerebellum	12 4	6 0	4 4	8 4	5 6	7 4	1 360 ± 0 601
(8) Pons	7 6	4 0	—	4 8	6 0	5 6	
(9) Olfactory bulbs	1 6	0 8	0 8	1 9	1 1	1 2	

Acetyl-^{3}H choline (5 μc) was injected into the right lateral ventricle and the animals were killed 30 min later Various regions of the brain were assayed for total radioactivity

* Data from Z Fink cited by Z Votava[1]
† Arranged according to regions of decreasing concentration of acetyl-^{3}H choline
‡ Listed as cortex

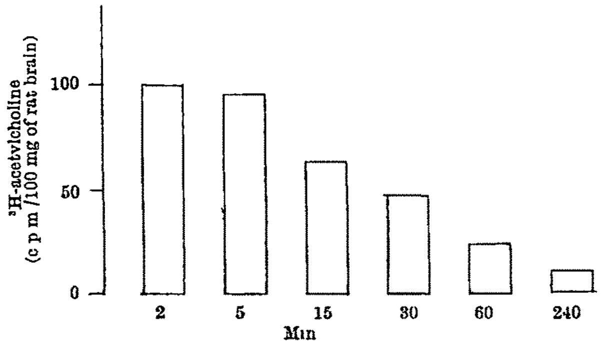

Fig 1 Rate of disappearance of acetyl-³H choline from the whole brain of rats Each point represents the mean of results in three rats each injected with 5 μc of acetyl-³H choline and decapitated at the times indicated on the abscissa

tration of acetylcholine, accumulated relatively large concentrations of acetyl-³H choline The observation by Votava that the cerebral cortex had more acetylcholine than did the basal ganglia cannot be compared with ours because the cerebral hemispheres we analysed included other telencephalic structures We found that the mesencephalon accumulated about 44 per cent as much acetyl-³H choline as the basal ganglia which is lower than the 95 per cent endogenous acetylcholine reported by Votava We are unable to account for these differences.

Although this study indicates that the brain takes up acetyl-³H choline, it is not yet possible to decide whether this is of physiological significance Passive uptake of ¹⁴C-acetylcholine by the mitochondrial, pinch-off nerve terminal, and synaptic vesicle fractions in rat brain[6] has been demonstrated The marked and consistent differences in regional distribution in our experiments suggest that the differential accumulation of acetyl-³H choline in various brain regions is not fortuitous, and may reflect the degree of involvement of acetylcholine in the activity of these regions

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[1] Votava, Z , *Ann. Rev Pharmacol* , 7, 223 (1967)
[2] Whittaker, V P , in *Progress in Brain Research*, 8 *Biogenic Amines* (edit by Himwich, H E), 90 (Elsevier Publishing Co , New York, 1964)
[3] De Robertis, E , in *Progress in Brain Research*, 8 *Biogenic Amines* (edit by Himwich, H E), 118 (Elsevier Publishing Co , New York, 1964)
[4] Bhagat, B , and Pradhan, S N , *Pharmacologist*, 8, 188 (1966).
[5] Hebb, C O , Morris, D , and Bull, G , *Nature*, 207, 1295 (1965)
[6] Burton, R M , *Intern J Neuropharmacol* , 3, 13 (1964)

Human Rod Visual Delay

In 1922, Pulfrich[1] noticed that when a swinging pendulum bob is viewed binocularly with a neutral density filter over one eye the bob appears to describe an ellipse The effect is caused by the extra delay associated with the lower illumination level in the filtered eye (Fig 1) This promoted interest in finding the relation between visual delay and intensity

Variations of visual delay with intensity have been measured electrophysiologically[2,3] It has been shown for animals that, whereas the total visual delay depends on the measuring point along the optic pathway, its variation with the intensity of stimulus is everywhere the same.

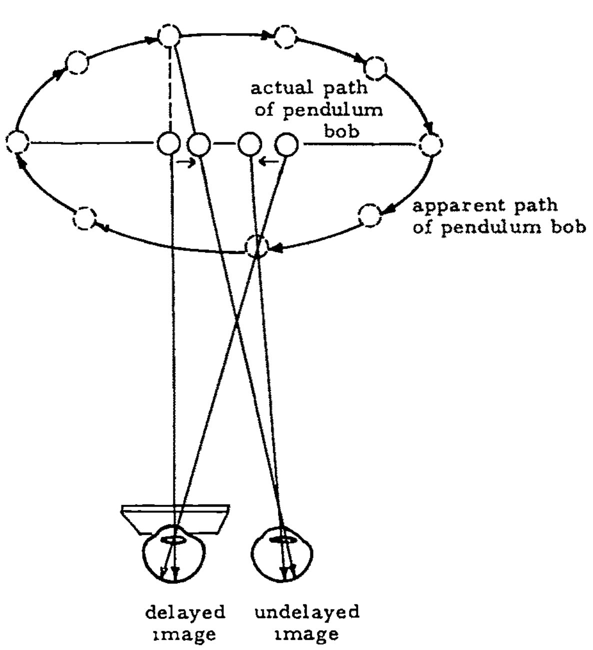

Fig 1 The Pulfrich pendulum for gaze centred straight ahead (Adapted from Gregory[7])

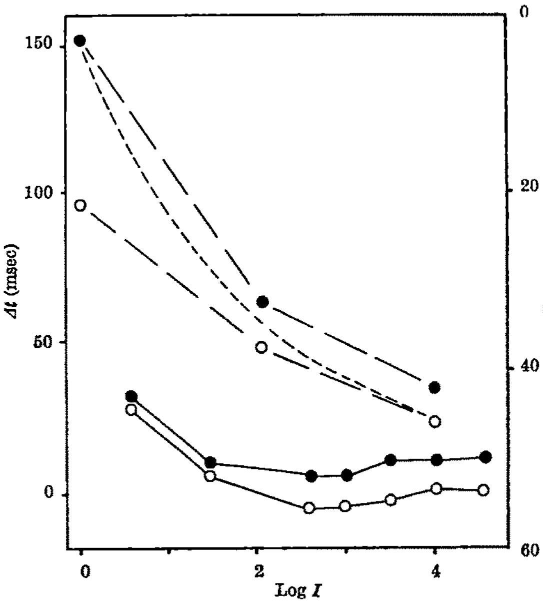

Fig 2 Delay versus intensity curves for rod vision obtained by Arden and Weale Their caption The change in the latent period of vision with intensity Ordinates on the left Δt in msec, on the right, degrees of episcotister scale Abscissa the logarithmic intensity scale expressed in multiples of the absolute threshold of vision, as measured with a 3 min field at 8° in the right visual field of the left eye ●, Observer G B A , ○, R A W Continuous line, foveal data, broken line, extra-foveal data, dashed curve, Bernhard's data (For details see text)

Thus variations must take place in the retina peripheral where all electrophysiological measurements have been made In 1954, Arden and Weale[4] measured human rod visual delay versus intensity by flashing a 3 min field for 3 msec at 8° off the centre of gaze Their methods relied on subjective reporting of simultaneity between two flashes Fig 2 shows that they agree well, however, with Bernhard, who arrived at his results by relating human EEG blocking times to stimulus intensities Weale[5] and Bornschein[6] had earlier verified that rods were active in photopic conditions, and that rod activity can be suppressed only at the highest levels of luminance So Arden and Weale further investigated the Pulfrich

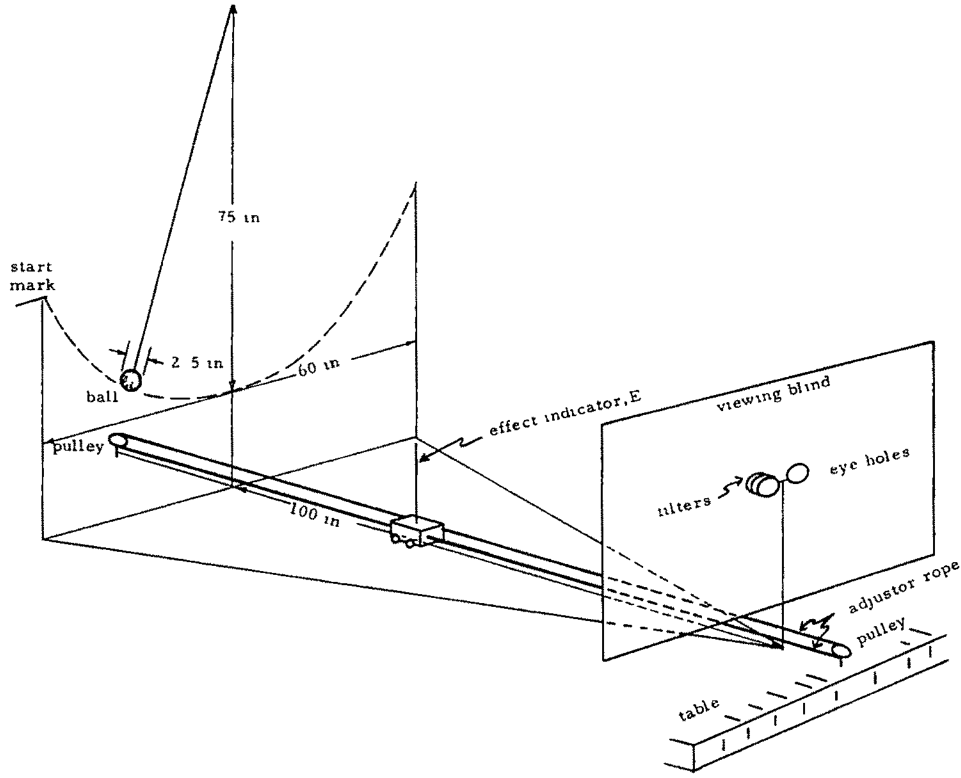

Fig. 3. Experimental apparatus

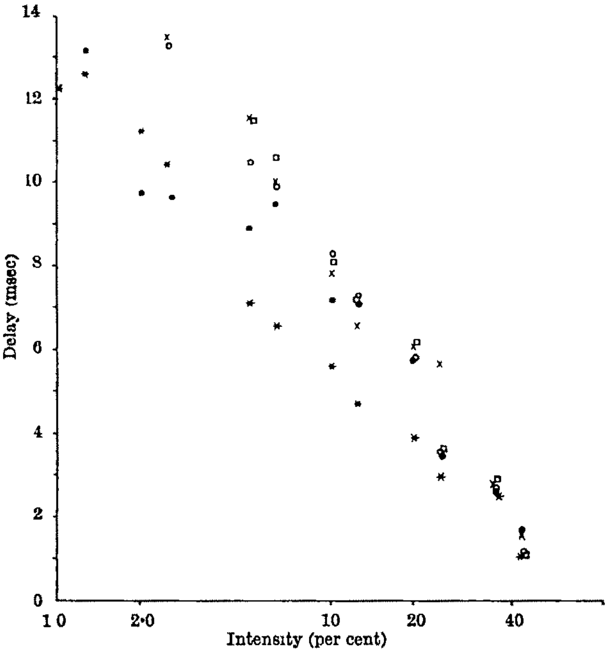

Fig. 4. Delay versus percentage of right eye light intensity admitted to left eye for five subjects. O, Mo; ×, Jane; □, Nancy; ●, Pam; *, Jeff

pendulum effect using only the shapes of their rod and cone delay curves and coloured eye filters. They concluded that the effect was predominantly the result of rod vision. They tacitly assumed that the curve of rod vision delay-versus-intensity is the same in natural visual conditions as it is for dark adapted eyes looking at 3 min spots for 3 msec in a darkened room. The multifarious effects of lateral inhibition in the retina[7-10] would seem to dictate against this unless variations in rod visual delay are wholly mediated within the rod receptor layer. We decided to use the Pulfrich pendulum to check this possibility.

Our experimental set-up is shown in Fig. 3. The 1·4° bright blue ball had a swing period of 2·9 sec and was boosted back to the start mark by an experimenter every three swings. The subject was asked to focus on the effect indicator, *E*, and by means of the adjustor ropes, to move *E* until it appeared to be just under the nearest point of the ball's apparent trajectory. This assured rod vision results. The experiment room was a small classroom, and was lit during each test by fluorescent overhead lights and early afternoon sunlight through windows to the left of and behind the ball. The illumination level was about 40 ft.-candles so, according to Rushton[11], our well adapted subjects had equilibrated at about 10 per cent rhodopsin bleach. Each subject performed an *E* alignment for each case over a random sequence of neutral density filter strengths. The tests on each subject were repeated several times during several days with the usual precautions. All subjects were between 20 and 40 yr old. The greatest effect was obtained with Nancy, who set *E* at 40″ on her side of the pendulum plane with a 320 reducing filter. At the point of fastest movement of the ball image on the retina, the image covered each rod for more than 20 msec. The ball image definitely produced an "on" effect in an otherwise static visual field filled with a variety of textures, colours and forms.

Our results are shown in Fig. 4. If percentage intensity were plotted on a linear instead of logarithmic scale, the shape of our curves would approximate Arden and Weale's, and Bernhard's. We must therefore conclude that the rod vision delay-versus-intensity relationship involves more than just 3 msec throughput receptor characteristics. Lateral interactions over the entire retinal field are centrally important.

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[1] Pulfrich, C , *Naturwissenschaften*, 10/25, 553 (1922)
[2] Adrian, E D , and Matthews, R , *J Physiol* , 64, 279 (1927)
[3] Bernhard, C G , *Acta Physiol Scand* , 1, Suppl 1 (1940)
[4] Arden, G V , and Weale, R A , *Proc Roy Soc* , B, 142, 258 (1954)
[5] Weale, R A , *J Physiol* , 114, 45 (1951)
[6] Bornschein, H , *Z Biol* , 105, 454 (1953)
[7] Gregory, R L , *Eye and Brain* (World University Library, 1966)
[8] Barlow, H B , *J Physiol* , 141, 337 (1958)
[9] Land, E H , *Sci Amer* , 84 (1959)
[10] Lettvin, J Y , *Proc Institute Radio Engineers*, 47, 1940 (1959)
[11] Rushton, W A H , and Campbell, F W , *Nature*, 174, 1096 (1954)

Distribution of Excitation and Inhibition in Receptive Fields of Lateral Geniculate Neurones

It has become apparent that the composition of receptive fields of cells in the visual system vary considerably with the species studied, the level in the visual system examined and the stimulus parameters used In spite of this variability, one characteristic form of receptive field has frequently been found at low levels in the visual system It is usually described as roughly circular in shape and is made up of concentric zones which have the same spectral sensitivity The important features of a unit with such a receptive field are that light stimulation in the centre of the field leads to either an increase or decrease in the cell's rate of discharge, while stimulation in the surround leads to a rate change in the opposite direction If both of these regions are stimulated together, the resultant response is a combination of the separate responses of the regions[1]. Thus it appears that at some peripheral point there is a combination of inputs so that the response of the cell indexes the amount of activation received by an excitatory system minus the amount of activation received by an inhibitory system

Two general models have been proposed to show how spatially distributed excitatory and inhibitory effects might be combined to produce the characteristics of the receptive fields described here The basic difference between these two schemes concerns the distribution of the surround mechanism, some assume[2-4] that the centre and surround mechanisms are spatially co-extensive, while others have suggested that there is a spatial discreteness for the centre and surround mechanisms with some overlap at the borders between the two[5] It has been specifically suggested that not only are the centre and surround mechanisms co-extensive, but that the surround mechanism has maximal sensitivity at the same point as the centre mechanism, so that it is chiefly the rate of fall-off in sensitivity that differentiates the two mechanisms The experiment reported here was designed to determine which of these two possibilities better describes the input to cells in the lateral geniculate nucleus of the squirrel monkey (*Saimiri sciureus*)

The activity of single cells in the lateral geniculate nucleus of lightly anaesthetized squirrel monkeys was recorded using micropipette electrodes and conventional recording methods, permanent records of cell activity and stimulus sequences were obtained for later analysis Receptive fields were analysed by presenting various light stimuli on a translucent tangent screen 1 5 m from the monkey Two types of stimulation were used narrow-band spectral stimuli were obtained from a grating monochromator behind the screen, and geometric forms (annuli, spots and so on) were projected on to the front of the screen using tungsten-filament projectors. In addition, full screen illumination was used to provide photopic adaptation conditions

Receptive fields of cells of the lateral geniculate nucleus were initially mapped using flashed spots of monochromatic light (duration, 1 sec, diameter, 20 min.) This procedure provided information on the spatial extent of the centre and the surround of the receptive field In addition, the spectral sensitivity of these two regions was determined, and only those cells that had the same spectral sensitivity throughout were used in this experiment Such cells are of the class previously studied in conditions of diffuse light stimulation, and are termed non-opponent or broad-band[6].

After this initial mapping, responses were obtained by presenting a 20 min spot, centred as accurately as possible in the middle of the centre region of the field Next an annulus was projected onto the screen and positioned so that its centre covered the centre of the receptive field (an area with a diameter of approximately 0 5°–1 2° for such cells) Sufficient time was allowed for the visual system to adapt to the various annular luminance levels, and responses to the centred spot were recorded for each such level In this way, responses were obtained for an annular luminance level equal to the background luminance and for several other levels up to as much as 2 5 $\log_{10}$ units higher

The two models of receptive field organization predict different results from this procedure If the surround and centre mechanisms are spatially discrete, the response to the centred spot should become no greater when annular adaptation is added On the other hand, if the centre and surround mechanisms are spatially co-extensive, adaptation to the surround at some levels might be expected to lead to an enhancement of the response to the centred spot because there should be a range of luminance levels that would affect the surround mechanism but have little effect on the centre mechanism Any adaptation of the centre mechanism, either in the surround from annular stimulation or because of light scatter back into the centre, could only lead to an attenuation of the response to centre stimulation

All cells tested gave a qualitatively similar outcome The results from six such cells are shown in Fig. 1, where the mean response of each cell to centre stimulation is plotted as a function of the annular luminance level and relative to the response without annular adaptation As can be seen, there is a range of luminance levels where the centre response is markedly enhanced over that obtained in the absence of annular adaptation Two points should be noted First, the enhancement effect typically occurs for annular adaptation levels only slightly

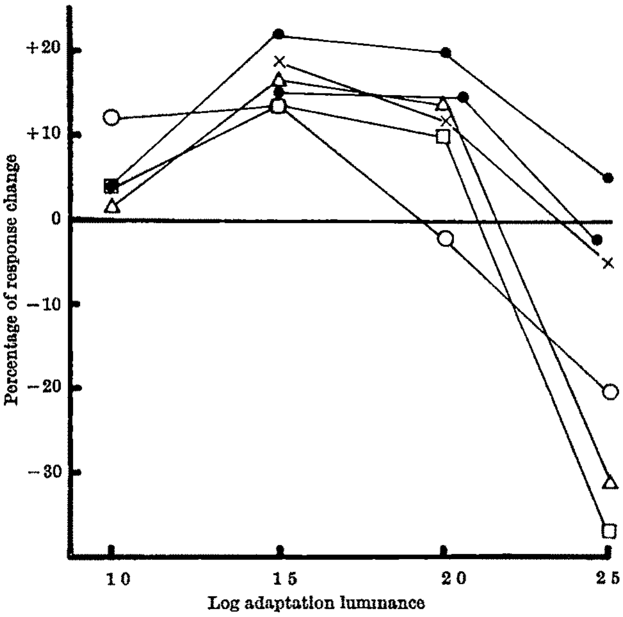

Fig 1 Change in response to centre stimulation for six lateral geniculate nucleus cells as a function of annular adaptation level Response change was calculated from a baseline condition consisting of no annular adaptation

above the background level. At some point above that the response to centre stimulation begins to decline, presumably because at those levels the annular light is also affecting the centre mechanism. Second, although there is variability from cell to cell in the intensity of annular adaptation necessary to produce enhancement, the magnitude of the maximum enhancement effect is roughly the same across cells. The latter feature would seem to indicate that the difference in sensitivity of the centre and the surround mechanisms in the surround is similar from cell to cell. That conclusion, however, seems unlikely in view of the fact that the balance between the centre and the surround of these cells seems to vary rather widely.

The results of this experiment suggest that the centre and surround mechanisms for these lateral geniculate nucleus cells must be to a large extent spatially co-extensive. It should be emphasized that the situation for lateral geniculate nucleus cells possessing spectrally selective receptive fields may be different from the cells examined here, although there is evidence to support the notion that some such cells may also have spatially co-extensive regions[5,7,8]. Finally, there is also the distinct possibility that even cells with the same sorts of receptive fields may be differently constructed in different animals; it has, for example, been reported that for lateral geniculate nucleus cells of this type in another monkey genus, *Macaca*, a centre stimulus is not made more effective by projecting a steady annulus on to the surround[5].

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[1] For example, Kuffler, S. W., *J. Neurophysiol.*, 16, 37 (1953).

[2] Rodieck, R. W., and Stone, J., *J. Neurophysiol.*, 28, 833 (1965).

[3] Bishop, P. O., and Rodieck, R. W., in *Information Processing in Sight Sensory Systems* (edit. by Nye, P. W.) (California Inst. Tech., 1965).

[4] Lamarre, Y., and Poggio, G., *Physiologist*, 10, 227 (1967).

[5] Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, 29, 1115 (1966).

[6] Jacobs, G. H., *Vis. Res.*, 4, 221 (1964).

[7] Wagner, H. G., MacNichol, jun., E. F., and Wolbarsht, M. L., *J. Opt. Soc. Amer.*, 53, 66 (1963).

[8] Michael, C. R., *Science*, 152, 1092 (1966).

Selection for the Absence of Scutellar Bristles

IN stocks of *Drosophila melanogaster* with a variable number of scutellar bristles, directional selection can increase the number of such bristles[1-3]. Directional selection for the removal of scutellar bristles, however, is rarely successful in the absence of either a sex linked mutant scute allele (*sc*) or a major mutant which similarly reduces the scutellar bristle number to less than four[1,2].

The base population for this experiment, *Vg*6, was homozygous for the recessive mutant vestigial wings (*vg*). This stock had been successfully selected for high sternopleural chaeta number for ninety-six generations[4] and subsequently maintained in mass culture for 6 yr.

Scutellar bristle number assays of *Vg*6 at 25° C showed that 4·8 per cent of females and 0·8 per cent of males had less than four scutellar bristles. Three females and one male each with three bristles were mated together and 125 flies of each sex were taken at random from the resulting progeny and assayed for scutellar bristle number. The female and male flies with the lowest number of scutellar bristles, regardless of the position of the missing bristles, were chosen as the two parents for the next generation. This selection and assay regime was repeated each fortnightly generation. Four single pair insurance cultures were also set up each generation from the flies with the next lowest bristle numbers to insure against the possibility that the best pair should fail to breed. All cultures and assays were made at 25° C.

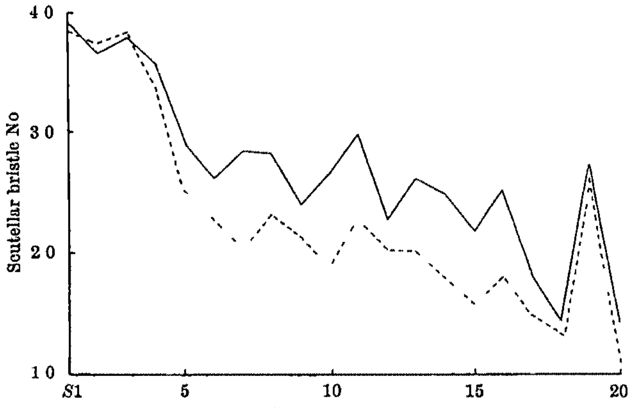

Fig. 1. Response to selection for low scutellar bristle number. ——, Females; - - - -, males.

The results (Fig. 1) show that selection was very effective in reducing the mean scutellar bristle number of both females and males from the four bristles characteristic of the base population. Male flies without any scutellar bristles appeared after only five generations of selection, and from *S*8 onwards neither of the two flies selected for the primary culture had any scutellar bristles.

It is interesting that even after this period, when the selection intensity was inevitably zero, the mean scutellar bristle number of the line continued to fall. The regression of mean scutellar bristle number on generations from *S*8 to *S*20 is negative and significantly different from 0 ($0·05 > P > 0·02$). It seems likely that for these thirteen generations natural selection increased the relative viability of low scutellar bristle number flies. Thus the proportion of flies with a low number of scutellar bristles among the progeny of selected parents increased and the mean bristle number of the line fell.

Each generation the number of bristles missing from the two anterior and the two posterior sites were separately scored. During the early generations of selection only anterior bristles were missing and in the whole experiment the proportion of missing posterior bristles never rose above 38 per cent (Fig. 2).

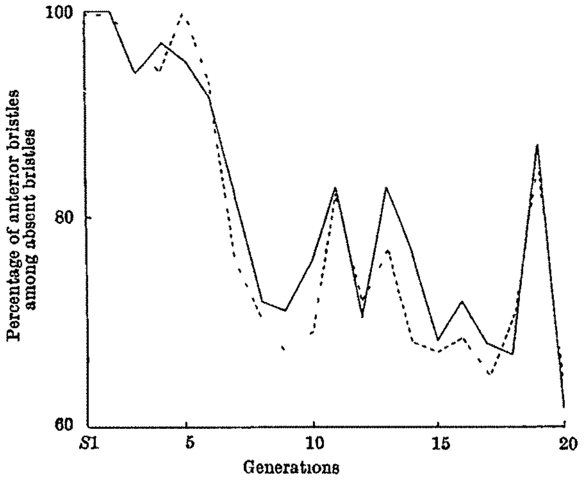

Fig. 2. The proportion of missing bristles at anterior sites in the selection line. ——, Females; - - - -, males.

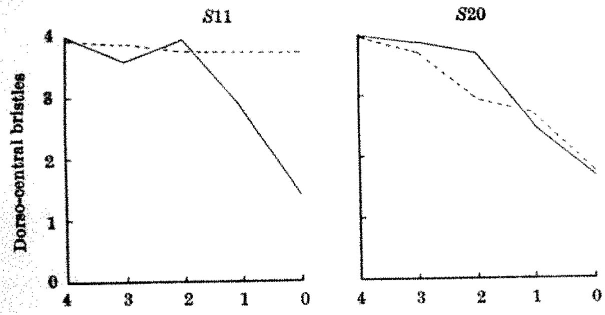

Fig. 3. The relationship between the number of scutellar bristles and the number of dorso-central bristles at generations S11 and S20. ——, Females; – – – –, males.

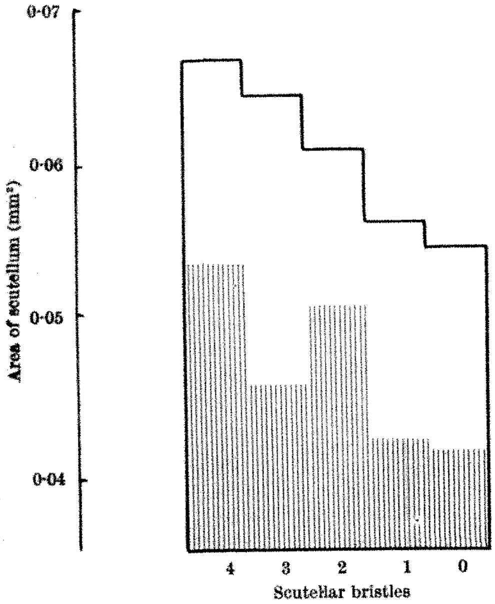

Fig. 4. The area of the scutellum in female flies of different scutellar bristle number phenotypes; shaded area, males.

This apparent gradient in the removal of scutellar bristles was reflected in the chaetaxy of the dorsal surface of the thorax. At generations *S*11 and *S*20 ten flies of each sex and scutellar bristle number phenotype were assayed for the number of dorso-central bristles on the thorax. These assays showed that selection for low scutellar bristle number had a correlated response in the dorso-central bristles which were reduced in number from the four bristles invariably present. Dorso-central bristle number was closely related to scutellar bristle number in females but less closely in males at *S*10. By *S*20 the correlation was similar in both females and males (Fig. 3). It was significant that 68 per cent of the missing dorso-central bristles were anterior in position.

The removal of scutellar and dorso-central bristles was associated with changes in the size of the flies. Flies with no scutellar bristles had a smaller mean scutellum size than flies with four bristles (Fig. 4 and Table 1). There were no corresponding changes, however, in the area of the thorax in the five scutellar bristle number phenotypes.

Table 1. ANALYSIS OF VARIANCE OF SCUTELLUM AREA FOR FLIES OF THE FIVE SCUTELLAR BRISTLE NUMBER PHENOTYPES (LOG TRANSFORMATION)

Source	*M.S.*	*d.f.*	*F*	Probability
Sex	0·2429	1	120·48	$P < 0{\cdot}001$
Scutellar bristle number phenotypes	0·0291	4	3·51	$0{\cdot}05 > P > 0{\cdot}01$
Sex x phenotypes	0·0013	4	< 1	$P > 0{\cdot}05$
Between individuals within sex and phenotypes (error)	0·0083	65		
Total		74		

Table 2. ANALYSIS OF VARIANCE OF CELL HAIR COUNTS PER UNIT AREA OF SCUTELLUM

Source	*M.S.*	*d.f.*	*F*	Probability
Sex	76·76	1	6·22	$P < 0{\cdot}05$
Scutellar bristle number phenotypes	13·69	4	1·11	$P > 0{\cdot}05$
Sex x phenotypes	11·94	4	< 1	$P > 0{\cdot}05$
Between individuals within sex and phenotypes (error)	12·34	20		
Between measures within individuals	2·95	120		
Total		149		

Table 3. MEAN SCUTELLAR BRISTLE NUMBERS IN ASSAYS OF *Vg*6 CHROMOSOMES ON A *y*, *bw*, *st* BACKGROUND IN MALES

		(Males hemizygous for a *y* marker *X* chromosome) Number of *Vg*6 third chromosomes	
		1	2
Number of *Vg*6 second chromosomes	1	4·0	4·0
	2	3·6	2·7
		(Males hemizygous for a *Vg X* chromosome) Number of *Vg*6 third chromosomes	
		1	2
Number of *Vg*6 second chromosomes	1	4·0	4·0
	2	3·5	2·6

Each epidermal cell in *Drosophila melanogaster* produces a single cell hair (Fig. 5) so that estimates of epidermal cell number can be made by counting the cell hairs in a defined area of the scutellum. The analysis of variance of cell hair assays (Table 2) shows that the only significant degree of freedom is associated with the difference in cell number between females and males. Thus the change in area of the scutellum resulted from a decrease in cell number rather than a decrease in cell size.

Crosses between female flies from the selected line and males hemizygous for sc^1 did not produce any flies with less than four scutellar bristles. Clearly the selection response was caused by factors other than mutant scute alleles which might have occurred and been selected for in the line. Whole chromosome analyses based on male flies from the F_2 of a cross between flies from the line and a standard stock homozygous for the recessive mutants *y*, *bw* and *st* (*X*, second and third chromosome markers respectively) confirm this finding (Table 3). None of the chromosomes derived from *Vg*6 had appreciable individual effects on scutellar bristle number relative to *y*, *bw*, *st*, when heterozygous or homozygous. Male flies heterozygous for the *Vg*6 third chromosome and homozygous for the *Vg*6 second chromosome, however, had a mean scutellar bristle number of 3·6 bristles/fly (Table 3). When the *Vg*6 third chromosome was also homozygous the mean was reduced to 2·7. Substitutions of *X* chromosomes from *Vg*6 did not have a significant effect on mean scutellar bristle number in males.

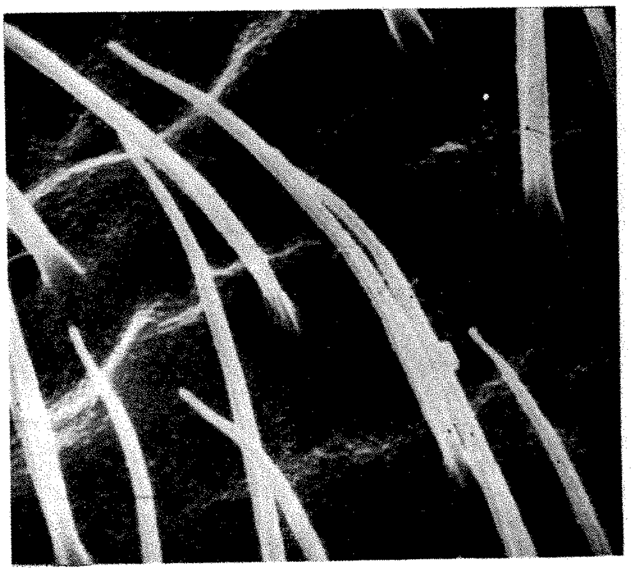

Fig. 5. Epidermal cell hairs on the scutellum (× *c*. 5,360).

These data confirm that by itself the mutant *vg* has little effect on scutellar bristle number in the line although some *vg* alleles do alter the orientation of the scutellar bristles. Nevertheless, the *vg* mutants affect the development of part of the same pair of dorsal mesothoracic imaginal disks that give rise to the dorso-lateral regions of the thorax and the scutellum. Thus the dorso-central bristles and the scutellar bristles derive from a common developmental pathway. As the scutellum was reduced in size but not the thorax, the data emphasize that fly size genes do not necessarily have general effects (compare with ref. 5).

Rendel[3] has shown that canalization of the number of scutellar bristles at four can be changed by the presence of mutant scute alleles. It may be that the mutant *vg* has a similar effect in the present line, for one of the located effective factors is closely linked to the *vg* locus. It is possible, however, that the long history of directional selection for high sternopleural chaeta number in *Vg6* has reduced the canalization of the scutellar phenotype to make the line responsive to directional selection for scutellar bristle number.

In view of the positive correlation between cell number and scutellar number at the end of the selection experiment, we can question whether the changes in canalization, revealed in this experiment, are in any direct way to be regarded as involving changes of the canalization system of scutellar bristle number and pattern. Rather, it seems that the factors determining the pre-pattern[6] of bristle number may have remained constant, but changes of cell number may have "removed" the possibility that the constant bristle pre-pattern be revealed, so that the canalization of scutellum shape and cell number may be the variable that has been manipulated.

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[1] Fraser, A., *Genetics*, **48**, 497 (1963).
[2] Payne, F., *Ind. Univ. Stud.*, **5**, 1 (1918).
[3] Rendel, J. M. R., *Evolution*, **13**, 425 (1958).
[4] Thoday, J. M., and Boam, T. B., *Genet. Res.*, **2**, 161 (1961).
[5] Spickett, S. G., *Nature*, **199**, 870 (1963).
[6] Stern, C., *Amer. Sci.*, **42**, 213 (1954).

Response of Neurones in the Motor Cortex during Performance of a Simple Repetitive Arm Movement

ANALYSIS of the firing pattern of single cells in the brains of unanaesthetized animals promises to be a useful approach towards understanding brain mechanisms involved in the control of movement. Evarts[1], studying reaction time in monkeys, observed that the firing rate of some pyramidal-tract neurones changes before the electromyographic potential associated with the behavioural response. Evarts's study provided valuable data on the response latency of pyramidal tract cell discharge before a conditioned arm movement. The reaction time task with non-human subjects has the disadvantage that protracted training is necessary to establish fast and stable responses[2], and relatively few behavioural responses are available each day for analysis in relation to unit activity.

We described earlier a technique for concurrent recording of physiological and behavioural data on a two-channel magnetic tape in which the tape recorder was modified so that it could be played backwards[2]. We used a backward summing technique as part of an analysis of muscle potentials occurring just before the behavioural response in a reaction time task. Here we describe an extension of the backward summing technique that shows changes in firing rate of units in the motor cortex of a monkey before and after the animal presses a lever.

A macaque monkey (*Macaca mulatta*) was kept in a primate restraining chair. The animal was first trained to depress a telegraph key to deliver a small food pellet. When the lever pressing response had been well established further responses were reinforced after a variable interval of time (with a mean of 32 seconds) had elapsed since the previous reinforcement (VI 32 schedule). After 2 weeks of training the animal was responding at a rate of about 1,000 responses/h throughout a 3 h daily session. When performance was stable, a stainless steel adaptor was implanted in the skull under general anaesthesia for later coupling with a microelectrode drive unit. The adaptor was positioned over the hand and arm area of the precentral cortex contralateral to the hand used in the training. The dura was left intact and was protected by a silastic rubber plug inside the steel adaptor. Tungsten electrodes etched in the manner described by Hubel[3] were insulated by repeatedly dipping them tip down in thickened Isonel 31 (Schenectady Chemicals, Inc., Schenectady, NY) and baking them tip down between each immersion. Six coats of 'Isonel' applied by this technique covered the electrode up to 10–15μ from its tip, and provided firm insulation which withstood repeated penetration of the dura.

During each recording session the monkey's head was loosely restrained to prevent gross movements. A remotely controlled hydraulic micromanipulator rigidly attached to the steel bone adaptor advanced the tungsten electrode through the rubber plug and dura into the brain. The electrode carrier was so arranged that the angle of penetration could be varied, permitting us to sample neurones within a circle of about 2·5 mm radius using the same skull mounted adaptor. Electrode penetrations were carried out daily while the animal was actively bar pressing. It was difficult to determine when the electrode actually entered the cortex, so the micromanipulator reading that corresponded to the first clearly identifiable unit activity was noted. Cells encountered deeper in the cortex were referenced to this first identifiable unit measure in order to provide a rough measure of each unit's relative depth. When a cell was sufficiently isolated, unit activity was recorded on one channel of a stereo tape recorder while the bar presses were recorded concurrently on the second channel. Data have been gathered on twenty-five well isolated units obtained in seven electrode penetrations. The spikes recorded were subsequently coded onto paper tape through a Schmitt Trigger circuit, and the pattern of cell activity was analysed by computing average firing rates in 20 msec class intervals from 500 msec before the bar press to 500 msec after the bar press. Cell firing prior to the bar press was analysed by playing the tape backwards and using the bar press time mark to initiate the computer's 500 msec analysis period. Cell firing after the bar press was similarly analysed by playing the tape forward. The number of behavioural responses used to establish the average firing rates varied from 38 to 200 depending on how long the cell was held.

Fourteen of the twenty-five cells from which we recorded changed their firing pattern before the behavioural response. This change took one of two forms: some cells that had a relatively low resting rate of discharge were phasically activated, while others that were tonically active transiently lowered their firing rate prior to the response. Representative single cell records and their averaged firing rates are shown in Fig. 1.

Although our measure of depth in the cortex was crude, it was sufficient to show that nearly all cells

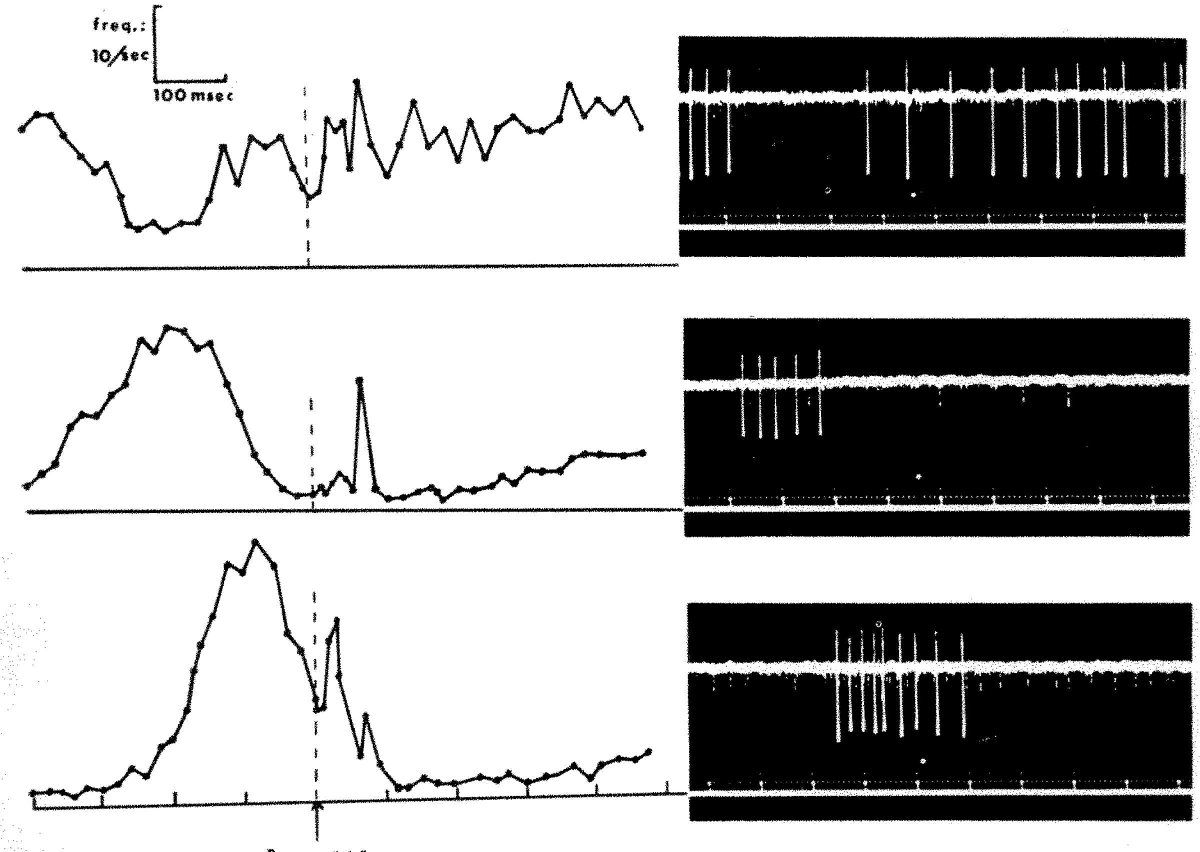

Fig. 1. Average firing rates and representative single cell recordings of three cells recorded in the same penetration. The oscilloscope photographs were made from taped records. The negative portions (downwards) of the units have been brightened by applying the negative portion of the input signal to the oscilloscope's intensity-modulation system. The bright dot above each time line indicates the occurrence of the bar press. Time marks are 10 and 100 msec. These three cells, recorded in the deeper layers of the cortex, showed consistent modification of their firing pattern before the response. Two cells isolated in the same penetration at more superficial levels had no apparent correlation of firing pattern with the response. The sharp post-response deflexion seen in the middle graph consists of a single discharge at a fixed interval after the response. Three cells of the sample had this characteristic.

consistently modified by the response were relatively deep in the cortex. The first few cells in any penetration had very low spontaneous rates, and became intermittently active in bursts with no apparent correlation to the monkey's bar pressing.

Although we do not know whether the deep cells were pyramidal tract neurones or not, the general form of the response patterns confirms the "tonic" and "phasic" distinction made for pyramidal tract cells by Evarts[4]. Moreover, their depth in the cortex suggests that the recorded cells lie in the layers of the precentral cortex which give rise to cortical efferents. Even the most strongly modulated phasic cells did not fire faster than 50/sec. This supports Evarts[5] suggestion that precentral cells in the awake animal may be subject to a frequency-limiting mechanism.

Our technique of recording and analysis offers some advantages for study of brain mechanisms in movement. The method may be readily adapted to other sites in the brain or to other movements.

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[1] Evarts, E. V., *J. Neurophysiol.*, **29**, 1011 (1966).
[2] Luschei, E., Saslow, C., and Glickstein, M., *Exp. Neurol.*, **18**, 429 (1967).
[3] Hubel, D. H., *Science*, **125**, 549 (1957).
[4] Evarts, E. V., *J. Neurophysiol.*, **28**, 216 (1965).
[5] Evarts, E. V., *J. Neurophysiol.*, **27**, 152 (1964).

MICROBIOLOGY

Chemotaxonomic Studies of some Gram Negative Bacteria by means of Pyrolysis–Gas–Liquid Chromatography

THERE has recently been a great need in medical microbiology for more rapid and objective methods of differentiating pathogenic micro-organisms. Earlier work[1,2] indicated that sub-species differences among diverse taxa could be detected by means of gas-liquid chromatography of their whole cell pyrolysis products. The present study confirms these results and shows that the distribution of pyrolytic products is a constant factor and is directly related to the chemical structure of the micro-organism.

The biochemical and serological procedures employed in the characterization of the cultures used were the same as those described by Ewing[3] and Edwards and Ewing[4], which were similar to those given by Kauffmann[5,6] and Ørskov[7]. Smooth cultures of *Escherichia coli* and *Shigella* serotypes as well as representative examples of other genera were inoculated into trypticase soy broth. The tubes were incubated with agitation for 6 h at 37° C in a water bath. Growth from these cultures was used to inoculate large (200 × 35 mm) plates, each of which contained either 350 ml. of freshly prepared meat infusion agar medium or a simple peptone yeast extract medium. A sample (1·2 ml.) of the broth cultures was spread on the surface of each plate. After incubation for 21 h at 37° C, the growth from each plate was collected in 10 ml. of distilled water containing 0·5 per cent formalin, centrifuged (10,000*g* for 15 min), resuspended in the same diluent and recentrifuged. The cells were washed three times in this way. The heavy suspensions of washed cells were lyophilized and stored at room temperature in screw-capped bottles.

Details of the pyrolysis–gas–liquid chromatography (PGLC) procedure and equipment used have been described elsewhere[2]. In brief, the method consists of subjecting a pellet of dry bacteria (100–275 ± 1·0 μg) to a rigidly controlled pyrolysis. A gas chromatographic system directly connected to the pyrolyser separates the thermal fragmentation products. A pyrogram or graph showing the pattern of these products is traced on a strip chart recorder.

One question which arises is whether the criteria used for distinguishing organisms are in fact related to differences in their chemical structure and not related to variations inherent in the technique. Instrument variation is not a significant factor, as numerous tests have shown. In a typical example, a single sample of *E. coli* 0105 was analysed five times in succession. Weights of samples varied from 143 to 200 μg. Individual peaks had retention times ranging in mean absolute deviation from 1 to 4 sec. Amplitudes of individual peaks (concentration) indicated good precision as well. The following is more important, however, from the standpoint of diagnosis: pyrograms from a variety of micro-organisms (2,400 analyses) show that certain areas or peaks of the profile are characteristic. These characteristic peaks, used to distinguish organisms, are constant and reliable. Such peaks seem to be unaffected by a number of factors, and these will be discussed.

The investigation involved sixteen 2 year old cultures analysed previously and thirty-three fresh cultures. The fresh cultures were coded and their antigenic schema was not available to the analyst before all pyrograms were completed. Every sample was analysed at least twice on each gas–liquid chromatography column. Although columns were prepared in an identical way, pyrograms of a single organism, analysed on different columns, may show some modification in pattern. In all, 316 analyses were recorded.

When the analyses were completed, pyrograms of the coded samples were sorted into five groups. These divisions were based on the ratios of two characteristic peaks: peak 8 (R_T, 900 sec) and a double peak, 9*a* and 9*b* (R_T, 1,010 sec). Group 1 consisted of nine profiles with peak 9 taller than peak 8. All of these were later found to be 055 serotypes. Group 2 comprised four pyrograms with peak 9, in this case, shorter than peak 8; group 3 was made up of five pyrograms with peak 9 equal to peak 8. Groups 2 and 3 proved to be *E. coli* 0111 serotypes. Group 4 included fifteen samples in which peak 8 was missing altogether. This group was further divided into those pyrograms with (1) 9*a* taller than 9*b*, and (2) 9*a* equal to 9*b*. The latter were found to belong to *E. coli* 0 group 105 (formerly designated 0115) (ref. 1) and *Shigella boydii* 10. In addition, some groups contained pyrograms which were identical in every respect (obviously duplicates). Conversely, some pyrograms within a group had small differences. At this stage, it could not be established whether these small differences were consistent, thus characteristic ones, so no further subdivisions were made.

When the identity of the samples was disclosed, nine serotypes had, in fact, been represented in the five groups. Now, however, additional subdivisions, based on small differences in the profiles already recorded, could readily be made. The small differences proved to be characteristic of the serotypes in question.

Table 1. DIFFERENTIATION OF PYRO-CHROMATOGRAMS

GLC column 1

E. coli culture and analysis No.[a]	6 Peak *a, b, c, d*	4 Peak $4a \lessgtr 4b$	Peak 8 : 9	$9a \gtrless 9b$
0111*a*, 011*b* : *B*4 : *H*12† 180^{c}, 181^{c}, 190^{e}, 191^{e}, 220^{d}, 221^{d}	. . .	=	>	>
0111*a*, 0111*b* : *B*4 : *H*12* 176^{c}, 177^{c}, 184^{e}, 185^{e}, 231^{d}, 232^{d}	. . .	=	>	>
0111*a*, 0111*c* : *B*4 : *H*4 194^{e}, 195^{e}, 214^{c}, 215^{c}	. . .	=	>	>
055 : *B*5 : *H*6 + 212^{c}, 213^{c}, 224^{e}, 225^{e}	. . .	=	<	>
055 : *B*5 : *H*6 + + 206^{c}, 207^{c}, 226^{e}, 227^{e}	. . .	=	<	>
055 : *B*5 : *H*7 192^{e}, 193^{e}, 198^{c}, 199^{c}, 208^{d}, 209^{d}	. . .	=	<	>
0127 : *B*8 210^{d}, 211^{d}, 216^{e}, 217^{e}	. . .	⩾	. . .	>
01 : *K*1 : *H*7 188^{e}, 189^{e}, 196^{d}, 197^{d}, 202^{c}, 203^{c}	$b \gg a$	=	. . .	>
06 : *K*13 : *H*1 174^{d}, 186^{d}, 187^{d}, 204^{c}, 205^{c}, 175^{e}	. . .	<	. . .	>
0105 222^{d}, 223^{d}, 228^{e}, 229^{e}	$a > b$	=	. . .	=
S. boydii 10 218^{d}, 200^{c}, 201^{c}, 182^{e}, 183^{e}, 219^{d}	$a < b$	=	. . .	=

GLC column 2

E. coli culture and analysis No.[a]	Notched peak 6	Single peak 6	Peak 8	$8 \geq 9$ $\lessgtr$	9*a*/9*b* ratio
0111*a*, 0111*b* : *B*4 : *H*12† 352^{b}, 352^{b}, 356^{c}, 357^{c}, 384^{e}, 390^{d}	+	−	+	>	1·6
0111*a*, 0111*b* : *B*4 : *H*12* 354^{c}, 355^{c}, 385^{e}, 389^{d}	+	−	+	>	1·6
0111*a*, 0111*c* : *B*4 : *H*4 403^{c}, 383^{e}, 391^{d}	+	−	+	≤	2·2
055 : *B*5 : *H*6 + 360^{c}, 380^{e}, 381^{e}, 394^{d}, 395^{d}	+	−	+	≤	2·0
055 : *B*5 : *H*6 + + 363^{c}, 379^{e}, 396^{d}	+	−	+	≤	2·0
055 : *B*5 : *H*7 359^{c}, 382^{e}, 392^{d}, 393^{d}	+	−	+	≥	2·0
0127 : *B*8 371^{c}, 376^{e}, 399^{d}, 400^{d}	−	+	−	. . .	2·0
01 : *K*1 : *H*7§ 370^{c}, 377^{e}, 398^{d}	−	+	−	. . .	2·0
06 : *K*13 : *H*1 372^{c}, 373^{c}, 374^{e}, 401^{d}	−	+	−	. . .	2·0
0105 367^{c}, 369^{b}, 378^{e}, 397^{d}	−	+	−	. . .	1·0
S. boydii 10 361^{b}, 368^{c}, 387^{e}, 388^{d}	−	+	−	. . .	1·0

[a] Analysis No.—denotes sequential order of analysis.

[b] Old BAB, samples stored 2 yr. BAB, blood agar base.

[c] New BAB; inoculation of same cultures as *w*, 2 yr later.

[d] 3 week BAB (cultures inoculated afresh, 3 weeks after [c]).

[e] Peptone media.

§ Prominent peak between 6 and 7.

* 492–64 0111*a*, 0111*b* : *B*4 : *H*12.

† 1004–64.

+ 881–63 055 : *B*5 : *H*6

+ + 2032–63.

Typical pyrograms of each group are shown in Fig. 1. On both chromatography columns, pyrograms fall into two principal patterns: on column 1, those with a peak at position 8 were *E. coli* 0 groups, 111 and 55. All others lacked this peak. The same divisions appeared in analyses performed on column 2. Those profiles having peak 8 on column 1, however, now had a double peak 6 on column 2; those lacking peak 8 on column 1 now had a single peak 6. On both columns these characteristic peaks always had a retention time close to 18 min. *E. coli* 0 group 1, unlike all the others, showed a prominent peak between 6 and 7. These findings indicate a relationship between organisms within each of the two principal groups. As far as is known, such a relationship has not been established by serological or other methods currently used.

In this investigation, experiments were designed to achieve several objectives, as follows. In a series of experiments on coded samples, one test was designed to measure how well closely allied organisms could be distinguished. Two samples of *E. coli*, 0111*a*, 0111*b* : *B*4 : *H*12 and 0111*a*, 0111*c* : *B*4 : *H*4, which differ only in the

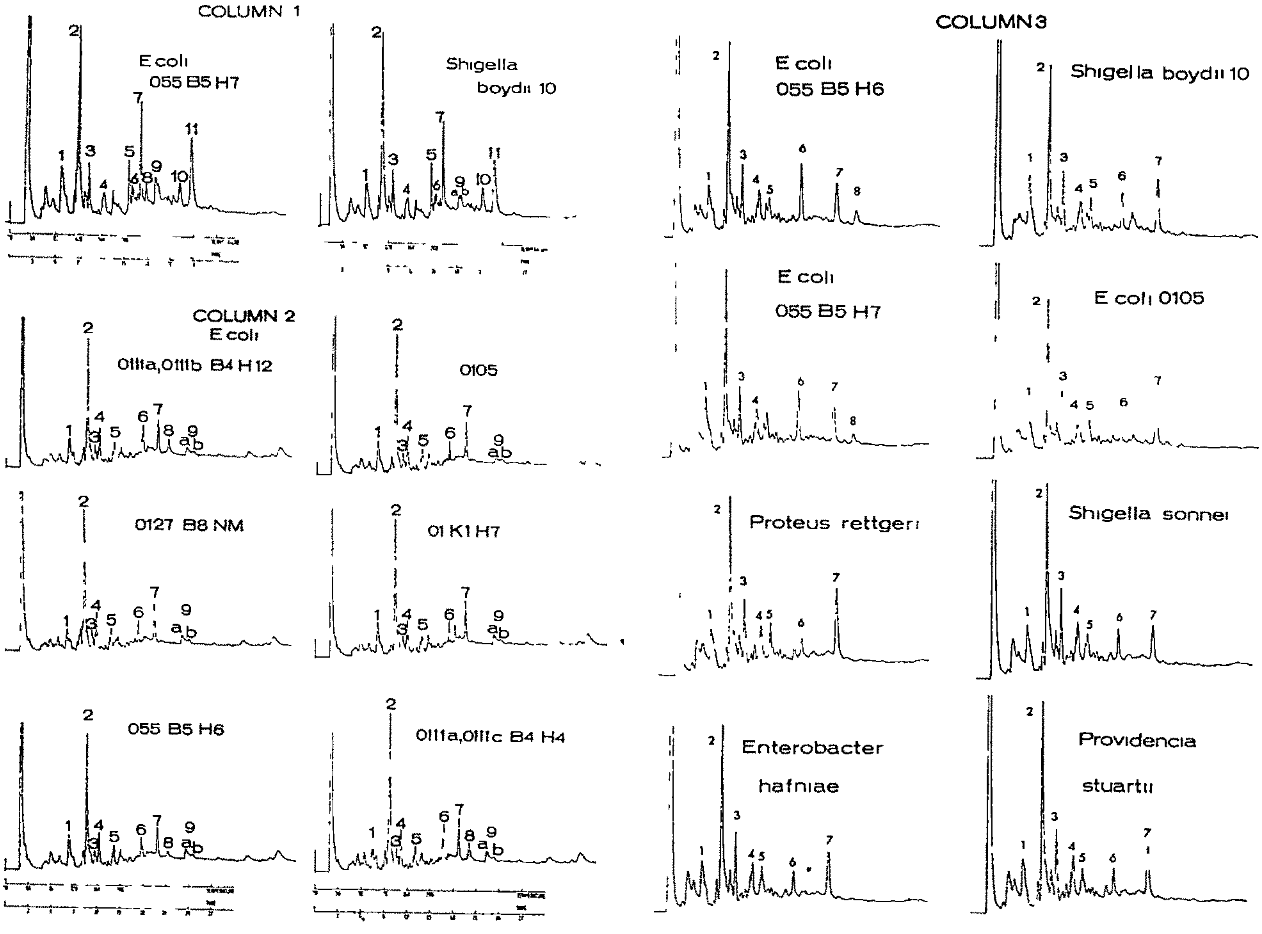

Fig. 1

somatic antigen sub-type and in the kind of flagellar antigen, were analysed Pyrograms showed consistent differences in ratios of peak heights of peaks 9*a* and 9*b* (see Table 1, gas–liquid chromatography column 2)

In another example (Fig 1) are shown pyrograms of *E coli* 0105 and *Shigella boydii* 10 Because these organisms share O and K antigens, serological differentiation is very difficult On the other hand, in their respective pyrograms, recognizable differences in pattern are shown between peaks 6 and 7

In addition, *Shigella sonnei*, which has no serological relationship to the other two, differs only between peaks 6 and 7. *A priori*, if pyrolysis profiles are indeed a reflexion of the original antigenic structure of the micro-organism, a much greater difference should be expected in a comparison of *S sonnei* and *E coli* 0105 than with *S boydii* 10 and *E coli* 0105 Such differences, however, are not revealed (Fig 1), it is therefore concluded that pyrograms do not always show a direct association with serological findings and therefore they must be showing other differences

Serological data are lacking for *Enterobacter hafniae* Together with *Providencia stuartii*, they give similar pyrograms (Fig 1; slight differences at peaks 4 and 5) These are, in turn, quite different from the profiles obtained from all cultures of *E coli* which have been studied

In a second test, two cultures of *E coli* 0111*a*, 0111*b* *B*4 *H*12, each isolated from different sources (human), were analysed Their PGLC profiles were superimposable

In another example, two organisms which were of the same serotypes, 055 B5 *H*6, but derived from different human sources, again yielded pyrolysis profiles which were identical when superimposed In addition, profiles from a variety of non-enteric pathogens, obtained in the same conditions, also have given superimposable pyrograms

A third set of tests concerned the following effects changing the culture media (blood agar base to peptone yeast extract), inoculating and collecting cells at different times (during 3 weeks), varying the storage period of lyophilized cells (days, up to 2 yr) In each instance, pyrolysis profiles, when judged by their characteristic peaks (peaks which impart uniqueness), remained unchanged by these modifications

A fourth test was designed to see whether the method could permit differentiation between even more closely related organisms Two *E coli* cultures of known serotype, 055 *B*5 *H*6 and 055 *B*5 *H*7, were chosen for comparison As far as is known, they differ only in their flagellar, H, antigens The H antigens are thought to be composed almost entirely of a protein, flagellin, which represents up to 2 per cent of the dry weight of the cell[8]. The analytical results, illustrated in Fig 1, show that a small peak, 2*a*, which follows the notched peak 2, appears below the notch on H6; above the notch on H7 Peaks 4 and 5 also show different relationships These small differences appeared consistently in every pyrogram (twenty-four) of the two samples

The fine distinction between these organisms, based on what seems to be a minute amount of dissimilar protein, raises the interesting point as to what specific chemical structures or combinations are contributing to the total pyrolysis pattern This question, as well as investigations on cell wall antigen structures and electronic data processing, is under study

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[1] Reiner, E , *Nature*, **206**, 1272 (1965)
[2] Reiner, E , *J Gas Chromat* , **5**, 65 (1967)
[3] Ewing, W H , *Pub Health Surv Publ No. 734* (US Gov. Print Off , Washington, DC, 1960, rev 1962)
[4] Edwards, P R , and Ewing, W H , in *Identification of Enterobacteriaceae* (second ed) (Burgess Publishing Co , Minneapolis, Minn , 1962)
[5] Kauffmann, F , *J Immunol* , **57**, 71 (1947)
[6] Kauffmann, F , in *Enterobacteriaceae* (second ed) (Munksgaard, Copenhagen, 1954)
[7] Ørskov, F , *Acta Path Microbiol Scand* , **29**, 373 (1951), **32**, 241 (1953).
[8] Rose, A H , in *Chemical Microbiology*, 7 (Butterworth and Co , Ltd , London, 1965)

BIOLOGY

Natural Selection by Glow-worms in a Population of *Cepaea nemoralis*

There are very dense populations of *Cepaea nemoralis*, the polymorphic land snail, on the sand dunes of the nature reserve of Newborough Warren in Anglesey, Wales They occur sporadically, chiefly in areas of dune colonized by *Salix repens*, and contain individuals of the usual phenotypes—yellow or pink and banded or unbanded Some populations also contain considerable numbers of the brown phenotype While sampling one of these populations with Dr K Isarankura, glow-worms were occasionally to be found feeding on the snails During a survey of the area at night, about thirty-three glow-worms were seen in an area of 10 m square, the actual population no doubt being denser than this Glow-worms may therefore well be an important predator of *Cepaea* and if they preferred one phenotype to another they might have a strong selective effect. An experiment was set up to test for any preferences the glow-worms may have for particular phenotypes A glow-worm was put in a bowl with a choice of two snails to eat The choices were either of brown and banded yellow or of unbanded and banded yellow The experiments were carried out in the autumn of 1965 and 1967 Table 1 gives the data when the choice was of brown and banded yellow and Table 2 the data for unbanded and banded yellow The results show that both brown and unbanded yellow are preferred to banded yellow but that there is heterogeneity in the results for unbanded and banded yellow If the data in Tables 1 and 2 are combined, the overall preference for brown or unbanded yellow becomes very significant ($0{\cdot}005 > P > 0\ 001$), the heterogeneity in the combined data is not quite significant If there is significant variation in the results of the experiments, it could be caused by a varying preference in different glow-worms The data for 1967 were obtained by using some of the glow-worms more than once The sequences of their choices were recorded the data and their analysis are shown in Table 3 There is no evidence for a correlation between successive choices of a glow-worm and no evidence therefore for variation in preference between glow-worms

Table 1 NUMBER OF SNAILS EATEN WITH A CHOICE OF BROWN AND BANDED YELLOW

	Brown	Banded yellow	Totals
1965 Adults	14	10	24
1965 Young	15	8	23
1967 Adults	15	8	23
Totals	44	26	70

Effect tested by χ^2	Degrees of freedom	Value of χ^2	Probability
Deviation of observed Nos eaten from 1 1 ratio	1	4 6286	0 05 > P > 0 025
Heterogeneity (variation between experiments)	2	0 3201	0 9 > P > 0 8

Table 2 NUMBER OF SNAILS EATEN WITH A CHOICE OF UNBANDED AND BANDED YELLOW

	Unbanded yellow	Banded yellow	Totals
1965 Adults	17	2	19
1965 Young	13	15	28
1967 Adults	8	4	12
Totals	38	21	59

Effect tested by χ^2	Degrees of freedom	Value of χ^2	Probability
Deviation of observed Nos eaten from 1 1 ratio	1	4 8983	0 05 > P > 0 025
Heterogeneity	2	9 1823	0 025 > P > 0 01

Table 3. SEQUENCES OF PHENOTYPES EATEN BY GLOW-WORMS THAT FED MORE THAN ONCE

No of glow-worm in experiment	Sequence eaten
*G*1	Bd Y , Unbd Y , Unbd Y., Unbd. Y.
*G*7	Bd Y , Unbd Y
*G*10	Bd Y Bd Y
*G*11	Bd Y , Bd Y
*G*20	Brown, Brown, Brown
*G*21	Brown, Brown, Bd Y , Brown
*G*22	Bd Y , Bd Y
*G*24	Brown, Brown
*G*25	Brown, Brown

Phenotype of second snail of a pair to be eaten	Phenotype of first snail of a pair to be eaten		Totals
	Brown or Unbd Y	Bd Y	
Brown or Unbd Y.	7	3	10
Bd Y	1	3	4
Totals	8	6	14

Fisher's exact test for both tails of the distribution gives a probability of $P = 0\ 245$ which is without significance

The preference shown by the glow-worms in these experiments may explain a sharp cline in the frequencies of the brown phenotypes The population we studied extends along a small dune for about 150 yd In the area where the glow-worms are found at the northern end of the population browns occur with a frequency of about 10 per cent But at the extreme southern end of the population about 40 per cent are brown, the frequency of brown decreasing sharply over about 80 yd The glow-worms are not to be seen where the browns are common Because there is almost a 2 to 1 preference for brown by the glow-worms it is tempting to suggest that the cline of decreasing brown is caused by increasing glow-worm predation It is therefore possible that the glow-worm predation may be a factor affecting the "area effects" that were extensively studied by Cain and Currey[1] They showed that in certain areas the frequencies of the different phenotypes in populations of *Cepaea* were not correlated with the background of the environment from which the populations were sampled Visual selection of the phenotypes by birds was presumably not the factor determining the frequencies Glow-worms might, however, be a selective predator Cain and Currey found populations with high frequencies of browns and noted that they were usually in areas where cold air might be expected to accumulate on clear nights suggesting that brown might be hardier, but it would be interesting to know if glow-worms were present or not

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[1] Cain, A J , and Currey, J D , *Phil Trans Roy Soc* , B, **246**, 1 (1963)

Production of Auxin by Detached Leaves

In senescent leaves proteins are hydrolysed to amino-acids and peptides[1], which might be expected to release protein-bound auxin and also to provide considerable amounts of tryptophan which can be converted by many plant tissues to the auxin indolyl-3-acetic acid (IAA). We have therefore investigated the concentrations of auxin in senescent leaves.

Mature trifoliate leaves from plants of *Phaseolus vulgaris* and leaves from young plants (2–3 weeks old) of *Avena sativa* were detached and placed with their petioles or bases in distilled water in the dark at 25° C. In these conditions, the leaves become senescent and turn yellow. Samples were taken at various times (at intervals of 1 or 2 days), weighed and stored in the deep freeze until they were extracted with peroxide-free ether for 3 h at 0° C. The ether extract was partitioned and the acidic fraction was run on paper chromatograms with isopropanol : ammonia : water (8 : 2 : 1 v/v). The zone corresponding to IAA was eluted and the auxin was estimated using an *Avena* coleoptile straight growth bioassay. The amounts of auxin extracted from the leaves at various times are shown in Figs. 1 and 2.

It can be seen that in both cases there is a large increase in the amount of auxin present over a period of 6 days. The amounts measured represent the resultant of auxin production and auxin destruction: in the case of *Avena*, after about the fourth day the rate of destruction exceeds the rate of production. The fall in total auxin was observed in each of six experiments. Libbert *et al.*[2] maintain that most of the IAA which can be extracted from plants is formed by epiphytic bacteria, but because plants do not normally grow in sterile conditions this alleged bacterial production of IAA is likely to be very significant physiologically. Thus although the experiments reported here were not performed in sterile conditions, the results are relevant whether or not the auxin is formed in part by epiphytic bacteria.

Young expanding leaves are an important source of auxin in the plant and concentrations of auxin in mature leaves are low[3-5]. The concentrations of auxin in attached senescent leaves, however, have received little attention, and such measurements as have been made have been based on relatively infrequent sampling (usually at intervals of about 10 days). A peak of auxin production during leaf senescence could therefore have been missed. The level of auxin in leaves and petioles is involved in the control of abscission[6] so the production of auxin by senescent leaves, if it is a general phenomenon, may be an important factor which so far has been overlooked.

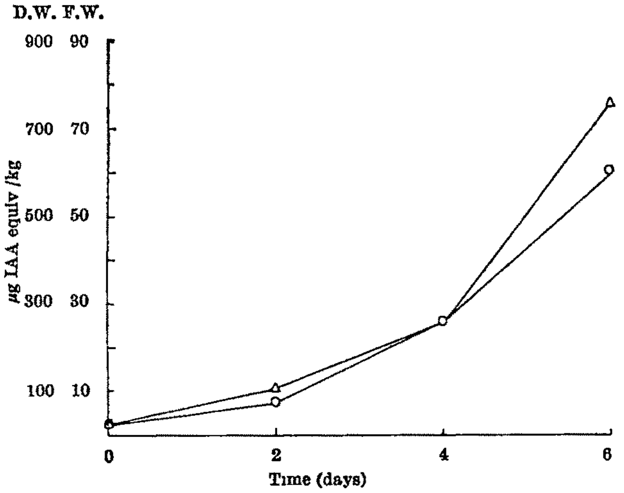

Fig 1. Auxin levels in detached *Phaseolus vulgaris* leaves. Expressed on dry weight (D W, ○) and fresh weight (F W, △) basis.

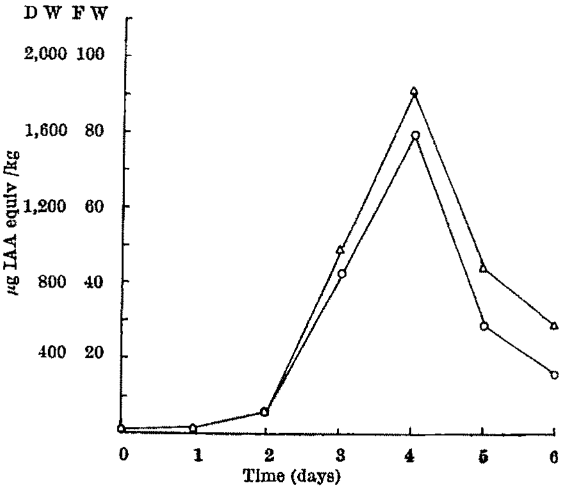

Fig 2. Auxin levels in detached *Avena* leaves. Expressed on dry weight and fresh weight basis as in Fig 1.

We have suggested elsewhere[7] that dying cells are a principal source of auxin in the plant. Because cell death is frequent in normal growth, differentiation and development of the plant, the production of auxin by senescent, dying leaves may be only one example of a much more general process.

One of us (A. R. S.) thanks the Science Research Council for a grant during the tenure of which the work was done.

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[1] Chibnall, A. C., *Protein Metabolism in the Plant* (Yale Univ. Press, New Haven, 1939).
[2] Libbert, E., Wichner, S., Schiewer, U., Risch, H., and Kaiser, W., *Planta*, 68, 327 (1966).
[3] Goodwin, R. H., *Amer. J. Bot.*, 24, 43 (1937).
[4] Shoji, K., Addicott, F. T., and Swets, W. A., *Plant Physiol.*, 26, 189 (1951).
[5] Jacobs, W. P., and Morrow, I. B., *Amer. J. Bot.*, 44, 823 (1957).
[6] Jacobs, W. P., *Ann. Rev. Plant Physiol.*, 13, 403 (1962).
[7] Sheldrake, A. R., and Northcote, D. H., *New Phytol.*, 67, 1 (1968).

GENERAL

"Least-squares" Estimation of Distribution Mixtures

The statistical estimation of the unknown fractions Π_i ($i = 1, \ldots k$), and, when unknown, further parameters $\theta_j (j = 1, \ldots m)$, in a mixed population which is denoted by its cumulative distribution (taken to be univariate)

$$F(x) = \sum_{i=1}^{k} \Pi_i F_i(x)$$

is, in general, cumbersome. It therefore seems worth noting the convenience and comparative efficiency, at least in relation to the estimation of the Π_i, of estimators of "least-squares" type, defined by the minimization of the integral

$$\int (\mathrm{d}F_s - \mathrm{d}F)^2/\mathrm{d}G$$

where $F_s(x)$ denotes the cumulative distribution of the empirical sample (from, for example, n independent observations), and $G(x)$ is a suitable increasing function of x. Taking for simplicity the case $m = 0$, the estimators of the Π_i are automatically unbiased with exact variances readily calculable, and with asymptotic normality. For example, if $k = 2$, we have an estimate p_1 for Π_1,

$$p_1 = \frac{\int (\mathrm{d}F_s - \mathrm{d}F_2)H}{\int (\mathrm{d}F_1 - \mathrm{d}F_2)H}$$

for example, where $H=(\mathrm{d}F_1-\mathrm{d}F_2)/\mathrm{d}G$, with the expected value $E(p_1)=\Pi_1$, and variance

$$\sigma^2(p_1) = \frac{\int H^2\mathrm{d}F - (\int H\mathrm{d}F)^2}{n[\int(\mathrm{d}F_1 - \mathrm{d}F_2)H]^2}$$

A considerable choice of H is possible   Thus, considering for definiteness the density case, we define the unweighted least-squares estimator of Π_1 by the choice $\mathrm{d}G=dx$, $H=f_1-f_2$ We should, however, expect weighted estimators to be better   In fact, because the variance of $\mathrm{d}F_s$ is fdx/n, we should try to choose $\mathrm{d}G=fdx$, the term $\int H\mathrm{d}F$ in the expression for $\sigma^2(p_1)$, arising from the covariance of $\mathrm{d}F_s(x)$ and $\mathrm{d}F_s(y)$, then vanishes, and $\sigma^2(p_1)$ becomes identical with the reciprocal of the information function $I(\Pi_1)$ If we take $\mathrm{d}G=\Pi_0\mathrm{d}F_1+(1-\Pi_0)\mathrm{d}F_2$, then this weighting will be most efficient when Π_0 is close to Π_1 Thus if we suspect Π_1 to be near 1, $\frac{1}{2}$ or 0, suitable choices for Π_0 would be 1, $\frac{1}{2}$ or 0, respectively. An alternative to $\frac{1}{2}(\mathrm{d}F_1+\mathrm{d}F_2)$ is the geometric mean of $\mathrm{d}F_1$ and $\mathrm{d}F_2$, and another is $\max(\mathrm{d}F_1, \mathrm{d}F_2)$. The geometric mean has some convenience over the arithmetic mean in theoretical investigations of efficiency (for example, for f_1 and f_2 normal), but the latter (or alternatively $\max(\mathrm{d}F_1, \mathrm{d}F_2)$) has the advantage of approximating to the maximum likelihood estimator, for any value of Π_1, in both the extreme cases of f_1 and f_2 well separated and of $f_1\rightarrow f_2$ It has been shown by Hill[1] that the information function $I(\Pi_1)$ for Π_1 may be written $n[1-S(\Pi_1)]/(\Pi_1\Pi_2)$, where

$$S(\Pi_1) = \int(f_1f_2/\mathrm{f})\mathrm{d}x$$

If f_1 and f_2 differ by some parameter μ (for example, the mean), and $\Delta\mu=\mu_2-\mu_1$, then also as $f_1\rightarrow f_2$,

$$I(\Pi_1) \sim n(\Delta\mu)^2 I(\mu)$$

where $I(\mu)$ is the information function for μ Thus for the maximum likelihood estimator $\hat{\Pi}_1$ the variance, which is $\Pi_1\Pi_2/n$ for f_1 and f_2 well separated, is $1/[n(\Delta\mu)^2 I(\mu)]$ as $f_1\rightarrow f_2$

In the case of further unknown parameters θ_j the problem is obviously more complex, because of the non-linear dependence of f on θ_j, but linearization of the estimation equations may sometimes be convenient   For example, for two populations with unknown parameters θ_1, θ_2, respectively, we might adopt the standard least-squares procedure of taking provisional values v_1, v_2, say, so that in the "sum of squares" to be minimized we write in the numerator

$$f \sim f_2(v_2) + \Pi_1(f_1(v_1) - f_2(v_2)) + \varphi_1\partial f_1/\partial v_1 + \varphi_2\partial f_2/\partial v_2$$

in the unknowns Π_1, φ_1 and φ_2, where

$$\varphi_1 = \Pi_1(\theta_1 - v_1),\ \varphi_2 = \Pi_2(\theta_2 - v_2)$$

(retaining the provisional values v_1 and v_2 in the weighting-function $\mathrm{d}G$)

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[1] Hill, B M, *J Amer Stat Assoc*, 58, 918 (1963)

Speculations on the Use of Orthonormal Functions in the Study of Morphogenesis

THE recent proposal[1] to analyse shapes of biological objects by means of orthonormal functions opens up some interesting possibilities   In the work cited, a particular set of two-dimensional orthonormal functions synthesized from Walsh[2] functions which take only the values +1 and −1 was used   As is well known, however, there is a vast number of other complete sets of orthonormal functions and, in fact, they could be constructed indefinitely   There are also standard linear methods for transforming from one set of orthonormal functions to another, these procedures can be realized as computer programmes

The Walsh-derived functions are most suitable as an initial set because of their simplicity   Their characteristic chequerboard patterns, however, are not the most natural to use for the analysis of familiar kinds of biological objects, this was indicated by the fact[1] that, for good accuracy, as many as 256 terms might be needed in the expansion for a leaf   It might be possible to construct a different orthonormal set of basic patterns which would be more "leaf-like" in shape, ideally, a development series of leaves would all be adequately described by merely two or three terms, say   What is required then is to devise computer programmes which, for a given series of input shapes, would find the best orthonormal set—using the transformation procedures referred to—best in the sense that fewest terms would be required for adequate description   When this had been achieved, one might hope to discover laws of a general type from the experimental data, for example, by studying the changes of the weights (that is, coefficients in the orthonormal expansion) that occur in development or because of experimentally applied external agents   If this were achieved, the basic patterns would presumably have genuine biological significance

What follows is rather more speculative   It is well known that most of the standard orthonormal functions of mathematical physics (sinusoidal or Bessel, for example) arise naturally in the solution of differential equations with boundary constraints   Thus a given set of orthonormal functions characterizes a given differential equation with its associated boundary conditions   If then an orthonormal set which was particularly adequate in the sense of the previous paragraph was found, this set might well have a special significance and be indicative of some differential equation or, maybe, difference equation or other type of equation, which characterizes in a general way the development of the patterns concerned   It might thus be possible, merely from examining the optimum orthonormal sets generated by the computer programmes, to draw conclusions about or even to derive the fundamental laws—in the form of differential equations or something similar—which govern the development of form.

Another issue, which it may be fruitful to investigate, is the biological meaning, if any, of the maximal weights previously discussed[1]. If one attempted to reconstruct a form from the maximal weights directly, one would fail, because in general the constructed function would have other values than 0 and 1. Nevertheless, this function, because it contains all the information about the degrees of resemblance of the form concerned to the basic pattern forms, should have a significance which might be usable—especially in those cases where the number of basic patterns effectively used is very small, as discussed previously

It would also be useful to generalize the orthonormal expansion method by allowing the form function to have more than two values   For example, a representation of the veins of a leaf might be included with a value of 2 being assigned at locations on a vein   This would provide a three-valued function, but many-valued functions could be used also for other purposes such as giving the distribution of colour as well as shape of an object, when a suitable numerical coding of the colours had been chosen

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[1] Meltzer, B, Searle, N H, and Brown, R, *Nature*, 216, 32 (1967)

[2] Walsh, J L, *Amer J Math*, 45, 5 (1923)

BOOK REVIEWS

PHYSICS OF HADRONS

Algebraic Theory of Particle Physics

Hadron Dynamics in Terms of Unitary Spin Currents By Yuval Ne'eman (Frontiers in Physics). Pp xvi + 334 (New York and Amsterdam W. A Benjamin, Inc, 1967) $10 cloth, $5 95 paper

The Interactions of Hadrons

By Hartmut Pilkuhn. Pp xi + 375 (Amsterdam North-Holland Publishing Company, 1967) 100*s*

THE past decade has seen three major advances in hadron physics The first was the discovery of parity violation in Weak interactions, with its elegant explanation in terms of the two-component neutrino This implies equal strengths for axial and vector lepton currents and suggests that the axial and vector hadron currents in the Weak interaction would also appear with equal coupling constants if Strong interaction effects could be switched off

The second advance culminated in the discovery of Ω^-, which in turn established that the Strong interactions are approximately invariant with respect to operations of the group $SU(3)$ The symmetry breaking interaction is about one order of magnitude weaker than the dominant Strong interaction, and is still invariant with respect to the subgroup $U(2)_I$ (isotopic spin and hypercharge) $SU(3)$ is an eight parameter group, and so it gives rise to eight currents which in the basic (quark) representation and conventional notation are written

$$J^i_\mu(x) = \bar{\psi}(x)\gamma_\mu \lambda_i \psi(x) \text{ with } i = 1, \quad , 8$$

These are all approximately conserved, and those corresponding to isotopic spin and hypercharge are exactly conserved apart from electromagnetic and Weak interaction effects

The third major line of progress has been the establishment of a number of links between the currents arising from the Strong symmetry and those appearing in the electromagnetic and Weak interaction Lagrangians

(i) The crucial consequence of the (correct) 8-fold-Way version of $SU(3)$ is that the electromagnetic current can be expressed as a linear combination of those $SU(3)$ currents which are related to isotopic spin and hypercharge

(ii) The CVC hypothesis states that the vector hadron currents appearing in the Weak interaction are also the appropriate components of the $SU(3)$ octet of currents The fact that the isotopic spin current is conserved implies that the corresponding vector coupling constant is not renormalized by Strong interaction effects

(iii) The PDDAC hypothesis asserts that the Pion Dominates the Divergence of the Weak interaction Axial-vector Current, in the sense of dispersion relations This means that the divergence of the axial current behaves like the pion field, with a constant of proportionality which depends on the particle masses, the Strong coupling constant and the ratio of the observed axial to vector Weak coupling strengths, G_A/G_V (This is often called PCAC)

(iv) By far the most adventurous of this chain of assumptions is that which asserts that the hadron currents appearing in the Weak interaction satisfy the algebra (or the commutation relations) obtained from the simple form of vector $SU(3)$ currents $J^i_\mu(x)$, and for the axial-vector currents the similar expressions with γ_μ replaced by $\gamma_\mu\gamma_5$ This has led notably to the Adler–Weisberger relation which depends on $SU(2)$ invariance of the Strong interactions and all the properties of the Weak interaction mentioned above It correctly relates the ratio G_A/G_V to pion–nucleon scattering, and appears to confirm this chain of assumptions about the Weak interaction It throws no new light on the symmetry properties of Strong interactions

On the basis of these successes, the problem which has received most attention in recent years is the further elucidation of the hadron spectrum, which can be approached either directly or indirectly through high-energy scattering and its analysis in terms of fixed or Regge poles The problem is to find the relation between the observed hadron masses, their spins and $SU(3)$ multiplet properties A considerable advance appeared to have been made with the proposal of $SU(6)$ which correctly predicts both $SU(3)$ and spin properties of the low-lying mesons and baryons and the ratio of proton to neutron magnetic moment But there appear to be fundamental difficulties with any symmetry which couples internal ($SU(3)$) properties to the external (space–time) properties of parity and spin

To understand the present paradoxical situation, it is instructive to consider a model world with only two spatial dimensions Thus we can visualize the flat-land physics which would be developed by two-dimensional physicists confined to a plane If there were no preferred position or direction in the plane, they would observe invariance of physical laws with respect to displacements and rotations about an axis perpendicular to the plane. Consequently they would find conservation of linear momentum and simultaneously angular momentum in the plane about an axis through the centre of mass. They would find intrinsic spin (in the plane) which in units of h could take on integer or half integer values m; but there would be no reason to expect any multiplet structure relating particles with different m values

Suppose now that it was found experimentally that the particles did in fact group themselves naturally into multiplets of approximately equal mass with m values which were observed to satisfy a relation

$$-j \leqslant m \leqslant j.$$

The physicists would interpret this as some internal symmetry, but to the mathematically minded flat-landers it would immediately suggest three-dimensional rotations $O(3)$ They could perfectly consistently postulate that single particle states were invariant with respect to three-dimensional rotations, since in the rest frame these rotations would not take the particle out of the plane (In a three-dimensional relativistic theory $O(3)$ would be replaced by $SU(6)$ or $U(6) \times U(6)_{\gamma_0}$, and I will continue to indicate in parentheses the corresponding generalizations)

To explain the origins of the $O(3)$ rule, they might assume that relative motion can be neglected in Strong short range interactions and that the remaining spin–spin interactions are invariant with respect to three-dimensional rotations This would be logically consistent, but would predict that spin (not angular momentum) in the physical plane would be conserved It would forbid, for example, the decay of a high-spin object into any two objects of smaller spin That would require orbital angular momentum to give overall conservation ($SL(6,C)$ or $U(6,6) = \widetilde{U}(12)$)

If this were contrary to observation, the two-dimensional physicists might conjecture that "reality" was in fact three-dimensional, invariant with respect to three-dimensional rotations—of which they were observing a two-dimensional section ($IU(6,6)$) To write down momentum-dependent and invariant interactions, it would be necessary to introduce momentum as a 3-vector (not just a 2-vector) and then to impose the condition that, for all physical processes, the momenta lie entirely in the physical plane The extra dimension is not in fact a serious problem The only physical implications of the assumed higher symmetry apply to collinear processes, such as forward scattering or two-body decay, which in their rest frame involve one direction These must now be invariant with respect to rotations about that direction—an axis in the physical plane defining rotations which do not remove the process from the plane If these latter are called W-rotations, we may recover all the physical implications of the wider symmetry by postulating $O(3)$ invariance for single particle states and W-rotation invariance for collinear processes, without ever mentioning three-component momenta $(SU(6)_W)$ The two statements have identical physical content The formulation which refers to extraphysical dimensions is intuitively clearer; the latter sacrifices some intuitive clarity for the advantage of not even referring to unphysical quantities.

There is, however, a serious difficulty with probability, which must still be conserved in the real two-dimensional world. The consequent unitarity of the S-matrix enables one to express the imaginary part of a forward scattering amplitude as a sum over all energetically possible processes. These latter are not in general collinear and thus do not have the extra W-spin symmetry required for the forward amplitude. Consistency between W-spin invariance and unitarity is only possible if the dynamics of the hadrons leads exclusively to forward (or backward) scattering. But since forward scattering is the limit of non-forward the simplest analyticity requirement on the S-matrix excludes non-vanishing forward scattering if scattering through any finite angle is zero

The two-dimensional model accurately illustrates the problems of higher symmetries which combine internal and external transformations A possibility which is not apparent from this simple model is to assume that the particles form infinite unitary multiplets of the non-compact groups which arise when the theory is made relativistic. This is clearly a very drastic approximation and, in fact, leads to further troubles

One can try to avoid the problem by noting that the bottom end of the meson spectrum is just that to be expected from a quark–antiquark pair bound in any simple potential. This model treats an extreme relativistic situation in which about 80 per cent of the original rest mass has disappeared into binding energy as though it were non-relativistic nuclear physics. It fails to explain the baryon states since pairs of quarks must not bind, and three quarks bind in mutual p-states—not natural properties of simple potentials. It purports to replace group theory by specific dynamics, but the dynamical model fails already for one particle states and is quite unbelievable for scattering, where the real difficulties arise It may yet "predict" the correct meson spectrum, but it does not solve the problem.

The two books by Dr Pilkuhn and Dr Ne'eman approach this material with very different intentions, but I cannot enthuse about either of them. Dr Pilkuhn has restricted himself to the well-established parts of the subject, and has written an introductory text suitable for someone starting research in this field He deliberately avoids the use of operator fields, which to my mind is a disservice to the student, for fields provide much the simplest vehicle for discussing both the invariance and the analytic properties of the S-matrix, particularly when antiparticles are involved. They do not depend on weak interactions or a Lagrangian Consequently anti-particles are here introduced almost apologetically and the important CTP theorem is not covered

The book deals with the phenomenology of pion-nucleon and kaon-nucleon scattering, but the Poincaré group is not mentioned and the formalism excludes the introduction of operator currents. As a result, the treatment of Weak interactions is clumsy, and generally the text does not lead naturally into those fields which are now of most active theoretical interest

Dr Ne'eman's book, on the other hand, is aimed at research workers who already have some experience in the field, and sets out to give an up-to-date account of the application of group theory to hadron physics He is thus directly concerned with the material summarized above The book is interesting since the subject matter is extremely interesting, but the treatment is obscured by the author's personal jargon and a partisan approach Thus current algebras are referred to throughout as TOA, Weak becomes "atonous", and internal "endo" with no increase in clarity The $U(6,6)$ theories are introduced with the revealing phrase—"one should perhaps mention the attempt to work with 'redundant components' ". It is not explained that the redundant components referred to are just the four components of Dirac theory which generalize the two component non-relativistic spinors, and are precisely the mechanism whereby one can produce an $SU(6)$ static limit and at the same time preserve all the attractive features of a local causal theory—crossing symmetry, CTP and the correct spin–statistics relationship The parallel work of Rühl on $SL(6,C)$ is not mentioned. The groups $U(6) \times U(6)_{\gamma_0}$ and $SU(6)_W$ for the Strong interaction emerge rather unconvincingly as subgroups of the algebra produced by commuting the Weak currents The successes of the theory are presented, but I failed to find the bad results (Hussein and Rotelli, Winternitz *et al*) on $p\bar{p}$ annihilation at rest. Fortunately, as explained above, the formulation of this theory is not as important for the progress of physics as the solution of the questions which it raises The problem of the hadron spectrum still stands where it did in the late summer of 1966, when the manuscript of this book was completed P T MATTHEWS

LIQUID STATE PHYSICS

An Introduction to the Liquid State

By P. A Egelstaff Pp. xvi+236. (London· Academic Press, Inc (London), Ltd , New York Academic Press, Inc , 1967.) 57*s*. 6*d* ; $10

THE last decade has seen the creation of a subject that may be called liquid state physics, and this book is one of the first to attempt an account of the new field.

X-ray and neutron diffraction have been used for many years to study the equilibrium structures of solids and, less frequently, of liquids Recent improvements in the accuracy of the work on liquids, recent advances in equilibrium theory, and the use of computers for simulation experiments have at last brought some order into what was previously a confusing situation. We can now claim to have some degree of quantitative understanding of the structure and thermodynamic properties of simple liquids

These advances are described by Egelstaff in the first ninety pages of this book, but it is clear that his real interest is in the dynamic properties. These can be studied in experiments of neutron and optical scattering in which the change of energy and angle of a monochromatic beam is observed The incoherently and coherently scattered neutrons are related, respectively, to the evolution in time of the distribution functions of single molecules and of molecular pairs It is the uncompleted task of non-equilibrium statistical mechanics to relate the space, time and velocity distribution functions to the transport properties of a fluid

These advances of technique and theory have come mainly from those who both have access to powerful sources of neutrons, and have done theoretical work on the parallel problems in solid state physics In this country these conditions have been met at Harwell where Egelstaff and his colleagues have been amongst the most successful both in obtaining and interpreting new results

The liquids discussed in this book are mainly "single particle" fluids, such as the inert gases and liquid metals, although the author spares a few pages for other problems

—molecular fluids, quantal fluids and even for a brief discussion of the critical point. These pages are very few, and might have been better used to allow less terse accounts of the main subjects

The book is far from elementary, in spite of its title The author explains in his preface that it arose from a course of lectures to postgraduate physicists, and it is unfortunate that the publishers' summary on the dust jacket claims that it is suitable for undergraduates The subject is inherently difficult, but those who have heard the superb reviews that the author has given at more than one conference will not be surprised by the many ingenious diagrams and other aids that he uses to mitigate these difficulties The book is recommended to those who are willing to work hard at it

J S ROWLINSON

TWO ON FLUID DYNAMICS

An Introduction to Fluid Dynamics

By Frank Chorlton Students' paperback edition. Pp University Press, 1967) 75*s* net; $13 50

Textbook of Fluid Dynamics

By Frank Chorlton Students' paperback edition Pp xiv+399 (London D Van Nostrand Company, Ltd , Princeton, N J D Van Nostrand Company, Inc , 1967) 35*s*

THE longevity of fluid mechanics as a scientific discipline is due primarily to the multitude of applications of the same basic ideas and partly because the correct interpretation of the theory is frequently not intuitively obvious. The experimenter is continually challenging fundamental tenets of the theoretician to the advantage of both and to the lasting benefit of the discipline These features of fluid mechanics, however, make the writing of introductory texts extremely difficult, for the author has to steer a narrow course between a phenomenological approach, in which the actual behaviour of fluids is properly described but the theory is presented in a piecemeal and unconvincing manner, and a mathematical approach in which emphasis is laid on elegant solutions of the basic equations without consideration of their relevance to observation.

Only a few books written this century have managed to achieve a proper balance and their authors have in consequence become household names in the world of fluid mechanics—Lamb, Goldstein, Prandtl Let me say at once that the new book by Professor Batchelor is truly worthy of standing next to them on the shelf of honour No instructor of an elementary course in fluid dynamics will be able to prepare his lectures without first seeing what Batchelor has to say about it, lest his students repair the omission for him The book is firmly embedded in the realities of fluid dynamics, but nevertheless the author develops the mathematical arguments in a coherent way so that the novice applied mathematician will feel that there is an important part for him to play in the discipline

The author rightly confines himself almost entirely to the study of incompressible fluids in laminar motion, examining in depth the significance of the interplay between inertial, pressure and viscous forces The opening chapter, however, on the physical properties of fluids, examines a wider spectrum and in some ways is slightly less satisfying than the remainder—no doubt because the author has to compress a number of complicated theories, such as thermodynamics, into a very few pages After that introduction the book gets properly under way with the kinematics of flow, governing equations, viscous effects at small and large Reynolds number, irrotational inviscid flow, and ending up with a study of inviscid rotational flow which includes a considerable section on fluids having a rigid rotation at infinity The explanation is careful, the style is pellucid and the diversity, excitement and beauty of the subject are made manifest to the reader The book includes a number of examples, some of which most students might find intimidating and the instructor may well have to supplement them with a set of his own

Alternatively he might turn to the extensive set in Dr Chorlton's book This second book under review is entirely mathematical in content and I could detect no comparison at any stage with experiment. The dangers of this approach have already been referred to and the author can be criticized for over-emphasizing mathematical beauty at the expense of physical truth However, provided the instructor emphasizes the physics of fluid mechanics his students will find Chorlton's book useful, for it presents the mathematical arguments in a clear and concise way

K STEWARTSON

HEREDITY AND CANCER

Hereditary Factors in Carcinoma

By Henry T Lynch (Recent Results in Cancer Research, Vol 12.) Pp xiv+186 (Berlin and New York Springer–Verlag, 1967) 24 DM ; $6.

As early as the seventeenth century, the physician Hoffmann suggested that a disposition to cancer might be inherited, basing the suggestion on family histories with a high incidence of malignant disease. The enquiry conducted by the Society for the Investigation and Cure of Cancer in 1802 asked the physicians in this country "Is cancer inherited ?" which shows that such a possibility had already been considered by then. Throughout the nineteenth century many family pedigrees were reported in which several members died from cancer; these reports formed the basis of statistical investigations into the familial tendencies of the disease These studies, however, were incomplete, lacking a suitable control group for comparison, and were useless It is not surprising that owing to the unreliability of the data and the contradictory conclusions which had been drawn from their investigations, the clinicians were inclined to dismiss heredity as a possible factor in the development of cancer.

Recent enquiries into the familial incidence of malignant conditions are conducted on a truly scientific basis; the pedigree studies employ improved statistical methods and use more vigorous criteria in selecting the control groups What are the findings of these investigations ? The monograph under review is based on more than 700 reports published in the world literature which deal either directly or indirectly with the subject of "cancer genetics". The aim of the author is to bring together the evidence which supports a "genetic etiology" at least in some human malignancies The classical examples of those pathological conditions which are known to be inherited (for example, xeroderma, multiple polyposis of the intestine) and result in cancer are given prominence. Much information concerning the common types of cancer, such as breast, stomach and lung cancer, has been brought together, and the author stresses the increased risk which a high incidence of these cancers in the families indicates, for example, the risk is claimed to be fourteen times higher for a smoking member of a family in which another member had cancer

The monograph contains a brief summary of the author's study concerning the incidence of multiple cancer in fifty families, six of which are presented in more detail. He finds an incidence of 19 per cent as compared with 3 per cent in the control group and draws the conclusion that the "multiple cancer syndrome" has a genetic basis According to him an autosomal dominant gene is acting together with environmental factors The reader of the monograph is advised to consult the original reports which give more information about the respective role of the familial and environmental factors in the development of a particular malignant disease.

The true value of the monograph is that it serves as a rich source of information covering many and varied aspects of cancer which are related to heredity.

P. C. KOLLER

VIRUSES AND DISEASE

Clinical Virology

By R. H. A. Swain and T. C. Dodds. Pp. xii+318. (Edinburgh and London: E. and S. Livingstone, Ltd., 1967.) 75s. net.

WHEN so much virology is directed towards molecular biology, it is worth recalling that the first impetus in the study of viruses came from the investigation of disease. Nor can it yet be said that problems associated with virus infections no longer confront us. Viruses which are capable of inducing disease in some can be isolated on occasion from others who are perfectly healthy. Some viruses appear to have such growth requirements that it has not yet been possible to find a suitable substrate other than the human or animal host. As for the suppression of virus multiplication by drugs, only the fringe has been reached.

The appearance of this book, which sets out to correlate known facts on the aetiology, clinical manifestations, pathological effects and epidemiology of virus diseases affecting man, is timely. It should be useful both to students seeking basic information and graduates facing problems of diagnosis and the general handling of such diseases. The information provided is clearly set out and easy to read. It is concise but on the whole adequate for its purpose. Those who wish to read further are given guidance at the end of each chapter. There is an impressive array of charts and photographs, many in colour, in support of the aim to supplement mere words by illustration. This adds to the attractiveness of the book.

There are four sections, the first two comprising the major part. Respectively, these are concerned with (1) general considerations of viruses, their characters and properties, together with some of the standard procedures used in the study of newly isolated strains and for the related immunological tests, (2) descriptions of virus diseases affecting different systems of the body, the virus groups concerned, and the methods by which they are identified. The slowness of virus laboratories in reporting results of investigations is a worry to clinicians. The information given here may help towards a better understanding of laboratory problems. At the same time there is continual pressure for more rapid diagnostic tests and in a still developing subject a book does not contain all the latest information. For example, in the diagnosis of rubella, important because of its association with congenital deformity, there is no mention of the simple and rapid haemagglutination–inhibition test.

Section three deals with infections caused by bedsoniae, the group which includes psittacosis, lymphogranuloma venereum, trachoma and inclusion conjunctivitis, and the rickettsiae. Of the latter only Q fever is prevalent in Britain, but no reference to studies carried out here is quoted. In section four on the control of infections there is useful information on active immunization including schedule guides for babies and international travellers. There is also an account of the state of chemotherapy in virus diseases.

No book is free from errors or omissions. On page 176 is the surprising statement that there are thirty-two Coxsackie A serotypes. Some of the references have careless spelling faults, and a reference to a British manual of virus methods would have been helpful. Despite such mild criticism this should be a very useful book.

A. D. MACRAE

RESEARCH ON PROTOZOA

Research in Protozoology

In four volumes. Edited by Tze-Tuan Chen. Vol. 1. Pp. vii+428+16 plates. (Oxford, London and New York: Pergamon Press, Ltd., 1967.) 110s. net.

IN 1941 a collection of review articles edited by G. N. Calkins and F. M. Summers was published under the title *Protozoa in Biological Research*. It resulted from the co-operative endeavour of a number of protozoologists concerned about "the best means to stimulate further research on these unicellular animals". For twenty-five years "Calkins and Summers" has remained an authoritative work of reference covering a wide range of research studies on the protozoa. In *Research in Protozoology* the editor has set out to survey the progress of studies on protozoa in the quarter of a century since the earlier book by presenting a series of review articles spread over four volumes and covering the principal aspects of protozoan biology. This is a formidable undertaking, for the 1941 book comprised some twenty articles occupying 1,150 pages, and the spectacular advances in recent years in such fields as biochemistry and ultrastructure studies have had far-reaching effects on every aspect of protozoological research.

Reviews written for such a purpose might be expected to bring all aspects of a particular topic together, so that a biologist with only a general knowledge of protozoology may be able to gain a broad understanding of the subject; they might also be expected to extend up to the frontiers of current knowledge in the subject so that a research worker, perhaps in a closely allied field, may be able to find his way around the recent literature with confidence. To do both of these in the same article is a difficult task, and becomes more difficult as the scope of the article is extended. This is clear in the present volume where the articles with narrow subject limits can be comprehensive works for reference as well as being useful for teaching, but there is a tendency for the wide-range subjects to become little more than reference lists which mean little to anyone without a good knowledge of protozoology and an extensive reference library. There are periodicals which publish review papers whose function appears to be the provision of up to date reference lists, but the articles in a book such as this, if they are to stand the test of time, should be more or less comprehensible by themselves, and should therefore contain adequate explanations and illustrations.

The first of the five articles in this volume is by Everett Anderson and deals with a restricted range of aspects of the fine structure of protozoa, namely, a miscellany of cytoplasmic inclusions and those cytoplasmic organelles that are of membranous construction. Such structures as cell surface membranes and their modifications, cell walls, fibrous structures and nuclei will presumably be dealt with in later volumes. Dr Anderson gives the reader a fair impression of the information available in this field up to 1964, although he only quotes a small proportion of the relevant literature on the subject. The chapter includes twenty-four micrographs which illustrate more or less convincingly the structures described.

The article on "Motile Behaviour of Protozoa" by Theodore Jahn and Eugene Bovee occupies 160 pages and lists more than 1,000 references—it is almost a book in itself. The scope of the subject matter is so wide that even with this extensive treatment it was not possible to do justice to some aspects of protozoan motility; many things are inadequately explained and there are no illustrations at all in most sections of the chapter. There are clearly arguments in favour of attempting to integrate all information on the movement of protozoa within a single framework, but this material would fit better into the pattern of this sort of review if it had been broken up into several chapters covering such aspects as "Motor responses of protozoa" (comparable with the review by Mast in the 1941 volume), "Movement of protozoa by flagella, cilia and other fibrous organelles" and "Movement of protozoa by cytoplasmic streaming" There is an immense amount of information in this chapter, and Jahn and Bovee have produced an excellent guide to the literature, although the omission of such structures as haptonemata and axostyles from their account of motile organelles is regretted

In his review of "Respiratory Metabolism" William Danforth has given an admirable general introduction and followed this by a systematic survey of our knowledge of respiration in protozoa It is admitted that evidence is mostly too scanty for generalizations, which makes it more difficult to convey the information coherently The author makes it plain that protozoa have exploited this aspect of their biology to an extent comparable with that known for more overt features, and his article is a valuable source of information about the common laboratory species Dr Danforth must be disappointed that his article was delayed in publication, for his literature review was completed in mid-1963

Problems of osmotic regulation, ionic regulation and excretion, with special reference to the functions of contractile vacuoles, are discussed in a comprehensive and authoritative, but commendably brief, chapter by J A Kitching The author has taken pains to illustrate his article with well-chosen figures and micrographs, and the reader is led to feel that he can really understand what is known of the subject from this chapter alone

Information on "Nutrition and Growth of Protozoa", reviewed by R P Hall in the last article, must necessarily overlap parts of the article on respiratory metabolism, but it is valuable to see such information dealt with from different viewpoints in the two chapters Parts of Hall's chapter make rather dull reading, with lists of species which have been shown to require various minerals or growth factors; surely this information could have been given adequately in tables, and the author could have spent more time discussing such aspects as the sources and uses of the required materials There is a great deal of useful information in this chapter, but it could have been presented in a more palatable form

This book clearly must be present in every protozoological library, but it could have been made more widely useful in teaching as well as research if more thought had been given to the subject divisions and the method of presentation, it would then be a book that every protozoologist should have on his personal bookshelf The problem of keeping a library provided with information about research in protozoology could be solved more adequately by annual or biennial volumes of shorter review articles which would allow topics to be reviewed as frequently as advances in that topic demand, and could provide a more rapid means of publication The present attempt to produce a comprehensive contemporary review of protozoological research appears from the first volume to be only partially successful; it is to be hoped that the future volumes will contain articles as good as the best in this first volume

M A SLEIGH

EUGLENA AND ITS RELATIVES

Euglenoid Flagellates

By Gordon F Leedale (Prentice-Hall Biological Sciences Series) Pp xiii + 242 (Englewood Cliffs, N J, and London · Prentice-Hall, 1967) 56*s*

AFTER having been for a long time a type in elementary courses in both botany and zoology, *Euglena* still features in modern biological teaching At the same time, it continues to be the subject of advanced research in a variety of fields, notably the biochemistry of vitamin B_{12}, the role of non-nuclear DNA, and photoreception Its position in biology must surely be unique, and this book, which surveys *Euglena* and its relatives, therefore promises to be correspondingly useful

About one third of the book is devoted to the consideration of the position of euglenoid flagellates in the biological hierarchy and to matters of taxonomy. The system of classification presented is a revised one based, unfashionably but quite plausibly, on a phylogenetic scheme The characters of order and genera are described and illustrated by clear line drawings Together with a dichotomous key given as an appendix, these provide an introduction to the taxonomy which can readily be extended by means of the references given. A valuable feature is the inclusion of notes on individual species indicating, among other things, which are available in culture

The second part of the book deals with cytology and fine structure This is lavishly illustrated with original photomicrographs and electron micrographs which, as one would expect from an associate of Professor I Manton, are all of excellent quality The various cell components, including pellicle, flagella, Golgi bodies, mitochondria and chloroplasts, are described clearly and concisely, examples being taken from many different genera The inclusion of both light photomicrographs and electron micrographs of these various structures should be particularly useful to the teacher Their function is touched on but not dealt with *in extenso* (physiological aspects were reviewed by J J Wolken in his book *Euglena an Experimental Organism for Biochemical and Biophysical Studies*, 1961)

This book can be unreservedly recommended, both to the teacher wishing to refurbish his image of a familiar type and to the research worker seeking background information about his experimental organism Both will be stimulated to find out more about this fascinating group, among the members of which are a great variety of physiological types providing excellent material for the investigations of problems suited to tastes ranging from that of the ecologist to that of the molecular biologist

G. E FOGG

JUNIOR ENGINEERING

The Engineer in Wonderland

By E R Laithwaite Pp viii + 172 (London The English Universities Press, Ltd, 1967) 27*s* 6*d* net

IT was a clever stroke to ask Professor Eric Laithwaite of Imperial College to give the 1966 Christmas Holiday Lectures for schoolchildren at the Royal Institution Here was an engineer full of enthusiasm for his subject, yet able to make it comprehensible Publishing the lectures as a book might have been a much worse idea, but it has come off splendidly well—the book has a pace and excitement which a collection of essays would find it hard to match It is also very well illustrated.

For the first time, the 1966 lectures were televised by the BBC, and, as Professor Laithwaite points out, were seen by more people than had attended all the previous 136 series of lectures The subject of the lectures, each of which had as a title a quotation from *Alice in Wonderland*, was electromagnetic induction, still a fascinating subject despite the ubiquity of its applications Professor Laithwaite described and demonstrated a variety of electric motors of odd shapes and sizes, including the linear motor It is interesting, perhaps, that he does not claim that the linear motor will be cheaper than other forms of power It will, he says, be more reliable, having no moving parts, and this will come to have a much greater economic importance in the future Reliability will be the crucial factor in the twenty-first century, replacing efficiency and power factor as the quality engineers strive for

The most exciting moment in the series of lectures came when Professor Laithwaite and his colleagues succeeded for the first time in levitating a hollow aluminium sphere

30 in in diameter. Professor Laithwaite was determined to accomplish this for the first time in the lecture theatre of the Royal Institution, to make up for a disappointment five years earlier. Then, he writes, he had been asked to give a Friday evening discourse and had decided to try a new experiment, levitating a flat rectangular plate Difficulties with winding the coils had finally persuaded him not to proceed, but when he returned to the laboratory and carried out the experiment, he discovered that if he had gone on, the experiment would not only have succeeded but would have demonstrated a new phenomenon for the first time This disappointment made him determined to succeed with the large sphere, and how it was finally done makes an interesting chapter on its own. He used two coils, one inside the other, with the outer coil raised slightly above the level of the inner one

As befits lectures intended for schoolchildren, these contain little mathematics Particularly valuable for teachers are short appendices describing how the models can be made, and the book is likely to find a place in many school laboratories. Nigel Hawkes

University News : London

Professor P. J B Clarricoats, professor of electronic engineering in the University of Leeds, has been appointed to the second chair of electrical engineering tenable at Queen Mary College

Appointments

Professor M G P Stoker, at present professor of virology in the University of Glasgow and director of the Medical Research Council Experimental Virus Research Unit, has been appointed director of research of the Imperial Cancer Research Fund, in succession to Dr G F Marrian

Dr E M Thain has been appointed deputy director of the Tropical Products Institute

Announcements

The Steacie Prize for 1967 has been awarded to Professor Myer Bloom of the Department of Physics, University of British Columbia, for his work in molecular physics The prize, which consists of a cash award of $1,500, is named after the late Dr E W R. Steacie, who was president of the National Research Council of Canada between 1952 and 1962.

The committee set up by the Secretary of State for Education and Science, Mr Patrick Gordon Walker, to look into the organization of British national libraries has now had its first meeting The committee, under the chairmanship of Dr F S Dainton, Vice-Chancellor of the University of Nottingham, is considering how best to develop a comprehensive library system for Britain Its first request has been to ask interested individuals or groups to submit evidence, in the first place in writing. The committee is particularly interested in factual information about the adequacy and utilization of existing library facilities, and about the pattern of future demand for libraries Evidence on these or related matters should be sent to Dr N B W Thompson, Secretary, The National Libraries Committee, Curzon Street House, Curzon Street, London, W1, not later than April 30, 1968

The trustees of the Lady Tata Memorial Trust invite applications for fellowships and scholarships for research on leukaemia in the academic year beginning October 1, 1968 In view of the affinity between leukaemia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration The awards are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad Further details can be obtained from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, SW3)

Meetings

Newer Methods and Concepts of Polymerization, February 23–24, University of Bradford (The Registrar, University of Bradford, Bradford 7)

Analytical Chemistry and Applied Spectroscopy, March 3–8, Pittsburgh (Dr John O Frohliger, Department of Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15213)

Semimetals and Narrow Gap Semiconductors, April 2–4, University of Durham (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1)

Israel Physical Society special meeting, April 14–15, Hebrew University of Jerusalem (Mrs R. Rahman, Faculty of Science, The Hebrew University of Jerusalem, Israel)

Stereochemistry, April 28–May 4, Burgenstock (Professor J Sicher, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Flemingovo n 2, Praha 6)

Protides of Biological Fluids, May 4–5, Bruges (XVIth Colloquium on Protides of Biological Fluids, PO Box 71, Brugge 1).

Thyroid Cancer, May 8–10, Lausanne (Professor G Candardjis, Chairman of the Organizing Committee, Institut Universitaire de Radiologie Médicale, Hôpital Cantonal, 1005 Lausanne)

Continuous Cultivation of Micro-organisms, June 17–23, Prague (Dr Zdeněk Fencl, Head of the Department of Technical Microbiology, Institute of Microbiology, Budějovická 1083, Prague 4)

Microcirculation, June 24–29, Gothenburg (Professor Lars-Erik Gelin, Surgical Department 1, Sahlgrenska Sjukhuset, Gothenburg SV)

Erratum In the review of *Sex Chromosomes and Sex-linked Genes* by Dr Susumu Ohno (*Nature*, **216**, 891, 1967) the price of the book was misquoted at $95; it should be $9.50.

Erratum In the article "Regulation of Cytochrome Oxidase in Human Cells in Culture" by N Hakami and D A. Pious (*Nature*, **216**, 1087; 1967) the position of the words "aerobic" and "anaerobic" in Fig 1 should be reversed

Erratum In the communication "Cosmological Models containing both Radiation and Matter" by C B G McIntosh (*Nature*, **216**, 1297; 1967) equation (21) on page 1298 should read $t = \frac{2}{3}\frac{AB}{c}\{\varepsilon^{-3/2}(1-9\varepsilon) + 6\sqrt{3}\}$ (B = constant) Equation (24) should read $\rho_{r,0} = 6\ 8 \times 10^{-34}$ gm/cm^3 and equation (25) should read $\varkappa\rho_r c^4 = 9H^2\varepsilon$

Erratum In the communication "Radio Sources in Opposite Directions as a Cosmological Test" by Jan-Erik Solheim (*Nature*, **217**, 41, 1968) the fourth sentence of the legend to Fig 1 should read "The two models shown are based on a Hubble parameter $H_0 = 100$ km sec^{-1} Mparsec^{-1}, q_0 is the deceleration parameter and σ_0 a density parameter, $\sigma_0 = 4\pi G\sigma_0/3H_0^2$ where σ_0 is the mean density of the universe "

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, January 15

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Project Problems" opened by Mr A Draper

Tuesday, January 16

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP AND AGRICULTURE GROUP (at 14 Belgrave Square, London, SW1), at 10 30 a m —Symposium on "Apples"

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Electronics Group of the Institute of Physics and the Physical Society, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 2 30 p m —Colloquium on "M O S T Devices"

UNIVERSITY OF ASTON IN BIRMINGHAM (at Gosta Green, Birmingham, 4), at 5 30 p m —Professor D Hinton "Master of Science or Mistress of Arts ?" (Inaugural Lecture)

SOCIETY OF CHEMICAL INDUSTRY, HEAVY ORGANIC CHEMICALS GROUP, and the PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, SW1), at 6 p m —Mr C E Hollis "Chloroprene and Polychloroprene"

Wednesday, January 17

INSTITUTION OF ELECTRICAL ENGINEERS (at the Royal Aeronautical Society, 4 Hamilton Place, London, W1)—Colloquium on "Fault Analysis in the Design of Complex Systems"

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, SW1), at 2 15 p m —Dr W P Williams "Sub-Surface Navigation"

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5 15 p m —Dr M G. Kendall "The Future of Statistics"

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5 30 p m —Mr B J Rogers "Colour Television Receiver Design"

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion on "Mathematics for Management"

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College, London, SW7), at 6 p m —Mr M J B Shelton "Dust"

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, WC2), at 6 30 p m —Mr E M N Fitzgerald and Mr S A Marshall "The Simulation and Control of Steam Turbines"

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the New Engineering Block, University College, Gower Street, London, WC1), at 6 30 p m —Mr T R Bullett and Mr D Dasgupta "Characterization of Blast Cleaned Surfaces and Its Relation to Paint Performance".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMICAL METHODS GROUP (at "The Feathers", Tudor Street, London, EC4), at 6 30 p m —Discussion Meeting on "Pyrolysis"

Wednesday, January 17—Thursday, January 18

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY, MATERIALS AND TESTING GROUP, OPTICAL GROUP, and the STRESS ANALYSIS GROUP (at the National Physical Laboratory, Teddington, Middlesex)—Conference on "Holography—Recent Advances and Applications"

Thursday, January 18

CHEMICAL SOCIETY (in the Large Chemistry Lecture Theatre, University College, Gower Street, London, WC1), at 2 30 p m —Symposium on "Developments in Transition Metal Chemistry"

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 2 30 p m —Discussion Meeting on "Co-ordination of Thermal Ratings of Transmission Plant"

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W1), at 5 p m —Scientific Papers

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 5 p m —Dr P E Brandham "Movement of Algae", Dr Edward J Klekowski, jun "General Considerations of Fern Mating Systems"

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W1), at 5 30 p m —Mr T K Tylko "Possible Applications for the Plasma-Arc in the Oil Industry".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion on "Unusual Mechanisms and Linkages"

SOCIETY OF CHEMICAL INDUSTRY ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, SW1), at 6 p m —Dr T W Parker "The Philosophy of Assessment and Its Relation to Testing in the Agrement Scheme"

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, WC1), at 7 p m —Mr L Wilson "Scientific and Technical Information—Millstone or Keystone ?"

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W1) at 7 30 p m —Sir John Boyd, OBE, FRS address on the occasion of the centenary of the birth of Sir Leonard Rogers, FRS Dr D Morley "Factors Influencing Growth and Nutritional Status of Infants and Young Children in a Nigerian Village"

Friday, January 19

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, SW1), at 6 30 p m —Dr J M Sprague (USA) "Recent Advances in Diuretics"

Monday, January 22

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 10 a m. Colloquium on "Microwave Integrated Circuits", "Microwave Solid State Sources"

INSTITUTE OF ACTUARIES (at Staple Inn Hall, High Holborn, London, WC1), at 5 p m —Mr H A R Barnett "A Theory of Mortality".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Microwave Electrostatic Wattmeter" opened by Professor H E M Barlow, FRS

UNIVERSITY OF LONDON (in Lecture Theatre A, Mechanical Engineering Building, Imperial College of Science and Technology, Exhibition Road, London, SW7), at 5 30 p m —Professor Bertil Matern (Royal School of Forestry, Stockholm) "Statistical Models of Two-Dimensional Variation" (further lectures on January 24 and 26) *

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2), at 6 p m —Mr W B Gosney "Modern Refrigeration" (First of three Cantor Lectures Further lectures on January 29 and February 5)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

PRINCIPAL LECTURER in the DEPARTMENT OF CHEMISTRY AND BIOLOGY —The Principal, Barking Regional College of Technology, Longbridge Road, Dagenham, Essex (January 20)

LECTURER or JUNIOR LECTURER (preferably with a special interest in soil mycology) IN MYCOLOGY—The Secretary, Trinity College, Dublin 2, Republic of Ireland (January 24)

LECTURER (preferably with experience in non-numerical computing) in the DEPARTMENT OF MATHEMATICS in the field of computational mathematics—The Registrar, The University, Nottingham (January 29)

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF ZOOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (January 29)

DIRECTOR OF THE HILL FARMING RESEARCH ORGANIZATION—Professor S J Watson, Edinburgh School of Agriculture, West Mains Road, Edinburgh 9, Scotland (January 31)

LECTURER IN PHYSICAL CHEMISTRY at the University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, January 31)

LECTURER and a SENIOR DEMONSTRATOR in the DEPARTMENT OF ZOOLOGY —The Registrar and Secretary, University of Durham, Durham City (February 3)

LECTURER IN EDUCATION at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, February 3)

LECTURER or ASSISTANT LECTURER IN PHYSIOLOGY—The Registrar, The University, Sheffield (February 3)

LECTURER or SENIOR LECTURER IN CHEMISTRY at the University of Tasmania—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, February 5)

ASSOCIATE PROFESSOR IN CLINICAL BACTERIOLOGY IN THE FACULTY OF MEDICINE, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, February 9)

JUNIOR LECTURER IN MATHEMATICS (Pure or Applied)—The Secretary of Faculties, University Registry, Broad Street, Oxford (February 12)

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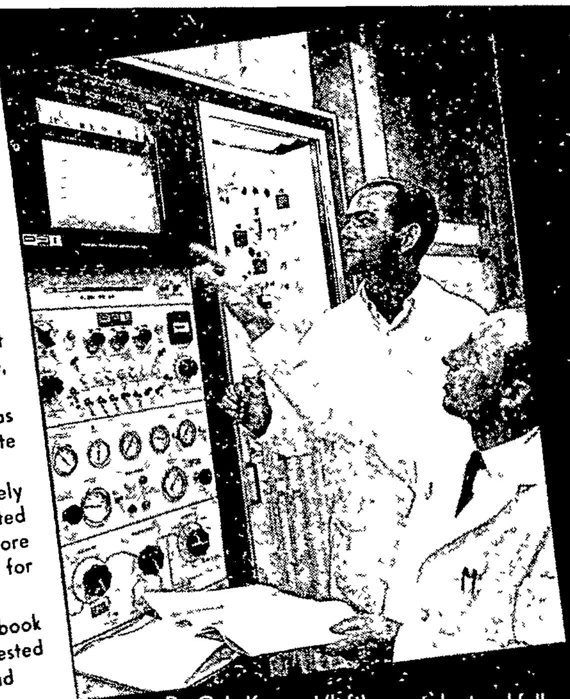

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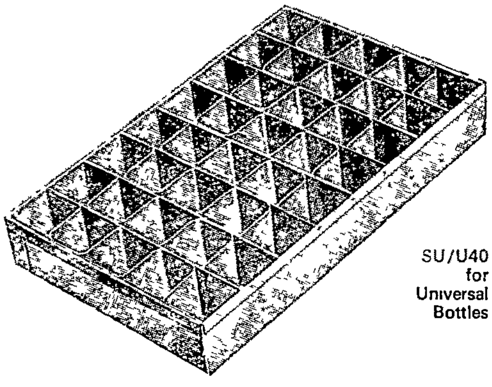

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JANUARY 20, 1968

What is Gulliver Doing?

THE OECD review of science policy in the United States is an unexpected document quite out of character with earlier attempts at the definition of national policies and even a little at odds with the supposed objectives of these delicate exercises in supra-national inquisitiveness Certainly the four examiners have been more concerned with Europe than with the United States They have been looking across the Atlantic for help and advice about a host of problems—the conditions of the universities, the technology gap and the brain drain One thing which comes out clearly from their report is the strength of what may be described as the European feeling which they share, and what they have to say about European problems is at once perceptive and courageous By contrast, their judgments on the United States are sometimes naive, sometimes superficial and sometimes both It is almost as if the examiners shrank at the beginning of their inquiry from a serious attempt to understand the machinery of science policy in the United States and from a clear identification of the serious strains which are now beginning to emerge, and there are many who will forgive them choosing not to rise to this great and time-consuming challenge Unfortunately, it is less easy to forgive the examiners for not producing a coherent summary of their joint conclusions As it turns out, their separate reflections on the condition of the modern world are interesting enough and sometimes stimulating, but they are shot through with idiosyncrasies and even prejudice Is it perhaps possible that the examiners did not have the time to put their busy heads together for long enough to produce a common report ? Or did they calculate that if they shot four separate arrows at the colossus, the chance of striking some vital target would be increased ? Whatever the reason, the OECD would be well advised, with future teams of examiners, to spend more time knocking heads together and less time producing background reports which are almost too big to be useful

The quality of what the examiners have to say about the administration of science in the United States is well illustrated by what it says—or does not say—about the function of the National Science Foundation The general view is that the NSF is intended to have a marginal existence, seeking to provide support for projects which the mission-oriented agencies neglect The examiners seem, however, to have overlooked the serious dangers of this state of affairs The present trouble is that when funds are scarce in Congress, the mission-oriented agencies shed their responsibilities for research when the NSF is unable to take up the slack In the long run, it is unthinkable that fundamental science in the United States can continue to prosper if the National Science Foundation remains the Cinderella that it is in comparison with the mission-oriented agencies or even the National Institutes of Health M Lefèvre comes closest to this view in saying that if there were an equivalent of the NSF in Europe, he would like to see it act more boldly One of the disappointments of the huge report which the examiners have produced is its failure to suggest ways in which the NSF might evolve into the more effective organization which circumstances require

The OECD examiners do, however, agree on the importance in the United States of the system by means of which universities depend on what are called project grants for the support of scientific research, and there is, of course, some truth in this Unanimity does not, however, imply accuracy, and there is at least room for doubt about the reasons which the different examiners give for believing that project grants give science in the United States some of its special qualities Professor Waddington, for example, considers the practice of awarding research grants to individuals in American universities for the carrying out of specific projects as the mainspring of competitiveness—good and bad—among scientists in the United States But is this really the way the system works ? There is at least a chance that the details of the machinery of how grants are awarded are symptoms and not causes of the character of academic science in the United States In any case, of course, research grants are awarded in large numbers for the support of individual research workers at British universities, in aggregate they account for roughly two-thirds of what is spent on academic research in science and technology and the terms of grant are frequently drawn in the finest detail It is true that research grants in the United States usually enable scientists to staff their laboratories in a condition which is considered to suit their status and that British research grants less frequently include an element of salary, but this is a question of degree and not of principle In reality, it is hard to see what would prevent the Science Research Council in Britain distributing what funds it has to spare with the precision, the ruthlessness and the daring which characterize the grant-giving agencies in the United States If British research councils pursue a different kind of policy, the blame must be levelled not at their constitutions but at the advisory committees which flourish in Britain as in the United States As with government, so with research councils—people get what they deserve

In the United States, competition between different agencies for the privilege of spending money in new and rapidly developing fields is probably a more important influence on the character of academic science than the details of the machinery for spending money as such, which is why it is perceptive of M Lefèvre to emphasize the implications of the American attachment to the federalist way of doing public business There is a splendid illustration of this in the contrast between the way in which American agencies compete with each other on common ground and the way in which British research councils deal with borderline territory either by implicit treaties of non-intervention or by collaboration with each other They may be right in choosing to blunt competition, but they are certainly responsible for doing so

In matters like these, the OECD report makes depressing reading The examiners have seen Gulliver but their view is of parts and not the whole But if they have learned only a little about the giant in the round, the shock of their encounter has taught them a great deal about themselves This is where their report is best

The first and most obvious lesson which the Europeans (or two of them, at least) confess to having learned is that European institutions are inflexible in comparison with the American Both M Lefèvre and Professor Waddington protest vigorously at the way in which Europe keeps young men waiting for old men to retire They are, of course, right M Lefèvre has a perceptive passage about the way in which industrial change is frequently frustrated by the force of habit, and it is, of course, absurd that in the middle of the most severe economic crisis for more than thirty years, the British Government should still be able to entertain proposals for keeping industries in being whose continued existence is not merely a public expense but an impediment to change elsewhere It is the same in the universities, and this is the tub which Professor Waddington has chosen to thump Movement, if ever perceptible, tends to be so ponderous that it is an active discouragement Although enlightened professors now accept that the old hierarchical concept of the university department is an anachronism, heads of departments are still kings—particularly on the mainland One way and another, OECD examiners are probably correct in their suggestions that Europe would profit if European institutions were more ready to accept change, and some of their eloquence on this theme may even help to foster change The difficulty, which the European examiners appear to recognize implicitly, is how to foster change in Europe without losing the peculiar qualities of life in the Old World of which they are plainly fond That would be a report worth writing

In separate ways, the examiners have also tumbled on what may be called the European solution—the view that the only sure remedy for the technology gap, the brain drain and the other symptoms of the imbalance of economic power between the two sides of the Atlantic is more effective European unity M Lefèvre and Professor Waddington have arguments on this perennial subject Although M Lefèvre clearly reflects the Belgian view that the abolition of customs barriers is not the end but only the beginning of the European Economic Community, his warning that there has so far been very little progress with the integration of science policy in Europe is at once forceful and timely For what sense can there be, for example, in arrangements which have persuaded three of the six nations in the EEC to support independent programmes of development in fast reactors, to say nothing of the quite separate programme being followed in the United Kingdom ? However bright the future may be for breeder reactors, there is nothing to suggest to people of discernment that the European market will be able to sustain four quite separate designs for reactors like these And exactly the same is true, of course, of the way in which there is no co-ordinated policy in Europe on the support of research in high energy physics CERN may be the most powerful of all proofs that collaboration can be made to work, but its success has not yet persuaded member nations to sink their national plans in the common pool Elsewhere, the machinery of the administration of funds for research will tend towards duplication and not complementarity, which is why there is great merit in Professor Waddington's proposal that decisions about the allocation of funds for research in Europe should be organized on an international basis even though—to begin with, at any rate—money never crossed national borders This is an entirely sound and practical suggestion, and much more likely to lead to something useful than the other suggestion of Professor Waddington's that a European analogue of the American Committee on Science and Public Policy should be established by agreement among the European academies There is every reason why the grant-giving agencies should pursue this matter urgently

Of the lessons which the European examiners have learned about themselves, in the long run the most important may be the undertone of disaffection from the United States which runs through a good deal of what the separate examiners have to say Even though the most useful suggestions in the report are European issues, Americans would be wise to pay close attention to what the examiners have to say about the relationship between the two sides of the Atlantic, for, in their separate and different ways, the four examiners accurately represent opinions and arguments which are growing in strength in the Old World And who, anyway, can fail to sympathize with M Lefèvre in his complaint that industry in the United States seems always to bring superior resources to bear in competition with whatever innovations seem about to flourish in Europe ? That may be what competition means, but it seems hard and it stimulates not merely the will to compete but resentment as well Professor Waddington's complaint that American scientists are less able to engage in contemplative studies but are at the same time inconsiderate of European achievements is necessarily more subjective This case would be hard to prove, and may well be unfounded But in a sense what matters is not the truth but the appearance of it In the quite short space of three years, there has been a marked change in the relationship between Europe and the United States There have been great political and economic strains, and it is not surprising that these are now beginning to have a perceptible influence on the character of the relationships among scientists and between scientific institutions If this should mean that European nations will seek to bring a halt to phenomena such as the brain drain by making Europe a more interesting and rewarding place, no harm will have been done But there is also a danger that the events of the past three years will permanently sour relationships between Europe and the United States from which everybody has profited enormously in the past half century That is something to worry about

Mr Wilson Trims his Sails

Mr Wilson, it is often said, is not a good hatchet man But there need now be no doubt that he is a dab hand with the scissors For the cuts in Government expenditure which he announced to the House of Commons on January 16 are a masterpiece of scrimping rather than saving, chopping rather than changing, putting off rather than cancelling altogether With two important exceptions—defence policy and the postponement of raising of the school leaving age to 16—it looks as if every sacred cow in sight has had its horns neatly trimmed

The most fundamental changes occur in defence policy, or—putting it more grandly (as the Opposition will)—in Britain's world role The order for fifty United States F 111 aircraft has been cancelled, some aircraft carriers are to be phased out sooner, British forces will leave the Far East and the Persian Gulf by 1971 Cancellation of the F 111 will save a total of £400 million by 1977–78—a figure which takes into account the likely cancellation charges The defence budget for 1969–70, which was to have been £1,970 million, will be £1,860 million (all at 1964 prices) By 1972–73, Mr Wilson said, the budget would be down to between £1,600 and £1,650 million at 1964 prices Civil Defence will be reduced to a "care and maintenance" basis, which will involve the disbandment of the Civil Defence Corps, the Auxiliary Fire Service and the Territorial Army Volunteer Reserve

None of these is a sacred cow But the decision to postpone the raising of the school leaving age to 16, and the imposition of prescription charges—at the rate of 2*s* 6*d* for each prescription—undoubtedly are Outsiders find it impossible to describe, or to understand, the horror with which the left wing of the Labour Party will greet the second of these decisions It is best simply to say that it is an emotional reaction springing from the best motives in the world, but hardened by now into a near-neurosis The re-imposition of the charges will bring in an extra £25 million a year, although there are to be exceptions—the old, the young, the chronic sick, and expectant and nursing mothers

The postponement of the raising of the school leaving age is potentially far more serious This is killing the goose with a vengeance—before it has even had time to give evidence of its capacity to lay eggs—but again it is only a deferment, to 1973, rather than an absolute cut It is a programme which Britain could neither afford to have nor to do without—an agonizing decision which has prompted the resignation of Lord Longford from the Government

Compared with this, the other cuts which education has suffered will feel like flea bites Secondary school children, for example, will no longer get free milk at school, capital expenditure in further education building, the youth services and public libraries will be cut by something like £2 0 million this year, £6 0 million next Universities will also feel the draught—their capital building programmes this year will be reduced to one-sixth of the planned level, a saving of £0·75 million—which will provide the University Grants Committee with a tricky problem For next year, 1969–70, the universities will have to make do with £2 5 million less for capital expenditure, at a time when vice-chancellors are already pining for more

Direct grant grammar schools will also be losers The grant which is paid to them, based on the number of pupils in the school, will be cut by £20 per pupil per year, an overall saving of £1 8 million And students will have to face the most extraordinary cut of all At the moment a committee is at work, deciding the amount by which student grants will be increased to keep up with the rise in the cost of living Whatever the committee decides will be cut by half, presumably on the principle that student living standards are only half expected to stay up with those of the rest of the community The grand total for savings on education amounts to £40 million in 1968–69 and to £59 million in 1969–70

The Ministry of Technology has been less harshly treated, and the Atomic Energy Authority, the greatest escapologist of them all, seems to have done it again The total savings in the ministry will be only £13 million in 1968–69 and £15 million the year after, and none of this will come from the programmes by which the ministry supports industries such as shipbuilding and computers The National Research Development Corporation will also come through unscathed The economies will come by maintaining the level of industrial research and development in Government establishments rather than by increasing it The Atomic Energy Authority and the defence research establishments will, in contrast, actually suffer cuts in the scale of support, although details of the figures are not yet available The ministry explains that this order of priorities has been decided on the principle that the support work for industry must not be allowed to suffer, so that long-term gains are again likely to be sacrificed in the interests of short-term economies.

Other cuts in the package prompt the same feelings. If the achievement of the present Government in house building had been better, it might have been easier to accept the need to reduce the number of houses built in 1969 by 15,000. And the building of roads has an economic importance far beyond its face value; despite this, expenditure on the roads is to be cut by £53 million this year and £69 million next. Happily there are to be no similar reductions in the building of hospitals.

It all makes a depressing catalogue. Only in the Stock Exchange was there enthusiasm for the programme. There was a surge of buying, reflecting a hope that this would be the last crisis measure for a little while at least. Elsewhere there was little but gloom.

Honesty is the Best Policy

The poor beleaguered British Government may yet derive some comfort, in the weeks that lie immediately ahead, from the way in which the Department of Education and Science has at last made public the documents on which it formed a view, some months ago, of the proposal to build a 300 GeV proton accelerator under the administrative umbrella of CERN. It is true, of course, that the Government would have done better still to make a clean breast of things right at the beginning, when people were still on tenterhooks wondering which way the cat would jump. That was the time to have made real friends. But even if, as events have turned out, the cat has not jumped at all, it is more than a historical courtesy to know just what Professor Michael Swann's working group told its masters, the Council for Scientific Policy—and to know who is prepared openly to vote against the proposal (see page 221). The whole issue has thus been endowed with a greater sense of reality than could otherwise have been attained. This will be a useful standby later this year, for, even if the issue of the 300 GeV machine is now quietly buried, it is plainly far from being dead.

That said, there are three important issues raised by the publication of these documents which deserve close study in Britain and in other countries which belong to CERN. The most immediate question is domestic, and concerns the relationship between bodies such as the Council for Scientific Policy and the Science Research Council and their joint masters—the British Government on the one hand and the professional community on the other. Constitutionally, of course, there is no problem to be solved. Membership of the two councils is in the gift of the Secretary of State for Education and Science, who is well placed to lay down conditions of membership. People invited to belong can say no, but it is not realistically within their competence to say that they will only serve if the councils conduct their business in some specified manner. In the hiring and firing of advisory committees, it is usually well understood that the minister has first call on whatever advice there is to be given, and that if he chooses he can keep this to himself. Unfortunately, however, there are a number of good reasons why this system functions badly in the provision of advice about the management of science and technology, not the least of which is that the advice which the scientific advisory councils provide is only worth having if the councils and their minister know that it credibly represents the opinions or the aspirations of the professional community. In some quite real sense, the members of these committees are not just wise birds in their own right but representatives of scientists elsewhere. In the circumstances, they are entitled to and should be given a greater degree of autonomy than most other kinds of advisory committees.

The right to publish is an important part of the respect the British Government owes the Council for Scientific Policy. The documents in the case of the 300 GeV accelerator are a perfect illustration of how everybody would benefit from the straightforward recognition of this principle. So much can be inferred by asking what would have happened if the advice of the two councils on the 300 GeV machine had been published last October, before the need to make some kind of response to the proposals of CERN had become urgent.

The Government would certainly not have been the loser. It has not been much of a believer in synchrotron escalation, even at its most enthusiastic. If the Government makes any clear impression at all on this issue, it is that of a sceptical but indulgent and cautious uncle, ready to give the physicists what they want if they have really set their hearts on a project and especially if there is afterwards some hope that they will have no further demands for money. There is no disgrace in this, for it is hard to see how responsible governments could act differently. But does it not therefore follow that the Government would have gained a more sensitive impression of what the scientific community is after if the sensible reports of the two councils had been published? Indeed, the physicists would also have gained, for, since the final version of the report was prepared four months ago, it has become plain that the original design produced at CERN in June can be substantially improved on. The case for the 300 GeV machine would have been stronger if it had been seen to be more flexible. Even the chemists would have profited from publication, for then there would have been more than rumour to suggest how vigorously they are opposed to the project. But if publication is beneficial for the 300 GeV proposals, surely the same rule should apply to a great many of the issues now having to be settled. The advisory councils should not merely be allowed but compelled to publish their arguments. If doing this should run counter to what the constitution says, then somebody should rechristen the committees.

The second issue is the course which the British Government should pursue towards the CERN proposals. The hiatus which has now arisen stems from devaluation and from the need for a further examination of plans at CERN. It is a welcome respite, but it will not last for ever. The chief conclusion of the documents which have now been published in Britain will most probably still be valid for most of the year ahead. In other words, it will probably be sensible that the British Government should participate. But there are grounds for asking that plans for the new machine should be more open-ended.

Whatever arrangements may now be made, however, they are likely only to be temporary. It is not too soon for governments to be thinking of the next machine. There is no assurance at this stage that reason will persuade all nations with an interest in high energy physics that a thoroughly international version of CERN would be the best solution. If there is a chance that super-conducting magnets or some other technical innovation could contain the cost of building more powerful machines, the United States and the Soviet Union might well elect to go ahead independently. In the circumstances, the greatest need is to ensure the closest co-operation within the members of CERN not merely on the building of big machines but in the less spectacular parts of high energy physics as well.

Hovercraft at Bay

Pegwell Bay, in Kent, is to be the site of a British hoverport. After a year's delay and a second session of public inquiry the Minister of Housing, Mr Anthony Greenwood, has granted planning permission for Hoverlloyd to build their hoverport, which is likely to cost nearly £1 million. Detailed plans of the scheme have also to be passed by the minister, as he is anxious to preserve as much as possible of the wild life of the bay. It has also been stipulated that no development can occur within 20 feet of the upper edge of the cliff, so that the geological features of the cliff will remain accessible. A fixed flight path has been approved for the hovercraft so that disturbance to bird life will be kept to a minimum. Dr Peter Gay, regional officer of the Nature Conservancy, thinks that about a quarter of the bay will be taken over from the birds, which include waders and wildfowl. The bay is an important feeding and resting ground for migrants, and a breeding ground for little terns which are becoming less common elsewhere. The flight path is to pass at least half a mile north of the breeding grounds at Shell Ness.

Hoverlloyd's first SR.N4 is due to be completed in the spring. Extensive sea trials will be required before a licence for operations can be granted by the Board of Trade, but it is hoped that the craft will be ready for service by the end of the year. A second craft should be ready in time for a full cross-Channel service to be operated in the spring of 1969.

Hoverlloyd originally suggested using Ramsgate harbour for their depot, but the entrance was thought to be too narrow for safe manoeuvring, particularly in rough weather. Pegwell Bay was then suggested, as having the best conditions for a hoverport. Planning permission was requested and a public inquiry was opened in January a year ago. The inspector's resulting report recommended that the application be granted, although it was thought the whole question of hovercraft travel should be examined within the context of a national transport policy. Following the announcement that British Rail was planning to run a hovercraft service from inside Dover harbour, the minister decided to reopen the public inquiry, to hear the relative merits of Dover against those of Pegwell Bay. It was found that in bad weather British Rail would be able to use their boats as an alternative service. Hoverlloyd, on the other hand, will operate hovercraft only. They will not be able to make a switch, and will therefore require an open site for their operations. The second inspector's report reinforced the view of the first, and, taking into account the effect of hovercraft on the national economy with particular reference to export potential, the minister has finally granted the required permission.

The new planning Bill, which is at present before Parliament, should prevent delays like this occurring again, as a preliminary inquiry should consider any possible sites. Further alternatives will therefore not arise after the public inquiry has taken place, as has been the case with the hoverport.

Pegwell Bay in Kent; future arrivals will be made by hovercraft.

No Reprieve for Stansted

MODIFICATIONS have now been made to the plans to develop Stansted as London's third airport. But they bring little comfort to those who believe that the airport should not be built at all; the changes provide for a re-alignment of the runways which it is hoped will reduce the noise suffered by those who live near the airport. One result will be that the existing runway at Stansted will not be usable in the long term, and will go out of use in the late seventies. Because of this, and because the new plans need an extra 500 acres of land, the cost of the airport will rise by between £8 and £10 million. Costs are now put at £45 to £50 million in the immediate future, rising to £80 to £90 million by the end of the seventies.

The changes have shifted the direction of the runways rather more towards the north. They will no longer lie in the south-west/north-east direction which makes the best of weather conditions in the area, but the change is said to make only a trivial difference to the problem of crosswinds. As a result of the changes, the Ministry of Housing and Local Government says that the number of people affected seriously by noise has been reduced from 28,600 to 10,500, although the number of houses which fall within the perimeter of the airfield increases slightly under the new plan. It is not yet clear how many runways are to be built at Stansted; while the first one is being built, the existing one will be used. But when the second runway is completed the first will be phased out, and it is possible that another two will be built. Much will depend on the imponderables of air transport—very large aircraft, for example, could have the effect of reducing the need for four runways at Stansted. Vertical take-off transport aircraft are also a distinct possibility in the long term, although it is unreasonable to expect them to influence the first part of the Stansted plan.

Most opponents of the Stansted decision will conclude that this is the end of the struggle. But a group in the City of London has in the last week come forward with an ambitious plan to build an airport at Foulness, in the Thames Estuary. The plan, for which no details have so far been released, originated with a firm of consulting engineers, Bernard L. Clark and Partners. Mr Clark, in collaboration with Sir John Howard, of the construction company John Howard, has put forward the idea. It would provide a combined airport and deep water docks on reclaimed land in the Thames Estuary. If the airport alone were developed, it would cost £45 to £50 million—with the docks, the total cost would be £250 million. So far, nobody is taking the plan very seriously—partly because Sir John Howard is away in Kenya on a business trip, and nobody else at his company is willing to comment—but also because of the great costs involved. Airports are not conspicuous money makers, and it is hard to see how the money could be raised. With the Government committed to Stansted, it is unlikely that it would provide either approval or financial support for the scheme.

New Computer Company

COMPUTER Technology is a new British computer company whose first machine will come off the production line in May. Modular 1, as the company's only model is called, will fill a niche only partly exploited by the large manufacturers and even then, its makers believe, will compete in both price and capability.

Based at Hemel Hempstead and at present employing thirty-five people, Computer Technology was founded two years ago by Mr Ian Barron, who had previously worked with Elliott-Automation. The company says that its machine can be made up from any desired number of compatible modules, each specific for such functions as processing, sorting and transmitting information. Modular 1 is a digital computer with integrated circuitry; its price depends on the number of modules required and can range from £10,000 to £1 million.

The company is financed by both British and international capital. The flexibility of the machine is claimed to be an assurance of a life of at least ten years without further research and development costs. It will be interesting to see how it fares among the Goliaths of the computer industry.

How to Make Engineers

A REPORT on the methods used to introduce secondary school students to engineering in Japan and America has recently been prepared for the Goldsmiths' Company by Mr G. C. Sneed. Mr Sneed's findings are of particular interest because, although in Britain university places in engineering may be too few, they are too many, it would seem, for British students. According to the 1966 report of the University Central Council for Admissions, there were 500 places left vacant in technology and 1,100 in pure science. The lack of interest among students may derive partly from the low social status of the engineer and partly from the lack of suitable instruction in schools.

An attempt to remedy this situation has been made by Mr Sneed of Ealing Grammar School, whose introductory engineering course, attended voluntarily by three-quarters of the science sixth form, has already been successful in encouraging highly able students to take degrees in engineering. Mr Sneed has now visited Japan and the United States to study the methods used there to encourage students towards the study of engineering at university.

In Japan, there is no lack of applicants for university engineering places. The value and national importance of engineers are publicly recognized. Japanese pupils in secondary schools are said also to be exposed for three hours a week to a subject called "industrial arts". The aim is to cover recent developments in Japanese science and technology, to impart some basic technical knowledge and to help children form a mental attitude towards modern life in which technology plays a major part.

Entrance to state universities in Japan is highly competitive, but it at least avoids the degree of specialization demanded by English universities. All students must pass examinations in mathematics, a scientific subject of choice, Japanese language and English language. For students attempting to enter a faculty of engineering, physics and chemistry are compulsory scientific subjects.

The training of science teachers is an important feature of Japanese education. There are now forty centres throughout Japan which are devoted to the training of science teachers. Mr Sneed reports that the centres are well equipped and staffed, and have met with undoubted success since their inception in 1960.

Mr Sneed says that the scale of Japanese education should not be underestimated In 1966 there were 346 four-year universities, of which 111 were operated by the state More than 70 per cent of Japanese children stay on at school until or past the age of 18, and 11 per cent receive a university education. In England, 7 3 per cent of boys and 3·2 per cent of girls go to university, these figures, however, are not of course strictly comparable

In the United States Mr Sneed says he has investigated the Engineering Concepts Curriculum Project (ECCP), a scheme which calls for the development of an introductory course in engineering and the training of teachers to conduct it The aim is not specifically to attract students to a career in engineering but, like the industrial arts course in Japan, to provide an understanding of the technical background of modern life There are signs that the popularity of engineering as a career in the United States is decreasing and, although it is too early yet to evaluate the course developed by the ECCP, its progress will be watched with interest

Education for Engineers

PLANS to bring the United States Commission on Engineering Education into the structure of the National Academy of Engineering were announced in Washington DC last week The commission was founded by the National Science Foundation in 1962 to meet the needs for improving undergraduate education in engineering It is supported by the NSF and by private means Since 1962, the work has covered areas such as the educational aspects of computers, manpower requirements for engineers in specific fields, the development of an Engineering Concepts Curriculum Project for high schools and the development of undergraduate engineering laboratories

The reason for the proposed association is that the commission recognized a need to establish "a more substantial basis of operation than that provided by its independent status" According to Dr Eric A Walker, president of the National Academy of Engineering, it is hoped that this relationship will encourage new ideas and new programmes as well as establishing co-operation with other organizations dealing with engineering education Formal dissolution of the commission as a corporation will take place no later than March 1, 1969 Meanwhile, the commission will continue to administer existing programmes, and new projects will be handled by the Committee on Engineering Education within the academy

Some Steam Ahead

THE award to a British company of a contract for a large turbine generator for Consolidated Edison in New York is a considerable coup at any time To beat General Electric and Westinghouse, and the highly effective Swiss company Brown Boveri, adds savour to the triumph But there must be some long faces in Associated Electrical Industries just the same If the order had come only two months ago, it might have been decisive in turning away the bid with which Mr Arnold Weinstock of the General Electric Company took over AEI Naturally AEI is trying very hard to play down the issue, despite its jubilation at winning the order Mr Weinstock, for his part, must be gratified that the turbine division of his new empire is already giving evidence of its competitiveness—it could mean one less problem for him, and keep alive hopes of maintaining the division through a difficult period The Central Electricity Generating Board is now cutting back so hard on new orders—despite the recent cold spell, it still had substantial spare capacity—that there is little hope of selling generating capacity in Britain for the next two years or so Export orders are therefore more than just an encouragement—they are a lifesaver

The contract which AEI has won is for one turbine generator with auxiliary equipment, worth £10 million, but it is more than likely that an order for another similar unit will be forthcoming Placing contracts in this way is a common practice in the United States, and AEI is confident of winning the option. The generator is of unprecedented size for a British company—1,100 MW from a single shaft, against the 660 MW units which the CEGB has reached The unit will be run from a boiling light water reactor, and will be a relatively low pressure, low temperature unit, running at 1,800 rpm These units are different from the ones the CEGB uses, which use a higher grade steam

Because the steam from the reactor will be saturated with water, and partially decomposed into hydrogen and oxygen, there are particular corrosion and erosion problems on the turbine blades The amount of erosion depends on the tip speed of the turbine blades, so it pays to keep this as low as possible AEI has managed to do this by using a device developed by one of its engineers—the Baumann multi-exhaust Although the other tenders for the Con Ed contract specified a blade size of 52 inches for the final set of blades, AEI says that its blades are much smaller The generator will use a combination of cooling techniques The stator will be water cooled, by a design which AEI itself developed But, although there had been speculation that the rotor would also have to be water cooled on a unit this size, AEI have provided hydrogen cooling This is not because it is technically impossible to cool the rotor with water—AEI is convinced that it knows how to do it—but merely because it might provide a weak link in the design Cooling by hydrogen gas is well proved, so AEI has decided to use it

The set will also be provided with a series of water separators, designed to remove as much water as possible from the steam In the design of these, AEI will be calling on the experience gained in the contract for the turbines for the steam generating heavy water reactor at Winfrith Heath in Dorset Although the SGHWR is very much smaller than the Con Ed contract, it shares some of the problems

The Lunar Society

from D G King-Hele

THE informal but influential meetings of the Lunar Society of Birmingham began about the year 1766, and its 200th anniversary was commemorated in

October 1966 (see *Nature*, **212**, 229, 1966) At Birmingham the bicentenary was celebrated with a fine exhibition at the City Museum and Art Gallery, seen by 90,000 people, and with a series of lectures, which has now been published in the *University of Birmingham Historical Journal* (Vol XI, No 1, pp 1–111; 1967) The texts of the seven lectures are prefaced by the address at the opening of the exhibition, given by the President of the Royal Society Professor Blackett stresses the important role of the Lunar Society in helping to bring about the Industrial Revolution, particularly through the work of Boulton, Watt, Keir and Wedgwood He also draws attention to the "all-importance of the marriage between science, technology and industrial management", so well exemplified by the members of the Lunar Society, and subsequently so sadly neglected in Britain until very recently.

The first of the lectures, on "The Origins and Lifespan of the Lunar Society", was given by Eric Robinson, who also wrote the greater part of the excellent catalogue of the exhibition The Lunar Society originated in the meetings of Erasmus Darwin, William Small and Matthew Boulton which, Mr Robinson concludes, were certainly taking place by 1766, often in Darwin's house at Lichfield. The end of the society, like its beginning, was ill-defined Mr Robinson shows that it survived until about 1807

The next two contributions are devoted to individual members of the society Lord Cohen of Birkenhead gives an interesting account of the life and work of Erasmus Darwin, well illustrated and with a useful bibliography of Darwin's published works This is followed by an appreciation of William Withering by Sir John McMichael, who shows that Withering's use of digitalis in the treatment of dropsy was subtle and far-sighted The next three lecturers discuss the influence of the Lunar Society on the development of chemistry, education and Birmingham Chemistry brings in Priestley, Keir, Watt and Wedgwood in education the chief contributors were Edgeworth and Darwin, and in Birmingham it is Boulton who reigns supreme In the final lecture, "The Lunar Society and the Industrial Revolution", R E Schofield skilfully sums up the contributions of the Lunar Society members in each branch of "industry", including medicine and agriculture, as well as technology in the narrow sense

More Trade

During this week, Academician V A Kirillin, chairman of the State Committee for Science and Technology of the USSR, has been visiting Britain The principal reason for his visit is to sign the technological agreement between the UK and the USSR, which has been negotiated over recent months (see *Nature*, **216**, 1060, 1967) From the British side the agreement will be signed by Mr Anthony Wedgwood Benn, Minister of Technology, and Mr Frederick Mulley, Minister of State at the Foreign Office

Meanwhile, the day before Mr Kirillin's arrival, another agreement between the UK and the USSR was being signed It is called the Anglo-Soviet trade agreement, and covers the import and export of consumer goods The agreement has been in force since 1959, and the most recent signing takes it forward to December 31, 1968 The agreement sets up quotas for the trade of consumer goods—the quotas now stand at £7 4 million for the import of Russian goods into the UK, and £6·7 million for the export of UK goods to the USSR These figures, the Board of Trade says, are 60 per cent larger than those agreed for the previous year They are still remarkably small, but fortunately do not represent the full amount of UK–USSR trade Although all trade is carried on within the agreement, the Board of Trade explains that over 90 per cent of it takes place outside the quotas

The USSR seems to be getting slightly the better of the balance of trade at the moment During 1966, UK imports from the USSR amounted to £125 6 million, while exports were just over £50 million Up to the end of November 1967, the balance was slightly more favourable In the 11 months, UK exports to the USSR were £61 7 million, while imports were down to £107 6 million

The new quotas agreed should offer instrument manufacturers a new chance The USSR authorities have agreed to import from Britain scientific instruments to the value of £1 1 million They are also prepared to import £1·08 million worth of chemicals and £820,000 worth of man-made yarns and fibres But it is clear that the vast majority of UK–USSR trade will continue to take place outside the quotas

Cost of Accidents

At a measurable cost to Britain of £246 million for 1965 alone, road accidents are expensive Mr R F F Dawson, of the Road Research Laboratory, has been making detailed investigations into the costs and has now produced figures which he believes to be more accurate than previous estimates made in 1938 and 1952 For the first time a value has been placed on human life and the prevention of suffering, and these subjective costs have been added to the measurable costs to give a figure which represents the total cost of accidents The figure of £1,020 is put forward by Mr Dawson, for use in economic calculations, as the average cost per accident for all areas This and other conclusions can be found in a report, *The Cost of Road Accidents in Great Britain*, which was published last week (RRL Report L R 79)

Costs of accidents are considered to arise from three sources injury to people, damage to property and from incidental expenses such as police and insurance administration Costs of insurance premiums are not included as these would not decrease even if the number of accidents was reduced Damage to property and incidental expenses are paid for by a diversion of current resources, and are comparatively easy to measure Insurance payments during 1963 put the cost of damage to vehicles and other property at £128 million Estimates of police man-hours spent in coping with accidents were made and the cost to the police for 1963 came out at £4 million Insurance administration costs were set at £20 million Injuries to people are the most difficult factor to evaluate Current costs of medical treatment and long term effects of loss of output can be estimated with a fair degree of certainty, but the additional cost to individuals of serious injury or fatal accidents is a matter for speculation Loss of output in 1963 was set at £34 million, £20 million of which resulted from fatal accidents

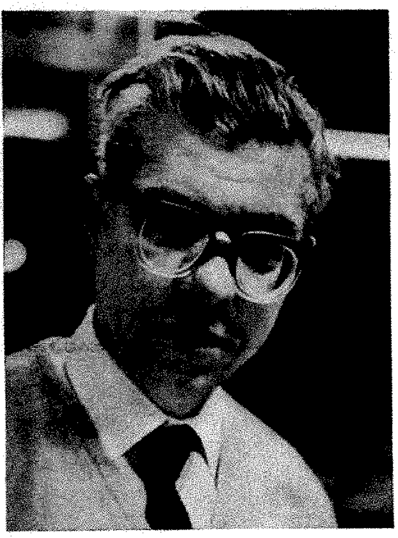
Medical treatment involving hospitals, doctors and ambulances was estimated to cost £10 million. There are no figures available for the costs incurred when vehicles are delayed by accidents and no estimate was included in the final accident costings. Mr Dawson suggested, however, that half an hour's delay per vehicle per year would cost the community £6 million.

The total measurable cost of accidents in 1963 came to £196 million. By 1965 this total had risen to £246 million, and the average measurable cost per accident had risen to £820 from the 1963 figure of £710 for all areas. This particular average is obtained by dividing the total cost of all accidents by the number of accidents in which personal injury occurred. Although this is not necessarily the perfect figure, it makes the best use of the available information. When road improvement schemes are evaluated, for example, public safety is of prime importance, and statistics are compiled with this in mind.

Subjective costs of fatal and serious injuries, which Mr Dawson has introduced for the first time, have been set at £5,000 for a fatality and £200 for a serious injury. In some cases, when a person is supported by the state, their death might result in financial gain to the community. The minimum value that can be put on people's lives is thus the amount of money the community pays to support them. It is on this premise that Mr Dawson made his calculation. When subjective costs are included in the total cost of accidents, the figure for 1965 rises to £306 million, £60 million above the measurable figure. This higher total produces the average cost per accident of £1,020 for all areas. When broken down into categories of road conditions, the estimates put urban accidents at £880, those in rural areas at £1,510, and those on motorways, where higher speeds cause greater damage, at £2,610.

Star Prizes

THE Royal Astronomical Society announced on January 12 the award of its Gold Medal to Professor Fred Hoyle for his contributions to theoretical astrophysics, especially the theory of stellar structure, nucleosynthesis and cosmology.



Professor Fred Hoyle.

Dr W. H. Munk has also received a Gold Medal for his contributions to oceanography and to the study of the variation of the rotation of the Earth.

The Eddington Medal has gone to Drs R. Hanbury-Brown and R. Q. Twiss for their invention and theoretical study of the intensity interferometer. This instrument has now led to accurate measurements of the angular diameters of a series of stars.

The Jackson-Gwilt Medal and Gift has been awarded to Dr J. G. Porter in recognition of his work on the orbits of comets and meteors and of his activities in the promotion of public interest in astronomy.

Fewer Fellows

THE decision reached in November 1967 to reduce the number of Harkness fellowships from sixty-five to twenty has been received in Britain without joy. These fellowships, named after Mr Edward S. Harkness, have enabled deserving young men and women from the countries of Western Europe and from New Zealand and Australia to continue their studies in the United States. The influence of the fellowships has furthermore extended to the growing number of departments in European universities concerned with American studies, where the staff are mainly former fellows.

The value of the fellowship varies, and the Commonwealth Fund in London says that it was difficult to quote an average figure. Despite the fact that the total value of the fellowships amounts to only one-seventh of the Commonwealth Fund—$7,059,190 for 1967—the directors of the fund in New York want to channel more money into medical research, which already receives the lion's share. At least Britain will not suffer quite as much as the other countries and from 1969 onwards will, in fact, be the only country to receive fellowships, with a reduction in the number from thirty to twenty.

There remains the question of where the axe will fall next. The number of fellowships awarded by the National Institutes of Health to United States postgraduates for research in Britain may be the next to be reduced or cut completely.

Sharing out Teachers

ACCORDING to an announcement made by Mr Patrick Gordon Walker on January 18, an improvement is expected in the total supply of school teachers and in their distribution in shortage areas. This week, a circular has been sent by the Department of Education to local education authorities, informing them what their allocation of certain kinds of teachers will be for next autumn. One thing which the circular points out is that some local authorities have been recruiting more than their share of teachers, so that some areas have gone short: authorities are therefore urged to exercise "closer control". The circular also stresses the importance of part-time teachers and of married women returning to teaching.

The fact that definite steps needed to be taken to improve the distribution of teachers was realized in 1956. This led to the introduction of a quota which laid down the total number of teachers to be employed by a local education authority. This quota scheme is confined to schools which are maintained by local education authorities and is a voluntary rationing scheme. It covers the majority of full-time teachers, but not married women returning to teaching after a break in service, and further excludes married women graduates from entering teaching for the first time some years after taking their degree. The purpose of the scheme is to ensure that each local education authority secures a fair share of the total number of teachers available, and the efficacy of the system depends very much on the accuracy of forecasts and the extent to which authorities observe the quota.

The policy of caution adopted in January 1967 was so successful that it is again being adopted for 1968–69. Because of the anticipated increase of 8,000 quota teachers, however, the total number of allocated teachers will naturally be bigger this year. In all, an increase of 10,000 teachers is expected, which will improve the ratio of teachers to pupils over the next few years. The date of operation of the quota is now being fixed for October instead of January, to improve the accuracy of the system.

Medicine Man

The University of Newcastle has appointed as its next vice-chancellor Dr Henry Miller, who is dean of medicine and professor of neurology at the university. There will be widespread pleasure at the appointment of Professor Miller, a cheerful extrovert, to the post. He succeeds Dr C. I. C. Bosanquet, who has been vice-chancellor since the university was constituted in 1963. Dr Bosanquet is retiring because he has reached the age limit for vice-chancellors (65) and Professor Miller, who is 55 this year, will be taking over on October 1.

Professor Miller is best known outside Newcastle for his trenchant criticisms of the National Health Service. He has frequently urged improvements in the standard of hospital care, and is chairman of the British Medical Association Planning Unit. He has held visiting posts in the United States and Canada, and his research work in recent years has been concerned principally with the demyelinating diseases. He is likely to prove an outspoken vice-chancellor, not one of the shy, retiring kind.

Professor Henry Miller.

Controlled Death for Plants

The patient horticulturists who remove the side buds from ornamental plants so that single prize winning blooms will grow may soon find themselves replaced by a man with a chemical spray. This was the impression left by Dr H. M. Cathey of the US Department of Agriculture Research Division, Beltsville, Maryland, when he spoke about what he calls chemical growth assassins to the Society of Chemical Industry at its symposium on plant growth regulators on January 9.

Chemical growth assassins can kill meristems, flowers, anthers or pistils without damaging the rest of the plant. There are three modes of action: hormonal chemicals such as auxin upset the endogenous growth substances; mechanically active chemicals, such as liquid plastic, cover parts of the plant and prevent pollination; and caustic chemicals, which include many aliphatic and aromatic compounds, penetrate and kill restricted parts of plants.

It was on these caustic chemicals that Dr Cathey concentrated. He described how the chrysanthemum cultivar 'Fred Shoesmith', which has between ten and twenty-four lateral buds to be removed for each flower which is allowed to develop, can be chemically disbudded by petroleum fractions containing a large percentage of naphthalenes, indenes, indanes and alkylbenzenes. These are sprayed on to the leaves together with a surfactant, after the plant has been exposed to fourteen eight-hour days. The lateral flower buds die, but the terminal buds are unaffected and flower at the same time as when the lateral buds are removed manually. Other chrysanthemums and other species are less responsive to this treatment than 'Fred Shoesmith'. The lower alkyl esters of the C8 to C12 fatty acids and the C8 to C10 fatty alcohols, however, can be used in aqueous emulsions to kill selectively the terminal meristems without damaging axillary meristems, foliage or stem tissue. Privet is one plant which can be pruned in this way, which, Dr Cathey remarked, will release a great deal of British labour for other tasks when privet hedges no longer have to be pruned by hand.

The selective action of the caustic chemicals depends on the use of a suitable surfactant to act as emulsifier, wetting agent and detergent and to make soluble the epidermal layer. They regulate the penetration of the organic compounds into the plant so that they can exercise their selective effects.

British Surveyors at Work

The Directorate of Overseas Surveys (part of the Ministry of Overseas Development), with headquarters at Tolworth, Surrey, gives aid to developing countries in land survey, air photography, basic mapping and land resource investigation. Some of the day-to-day work and some of the more unusual projects done by British surveyors are described in the annual report of the directorate, just published (HMSO, 10*s*.). The surveyors worked in sixteen countries during the year in conditions ranging from the Kalahari Desert in Botswana to a primary swamp jungle in Sarawak. Hazards encountered included cyclones in the Solomon Islands, a landslide in Dominica and inquisitive hippos in Tanzania.

The directorate's first task is basic survey and mapping, and since it was established in 1946 it has mapped over 1,654,000 square miles of territory, over 220,000 square miles of this have already been remapped This surveying and mapping is a continuing process, first, accurate maps are needed for planning development schemes, then as the plans become reality new and more detailed maps are required during the different stages of development The change from tribal to individual land tenure, together with the rapid urban development in overseas countries, has also led to more demands for large scale mapping, and an increasing proportion of the directorate's mapping activity has been spent on this type of work.

During the year, assistance was given to forty-six countries In 1966–67, 532 new maps were published, compared with 514 in 1965–66 180 of the new maps are at a scale of 1 5,000 or larger In addition, reproduction material of 96 new maps was supplied to overseas governments for local publication The maps are plotted at the headquarters at Tolworth from air photography and the surveyors' work The plots are then sent to the country concerned for completion in the field by the survey department of detail not visible on the photographs and for the addition of names The final maps are then scribed or fair-drawn and most are sent to the Ordnance Survey for printing

An important feature of the year, says the report, has been the continued expansion of the Land Resources Division to enable the directorate to meet the requests for scientific investigations to aid development planning overseas The division worked for sixteen countries and carried out field investigations in six Training attachments for officers from overseas technical departments form an important part of the directorate's aid to developing countries Senior members of the staff are also seconded to overseas governments At headquarters, research into new techniques, equipment and materials for mapping continued to be carried out, and exhaustive field tests were made on the MRA 3 and MRA 101 tellurometers in southern Nigeria

Safety in School

A NEWLY revised version of the pamphlet *Safety at School* has just been published by the Department of Education and Science (HMSO, 9*s*) It replaces a similar pamphlet first published in 1948 and refers in particular to fire precautions and electrical safety The pamphlet says that there are positive and negative sides to safety, the aim of safety precautions should not be to eliminate every possibility of accident but to avoid unnecessary risks so that children can face up confidently and sensibly to those which cannot, or should not, be avoided

Improvements in design have helped to make schools safer and more efficient Serious accidents are, fortunately, comparatively rare Figures for the total number of serious accidents do not appear to be available, though no doubt individual local authorities retain records The chapter on road safety is the most disturbing Road accidents are responsible for half the total of accidental deaths in childhood Between 1961 and 1965, fatal and serious casualties in the 5–19 age group increased by 29 per cent, compared with a rise of 15 per cent for the population as a whole

Schoolchildren seem to be taking to the air in sufficient numbers for the pamphlet to include a new chapter on that subject There is also a new appendix on precautions in the use of electricity, and the chapters dealing with safety in science laboratories and workshops and with safety afloat have been re-written and brought up to date The chapters on safety in science laboratories and workshops could be profitably read by practising scientists and technologists outside schools

How Eskimos Adapt

How have Eskimos become adapted to extremes of climate ? This is a question which an international study undertaken by the United States, Canada, France and Denmark may help to answer The idea for the study arose at a recent conference on circumpolar peoples held at Point Barrow, Alaska, under the sponsorship of the International Biological Programme (IBP) Eskimos are particularly interesting because over the centuries they have migrated considerable distances and have been exposed to extremely hostile conditions The purpose of the study, which will be conducted under the auspices of the IBP, is to measure the way in which genetically similar groups of Eskimos have adapted to the varying environments along the routes of migration to do this, research sites have been situated at points along these routes

Danish and French investigators will investigate Eskimo adaptation at Upernavik in north-eastern Greenland—one of the most distant points in the Eskimo migratory pattern, the US research will be directed in the vicinity of Wainwright, Alaska, while the Canadians will work at Iglook in the North-west Territories—the centre of the circumscribed migration route

The US study was developed by the Human Adaptability Sub-committee of the US National Committee for the IBP and is under the direction of Dr Frederick A Milan of the University of Wisconsin According to Dr Milan, it is hoped that their study at Wainwright will determine the way in which "an Eskimo community on the Arctic coast of Alaska has successfully perpetuated itself under severe climatic conditions with relatively meagre resources" In addition, by studying the patterns and habits of Eskimo life, an insight might be provided into human adaptability and evolution in general Scientists from the Universities of Wisconsin, Chicago, Indiana, Oregon, California at Los Angeles, the State University of New York at Buffalo and the Wisconsin State Laboratory of Hygiene will all participate in the US effort Among the aspects of research will be behaviour, psychology and mental illness, ecology, environmental studies, genetic markers, growth and development, local climate and geography, and population history By co-ordinating the data from each of the four countries, an overall picture of Eskimo migration can be built up

According to the IBP headquarters in London, similar studies are being carried out by the Russians and Scandinavians as part of the large-scale investigation of circumpolar peoples The Finns have already completed some work near the Russian border on the distribution and habits of Lapps and are hoping to extend this work

Scientific Libraries Examined

A recent report from the Library Association urges changes in the organization of libraries in Britain.

LIBRARIES in Britain have attracted considerable attention in recent weeks—the problems of the British Museum have made sure of that The report of a committee of the Library Association on scientific libraries (*Scientific Library Services*, Library Association, 16s) is unlikely to stir up public interest in quite the same way but it is none the less important for that

To quote the report, "Government departments, universities, colleges, research establishments and industries which have readily recognized the need for rapid expansion of their scientific or technological branches have often failed to appreciate the need for the same dynamic policy in their libraries Even where library development of this kind is acknowledged to be necessary, it is hampered by the very great shortage of librarians trained in one or other of the sciences" The report is apparently the first comprehensive attempt to review the problem of British scientific library services as a whole It is a result of a study made by a special independent committee set up by the Library Association in 1964, under the chairmanship of Major-General C Lloyd The committee set out to review the provision of specialist library services not merely for scientists and technologists but for other specialist workers in government departments, public authorities, universities, colleges, research establishments and industry

At the local level, the report says, "there exists among scientists and technologists a large degree of ignorance of the availability of library and information services Many are not even familiar with the services and journals available within their own particular field " The committee is disturbed about this lack of exploitation of the literature which is, or could be, available at local level and at the apathy towards local library and literature information services It complains that people rely too much on specialist services provided by research associations and learned societies which are usually available from only one national centre which is "geographically remote from the majority of members". To correct this situation the committee urges that more specialist library and information workers should be provided at the local level and that the local library authority be given a statutory responsibility for developing suitable services

Wherever possible, university libraries should be incorporated into the national scientific and technical library services One recommendation which should be relatively easy to put into practice is that which would allow members of one library free use of related libraries on a reciprocal basis which would do away with delaying procedures such as written applications and letters of introduction Many public libraries, after all, have introduced inter-availability of tickets

On a national level, the committee makes a comparison of the services provided by the National Lending Library, the Patent Office Library, the new Reference Library for Science and Invention and the National Central Library on the one hand with the services of the large state libraries of the Soviet Union and the All-Union Institute of Scientific and Technical Information (VINITI) on the other The committee adds its plea to all the others made in the past few years, that there should be no more delay in the full operation of the National Reference Library It also recommends that this library should be given the task of developing a National Co-ordinating and Referral Centre to "back up" and co-ordinate the specialized and local centres Aslib's national referral organization, operating through the Aslib Information Department and the Aslib Directory of sources of information, could possibly provide the referral services for this centre This would mean finding room for Aslib on the same site as the National Reference Library—a thought for the Dainton committee

The committee has drawn up a table with its recommendations on the structure of scientific and technical library and information services at local, regional and national levels It believes the aim should be that any organization needing scientific and technical documents and information should be able to satisfy at least half of its needs within its locality (half an hour's travelling each way), eighty per cent within the region (two hours each way), and virtually all its needs within Great Britain Central finance should be made available to enable libraries to provide a more efficient service, and the Department of Education and Science should "treat the co-ordinated development of information services as of national importance and urgency", now that it has wide powers in library matters

It goes without saying that specialist library staff are a necessary adjunct to the provision of efficient scientific and technical library services The committee makes several recommendations, some of which are in fact already receiving attention Soon after its establishment the committee asked the Library Association to devise some means for the formal education and admission to the register of scientists of a "mature" age It was pointed out that of approximately 1,000 scientists engaged in library and information work, only 3 per cent were on the register of chartered librarians, and that it was impossible for most of them to study full-time and so obtain formal qualification Accordingly, the association devised a scheme for mature graduate entrants

One way and another, the report covers a lot of ground, it uncovers many inadequacies of the present situation, although it is doubtful when any of the recommendations will be put into practice Major changes are of course affected by the economic situation, like everything else, and there is also uncertainty about what the Dainton committee will say It is to be noted that the views expressed in the report are those of the Scientific Libraries Committee and not necessarily those of the Library Association The association itself is in the middle of detailing a lengthy memorandum to the Dainton committee

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Professor of Chemistry, Texas Technological College, Lubbock, Texas

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Imperial Chemical Industries, Ltd, Petrochemical and Polymer Laboratory, Runcorn, Cheshire, England

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Division of Chemical Engineering, C S I R O, Melbourne, Australia

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Academic Press

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Such a wealth of knowledge now exists on this subject that the new edition appears in two volumes New chapters deal with glucagon, placental lactogens, hypothalamic releasing factors, aldosterone, renin and angiotensin and parathormone and calcitonin Five new introductory chapters describe new techniques and the section dealing with thyroid and thyroid-stimulating hormones has been expanded into two chapters

Treatise on Collagen

General Editor·
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NEWS AND VIEWS

Keepers and Readers

THE report of the Library Association on scientific library services (see previous page) is sensible enough in what it has to say about the organization of a national library network and the training of librarians, but what it has to say about the defects of the scientific literature is at once pedestrian and dangerous. To readers of *Nature* at least, the committee has given the show away with its assertion that among "advance announcement publications ... *Nature* is performing a useful function but it seems that, because of the overwhelming amount of material, lengthy delays are occurring in the announcement of even the briefest outline details". The association agrees that this is erroneous and has kindly undertaken so to inform the readers of its official journal. In fact, the time lag in publication is now only a few weeks and may be even less before the year is out. A part of the trouble seems to have arisen because the association's report has been in draft since 1965, which suggests that the committee's concern about delays in publication should be extended to its own operations.

What the committee implies about the character and quality of the scientific literature is, however, even more alarming. What, for example, is an "advance announcement journal" and what is its function? The committee says there is a need for some means of bridging the gap between "the completion of a report on a scientific task and the ultimate publication of the report", points to the danger that this system may be used as a means of "claiming priority by people who have no serious intention to follow up" and urges that more should be done to "gain speedy publication of full papers"—which is "not to say that there is no place whatever" for "advance announcement publications", among which are singled out *Physical Review Letters* and *Nature*. This is a curiously mistaken view of what rapid journals are for. To begin with, a great many communications in them are complete in themselves, and it would be folly to ask that they should eventually be published somewhere else at even greater length. In practice, the rapid journals have to be as discriminating as the more ponderous journals, if only to avoid being swamped by the demand for space. In the nature of things, referees play an essential part. The difference is that the rapid journals apply or should apply as rigorously as they can the test of whether an article sent for publication really deserves to be published rapidly. *Nature*, by tradition and inclination, is also keen to publish material which does not fall easily into accepted classifications. When the committee of the Library Association writes that public funds should be used to help societies start more rapid publications, it begs more questions than it knows.

The committee also shakes its head in sorrow about the delays which are supposed to occur "because scientists sometimes have to search for journals in which to publish" and goes on to say that if this belief is justifiable "then it appears that the state will have to intervene". If this is more than mere bluster, it can only imply passive acceptance of the Parkinsonian law that the volume of the literature will always grow so as to accommodate what authors choose to write. This in turn amounts to a denial of the central problem in the management of the literature—the devising of techniques by which authors can be helped or even persuaded to write what potential readers are willing and able to digest.

In the circumstances, the committee's proposals for the better management of journals cut very little ice. It is not true to say, as the report does, that nothing can be done to accelerate the refereeing of articles. With many journals, referees are asked not merely to make decisions about the content but to polish manuscripts for publication. Journals could and should employ professional assistance instead. The committee also raises the wrong hares in what it has to say about the mechanical problems of producing journals. It is hard, for example, to believe that "speed is sacrificed for the sake of a high standard of typographical appearance and the sake of retention of advertisers". In practice, electron microscopists are more demanding than any advertisers. In any case, there is no necessary conflict between speed and typographical appearance. In the same way the committee's fondness for "typewriter composition" in the interests of speed can only stem from innocence of the printing trade. Most journals would be slower and also more expensive if they were set in type with typewriters.

The committee of the Library Association is entirely within its rights to raise these questions, for they are interesting and important. It is a pity it has not done a more thorough job.

Protein Group

THE inaugural meeting of the Protein Group of the Chemical Society was held at Burlington House on Friday, January 12. Proteins and nucleic acids are the twin pillars of molecular biology and a forum for the discussion of the chemical aspects of protein structure and its relation to biological activity, a field which draws on disciplines ranging from physics to biology, is greatly needed The proposal to form such a group originated with Dr G T Young, and protein chemists and biochemists are much in his debt for his efforts in launching this new venture The attendance, well over a hundred, at this inaugural meeting, augurs well for the success of the group, which seems likely to do much to speed progress in an important field of research

The six papers read and discussed at this inaugural meeting covered a wide range of topics, X-ray crystallographic studies being the only aspect of current work on proteins not represented The study of conformations in solution by physical methods was represented by a paper, by Dr Haylock and Professor H N Rydon (Exeter University), on the conformation of poly-L-methionine as revealed by n m r spectroscopy at up to 220 MHz, in chloroform–trifluoroacetic acid mixtures the helix breaks down, after protonation of about one peptide group in four, to the unprotonated random coil The tactics and strategy of peptide synthesis were illustrated by Dr Morley (ICI Pharmaceuticals) by reference to the synthesis of a number of physiologically active polypeptides and of a sequence in the neighbourhood of the active centre (the "cleft") of lysozyme The Liverpool group (Dr Agarwal, Professor Kenner, Dr Millington and Dr Sheppard) described a synthesis of canine gastrin and the use of N-methylation to render peptides more volatile and so more amenable to study by mass spectrometry Sequence studies were represented by two papers one, by Dr Butler (MRC Laboratory for Molecular Biology, Cambridge), dealt with the use of maleic anhydride as a lysine blocking agent in degradations with trypsin, the other, by Dr Press (Oxford University), with sequence studies on immunoglobulins, with particular reference to those parts of the molecule concerned in immunological activity Dr Doonan (University College, London) described several interconvertible sub-forms of glutamic–aspartic transaminase and suggested that these were conformational isomerides with identical sequences

It was generally agreed that more inter-disciplinary meetings of this kind were desirable, and a resolution was passed asking the council of the Chemical Society to approve the formation of the group The following were elected to the group committee Professor H N Rydon (Exeter) (chairman), Dr G T Young (Oxford) (secretary and treasurer), Dr M J Crumpton (Mill Hill), Dr R C Sheppard (Liverpool), Professor C A Vernon (London) It is proposed to hold meetings twice a year, the next being at Keele University during the autumn meeting of the Chemical Society in September Membership of the group is not restricted to fellows of the Chemical Society and there is no subscription, those interested in joining should write to Dr G T Young at the Dyson Perrins Laboratory, South Parks Road, Oxford

Phospholipids and Electron Transfer

from a Biochemistry Correspondent

There has been a great deal of progress in the study of the structure and mode of action of enzymes which can be obtained in solution, but enzymes which are bound to membrane systems are at present much less well characterized The membranes are usually complex combinations of protein, lipid and sometimes carbohydrate, and it has proved difficult to disrupt the membrane without losing the activity The phospholipids would seem to be ideal compounds for biological membranes, as they have both lipid-soluble and water-soluble groups in the same molecule Surface active molecules of this type can form structures called micelles in which the lipid groups form a lipid phase separated from the surrounding aqueous medium by the water-soluble groups Recent work on the electron transport systems of microsomes (particles produced by breaking up the endoplasmic reticulum) indicates that some of the enzymes in the microsome depend on the presence of phospholipid micelles of a particular form

The oxidoreductase in microsomes which transfers electrons from reduced nicotinamide adenine dinucleotide (NAD) to cytochrome *c* was discovered by Strittmatter, who showed that the flavin group and cytochrome b_5 act as intermediate electron carriers MacLennan, Tzagaloff and McConnell (*Biochim Biophys Acta*, **131**, 59, 1967) recently described a preparation which was 10 to 15 times more active than the original microsomes which contained much phospholipid as well as flavin, cytochrome b_5 and non-haem iron P. D Jones and S K Wakil (*J Biol Chem*, **242**, 5267; 1967) have now shown that the reactions transferring electrons from the flavin to cytochrome *c* will only take place in the presence of very asymmetrical micelles of phospholipid Extraction of the microsomes with acetone removed 80 per cent of the lipid and reduced the NAD–cytochrome *c* reductase activity to 20 per cent of the original The NADP–cytochrome *c* reductase (which does not use cytochrome b_5) and NAD–ferricyanide reductase activities were not affected The extracted lipids were fractionated, and attempts were made to restore activity by adding the separated lipids to the inactive microsomes Activity was completely restored only when the lecithins and lysolecithins together were made soluble by sonic oscillations. This preparation increased the activity of intact microsomes and, if the lecithin lysolecithin ratio was adjusted, extracted microsomes were restored to much more than the original activity

It has been shown previously that sonic treatment of combinations of lecithin and lysolecithin forms a sol containing mixed micelles which are long helical structures containing much aqueous solvent The synthetic phospholipid γ-oleyl-β-butyryl phosphatidyl choline forms similar micelles and also restores enzyme activity to the extracted microsomes In a note added in proof the authors state that mixed micelles of lecithin and free fatty acids gave the extracted particles twice the enzyme activity of intact microsomes It seems that electron transport from the flavin to cytochrome *c* in this system depends on the presence of lipid material, but the physical form is more important than the chemical constitution

Titrating Proteins

from our Molecular Biology Correspondent

It is nowadays seldom possible to open a journal and not find at least one potentiometric titration curve of a protein, and indeed the behaviour of the ionizing groups and their interactions are matters of considerable interest to the protein chemist and the enzymologist

But it is difficult to disentangle the many individual ionizations which make up the titration curve, and consequently interest has recently focused on spectroscopic methods, whether infrared, ultraviolet or nuclear magnetic resonance, which may be sensitive to the ionization of one type of residue Perlmann, Oplatka and Katchalsky, however (*J Biol Chem* , **242**, 5163, 1967), have now made a new examination of just how much information can be garnered from simple potentiometric acid-base titration curves

They have applied to the data for the protein pepsinogen the analytical procedures previously devised by Katchalsky and his associates for polyampholytes It is known from independent measurements of optical rotation that there is a *p*H-region in which the protein undergoes a change in conformation If the titration curves are plotted in terms of a function known as the buffer-capacity, defined as the *p*H increment for an incremental addition of hydroxyl ions (in effect the slope of the conventional titration curve), one will expect the conformational transition to manifest itself The reason is obvious enough if the protein is pushed into a state favoured by high concentrations of hydroxyl ions, it will respond by ejecting such ions, that is to say there will be a downward shift of certain *p*Ks For a sharp transition, the buffer capacity will then be very high at the transition *p*H, and indeed sharp, relatively spiky maxima are observed The *p*H of the transition—which decreases as the temperature is increased—is given by the position of the maximum, and the area under the curve gives the number of ionizing groups involved The three histidine residues of the protein are evidently implicated in the present case The conformational transition so measured corresponds with that observed in the *p*H-dependence of the optical rotation It is demonstrated further that the temperature dependence of the buffer-capacity curves leads to a value for the enthalpy of the transition, which can here be equated with the heat of denaturation It has, of course, long been clear that titration curves contain such information, but the directness and economy of the methods by which it has been extracted are to be admired

An interesting *p*H effect in proteins has been described by Shinitzky and Goldman (*Europ J Biochem* , **3**, 139, 1967) Earlier work by Shinitzky, Katchalski and others has shown that charge-transfer complexes are formed in aqueous solution between tryptophan and the protonated imidazole ring of histidine One consequence of this association is the quenching of the ultraviolet fluorescence of the tryptophan When the imidazole ring is titrated, the capacity for charge-transfer interaction is lost and the tryptophan fluorescence is restored Shinitzky and Goldman have now shown that the existence of these associations is surprisingly widespread It occurs in small peptides of histidine and tryptophan, but also in a large number of proteins, as judged by increasing fluorescence with rising *p*H in the neutral range In nearly all cases the fluorometric titration gives an apparent *p*K between 6 and 7, which renders the involvement of histidine highly likely—though other charged groups can also quench Given this measure of uncertainty, the method offers the possibility of determining ionization states of strategic histidines Whether these charge-transfer complexes are functionally significant remains an unanswered question

Nitrogen Fixation

from our Microbiology Correspondent

IN 1966 it was found that the nitrogen fixing complex could reduce acetylene to ethylene, a process that might prove important as an index of nitrogen fixation. At the University of Wisconsin, R H Burris (one of the discoverers of acetylene reduction by nitrogenase) and his colleagues have applied the method to the estimation of nitrogen fixation in the field (Stewart, Fitzgerald and Burris, *Proc US Nat Acad Sci* , **58**, 2071, 1967) Their success is a great stimulus to ecological studies of nitrogen fixation since the existing analytical procedures, based on changes in total nitrogen or nitrogen-15 assimilation, lack sensitivity or are expensive in time and equipment Although studies with cell-free extracts have shown that acetylene reduction is catalysed by the nitrogen fixing complex, it is perhaps unwise to rely entirely on this secondary standard; the authors urge that critical studies be verified by the nitrogen-15 fixing method The acetylene reduction assay has overriding advantages, however relative cheapness of materials and measuring equipment, and a sensitivity that makes possible short incubation times and assay of short term changes in the ecosystem. Another technological benefit here is the fact that the nitrogen fixing system has a higher affinity for acetylene than for nitrogen at comparable partial pressures This means that air contamination is not as serious in the acetylene assay as in the nitrogen-15 method and consequently rather simpler apparatus can be used

Stewart *et al* have used the acetylene reduction method to determine nitrogen fixing capacities of lake waters, soils and nodulated plants Thus, diurnal fluctuations in the acetylene reduction of lake waters were followed conveniently and shown to be related directly to the abundance of the blue-green alga *Gloeotrichia* Similarly, day to day variations reflecting the development and disperal of algal blooms could be determined by the acetylene assay The relative ease of the assay also made possible stratigraphical analyses in the lake being studied Reduction rates again were related directly to the abundance of blue-greens of which *Gloeotrichia* dominated the upper 2 metre layer while *Anabaena* appeared to be restricted to deeper water Acetylene reduction by *Alnus*, *Comptonia* and soybean was restricted to their root nodules The data relating to *Comptonia* demonstrated the dependence of not only nitrogen fixation but also acetylene reduction on the availability of photosynthetic products from the higher plant, ethylene production by afternoon sampled nodules was about twice as high as that of nodules sampled in the morning

The correlation between nitrogen fixation and acetylene reduction rates has not been studied extensively so far, but such information is essential if the acetylene reducing assay is to be used to estimate nitrogen fixation *in situ* On the basis of the electron requirements for the reduction of acetylene and nitrogen it might be predicted that the formation of ethylene should proceed three times more quickly than that of ammonia Ratios of 1·59 to 1 25 for the production rates of ethylene to ammonia have been reported for free-living, nitrogen fixing, soil bacteria, but clearly more data are needed to define the variability of this ratio.

Valence and Reactivity

from a Correspondent

An international symposium on valence and reactivity was held in Oxford under the auspices of the Chemical Society on January 9–11. It was attended by nearly 200 members from more than 20 different countries.

Valence problems in the second half of the periodic table (Groups IV–VII) are bound up essentially with the role of *d* electrons. As early as 1931, Pauling had proposed sp^3d^2 hybridization to account for the octahedral shape of SF_6. Further, the existence of complex ions such as ClO_4^- and the non-existence of FO_4^- are argued as evidence that the lower-lying 3*d* orbitals of Cl can be used more readily than the less accessible 3*d* orbitals of F. Similar situations occur when reactivity is considered. Thus, as Professor R F Hudson of the University of Kent showed, some substitution reactions can occur around a silicon atom by a form of ring-closure which does not occur in corresponding carbon compounds, an explanation is possible in terms of *d*-orbital participation in Si.

But the really important question is are we forced to invoke *d* orbitals in these situations, or are there alternative explanations? A careful study of several types of microwave experiments by Dr E A C Lucken of Geneva showed that in many cases alternative explanations were possible.

Some situations, however, did seem to be fairly certain. Thus Professor E H Wiebenga of Groningen showed that a complete account of the polyhalogen molecules and ions (such as BrF_3, I_3^-) can be provided without any use of *d* orbitals, all that is needed is the concept of three-centre bonds and *p* electrons. This definite situation has to be balanced by the equally convincing arguments of Professor N L Paddock of British Columbia, whose account of the phosphonitrilic ring systems such as the chlorides $(PCl_2N)_n$ invokes *d* orbitals in no fewer than two planes.

A reconciliation of these views is possible, as was shown by Professor C A Coulson of Oxford, if we say that $d\sigma$ bonding is seldom important in ground states of molecules, but $d\pi$ is much more likely to be important. Nevertheless in certain excited states, as Professor H H Jaffé of Cincinnati pointed out, a study of ultraviolet spectra shows $d\sigma$ and $d\pi$ orbitals to be necessary.

But the size of these *d* orbitals is critical. They must not be too large, as they are calculated to be for an isolated atom, or their overlap will be small and their contribution to the bonding negligible. Professors D W J Cruickshank of Manchester and C Zauli of Bologna have calculated that both the presence of ligand groups around a central atom, and the mutual spin coupling arrangements of the valence electrons, can provide a mechanism for contracting the size of a *d* orbital. So also can the presence of a formal positive charge on the central nucleus. In this way there is a nice explanation for the greater likelihood of large valence numbers when highly electronegative ligands such as fluorine are used.

An important feature of this symposium was the all-round character of the discussion. Topics of this kind require a comprehensive view. In this case it was abundantly justified.

Discussing Nucleotides

from a Correspondent

Although bad weather cut down the attendance at the inaugural meeting of the nucleotide discussion group of the Chemical Society held on January 9 in the Chemistry Department of the University of Birmingham, the programme was followed mainly as planned. Professor M Stacey formally opened the meeting by welcoming the visitors and Dr A S Jones took the chair.

The first proceedings took the form of a business meeting to constitute formally the nucleotide group. Dr Jones reported that more than seventy people had expressed an interest in the formation of the group and that a provisional committee had met to make proposals about rules and the constitution. The object is "to encourage the discussion of the chemistry including the biological chemistry of nucleosides, nucleotides and nucleic acids". The committee will consist of a chairman, secretary-treasurer and four members who would normally serve for three years. The following were elected chairman, Dr A S Jones (Birmingham), secretary-treasurer, Dr D W Hutchinson (Warwick), committee members, Dr M G Blackburn (Sheffield), Dr D. M Brown (Cambridge), Professor K Burton (Newcastle) and Dr R T Walker (Birmingham). The committee is to arrange a meeting at some convenient time during the early summer.

The inaugural lecture at Birmingham was delivered by Professor Lord Todd, who drew attention to the large amount of work carried out on the synthesis of nucleotide analogues and suggested reasons for their relatively poor chemotherapeutic properties. He indicated that more information is needed about the role of rare bases in nucleic acids and that better methods of forming phospho-diester linkages in oligonucleotides are also required. Among the methods used to determine nucleotide sequence, he suggested that procedures by which one base might be rendered visible in electron micrographs by the use of a specific chemical marker would be worth developing. On the biochemical side he suggested that further work is necessary in order to establish more about the nature of the infectivity of viruses in particular to decide why some RNAs are infective while others are not.

There were also four contributed papers. Professor K Burton (Newcastle upon Tyne) gave some results on the effect of specific chemical reagents on the acceptor activity of transfer RNA and pointed to the need for a method of determining the tertiary structure of transfer RNA. Dr T L V Ulbricht (Twyford Laboratories) discussed the use of optical rotatory dispersion in the determination of the conformation of nucleosides and its application in the determination of the conformation of nucleic acids. Dr G Shaw (Bradford) discussed investigations of the chemical reactions involved in the biosynthesis of purine nucleosides, in particular the carboxylation and decarboxylation of some substituted imidazoles. Dr A S Jones (Birmingham) described the synthesis of polymeric analogues of polynucleotides, including polyacrylate derivatives with purine and pyrimidine side chains, and polycarboxymethylthymidine. These polymers interact in solution with polynucleotides.

The British View on the Big Machine

Should Britain support the plan to build a 300 GeV proton accelerator somewhere in Europe? A document just published shows that not everybody thinks it should

THE Department of Education and Science has now published the documents which have helped to form the policy of the British Government on the construction of the 300 GeV proton accelerator (HMSO, 7*s* 9*d*) At the last Council meeting of CERN on December 13, 1967, a final decision about the machine was postponed at least until March and probably until the end of 1968, partly so as to allow Britain and Spain to assess the implications of devaluation for their membership of CERN, but chiefly because there is a feeling—fostered by the Government of the Federal German Republic—that there may be profit in reconsidering the technical design of the 300 GeV machine put forward last year by the European Committee for Future Accelerators The publication of British documents, the chief items of which are opinions of the sub-committee of the Council for Scientific Policy under Professor Michael Swann, and of the Science Research Council, is of some importance because the Department of Education and Science was at first inclined to keep this material to itself

Devaluation

In his introduction to the report, Mr Patrick Gordon Walker, Secretary of State at the Department of Education and Science, says that the decision about the 300 GeV machine "cannot be considered by itself", but must rather be "part of the plan for all high energy physics, both national and international" Mr Gordon Walker also points out that the opinions which have guided the formation of policy on the 300 GeV machine were given before the devaluation of sterling The reduction of the external value of the pound will increase the direct cost of participation but not necessarily by the full amount of the devaluation because, "if the United Kingdom were to participate, its share of the cost of the project would in due course be re-assessed" Devaluation may also change earlier views about the advantages of siting the project in Britain, and has already made necessary a reconsideration of the British Government's involvement in projects overseas "The Government will announce its decision in due course"

Listed in the report are the members of the working group appointed by the Council for Scientific Policy Chaired by Professor Swann and working in close collaboration with the Science Research Council and its Nuclear Physics Board, the group was formed to examine the implications of the proposed accelerator and to assess the wider effects of implementing it The group includes Dr J B Adams, UKAEA, Dr F S Dainton, vice-chancellor of the University of Nottingham, Professor B H Flowers, chairman of the Science Research Council, Dr J C Kendrew, deputy chairman of the Medical Research Council Laboratory for Molecular Biology, Cambridge, and Professor A B Pippard, professor of physics at the University of Cambridge

In a letter to Mr Patrick Gordon Walker which is included in the report, Sir H S W Massey, chairman of the Council for Scientific Policy, states that he and his council members share the view of the SRC "on the scientific value of the 300 GeV project and on the importance for European basic science" It should be possible, he adds, to accommodate the project within a budget for nuclear physics rising at seven per cent a year for the next decade, but that it would be more than this in the early 1970s Should costs rise, however, he suggests that they should be met by savings from the budget for nuclear physics Sir Harrie also points out that the benefits to Britain of having the machine in Britain would be great despite the diversion of resources which would be necessary to establish and maintain it

Escalation

The council takes the line that an escalation of cost in building and operating the 300 GeV machine could seriously divert resources from the rest of science and that the project should not be undertaken without assurances that this will not happen (Lord Jackson, one of the fourteen members of the committee, is described as "having reservations about the practicability of achieving such safeguards") The council also emphasizes that long-term planning on behalf of nuclear physics will put the rest of science at a disadvantage unless there are long-term plans to foster developments elsewhere

According to the working group, it is essential for Britain to participate in the project if it is to continue with a high energy nuclear physics programme Reference is made to the possibility of a substitute for the 300 GeV machine, such as obtaining access to the 200 GeV machine in the United States Workers from the US would in return have access to the Intersecting Storage Rings (ISR) facility at CERN Such a scheme would obviously depend on assurance of access to a substantial proportion of the machine time—which is unlikely Another hazard which the group visualizes is that the cost of mounting experiments at such a great distance would be excessive There is also the danger of generating a further brain drain For these reasons, such a substitute would therefore be a poor one

Work at this level is of course expensive and there is no guarantee that it would have commercial applications If Britain were to participate, it would have to be recognized that the country would be committed for the next thirty years The group therefore considers that even on present price estimates, the project could only be financed if science resources rose at an annual average of about nine per cent for the next decade

Cost

The actual cost of the project falls into several parts the cost of constructing the machine together with ancillary costs which must be met by the host country, operational and experimental costs, the cost of necessary national facilities, and the necessary supporting costs for research staff in British universities to enable them to use the machine Assuming that Britain would contribute 25 per cent of the cost, its annual contribution would be as shown in Table 1 The host country would be responsible for providing houses, roads, hospitals and schools, while the machine construction, operation and experimental costs would be shared among nations according to their gross national product

Table 1. BRITISH CONTRIBUTION TO THE 300 GeV ACCELERATOR (£m = 12 m Swiss francs)

Construction phase	Machine proper	General laboratory facilities and services	Preparation for research	Operation of accelerator and experiments	Experimental equipment and facilities	Total £m (rounded)
1967	0 073	0 021	—	—	—	0 094
1968	0 156	0 042	—	—	—	0 198
1969	0 364	0 104	—	—	—	0 469
1970	1 260	0 573	0 104	—	—	1 938
1971	3 573	0 958	0 354	—	—	4 885
1972	3 833	1 115	0 521	—	—	5 469
1973	3 698	1 083	0 833	—	—	5 615
1974	2 969	1 635	1 510	—	—	6 115
1975	1 938	1 750	2 406	—	—	6 094
1976	1 532	1 708	2 885	—	—	6 125

The group acknowledges that the need to insure against the possibility of cost escalation is very real and therefore recommends that Britain should enter the project, but with some reservations First, there should be an assurance that science resources would be allowed to rise at a rate sufficient to allow the rest of science to develop properly Second, there should be a limitation of the construction and operation costs of the machine and ancillary equipment by previous international agreement Third, commitment on operation and equipment costs should be deferred until construction costs are clearly defined Finally, any increase in costs during the construction phase should be met by corresponding economies in the nuclear physics budget. Even if these safeguards were observed, the group suggests that Britain could only just afford to participate in the project and maintains that, if ever another machine were proposed, it would have to be built on a wider basis than that of collaboration within Europe

The working group's report also refers to the SRC's Forward Look for 1968–69 to 1972–73, in which the SRC demonstrates how the costs of the project could be maintained within the amount provisionally allocated to it by the CSP for 1968–69 and 1969–70, provided that the SRC received some of the CSP unallocated reserve Already, Britain's expenditure on nuclear physics research is more than £17 million a year, and if participation were agreed to, this would rise to £34 2 million by 1977–78—an annual rate of increase of seven per cent from beginning to end of the period The ECFA, which made a detailed design of the machine and an estimate of cost, did, however, state that the proposed equipment for the machine is at a level "entirely appropriate to the general scale and importance of the proposed accelerator" and that it is "far from extravagant"

If the figure of seven per cent were to be insufficient to support operation and growth of the 300 GeV accelerator and the CERN facilities, respectively, the group emphasizes that it might be necessary for Britain to withdraw from CERN (Meyrin)

Manpower requirements for the machine should be met without great difficulty, although it might not be possible to accommodate all the extra postgraduate staff required in university posts For this reason, the group states that the SRC should award essentially temporary postdoctoral fellowships at the universities so as to transfer acquired skills into industry As for the benefits, the group recognizes that although the project would be of extreme scientific interest and the results would be important, there are, on the other hand, many scientific activities of comparable interest which cost only a fraction of the amount required to finance the 300 GeV accelerator The project would, it is argued, introduce new dimensions into teaching physics and it would also encourage the flow of technical skills into industry as well as set a challenge to industry as new products and techniques will have to be developed There are also the general political benefits of participating in a European project

Economics

On the economic benefits of participation, Professor Wolfe and Professor Youngson of Edinburgh University maintain that there would be no overall benefit in having the machine sited in Britain The group therefore considers that, on balance, it is not worth offering an extra subscription so as to secure a site in Britain Indeed, there might be a loss incurred if the accelerator were sited in Britain, as the site which has been proposed—Mundford in Norfolk—is one in which there is full employment Drs Wolfe and Youngson furthermore have estimated that if the project were to absorb unemployed resources so that the project would lead to a total net addition to the national product, this would have an adverse effect on the Government stabilization policy, causing wages and prices to rise Overall, their view is pessimistic

This gloomy economic assessment will come as a surprise not merely to those in Britain who have taken it as axiomatic that local construction could not fail to be profitable but also to nations elsewhere in Europe which have been angling for an invitation to build To begin with, Professors Wolfe and Youngson say that they do not believe construction would be completed for £148 million even though they have used this figure in their calculations They also point out that the proposed British site at Mundford does not qualify, under the British legislation on the encouragement of projects in development areas, for subsidies which would amount to something like £6 million spread over the period of construction

The calculation in the report suggests that the external balance of payments of the United Kingdom would benefit to the tune of between £84 and £136 million during the 30 years which are likely to be occupied in construction and operation of the accelerator These comparatively modest sums are the figures obtained by setting off against the receipts of non-British currency to be expected the costs of imported equipment, remittances by foreign staffs and British tax refunds The direct cost of having the accelerator on a British site turns out to be substantially greater than this According to the calculations implicit in the report, the total cost of providing the land-access and service roads and utility supplies would work out at between £186 million and £336 million according to the extent to which resources would have to be diverted from other projects One way and another, the authors of this study conclude, there would be no significant advantage in building the 300 GeV accelerator in the United Kingdom Professors Wolfe and Youngson suggest that there is no reason to expect that the siting of the machine in Britain would substantially increase the effectiveness with which British companies tender for contracts with CERN

The chairman of the SRC at the time, Sir Harry Melville, also comments on the case for participation in the proposed 300 GeV accelerator Generally, the SRC supports the scientific case for building the accelerator and believes that the estimates of construction and running costs are reasonable The council recommended participation if certain safeguards are observed It thus supports previous views that, despite the importance of the project, it should not be allowed to imperil the development of other disciplines Another safeguard is that other countries should agree to participate on a sufficient scale, and finally that the CERN Convention which is now being negotiated should be revised to ensure adequate safeguards of the rights of participants in the 300 GeV laboratory

In considering the resources which would be required in the five years 1973–74 to 1977–78, the council has estimated that all the expenditure on nuclear physics in the second five years could be accommodated within a growth rate of some five per cent per annum this, however, makes no provision for unforeseen major projects It makes clear the fact that it would not be prepared to meet any deficits in the nuclear physics budget arising from escalation on this project, by re-allocation from other disciplines In the event of any such escalation, the council maintains that additional resources would have to be sought or else the nuclear physics programme would have to be reduced during the construction period with serious detriment to the whole programme Furthermore, during the operational period, total or partial withdrawal from CERN (Meyrin) would be necessary

Concerning the proposed UK site at Mundford, Sir Harry describes it in the report as consisting of flat sandy soil overlying chalk, it belongs mostly to the Forestry Commission British experts are at present conducting elaborate loading tests to determine the stability of the strata and these should be finished by August The SRC is also concerned with the cost to Britain of locating the project there, and maintained that this could be greater than assumed It does, however, suggest that some of the additional costs which would be involved might be offset by the "balance of payments advantage flowing to the host nation"

Sir Harry discusses the possibility of collaboration with the US and the USSR where big machines are already built, or are planned A meeting was, in fact, arranged in Vienna in 1964 of the Americans, the Russians and a party of CERN representatives They examined the possibility of a tripartite provision of a single world facility at the 200–300 GeV level As the report makes clear, however, nothing came of this

Dissent

The SRC view emphasizes that, for the project to be successful, it is essential that the four countries, France, Germany, Italy and the UK, which account for 76 per cent of the CERN budget, should participate Although two members of the council—Professor Sir Ewart Jones and Professor Sir Ronald Nyholm—dissented, the council as a whole supports the proposal and is in favour of an early decision

The dissenting case is brief

"We recognize that a good case, on purely scientific grounds, has been made for proceeding with this project But we are also acutely aware that there are other scientific fields which, cultivated and nurtured as nuclear physics has been in recent years, would yield still richer harvests Some of these are of no less scientific interest and have much greater potentialities for benefiting mankind. In several of these fields we are leading the world, we should be able to offer generous support where such ability is demonstrated, but this we cannot afford to do

"Our participation in this project means that for the next ten years the Science Research Council expenditure on a single branch of physics will continue to consume more than 40 per cent of the funds which seem likely to be available Thus the council's intention gradually to reduce the proportion of its resources going to nuclear physics will be completely frustrated We are convinced that to continue to spend such substantial sums in this direction is not in the national interest and that many scientists in this country, if they are properly appraised of the situation, will view the prospect with dismay Can we expect them calmly to accept a slowing down of their activities whilst we go ahead with a huge project which will directly benefit only two or three hundred academic physicists ?

"We cannot reconcile embarking upon this massive new commitment with our present unsatisfactory and worsening situation Can high energy nuclear physics justly claim to be of such overwhelming merit and importance ? We should urgently be seeking opportunities of investing comparable, and if possible larger, sums in projects which offer some prospect of material advantage to the community and which at the same time serve to train useful scientists and technologists (E R H Jones and R S Nyholm)"

The conclusions and recommendations of the Nuclear Physics Board of the Science Research Council are presented in the final pages of the report. In an introduction the board refers to nuclear or particle physics as one of the main growing points of science, and emphasizes the "outstanding and pressing case for constructing in Europe a 300 GeV proton accelerator" It estimates that the machine would take nine years to build and would serve the needs of European physicists for the next 25 years

The second section of the board's report deals with the present world situation in particle physics and lists the present proton and electron accelerators now in use and the way in which they operate The present position of Britain in the field of particle physics is, it suggests, a favourable one and the scientific and technical skills available indicate that Britain more than any other European country is able to exploit fully a machine such as the proposed 300 GeV accelerator If Britain is to remain an important contributor to this field of research on a world scale, the board argues that participation is essential

A brief description of the machine emphasizes its flexibility It has been designed to produce 10^{13} protons per second and will produce 10–20 GeV beams up to 1,000 times more intense than are at present available Using this machine, not only will the energy range of neutrinos be greatly increased but the time required for a particular experiment will be cut from weeks to days Following the 70 GeV machine in the USSR and the 200 GeV machine in the US, the 300 GeV accelerator would take a natural place in the development of high energy accelerators

Of the 22 sites originally proposed, nine in different countries are still being considered The board refers to the stringent geotechnical requirements of any site, this is because very accurate alignment of the large magnet ring and beam lines is essential and must be maintained in the presence of movable massive radiation shielding and experimental equipment The board holds a more optimistic view of the benefits to any nation of having the machine in its territory Among the benefits the board mentions are easier travelling to and from the site and therefore easier combination of research at the centre with university activities In terms of economic benefits, the host nation would have a near-monopoly of construction contracts, and a proportion of salaries and wages would be spent in that country, to the value of £12 million per annum

One suggestion which has been made concerning organization is that CERN and a new 300 GeV laboratory should both be under a single council, responsible for a single European programme The board recognizes the importance of securing a convention which safeguards the rights and interests of those states participating in the project, and of ensuring that a state could withdraw from CERN (Meyrin) while, remaining in the 300 GeV laboratory and in the general CERN Council

Confrontation in Paris

from our Special Correspondent

A POLICY for science is an elusive thing, not to be pursued by those who hope for quick and easy answers But this seems not to have deterred the members of the Committee for Science Policy of the Organization for Economic Co-operation and Development, who met in Paris last week The committee gathered to hear the last stage in the examination which the OECD has made of the science policy of the United States The meeting was a confrontation between the examiners who have been responsible for the report, and delegates from the United States who know at first hand how science is organized in the United States

The party from the United States was led by Dr Donald Hornig, President Johnson's Special Assistant for Science and Technology He was accompanied by Dr Ivan Bennet, Deputy Director of the Office of Science and Technology, Mr Herman Pollack, Director of International Scientific and Technological Affairs, Department of State, Dr Philip Handler, Chairman of the National Science Board, Congressman George P Miller, Chairman of the Committee of Science and Astronautics of the US Congress, Mr William Carey, Assistant Director, Bureau of the Budget, and Mi James A Shannon, Director of the National Institutes of Health

The report itself falls into two sections The first, prepared by the examiners, consists of four essays written from an individual point of view The second section, prepared by the secretariat, backs up these rather subjective views with some statistical data This is necessary because of the way in which the examiners carried out their survey—a ten day visit to the United States, packed with interviews at every turn, may capture the flavour, but cannot include all the facts The examiners' reports should not therefore be expected be to correct in every particular, nor to agree on every point

The confrontation meeting at the Chateau de la Mouette in Paris covered four broad areas The first, science policy proper, was concerned with the institutions, the problems of defining priorities, the role of Congress, the dangers of the "military-industrial complex", and the integration of science into foreign policy The second area of discussion covered the impact of science policy on academic research One question asked, for example, was whether the efforts being made to produce more qualified manpower could ever be successful Was there not a danger that increasing the output of qualified people would itself have a multiplying effect which would create a demand for more people than could possibly be supplied ? In the third and fourth areas of discussion, science policy and industry, problems such as the distribution of federal expenditure between firms, and the extent to which prosperity in some areas of industrial life relies on federal support, were discussed Professor Casimir raised the problem of the amount of spin-off which derives from large research projects Did one have to have a space programme to get the industrial benefits ? In the US, he said, companies tended to say that the effect of spin-off was negligible, while federal agencies said that the importance of spin-off could not be over-estimated Dr Hornig said that it was hard to reach the truth by polling industry, which tended to oppose large scale federal research and development The identifiable direct spin-off was small, he said, but the indirect effects were considerable

M Pierre Massé went on to discuss the ways in which federally supported science could be brought to bear on social problems Dr Hornig started his reply by saying that there was no problem in finding places for social scientists—lawyers already filled Congress, and economists had an important place in the US And large scale social problems were not as new to the United States as Europeans sometimes implied—there was Social Security and the New Deal in the thirties, for example But one of the difficulties was deciding what should be done—in the cities, for example, half the social scientists thought that the problems could be solved by increasing population density, while the other half thought that it was too high already Technology alone could not solve problems as complicated as this, though it could help Mr Carey added that they were relying heavily on faith and hope in turning to the social scientists—it was not yet clear how they could help, although they were eager to

M Pierre Massé brought the discussion round to the problem of the technology gap The rapid advance of technology had introduced a de-stabilizing effect into international trade relations, and there was a danger that if the US economy became too dominating, it would invite repercussions from Europe Did the United States feel that it was part of its duty to make sure that this did not happen ? In the short term, would it not be in the United States interest to support schemes of industrial good-neighbourliness, to enable Europe to catch up ? Dr Hornig thought that schemes of this sort were not really necessary—"the process of catching up is always easier than the process of getting away" Japan was a living example of this Not only were the differences of wage rates a stabilizing influence, but the rapid flow of technological information also tended to even things up Dr Hornig's recipe was that international relations should be stimulated by competition, mitigated by the effects of the transfer of information and by co-operation on some projects

All this was useful discussion, if at times rather imprecisely articulated What did emerge was at least some reassurance to the Europeans—the United States has not achieved its pre-eminence in science and technology by organizing it in a fundamentally more efficient way There are, of course, occasional felicities of organization which could be transplanted with advantage to Europe, although a number of them would require a more united Europe if they were to be applied with any hope of success But if the fact that the United States administration has not hit upon some uniquely happy form of organization is a reassurance to some European governments, it also means that American experience is unlikely to supply any short cuts to success

The short lesson, although the United States witnesses were far too tactful to put it this way, was that Europe should concentrate in putting its own house in order United States success should be seen as a spur to European success, not as a daunting reminder of failure And observers were left with several clear impressions of the way in which Europe could set about the task The principal need, it is probably fair to say, is for far more genuine collaboration in Europe—the formation of European learned societies would be a worthwhile start On the industrial front, the Economic Community should make sure that its organization provides for effective industrial mergers within Europe, instead of making it easy for United States companies to pick off European companies by lightning sorties with cheque book in hand

Does the United States have a Science Policy?

In Paris last Friday the OECD organized a confrontation between the authors of the review of United States science policy which it is to publish a month from now and a number of distinguished American officials. What follows is a summary of the review.

THE underlying tone of the document which the OECD has prepared is one of wonder at the scale on which science and technology are supported in the United States. In many places, this leads quite simply to suggestions—or hints—that the experience of the United States is not directly relevant to the problems of most nations in Europe. The four examiners appointed by the OECD are also somewhat preoccupied by the constitutional differences between the United States and most European nations. The result is that the published review will probably be more valuable to those outside the United States who seek a sensitive explanation of the relationship between the Administration, Congress and the external organizations such as the American universities than by those in the United States who may have been looking to the report for solutions to some of the problems which are now becoming prominent.

The tone of the review is set by the passage in the introduction which says that "by the magnitude of the resources which it deploys, the United States scientific and technical effort is today the most important in the world, and this immense effort, far from taking the form of a unified policy, displays an astounding plurality of methods, institutions and means. We could not begin to think of reviewing all the questions involved or even of going deeply into every aspect of each question. . . ." The four examiners go on to explain that their reports must be regarded as the "opinions of four European experts of very diverse background and culture . . . " and that they have decided that it would be "preferable that each of them should recount certain of his immediate and personal impressions" than that they should attempt to produce "a single comprehensive report". The result is that the review begins with a statement prepared separately by each of the examiners. What then follows is a more detailed survey of institutions and problems which has been prepared by the scientific secretariat of the OECD.

The Examining Team

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Netherlands

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State Minister,
Member of the Chamber of Representatives,
Former Prime Minister,
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PIERRE MASSÉ
Chairman of the Board of Management of
Electricité de France,
France

C. H. WADDINGTON
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Institute of Animal Genetics,
Edinburgh,
United Kingdom

The Belgian View

M. Théo Lefèvre, the Belgian examiner, begins with the flat statement that it is "impossible to speak of a science policy—in the singular—in the United States". He explains that research and development activities have developed "to meet challenges as and when they impinged on the American consciousness", but that programmes in fields such as atomic energy and space exploration have usually been so broadly conceived as to cover the "whole spectrum of the natural sciences". In this process, however, social science and the humanities have been neglected, and M. Lefèvre points out that it was only in 1965 that the Arts and Humanities Foundation was established "as a sort of puny offspring of the National Science Foundation".

In the United States, M. Lefèvre continues, "an ethics has been successfully created of research and development in the service of progress", with the result that research takes on the appearance of a duty. The response of the Federal authorities to a new challenge is to create a new agency which is usually responsible directly to the President. The creation of a centralized ministry of research would run counter to federalist principles, with the result that there is "no question of centralization as we understand it in our European states of Napoleonic mould". Indeed, M. Lefèvre continues, the same "Federalist reflex" leads some agencies to reserve for themselves only responsibility for programming and planning, leaving the execution of policy to industry and the universities. Although the United States has been able to steer clear of the obvious danger of an oligarchy of technocrats, the vertical organization of research and development has led to the emergence of "feudal fiefs which escape the demands of policy". Because these agencies have been able to interpret their mandates widely, M. Lefèvre considers that scientists in the United States have found them to be beneficial. One consequence is that scientists have been able to approach several agencies for support, with the result that there is only a small risk that valuable projects will be neglected for lack of support. From the outside, M. Lefèvre continues, this system has drawbacks such as duplication and its cost sometimes seems "very high".

Of the President's Special Assistant for Science and Technology (now Dr Donald Hornig), M. Lefèvre says that the lack of power of decision does not prevent this official from being the only person to have an overall view of Federal activities in science. As the Special Assistant's personal team has been strengthened over the years, his influence has increased, particularly in the fixing of the budgets of the various agencies.

M. Lefèvre is also impressed with the recent increase of congressional influence on science policy, and he singles out for special attention the setting up within the Library of Congress of a group of congressional advisers. The result has been to "loosen the stranglehold" of the Administration. One happy consequence has been that there may now be a more equitable distribution of federal funds. On the other side of the coin, the way in which pressures on Congress may subordinate national to parochial considerations may lead to a waste of resources.

M. Lefèvre is more perceptive than his fellow examiners about the National Science Foundation. He defines the foundation's function as that of taking over from the military agencies in the field of fundamental research and of balancing the needs of different sectors. "It is no doubt regrettable that the National Science Foundation does not seem to have succeeded fully in all its tasks. A newcomer, with less power than other agencies and no doubt because its directors have not sought to make it assert more power, its support has more often been complementary than propulsive. . . . In the event of the European countries pooling their efforts, they should equip themselves from the outset with a similar instrument but be prepared to display greater boldness."

In M. Lefèvre's view, although federalist principles and the policy of creating agencies have so far yielded good results, this is more by accident than design. Defects which are tolerable in a society of plenty would be unacceptable in a European society with limited resources. M. Lefèvre points out that Americans are beginning to recognize the importance of cost-benefit analysis of research programmes, and have accepted that science must in future be contained within fixed budgets. "The cost of the Viet Nam war can only bring nearer the moment of decision." The difference between the United States and Europe, according to M. Lefèvre, is that American resources are greater, the United States finds it easier to mobilize resources because of political unity (compared with Europe as a whole) and that the United States is able to set national goals for itself and then set out to achieve them. In science policy, European unity is a precondition of success.

On the issue of technological spin-off, M. Lefèvre considers that the electronics, chemical and instrument industries have benefited directly from the scientific and technical programmes supported by the Federal government, but that the chief influence of the large government programmes on the economic life of the United States has been indirect. For one thing, large projects have helped to create a climate in which research is regarded as an economic duty, in which industrialists are "research minded" and willing to take risks. Another indirect spin-off is the development of the multiversity, the growth of the larger universities and the promotion of smaller institutions into the category of the large. M. Lefèvre considers that research contracts have been chiefly responsible for these developments in higher education. A part of the same trend has been the appearance of large numbers of highly qualified executives in American industry, which has served to reinforce the economic prowess of the United States.

M. Lefèvre's view of the United States is not entirely cheerful, however. "Research sectors are neglected which are or seem to be irrelevant to the challenges which are felt—those who devote their lives to such research are stigmatized as "egg-heads". Regions are undeveloped in which there are no universities or industries which fall under the authority of the agencies. Those who cannot stand the pace are destitute, sometimes to an extent which is unimaginable in Europe. They are left to the paternalism of the churches and the charitable institutions, pending the arrival of the 'Great Society', the resources for which are lacking because they are swallowed up by the war in Viet Nam."

This line of argument leads M. Lefèvre to the conclusion that the growth of science and technology is likely to have an important influence on United States politics. The Federal Government's concern for the supply of scientists and technologists has already persuaded the Federal Government to intervene directly (in 1965) in the administration of education, previously the preserve of the states. He considers that problems of air and water pollution, surface transport and even public health campaigns will increase the tendency for the Federal government to intervene in matters which have traditionally been the prerogative of the states. To be sure, the Federal Government will hang back as far as it can. "But is there still room for such a concept in the web of contractual relationships which the Federal Government has spun around itself. . . . Will not the new forms of federalism which are tending to emerge quite simply be forms of transition towards a more centralized state ? The future will teach us whether science and technology do not

The Examining Board at the OECD meeting. Left to right: M. Pierre Massé, President of Electricité de Frnce; Professor C. H. Waddington, of the Institute of Animal Genetics at the University of Edinburgh; Professor H. B. G. Casimir, director of the Philips Laboratories in the Netherlands; M. Théo Lefèvre, former Prime Minister of Belgium; Mr Gerald Bell of OECD; Dr Jacques Spaey, Chairman of the Science Policy Committee of OECD (the last two were not examiners).

determine an irreversible movement of centralization and unification."

M. Lefèvre also sees as a portent the omnipresence of scientific advisers at all levels of the Federal Government. There are, he says, hierarchies of scientific committees which duplicate in their inter-relationships the hierarchical relationships between the agencies themselves. But the scientists on the advisory committees also retain their links with the universities or industry. This system may well be the best way of circulating new ideas, but there is also the danger that the scientific committees will acquire more than their democratic share of influence. "In the extreme case, is there not a danger that a *de facto* power may become gradually and imperceptibly established at the cost of the *de jure* power and that, in the long run, political democracy may be no more than a front ?"

M. Lefèvre is one of those who believes in the technology gap. Indeed, he considers that it can be traced back to the two world wars in the twentieth century, to the "decolonization operations" which have followed the second of them and which have given European nations a more realistic appreciation of the balance of power in the world. But Europeans have the feeling that the United States is now preoccupied with problems elsewhere, as is the Soviet Union. Europe in the larger sense exists, "in a larval state", with no common economic policy as yet and no policy for research and development. "Many people prefer to shine in their canton without troubling themselves about the whole. In any event, how can the whole be grasped so long as walls are erected between research, the management of the economy and the administration of the estate ?" All this can be seen in the universities, which are "ivory towers, First Empire style". In the same way, in European industry and commerce, men arrive "at the seats of power and at the age of retirement about the same date". There is an undeniable gap, but it is a "managerial gap". And "whereas the American concentrates on the future, on risk and on progress, the European seems to opt for safety first and sometimes even for stagnation". Europeans seem not to have heard of what the Americans know from practice, that "in the sphere of research and development, it is the young people who have the experience".

It is plain that M. Lefèvre envies on behalf of Europe the plasticity of American industry. European nations are tempted to keep dying textile and coal industries alive, but the United States is willing to "let the dead bury the dead". He suggests that there should be a more deliberate development of growth industries in which European countries may have an advantage, a combined European policy on research and development, and the judicious exploitation of "American know-how" under licence. But Europe will not be able to make these policies succeed without political unity—indeed, there is a danger that "if the Common Market does not move towards political unity, we would have created circumstances even more convenient for invasion by large American firms".

This view of the reasons for the disequilibrium between Europe and the United States finishes with the declaration that the United States profits from the brain drain, but that there is nothing to be done about it until Europe can offer scientists and technologists conditions for work comparable with those they find in the United States . . . that United States tariff policy widens the gap, as does the tendency of American industry "to launch into production lines where Europe is beginning to give proof of competence" and when "it divides us by favoured partner policy or by offers of technological co-operation to NATO". In the long run, for M. Lefèvre, there is only one way of dealing with the technology gap as he has defined it—"we must unite by means of common policies on both the scientific and the economic planes. This cannot be done without new trans-national institutions".

The British View

Professor C. H. Waddington echoes the opinion that the structure of the mission oriented agencies in the United States is an important determinant of science policy, and emphasizes the way in which the distinction between operational and research agencies is minimized. The fact that the National Institutes of Health are responsible to the Public Health Service, which is also charged with executive responsibilities for public health, contrasts with the way in which, in Britain, the Medical Research Council is responsible to the Department of Education and Science and not to the Ministry of Health. One result is an "extreme plurality" of administrative organizations which "has enormous merits and also a few disadvantages".

The United States Delegation at the OECD meeting. Left to right: Dr Ivan Bennet, Deputy Director, Office of Science and Technology; Mr Herman Pollack, Director, International Scientific and Technological Affairs, Department of State; Dr Philip Handler, Chairman of the National Science Board; Mr George P. Miller, Chairman of the Congress Committee on Science and Astronautics; Dr Donald F. Hornig, Director, Office of Science and Technology; Mr David Beckler, assistant to the Director, Office of Science and Technology; and Mr William Carey, Assistant Director, Bureau of the Budget.

Professor Waddington says that "American scientific leadership" is at present somewhat concerned about the functions which should be performed by research institutes operated directly or indirectly by the Federal Government (and which appear to cost $4,800 million a year). The difficulty is that most of these laboratories belong in some sense or another to mission oriented agencies, but that many of them have been in existence for such a long time that the "system of priorities in force at the time of their foundation" has been materially altered. The system of mission oriented planning would tend to force unwanted laboratories into decline, while encouraging the creation of others intended to meet new needs. Professor Waddington says that discussions are now taking place of ways by means of which a laboratory belonging to one agency may be enabled to put its resources at the service of some other agency, and he suggests that the vital factor is not the administrative arrangements which may be made but the "entrepreneurship of the laboratory director".

Like his fellow examiners, Professor Waddington has clearly been impressed by the importance of what is called the project grant system as a determinant of science policy in the United States. He also considers it to be the origin of several palpable disadvantages, mostly recognizable in the quality of scientific life in the United States.

The essence of the system is that attention is focused on individual items of scientific work rather than on general progress within a broad area—a system which is "in rather sharp distinction to the traditional practice in European universities in which in general the entity on which a judgment of value is passed has been a department covering a considerable field". Professor Waddington says that the American system has the advantage of making it possible rapidly to "encourage excellence wherever it may appear". Young men can obtain support directly, and do not have to apply through the heads of their university departments who, "for a variety of reasons, may not respond as readily as outside bodies would do". Professor Waddington says that this feature of the American system of awarding grants for research is "one of the main features which has enabled American science to advance so rapidly in the last 20 years".

The machinery for awarding project grants is held to be important. Professor Waddington points out that the National Science Foundation uses some 27 advisory committees, each with about 10 people appointed for comparatively short terms of office. The result is a rapid turnover of committee members and the "almost complete prevention of gerontocracy". Because there is direct contact between committee members and scientists seeking grants, it is "less easy for the professor, however wedded to the old ways, to stand in the light of a young man proposing a new idea". By the same test, the system effectively avoids the danger "of activities in particular fields being dominated by a self-perpetuating clique". Professor Waddington says that these dangers have acted and to some extent still act as a "brake on the rapid advance of European science". Even when European nations rely on committees for advice, the numbers of people involved are too small for the advantages of American practice to be replicated, and Professor Waddington goes on to suggest that it would be worthwhile if the panels which are appointed to review project applications in Europe were organized internationally even though their funds would never cross national frontiers.

Professor Waddington is clearly less enamoured of the effects of the American system on individual researchers. Although grant recipients are stimulated to work hard, this is paid for in unpleasant ways. "One hears of many scientists speaking of American science as a rat race and of the summer courses at Woods Hole or the Federation meetings as the slave markets." He adds the qualification that those who lose in these competitions are not bereft of all support, for "in the last few years, at any rate, any reasonably competent worker could be assured of support which would seem quite lavish in European eyes. The battle which rages so fiercely is for high status and extensive opportunities within a scientific community which is genuinely affluent".

This judgment begets a jaundiced view of the tensions within the scientific community in the United States. Professor Waddington says that it is not uncommon to find scientists who are unwilling to disclose at private seminars the results of work they have done until this has "been safely rushed into print". Because careers depend on recognition, priority of publication may have an "unfortunate importance". Although American scientists are scrupulous among themselves in awarding recognition for accomplishments, "this rule does not apply so strongly to non-American scientists" and this has had "a certain bearing on the picture which the world in general, and Europe in particular, has formed of American science". Because of publicity and the grant system, "American science, largely inadvertently, comes to present itself as even more dominant in the world than it really is. It might do no harm for Europe, with its less spontaneously outgoing methods, to take some consciously planned steps to present its image in a more just balance".

The truth is, in any case, that "project grant support makes it more difficult for Americans to find opportunities for the contemplative imaginative thought which is the seed bed for the most radical innovatory concepts". At the highest levels, Professor Waddington says, this may not be easily apparent, although even among Nobel prizewinners, "it may be remarked that quite a number of American Nobel prizewinners have received their awards for discoveries depending on large machines or the organization of large teams of research associates. There are not many of the major scientific institutions which have, in Kuhn's phrase, 'changed the scientific paradigms' . . ." This difference between the United States and Europe is, according to Professor Waddington, more apparent lower down the scientific hierarchy, for young scientists "and those in middle years who are doing quite well but have not yet hit the jackpot find it rather difficult to spend time mulling over a field of science". Professor Waddington considers that the greater opportunities for contemplative science in Europe arise either from the attainment of a stable university position at an early age, the lack of pressure in the system or even the sheer shortage of the equipment necessary for experimental work. He considers that a more constructive method of attaining the same ends would be to increase the opportunities for postdoctoral research.

On the machinery for the formulation of science policy, Professor Waddington says that in the United States the apparatus is outstandingly effective and farsighted in producing programmes for the development of particular broad fields of science, but there has not yet been developed a means by which "scientists can participate fully in directing the progress of science as a whole". Although no country has been successful in the second task, there are—according to Professor Waddington—special problems in the United States.

The machinery for making middle range strategy—the interlocking network of institutions which includes the President's Science Advisory Committee, the Office of Science and Technology (under Dr Donald Hornig), the committees of the National Science Foundation, the National Academy of Sciences and particularly its influential Committee on Science and Public Policy ("far from being a rubber stamp")—all command Professor Waddington's respect. Private organizations such as RAND and the Brookings Institute have a recognized influence, as have the private foundation, but Professor Waddington is a little worried that the foundations, "once wide open to novel suggestions so long as they were good enough", have more recently adopted policies determined by their senior staff and have become "as mission oriented as the governmental agencies" Of all these sources of policy on science and technology, pride of place goes to PSAC and COSPUP, but these are said to depend not only on the energy, judgment and imagination shown by the leaders on the main committees, but "would be impossible without the solid backing from an adequate supporting staff and the participation of a number of scientists and engineers throughout the country" By comparison, the scale of activities in Europe is "pitiful", and Professor Waddington considers once again that it would be sensible if Europe as a whole were to take a leaf out of the American book and try to establish a European analogue of the National Academy of Sciences—National Research Council, presumably by co-operation between European academies

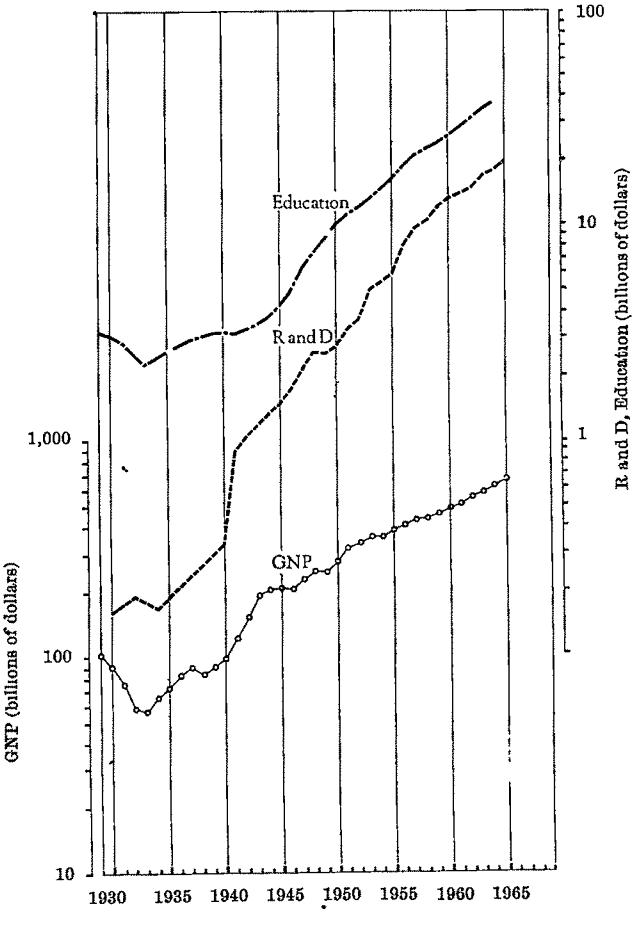
If the machinery for middle range strategy works well, however, Professor Waddington is less happy about the machinery for making decisions on the overall direction of science—questions such as the balance between research and development on the one hand and teaching on the other and the distribution of support between alternative fields of research, both applied and academic He says these problems are ultimately political and that the directions in which a nation emphasizes its support of science must be expressions of political desires and aspirations It follows that Congress must play an important part in the United States It is understandable that the beginnings of science policy in the United States can be traced to the establishment of land grant colleges and the development of agricultural research towards the end of the nineteenth century brought about by the recognition of the need for agricultural development In the same way, the scientific needs of the Second World War have set the scale for Federal expenditure on research and development, which now amounts to $17,000 million a year Since the war, Professor Waddington considers that American society has reacted quickly to the "challenge of ill-health" by the increase of expenditure on medical research, which amounted to $1,900 million in 1965, 64 per cent of it from Federal sources But all these developments are examples of what Professor Waddington calls "subjective programming"—the process of projecting present trends and possibilities into the future He would like there to be more "anticipatory programming", in which people set out first of all to decide what objectives they would like to attain and then seek the means of doing so

Professor Waddington also argues strongly for a greater involvement of scientists in the United States in the social problems thrown up by what he calls "great society science" Simply adding sociologists to existing committees will not help The fact that the social sciences inevitably raise political, ethical and religious questions when they are applied to practical problems impedes the integration of the social sciences into the machinery for making grand strategy Professor Waddington acknowledges that the amount of money spent on social sciences in the United States is quite large compared with what is spent in Europe but he considers there is something to be said for setting up a social science foundation with "a massive Federal grant of capital" and allowing the foundation thereafter "to employ the interest on its capital without further governmental tutelage" Only by such a means will there be any real prospect of applying the techniques of social anthropology within the United States—and even then there is the possibility that "it could not be undertaken satisfactorily by native American scientists, committed by their own upbringing to one of the very systems of social values which would have to be examined"

In education, Professor Waddington is impressed with the success of the Federal Government in bringing about a reform of the curriculum at the high school level He admires the flexibility of undergraduate courses in American universities, and the attempts now being made to improve the "matching of instruction to individual requirements" The emphasis on elective subjects contrasts, Professor Waddington says, with most European systems of university education Those in the Napoleonic tradition are rigidly and centrally prescribed in detail The traditional English system, exemplified by Oxford, requires that a student should follow only one course of studies Among professors at Scottish universities, Professor Waddington must be alone in admitting that the Scottish university system has a few defects, he says that the elective system in the Scottish universities which has been followed for several centuries resembles the American system but that the range of election is much narrower than in the United States

At the undergraduate level, Professor Waddington considers that Europeans have much to learn from the American system For one thing, it is much more able to accommodate advances in knowledge and to find places for interdisciplinary topics But Professor Waddington also considers that the American system provides a more valuable education "as opposed to mere specialist training"

Professor Waddington would also like to see European countries adopting some of the ways of handling graduate instruction now common in American universities Instruction courses for PhD students provide students with help in finding a "way through the mass of relevant information which is so rapidly increasing" Summer schools are in his view splendid innovations and there



Growth of United States expenditure on research and on education as a percentage of the GNP should be encouragement for the attempts being made in several European countries to organize advanced level teaching on a co-operative basis Organizations such as the European Molecular Biology Organization (EMBO), organized unofficially, show that a need exists and this could probably best be met by an international system within Europe which elicited the co-operation of important universities

On the financing of university research, Professor Waddington emphasizes that the project grant system provides flexibility in that it enables new opportunities to be exploited quickly By contrast, "in most European universities, adaptation to a science which is changing radically within small fractions of a working lifetime can only be done if, at the time of the retirement or death of some high-status figure, a professor or head of department, there happens to be a consensus among his surviving colleagues to strike out in a new direction" Apart, however, from its effect on personal relationships between scientists, the project grant system brings administrative headaches in American universities For one thing, it has meant the request by Federal agencies that academic research workers should account in detail for the time spent on various activities The way in which staff members are at liberty to supplement their income by accepting research grants or contracts during the summer is discriminatory against people working in the humanities and the arts, and in any case places the scientists themselves under considerable pressure There is also the problem that the system favours the stronger in his competition with the weaker Professor Waddington discerns the beginnings of a system in which American agencies will award large research grants on an institutional, not a personal, basis The lesson for Europe, he considers, is that there should be a deliberate attempt to "get the best of both worlds" by building up centres of excellence which are closely linked with universities

Like his fellow-examiners, Professor Waddington is impressed with the concentrated power of the very large universities Mass education, he says, is quite out of keeping with the structure and traditions of established European universities One of the problems is how to give young people, while still undergraduates, a sense of contact with experienced men of stature Attempts in the United States to create institutions with a high grade faculty and small numbers of highly selected students, of which "the most extreme example is the Rockefeller University in New York", have found it difficult to maintain excellence in a body which is stationary in size when other institutions are expanding rapidly For the most part, American universities have accepted wholeheartedly the need to become agencies for mass education, and there are those like Dr Clark Kerr who have urged that, under the pressure of public desires for higher education, universities must develop into multiversities More than this, universities are not merely places at which higher education is carried out but social institutions with such a great impact on society at large that "what the railroads did to the second half of the last century, and the automobile to the first half of this century, may be done for the second half of this century by the knowledge industry"

Professor Waddington points out that European universities may be more embarrassed than stimulated by being endowed with such an important part to play For one thing, at present they do not have the money But it does seem to him necessary that some European universities should develop along American lines, both in scale and in influence, and "that Europe should also develop a social system as profoundly influenced by higher education as the American" Indeed, the European need if anything is greater Professor Waddington is particularly concerned that universities should help to predict the ways in which society can change and guide society in moving in these directions He shakes his head a little over the price which the American university system has in his view been required to pay—a lack of unity—and he recommends that there should be attempts to create complexes of universities able to co-ordinate activities Professor Waddington points out that university systems such as the University of California have already gone some way in this direction and that European universities could profitably follow suit

TABLE 1 EDUCATIONAL EXPENDITURE AND GROWTH (1964)

	Education expenditure as percentage of GNP	Growth of education expenditure as percentage of GNP
Germany	3 23	14
France	4 28	50
Italy	4 86	73
Netherlands	5 66	69
United Kingdom	4 49	57
United States	6 18	50

Expenditure at current prices from OECD sources Private expenditure on education not included

On the contribution of American universities to the strength of the American economy, Professor Waddington has very little more to say than that American universities are usually much more closely related to industrial companies than their European counterparts For the most part, in his view, these links are usually beneficial Sometimes industry stimulates interest in pure science More often, academic science gains from the involvement in public affairs The climate in which an interaction like this is possible is also one of which universities and industry have a mutual appreciation for each other's potential contribution One way and another, Professor Waddington concludes, it would not be possible for universities in Europe acting alone to close the "technological gap" by their own efforts, if only because so much change would be required elsewhere in European society and industry.

On the brain drain, he takes the line that nobody has yet done the kind of cost–benefit analysis on which it would be possible to calculate precisely how much European nations lose by the emigration of PhDs On the other hand, there are equally intangible gains to be counted in the equations The pattern he would like to see is one in which European countries encourage young scientists to spend periods of postdoctoral work in other countries but take energetic steps to persuade them to come back again He goes on to say that those concerned with science policy in Europe should take it on themselves to counter-attack the attractions of emigration to the United States by more collaboration between academies in Europe, by collaborative plans between grant-giving research councils and, most promising of all, more collaboration between European scientists as in CERN and EMBO

The Dutch View

Dr H B G Casimir has produced the shortest of all the four examiners' reports and is principally concerned with industrial matters He points, for example, to the scale on which American companies operate and argues that, if European firms are to be able to look their American competitors in the face, they "must operate on an international market"

He points out that at present there is in the United States a "definite tendency against fundamental research" There has been some disappointment with expectations of the benefits of fundamental research which is, in any case, no longer cheap Then putting men on the moon has drained funds away from fundamental science This view, however, is partly offset by the way in which organizations such as the Bell Telephone Laboratories attach great importance to fundamental research carried out in an industrial setting where an "unexpected windfall" is not the chief aim Yet the contribution of industrial research laboratories other than the enlightened handful to the progress of fundamental research has been small

In the progressive industries, on the other hand, companies in the United States appear to do more than their European counterparts to apply scientific methods to the development of existing products and methods.

Dr Casimir is impressed with the way in which it is possible for quite small companies to have access to a large market in the United States without having to equip themselves with an elaborate sales organization. Staff is more mobile, between companies, between universities and industry and between industry and government. Government contracts have also helped to foster technology in the United States even though "we in Europe are inclined to overestimate this influence". Although military contracts have been particularly influential in fostering the development of electronic components of various kinds, it is clear that the influence of the Federal Government has been negligible in the development of motor cars, small in power engineering except for atomic energy and only slight in industries concerned with chemicals, metals and plastics.

To Dr Casimir, the term "technology gap" is inadequate and misleading. As far as industry is concerned "no fundamental science gap can exist". There is, however, a difference in the "scale of operation at the level of technological diffusion". Dr Casimir is all for better management, less complacent industry in Europe and more training in modern business techniques.

The French View

M. Pierre Massé has a more philosophical view of the contrast between the United States and Europe than his three fellow examiners. The starting point for his argument is that the American economy is a dominant force in the world but that science policy is not the prime cause of this state of affairs. In the same way, the "technological gap" is only one aspect of "a whole complex of gaps".

M. Massé shares the view of his colleagues that the way in which the Federal Government contributes two-thirds of the cost of research and development carried out in the United States but spends only one-sixth of the total sum available within its own establishments is a source of flexibility. Whether this great expenditure of federal money does really lead to tangible and valuable "spin-off" is, in M. Massé's view, a controversial point. On the whole, he has the feeling that the economic importance of the big federal programmes is more important than industrialists are usually prepared to admit. One of M. Massé's points is that the techniques of budget control introduced within the Department of Defense and now within the Bureau of the Budget have served as a powerful means of undermining the old assumption that public expenditure is bound to be less efficient than private expenditure so that in the future the American proclivity to decentralize may be diminished. However that may be, M. Massé follows Galbraith (without mentioning him) by pointing out that the growth of large American corporations may have introduced an element of instability into national and international economic equilibrium by eroding "the concealed stabilizer of economic life—the law of diminishing returns". This leads M. Massé to the conclusion, which all his colleagues share, that European unity is the only long-term answer to the technology gap.

Normal Immune Responses of Bursaless Chickens to a Secondary Antigenic Stimulus

by
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Hormonal bursectomy—inhibition of the development of the bursa by testosterone—has been more effective than surgical bursectomy coupled with irradiation in depressing the primary response to soluble antigens. None of the treated birds was agammaglobulinaemic, and all formed some antibody.

THE failure of bursaless chickens to respond to certain antigenic stimuli (most recently reviewed by Warner[1]) has led to the concept of the "dissociation of immunological responsiveness"[2] which predicates the existence of two populations of immunologically competent cells: one thymus dependent, mediating delayed hypersensitivity reactions and homograft rejection; the other bursa dependent, responsible for specific antibody formation and immunoglobulin synthesis.

The results of experiments showing the absence of immunoglobulins and of specific antibody in bursectomized chickens, however, are not at all straightforward. An unpublished study of ours involving the sera of fifty hormonally bursectomized (HBX) birds and a similar number of controls showed that only half of the treated birds had significantly reduced contents of gamma globulin (that is, ranging from one-third to one-tenth of the normal content) and that of these all had readily detectable gamma globulin, that is, more than 0·1 mg/ml. Large variations in the immunoglobulin contents of HBX birds have been found before[3,4]. In our HBX birds, natural agglutinins to sheep red cells were found 3 weeks later than in the normal controls, but then increased to similar concentrations. Similar results had already been obtained with natural agglutinins to rabbit red cells in surgically bursectomized (SBX) birds[5].

There are recent reports of antibody production by bursaless chickens, either as a result of more than one antigenic stimulus[6] or by detection with a very sensitive test[7]. The presence of antigen in the circulation of BX birds several days after its clearance by normal controls—the best criterion of unresponsiveness—has only been demonstrated in HBX birds after primary stimulation with BSA[4,8].

Investigation of the secondary responses of BX chickens is hindered by the following factors. (1) The low and variable responses of chicks less than 6 weeks old, and the long interval that is required between the first and second

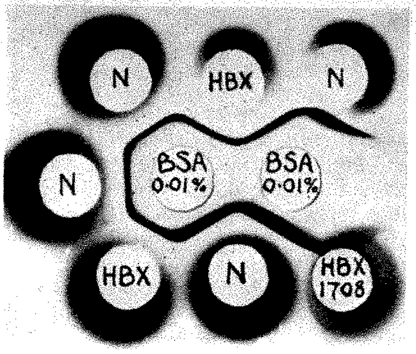

Fig. 1. Reactions in agar (8 per cent sodium chloride) of secondary anti-BSA sera from HBX and control birds against 0·01 per cent BSA.

injections of antigen to elicit a secondary response[9]. (2) Hormonal bursectomy, which is generally considered more effective because it occurs earlier, produces high mortality during the first weeks of life; the survivors may therefore be suspected of having bursal remnants unless these are shown to be absent at examination *post mortem*. (3) These examinations must be made before the bursae of the normal control birds are involuted; the age at which this occurs varies with breed, but bursal regression begins between 3 and 7 weeks and is complete at around 20 weeks[10,11].

Total body irradiation of 2 day old chicks, SBX on the day of hatching, has produced unresponsiveness to repeated injections of BSA, haemocyanin and *Brucella abortus*[12]. These birds were also reported to lack immunoglobulins.

We have studied the primary and secondary responses to soluble antigens injected, either intravenously or subcutaneously in Freund's complete adjuvant, into HBX and SBX chickens—some of which had also been irradiated—and into suitable controls. Primary responses to sheep red cells, tuberculin reactions, immunoglobulin concentrations and changes in peripheral lymphocytes were also examined. The antibody responses of thymectomized (TX) and TX irradiated birds which were included in the study were indistinguishable from those of the normal controls and not reported.

Pure line Light Sussex (LS) or Rhode Island Red (RIR) chicks were used in the experiments involving hormonal bursectomy, and RIR chicks were used in all the others. For hormonal bursectomy, 7 day embryos were injected with 2 mg of testosterone propionate. Surgical bursectomy[13] was performed by Dr B. M. Freeman at hatching or on the following day and thymectomy[14] was performed on the day of hatching. Irradiation with 600 or 700 r. was done by Mr E. A. King, Department of Radiotherapeutics, the conditions being 119 r./min, 220 kV, 15 mamp with a 1 mm aluminium filter.

White blood cell counts[15] were made at approximately weekly intervals for up to 4·5 weeks after hatching. The effectiveness of bursectomy or thymectomy was checked after death and, in some cases, also by histological examination of tissues fixed in formol saline and stained with haematoxylin and eosin. To test for hypersensitivity reactions of the delayed type, chicks were injected intramuscularly with 1 mg of heat-killed *Mycobacterium avium* suspended in liquid paraffin at 4 or 5 weeks of age. At 8 or 9 weeks 0·1 ml. of avian PPD was injected into the wattles and the reactions were noted 24 and 48 h later.

Human serum albumin (HSA) (fraction V, Calbiochem), bovine serum albumin (BSA) (Armour), 2,4-dinitrophenyl bovine gamma globulin (DNP-BGG) prepared according to Eisen[16] and sheep red cells (SRC) were used as antigens; injections were either subcutaneous or intravenous. For subcutaneous injection (BSA, HSA or DNP-BGG), 10 mg of antigen in Freund's complete adjuvant (FCA, Difco) were given twice with an interval of 5 weeks and blood was taken 3 weeks after each injection. For intravenous immunization 30–40 mg/kg body weight of HSA was injected twice with a 7 weeks interval. For the first injection, SRC were suspended in the HSA so that each bird received 1 ml. of a 15 per cent suspension of SRC/kg body weight. Blood was taken 8 days after each intravenous injection.

Tests for precipitating antibodies were made in agar containing 8 per cent or 0·85 per cent sodium chloride to provide a rough guide to the type of antibody formed. Wells 6·0 mm in diameter and 12 mm (centre to centre) apart were used. In passive haemagglutination tests[17], fowl red cells treated with tannic acid and a diluent containing 1·5 per cent heated normal fowl serum were used. Antibodies to SRC were detected by direct and indirect agglutination[18].

Responses of Hormonally Bursectomized Birds given Antigen in Adjuvant

Ten birds (six HBX and four controls) received BSA when 5 and 10 weeks old. Twelve birds (six HBX and six controls) were given DNP-BGG at 12 and 17 weeks of age. The sera obtained 3 weeks after each injection were tested against BSA or BGG and DNP-ovalbumin in Ouchterlony plates. The results obtained in 8 per cent sodium chloride after the second injections are shown in Figs. 1 and 2.

Of the primary anti-BSA sera only two, one HBX and one normal, reacted with antigen. All the secondary sera gave reactions, all but one of which (HBX 1708) were strong.

When primary sera were tested against BGG, 5/6 normal and 3/5 HBX sera formed precipitates. When tested against DNP-ovalbumin none of the control sera and 2/5 of HBX sera gave reactions. All the secondary sera as seen in Fig. 2 had antibodies to DNP, but 2/6 normals and 4/5 HBX sera failed to react with BGG.

Responses of Hormonally Bursectomized and Irradiated Birds given Antigens Intravenously

Approximately half the chicks in this experiment which included thymectomized (TX) birds were irradiated with 600 r. when 2 days old. Mortality was much higher (68, 50 and 75 per cent, respectively) in the TX irradiated, HBX and HBX irradiated groups than in the normal, irradiated or TX groups. At 12 weeks of age, the chickens received an intravenous injection of SRC suspended in HSA and were bled 8 days later. The results of antibody estimations on these sera are shown in Table 1. At 18 weeks the surviving birds were killed and the cloacal region was examined for bursal tissue; the results are included in Table 1.

Immunoelectrophoresis of the sera showed reductions in the gamma globulin bands formed by the sera of 6/9 of the HBX and HBX irradiated birds, and of 2/5 of the

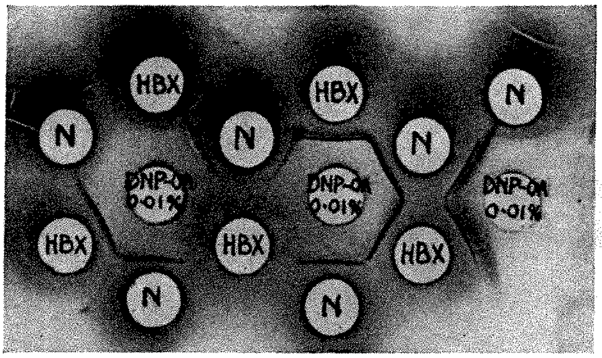

Fig. 2. Reactions in agar (8 per cent sodium chloride) of secondary anti-DNP-BGG sera against 0·01 per cent DNP-ovalbumin.

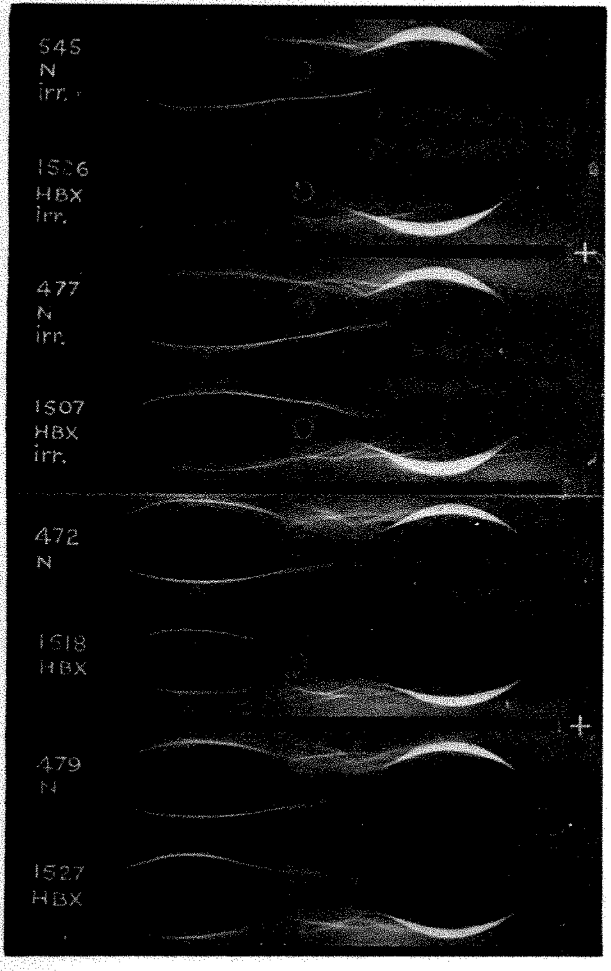

Fig. 3. Immunoelectrophoretic analysis of some representative chicken anti-sera described in Table 1. Rabbit anti-sera to whole fowl serum and to chiefly fowl gamma globulin in alternate troughs. Note varying amounts of gamma globulin detected in HBX and HBX irradiated birds.

irradiated group. Even in these the gamma globulin band could be seen (Fig. 3).

The results in Table 1 show that the response to HSA in the HBX and HBX irradiated groups was much more seriously impaired than that to SRC. Two of these birds (1504 and 1507) had very high titres of anti-HSA by passive haemagglutination but still lacked precipitins. Irradiation alone also seemed to reduce the response to HSA.

Responses of Surgically Bursectomized and Irradiated Birds

The chicks used in this experiment were operated upon on the day of hatching, irradiated on the next day and given their first injection, either of HSA and SRC (intravenous) or of HSA in Freund's adjuvant, when 8 weeks old. Because the primary responses to SRC were identical in all groups (Table 1) the secondary response to SRC was not studied. The second intravenous injection of HSA was given at 15 weeks, and the second adjuvant injection at 13 weeks. Results of antibody estimations for secondary sera are shown in Table 2. After two injections of HSA all twenty-two normal and irradiated birds had formed precipitins compared with 13/22 in SBX+SBX irradiated groups. Of the seven sera which did not precipitate with antigen, two (789 and 792) also had very low passive haemagglutination titres. These two birds were given a single intravenous injection of 1 ml. of 15 per cent SRC to which they responded normally.

Primary responses to HSA in adjuvant were equally low in operated and control birds. Primary responses after intravenous HSA again showed that many more birds were without precipitating antibody in the operated than in the control groups. Mean anti-HSA titres of the SBX and SBX irradiated groups did not differ significantly from those of the control groups, although a few individuals had lower titres than any found in the controls.

No reductions in gamma globulin concentrations, such as those found in some of the HBX sera, were seen even in the sera of the least responsive SBX or SBX irradiated birds.

Effect of Irradiation, Bursectomy and Thymectomy on Peripheral Lymphocytes and Body Weights

The results in Table 3 show that irradiation of normal chicks with 700 r. instead of 600 r. produced a much greater and longer lasting reduction in lymphocytes and substantially lower body weights at 15 weeks. This difference was also found in operated irradiated birds.

Lymphocyte numbers after thymectomy alone had not returned to normal by 25 or 31 days. Neither hormonal nor surgical bursectomy alone had a lasting effect. Thymectomized birds were on the whole more seriously affected with both doses of radiation than their normal controls.

The greatly enhanced susceptibility of the TX birds to irradiation as shown by the depletion of peripheral

Table 1. ANTIBODY RESPONSES OF NORMAL, IRRADIATED, HBX AND HBX IRRADIATED BIRDS AFTER ONE INTRAVENOUS INJECTION OF SRC AND HSA

Group	Bird No.	Anti-HSA Precipitation 8 per cent NaCl*	Anti-HSA Passive haemagglutination	Anti-SRC Direct agglutination	Anti-SRC Indirect agglutination	Gamma globulin levels†	Post-mortem findings Mean bursa weights (g)
Normal	472	+	320	2,560		Normal	4·0
	475	+ +	640	> 5,000		Normal	
	479	+ +	5,000	2,560		Normal	
	548	+ +	2,560	5,000		Normal	
	1449	–	160	640		Normal	
	1450	+	320	> 5,000		Normal	
Normal irradiated	473	(+)	80	5,000		Normal	4·4
	477	(+)	160	> 5,000		Normal	
	480	–	640	80		Reduced	
	484	–	80	2,560		Normal	
	545	–	320	640		Slightly reduced	
HBX	1504	–	10,000	> 5,000	100,000	Normal	Bursa not visible
	1518	–	10	80	3,200	Reduced	
	1523	–	< 10	160	1,600	Slightly reduced	
	1525	–	< 10	320	3,200	Reduced	
	1527	–	< 10	2,560	6,400	Normal	
	1535	–	< 10	160	6,400	Reduced	
HBX irradiated	1507	–	5,000	> 5,000	200,000	Normal	Bursa not visible
	1522	–	< 10	40	1,600	Slightly reduced	
	1526	–	< 10	20	1,600	Very low	

* All sera were also tested in 0·85 per cent sodium chloride and found negative.
† Estimated from immunoelectrophoretic pattern such as shown in Fig. 3.

Table 2. PRIMARY AND SECONDARY ANTIBODY RESPONSES OF NORMAL, IRRADIATED, SBX AND SBX IRRADIATED BIRDS AFTER INTRAVENOUS OR SUBCUTANEOUS IN ADJUVANT INJECTIONS OF HSA; ANTI-SRC AGGLUTINATION TITRES AFTER A SINGLE INTRAVENOUS INJECTION

		Intravenous injection							Subcutaneous injection with adjuvant				
		Primary response			Secondary response				Primary response		Secondary response		
		Anti-HSA		Anti-SRC	Anti-HSA				Anti-HSA		Anti-HSA		
					Precipitation						Precipitation		
Group	Bird No.	Precipitation 8 per cent NaCl*	Passive haemagglutination	Agglutination	8 per cent NaCl	0·85 per cent NaCl	Passive haemagglutination	Bird No.	Precipitation 8 per cent NaCl*	Passive haemagglutination	8 per cent NaCl	0·85 per cent NaCl	Passive haemagglutination
Normal	209	+ +	640	2,560	+ +	+ +	≥10,000	203	–	40	+	(+)	5,000
	216	(+)	160	320	+	(+)	1,280	213	–	10	+	(+)	2,560
	224	(+)	640	10,000	Dead			214	+ +	1,280	+ + +	+ + +	> 10,000
	225	–	80	320	+ +	+	1,280	219	–	80	+ + +	+ +	5,000
	231	–	80	160	+	(+)	1,280	220	–	20	(+)	?	640
	239	+	640	1,280	+ +	+ +	10,000	229	–	10	+ +	+	5,000
	241	+ +	≥5,000	640	Dead			236	(+)	160	+ + +	+ + +	5,000
Normal irradiated	202	–	80	1,280	+ +	+	10,000						
	207	–	320	2,560	+	(+)	1,280	204	–	40	+ + +	+ + +	10,000
	212	+ +	1,280	640	+	(+)	2,560	211	–	20	+ + +	+ +	10,000
	222	(+)	320	320	+ +	+ +	10,000	227	–	640	+	+	2,560
	223	+ +	1,280	1,280	+ +	+	10,000	228	–	320	+ +	+	5,000
								232	Not done	Not done	+ + +	+ + +	10,000
SBX	460	+	1,280	1,280	+ + +	+ +	5,000	464	–	80	–	–	10,000
	571	–	2,560	2,560	+ +	+ +	10,000	554	–	160	+ +	(+)	5,000
	572	–	≥5,000	640	+ +	+ +	≥10,000	565	–	< 10	(+)	–	640
	573	–	20	> 5,000	+ +	+ +	10,000	789†	–	10	–	–	16
	575	–	640	2,560	+ +	+ +	10,000	793	–	20	–	–	1,280
	576	–	< 10	1,280	(+)	(+)	2,560	794	–	320	+ +	+ +	10,000
	595	–	< 10	2,560	–	–	640						
SBX irradiated	457	–	160	2,560	+ + +	+ +	10,000	558	+	640	+ +	+	10,000
	570	–	640	640	+ + +	+ +	10,000	581	–	320	+	–	1,280
	574	–	320	2,560	–	–	2,560	589	–	10	Dead		
	582	–	2,560	5,000	+ + +	+ +	≥10,000	597	–	1,280		–	2,560
	799	–	40	1,280	+	(+)	2,560	792†	–	10	–	–	16

* These sera were also tested in 0·85 per cent sodium chloride and all were found negative.

† These two birds, having no anti-HSA after two injections, were given a single (primary) injection of SRC to which they responded with agglutination titres of 1,280 and 160.

Table 3. EFFECT OF IRRADIATION (600 OR 700 R.) ON THE MEAN LYMPHOCYTE COUNTS AND BODY WEIGHTS OF NORMAL, BURSECTOMIZED AND THYMECTOMIZED CHICKENS

Days after irradiation	Normal	Irradiated 600 r.	HBX	HBX 600 r.	TX	TX 600 r.
	No. of lymphocytes × 10^2/mm³					
5	78 ± 9	26 ± 12	55 ± 12	17 ± 3	94 ± 10	10 ± 1
11	81 ± 3	74 ± 5	110 ± 27	68 ± 15	111 ± 23	31 ± 1
19	200 ± 25	134 ± 22	110 ± 8	109 ± 26	85 ± 18	78 ± 7
25	152	148	132	118	91	87
	Mean body weights (g) at 12 weeks					
	1,220 ± 84 (11 birds)	1,160 ± 70 (5 birds)	1,120 ± 84 (6 birds)	1,000 ± 106 (3 birds)	1,220 ± 79 (5 birds)	770 ± 65 (14 birds)
	Normal	Irradiated 700 r.	SBX	SBX 700 r.	TX	TX 700 r.
	No. of lymphocytes × 10^2/mm³					
5	90 ± 8	8 ± 1	88 ± 16	9 ± 2	62 ± 15	5 ± 2
11	250 ± 40	66 ± 15	217 ± 41	88 ± 12	80 ± 6	37 ± 11
23	136 ± 8	97 ± 14	122 ± 8	106 ± 22	79 ± 14	62 ± 17
31	390 ± 34	228 ± 45	310 ± 45	182 ± 31	160 ± 28	135 ± 13
	Mean body weights (g) at 15 weeks					
	1,660 ± 65 (19 birds)	1,190 ± 55 (16 birds)	1,700 ± 84 (13 birds)	1,300 ± 45 (9 birds)	1,950 ± 90 (17 birds)	1,290 ± 73 (18 birds)

lymphocytes and reduced body weights (Table 3) occurred despite the presence of residual thymus tissue in most of the birds. It is also surprising that the TX irradiated (600 r.) birds produced similar amounts of antibody to HSA and SRC as the normal and TX control groups. This is in contrast to the findings of Cooper *et al.*[19].

No fewer than six birds from each of the six groups in the two experiments which included irradiation with 600 or 700 r. were tested for delayed hypersensitivity. Only two failed to react; both were in the TX irradiated (700 r.) group and they also lacked detectable thymus tissue when examined at 22 weeks. There were, however, five additional birds, also devoid of detectable thymus tissue, which gave positive reactions. This differs from the results of Cooper *et al.*[12], who obtained delayed hypersensitivity wattle reactions to diphtheria toxoid in only two out of fourteen TX irradiated birds.

In both experiments, surgical thymectomy combined with irradiation provided more birds (11/32) without detectable thymus tissue at 20 or 22 weeks than surgical thymectomy alone (3/28). The mean weights of the residual thymus tissue were 0·17 g in the TX group given 700 r. and 0·54 g in the corresponding TX group; mean values of the thymus weights of the normal and irradiated control groups were 6·9 g and 4·4 g, respectively.

The results indicate that the inhibition of bursal development with testosterone *in ovo* was more effective than surgical bursectomy combined with irradiation in depressing the primary responses to HSA and SRC. Both procedures suppressed the formation of anti-HSA precipitin, but in addition in the HBX birds only very low concentrations of antibodies to HSA could be detected by tanned cell agglutination; they had reduced anti-SRC titres and 6/9 of them had reduced gamma globulin concentrations, whereas SBX and SBX irradiated birds were normal in these respects.

Inability to form precipitating antibody to BSA after one injection of antigen has been demonstrated in birds bursectomized up to the tenth day after hatching and immunized as late as 22 weeks[8]. In our experiments, the formation of normal amounts of precipitating anti-BSA by 13 week old HBX birds (Fig. 1) after two injections of antigen must therefore be attributed to the mode of immunization. The effect was not a result of the use of adjuvant, because, as Table 2 shows, a second intravenous injection of HSA 7 weeks after the first also produced a normal response.

The presence of natural antibodies to erythrocytes in normal fowl sera is probably caused by unidentified but repeated antigenic stimuli. The response to a first injection of red cells can therefore be considered not to be a true primary response and this may account for the formation of antibody to SRC by our bursaless birds. Janković and Isaković, however, obtained no haemagglutinins after one injection of human O cells[6] although anti-O agglutinins are usually present in fowl sera.

Experiments on antibody production by bursaless chickens involve so many variables (for example, breed, mode of bursectomy, age of immunization, nature of antigen and method of detecting antibody) that they are not strictly comparable. It seems, however, that antibody formation to viruses[20,21], resistance to coccidiosis[22] and the clearance of fowl red cells[8] labelled with chromium-51 and of goat red cells[23] were completely unaffected by bursectomy. The formation of agglutinating antibody to *Leptospira icterohaemorrhagiae*[24], *Salmonella typhimurium* and *Spirochaeta gallinarum*[25] after infection or multiple injections was impaired and possibly delayed but not abolished. The complete absence of detectable antibody after a single antigenic stimulus has been reported for a variety of protein and bacterial antigens[1] The only report of a secondary response to BSA in SBX birds[26] shows that, while primary peak antibody concentrations (at 22 weeks) were 7 per cent of those of the normal controls, this proportion rose to 43 per cent after a second injection at 34 weeks

In our experiments, bursaless birds had seriously impaired primary responses, especially to soluble protein antigen They cannot, however, be considered unresponsive, for they all formed some antibody either to SRC or to soluble protein after a second injection, and they were not agammaglobulinaemic It is apparent that only hormonal (as opposed to surgical, even combined with irradiation) bursectomy early in incubation can produce a marked effect on gamma globulin concentrations and antibody production and that only a small proportion of HBX animals is permanently affected Dr N L Warner (personal communication), starting with thirty-four HBX chicks, had nine still alive at 9 months, only two of which were considered to be agammaglobulinaemic although five did not respond to a fourth injection of *Brucella* organisms

The massive, rapid and transitory antibody response of the fowl to a single intravenous injection of a soluble protein antigen is unlike that of any other species It differs also from subsequent responses of the fowl in the type of immunoglobulins formed[27] It is this unusual type of primary response that seems to be affected by bursectomy

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[1] Warner, N L , *Folia Biol* **13**, 1 (1967)
[2] Warner, N L , Szenberg, A , and Burnet, F M *Austral J Exp Biol Med Sci* , **40**, 373 (1962)
[3] Carey, J , and Warner, N L , *Nature*, **203**, 198 (1964)
[4] Pierce, A E , Chubb, R C , and Long, P L , *Immunology*, **10**, 321 (1966)
[5] Graetzer, M A , Cote, W P , and Wolfe, H R , *J Immunol* , **91** 576 (1963)
[6] Janković, B D , and Isaković, K , *Nature*, **211**, 202 (1966)
[7] Claflin, A J , Smithies, O , and Meyer, R K , *J Immunol* , **97**, 693 (1966)
[8] Mueller, A P , Wolfe, H R , and Cote, W P , in *The Thymus in Immunobiology* (edit by Good, R A and Gabrielsen, A E), 359 (Hoeber-Harper, New York, 1964)
[9] Blazkoveć, A A , and Wolfe, H R , *Intern Arch Allerg Appl Immunol* , **26**, 80 (1965)
[10] Glick, B , *Poultry Sci* , **35**, 843 (1956)
[11] Glick, B , *Poultry Sci* , **39**, 130 (1960)
[12] Cooper, M D , Peterson, R D A , South, M A , and Good R A , *J Exp Med* , **123**, 75 (1966)
[13] Mueller, A P , Wolfe, H R , Meyer, R K , and Aspinall, R L , *J Immunol* , **88**, 354 (1962)
[14] Aspinall, R L , Meyer, R K , Graetzer, M A , and Wolfe, H R , *J Immunol* **90**, 872 (1963)
[15] Natt, M P , and Herrick, C A , *Poultry Sci* , **31**, 735 (1952)
[16] Eisen, H N , *Meth Med Res* , **10**, 94 (1964)
[17] Daniel, T M , and Stavitsky, A B , *Meth Med Sci* , **10**, 152 (1952)
[18] Orlans, E , *Immunology*, **12**, 27 (1967)
[19] Cooper, M D , Peterson, R D A , and Good, R A , *Nature*, **205**, 143 (1965)
[20] Cho, B R , *Amer J Vet Res* , **24**, 832 (1963)
[21] Glick, B , in *The Thymus in Immunobiology* (edit by Good R A , and Gabrielsen, A E), 343 (Hoeber-Harper, New York, 1964)
[22] Pierce, A E , and Long, P L , *Immunology*, **9**, 427 (1965)
[23] Solomon, J B , *Immunology*, **11**, 97 (1966)
[24] Kemenes, F , and Pethes, G , *Z ImmunForsch Exp Ther* , **125**, 446 (1963)
[25] Perek, M , and Drill, A E , *Brit Vet J* , **118**, 390 (1962)
[26] Mueller, A P , Wolfe, H R , and Meyer, R K , *J Immunol* , **85**, 172 (1960)
[27] Benedict A A Heisch, R T , and Larson, C *J Immunol* , **91**, 795 (1963)

Genetic Influence on Alimentary Lipaemia in Rats

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The response of the concentration of postprandial plasma triglyceride to ingested oil is exaggerated in certain rats. This characteristic can be passed on to the offspring for at least two generations

WHEN studying alimentary lipaemia in the rat[1,2] we noted a few individuals showing very high postprandial plasma triglyceride (PPTG) and a few others showing only a minimal PPTG response When retested, these rats consistently exhibited the same responses, although their fasting or preprandial plasma triglyceride values were unexceptional

We were curious as to whether these opposite responses to oil feeding might be genetically determined, and therefore bred both types through two generations The results of this study are described here

Twenty mature male rats and thirty mature female rats (Long–Evans strain) were each given 3 ml of cottonseed oil by stomach tube once each week for 3 successive weeks The concentrations of plasma triglyceride (TG) before the administration of oil and 6 h after were obtained each week by the method of Van Handel and Zilversmit[3] During these 6 h the rats were housed in the central area of the laboratory to ensure maximal lipaemia[2] The individual TG values of males and females were averaged separately The pre-feeding plasma TG averaged 22 and 24 mg/100 ml and the 6 h PPTG, 98 and 88 mg/100 ml , respectively, in the two groups

Five rats of this male group which exhibited an average concentration of PPTG (143 mg/100 ml) greater than the mean of the total male group by four standard errors were selected and bred with five rats of the female group which exhibited an average PPTG (155 mg/100 ml) greater than the mean of the total female group by four standard errors Five male and five female rats with average PPTG less than the respective means of their groups by four standard errors (average PPTG of each group of five rats 76 and 48 mg/100 ml , respectively) were bred similarly

From these breedings, five litters of rats (forty-eight surviving) were obtained from the "hyperlipaemic" parent rats and a similar number of litters (fifty-two surviving) from the "hypolipaemic" parent rats These offspring will be designated as first generation (F_1) From 95 to 115 days after birth, the F_1 rats were fed oil and their pre- and postprandial plasma TG values were measured each week for 3 weeks For control purposes, rats routinely obtained for general laboratory use were treated in the same way Then, as already described, the eight male rats with the highest concentration of PPTG (average PPTG 339 mg/100 ml) were bred to the eight female rats also with the highest PPTG (average PPTG 205 mg/100 ml) The six F_1 males with the lowest PPTG (average PPTG 31 mg/100 ml) were bred to the six females with the lowest PPTG (average PPTG 55 mg/100 ml)

From this breeding of F_1 rats, eight litters (sixty-seven surviving) were obtained from the hyperlipaemic parents and six litters (fifty-six surviving) from the hypolipaemic parents These second generation offspring (F_2) were allowed to mature and then the three weekly studies of their PPTG values before administration of oil and 6 h after were carried out as already described Again control studies were carried out on rats routinely obtained for general laboratory purposes

When it became apparent that the F_1 rats born of hyperlipaemic parents also showed postprandial hyperlipaemia and those of hypolipaemic parent rats a postprandial relative hypolipaemia, various other investigations were carried out First, ten male F_1 rats born of hyperlipaemic parent rats and nine male F_1 rats born of hypolipaemic parent rats were given 200 mg of tyloxapol ('Triton *WR* 1339') by intravenous injection to determine whether the observed plasma TG responses of each group to oil were a result of differences in their respective rates of hepatic discharge of TG Blood samples obtained before and then 6, 12 and 24 h after injection of tyloxapol were analysed for TG and the pre-administration of 12 and 24 h samples also were analysed for cholesterol[4]

We observed earlier[2] that the environment markedly influences the response of the rat's plasma TG to ingested oil, twenty-four F_1 rats (twelve born of hyperlipaemic and twelve of hypolipaemic parents) were housed in an isolated sound-proof room[2] and again the pre-administration and 6 h PPTG concentrations were measured

To determine whether the observed differences in the PPTG values between offspring of hyperlipaemic and hypolipaemic parents could be the result of differences in TG absorption, fifteen F_1 rats (eight born of hyperlipaemic and the rest of hypolipaemic parents) and eighteen F_2 rats (ten born of hyperlipaemic and the rest of hypolipaemic parents) were given 3 ml. of cottonseed oil which had been labelled by mixing with glyceryl-tri-1-^{14}C-palmitate, containing a total of 300,000–600,000 d p m The rats were bled and killed 6 h after the administration of the labelled oil The entire gastrointestinal tract and liver were removed from all rats (an epididymal fat pad also was removed from the F_1 rats) The amount of fat absorbed from the intestine and the amount of this absorbed fat present as fatty acids in the blood, liver and epididymal fat were calculated as described before[2]. In brief, this consisted of hydrolysing the tissue or plasma sample with 30 per cent KOH 95 per cent ethanol in a 1 1 ratio by volume, extracting the acidified hydrolysate with petroleum ether, evaporating a portion of the petroleum ether extract, dissolving this residue in scintillator solution and counting in a Packard model 3003 scintillation counter

Finally, the blood pressure of twenty-two F_1 rats (ten born of hyperlipaemic and twelve of hypolipaemic parents) and twenty-four F_2 rats (twelve born of hyperlipaemic and twelve of hypolipaemic parents) was measured

Pre- and Postprandial Plasma Triglyceride Values in the Offspring

It was apparent (see Table 1) that the male offspring of hyperlipaemic parents exhibited a far greater average 6 h concentration of PPTG (183 mg/100 ml) than the F_1 male offspring of hypolipaemic parent rats (78 mg/100 ml) and also a significantly greater concentration of PPTG ($P < 0\ 001$) than the ordinary stock control rats (90 mg/100 ml). The average fasting plasma TG of these male rats was, however, no greater than that of the male offspring of the hypolipaemic parent rats or of the control rats This hyperlipaemic response of the F_1 rats, however, was not further intensified (Table 1) in the rats of the second generation

The F_1 male offspring of hypolipaemic parent rats, while exhibiting a much lower 6 h PPTG (78 mg/100 ml) than the offspring of hyperlipaemic parents, did not show (Table 1) a significantly lower value than did the controls (90 mg/100 ml) The F_2 male offspring of hypolipaemic parent rats similarly failed to exhibit a 6 h PPTG significantly lower than that of the control rats No differences were found in the fasting plasma TG values of F_2 and stock rats.

The F_1 female offspring of hyperlipaemic parent rats, while not exhibiting (Table 1) as dramatic a rise in PPTG as did their male counterparts, nevertheless showed an average value (115 mg/100 ml) significantly higher ($P < 0\ 001$) than that (59 mg/100 ml) of the F_1 female offspring of hypolipaemic parent rats or than that (78 mg/100 ml) of the female stock control rats. Again, the F_2 female offspring, like the F_2 male offspring, of hyperlipaemic parent rats failed to exhibit (Table 1) a 6 h PPTG any greater than that shown by their F_1 counterparts The fasting plasma TG values were essentially the same in all groups of rats

The F_1 female offspring of hypolipaemic parents exhibited a 6 h concentration of PPTG (59 mg/100 ml) much less (Table 1) than that shown by F_1 offspring of hyperlipaemic parents and significantly ($P < 0\ 001$) less than that observed in the female control stock rats. The 6 h concentration of PPTG in the F_2 female offspring of hypolipaemic parents was even lower (41 mg/100 ml).

Table 1. ALIMENTARY LIPAEMIA OF OFFSPRING OF HYPERLIPAEMIC AND HYPOLIPAEMIC RATS

	Control stock rats		Offspring of hyperlipaemic rats*				Offspring of hypolipaemic rats†			
			First generation		Second generation		First generation		Second generation	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No of litters	—	—	5	5	8	8	5	5	6	6
No of rats	40	48	23	25	30	37	25	27	31	25
Average weight	310	225	314	210	350	214	304	212	368	220
Average serum cholesterol (mg/100 ml)	95	88	78	81	86	96	72	86	69	81
Range	(68–94)	(58–88)	(71–88)	(50–84)	(74–100)	(75–132)	(64–100)	(61–92)	(53–91)	(58–141)
S E of mean	±1 8	±2 1	±1 7	±4 1	±1 8	±3 1	±2 1	±3 6	±2 2	±4 8
Average plasma TG ‡ (mg/100 ml)										
Before oil	21	22	22	18	20	19	30	22	20	18
	(10–30)	(11–34)	(12–41)	(11–38)	(9–47)	(6–34)	(11–39)	(13–37)	(9–47)	(6–34)
	±2 1	±2 4	±2 8	±2 3	±2 4	±2 1	±3 1	±2 7	±2 4	±2 1
6 h after oil	90	78	183	115	197	112	78	59	73	41
	(69–177)	(64–98)	(78–382)	(26–277)	(53–352)	(35–301)	(28–198)	(31–256)	(29–114)	(23–83)
	±6 6	±4 4	±16 1	±13 6	±12 2	±9 1	±5 0	±3 1	±4 1	±2 9

* The average 6 h concentration of PPTG was 143 and 155 mg/100 ml , respectively, in the male and female hyperlipaemic parents of the rats of the first generation The average 6 h concentration of PPTG was 339 and 205 mg/100 ml , respectively, in the male and female hyperlipaemic parents of the rats of the second generation

† The average 6 h concentration of PPTG was 76 and 48 mg/100 ml , respectively, in the male and female hypolipaemic parents of the rats of the first generation The average 6 h concentration of PPTG was 31 and 55 mg/100 ml , respectively, in the male and female hypolipaemic parents of the rats of the second generation

‡ Three separate determinations of plasma TG concentrations were made on 3 successive weeks for each rat, these values were then averaged to obtain the values shown

The serum cholesterol values were approximately the same in the stock control rats, and in both generations of offspring of either type of parent rat.

Response of Plasma Triglyceride and Cholesterol of First Generation Offspring to Tyloxapol

Essentially no significant differences were found between the response to tyloxapol of the nine F_1 offspring of hyperlipaemic parents and those of the nine F_1 offspring of hypolipaemic parents. Although the former group had previously shown an average 6 h concentration of PPTG of 260 mg/100 ml after oil ingestion and the latter group had shown 68 mg/100 ml, their plasma TG values 6 h after injection of tyloxapol were 981 and 959 mg/100 ml, respectively. After 12 h, the respective values were 2,008 and 1,783 mg/100 ml. Similarly, the average serum concentration of cholesterol in these two groups was 342 and 335 mg/100 ml twelve hours after injection of 'Triton', and 483 and 514 mg/100 ml twenty-four hours after injection. These results indicate that the rate of hepatic discharge of triglyceride into blood was approximately the same in the two types of offspring[5].

Effect of Sound-proof Room on Postprandial Responses of First Generation Offspring

Whereas housing twelve F_1 rats of hypolipaemic parents in the isolated sound-proof room did not alter their PPTG response, such housing markedly lowered the PPTG response of twelve F_1 rats of hyperlipaemic parents. Thus the average 6 h concentration of PPTG of the descendants of hypolipaemic parents housed in the central area of the laboratory was 33 mg/100 ml (range 22–57) and was 34 mg/100 ml (range 14–123) when tested in the sound-proof room. On the other hand, the average 6 h PPTG of the twelve rats born of hyperlipaemic parent rats was 169 mg/100 ml (range 96–382) in the central area of the laboratory and only 62 mg/100 ml (range 24–121) in the sound-proof room. In short, the plasma TG responses of these two groups which were so different ($P < 0.001$) when they were tested in the central area became far less so ($P < 0.05$) when outside environmental stimuli were eliminated as far as possible.

Intestinal Absorption and Tissue Distribution of Exogenous Fat

As Table 2 shows, intestinal absorption of fat was not significantly different in first and second generation offspring of hyperlipaemic parent rats and first and second generation offspring of hypolipaemic parent rats. The marked difference that was found in their plasma TG response to ingested oil could not therefore be ascribed to the faster or greater absorption of dietary fat in one of the two groups.

Table 2 ABSORPTION AND DISPOSITION OF FED FAT IN OFFSPRING OF HYPERLIPAEMIC AND HYPOLIPAEMIC RATS

				Absorbed dietary triglyceride fatty acid/6 h		
No of rats	Average weight	6 h PPTG (mg/100 ml)	TG absorbed* (mg/6 h)	Total plasma volume (mg)	Total liver (mg)	Fat pad (mg/g of pad)
A, Offspring of hyperlipaemic rats						
(1) First generation						
8	403	233	1,135	29	88	—
(2) Second generation						
10	405	439	798	50	97	1.54
18	Av 404	347 (103–894) ±58	948 (440–1,623) ±70	41 (10–103) ±6.9	93 (48–130) ±6.6	1.54 (0.57–3.24) ±0.2
B, Offspring of hypolipaemic rats						
(1) First generation						
7	376	57	1,206	7	56	—
(2) Second generation						
8	438	51	660	6	56	1.12
15	Av 408	54 (32–79) ±3.7	914 (360–1,650) ±105	6 (4–9) ±0.6	56 (30–100) ±5.0	1.12 (0.43–2.26) ±0.2

* For method of measurement see text

Table 2 also illustrates that the plasma of the offspring of the hyperlipaemic parent rats contained more than six times as much exogenously derived fatty acids as the plasma of the offspring of the hypolipaemic parent rats despite the approximate equivalence of fat absorption in these two groups. Also the content of exogenously derived fatty acids in the liver was significantly greater in the first than in the second group, although the epididymal fat pads of each group contained approximately the same amount of fed fatty acids.

Blood Pressure in the Two Generations

This measurement was taken because the offspring of the hyperlipaemic parent rats usually appeared to be considerably more excitable than the offspring of the hypolipaemic parent rats. The average blood pressure of the twenty-two male offspring rats of hyperlipaemic parent rats (ten were of the first and the rest of the second generation) was 136 mm of mercury (range. 126–162) and that of twenty-four male offspring of hypolipaemic parents (half of the first and half of the second generation) was 118 mm of mercury (range 98–140). This difference was significant ($P < 0.001$).

Our observations indicate that an exaggerated PPTG response, which is found in certain rats, can be passed on for at least two generations to their offspring (particularly the male members). These observations also suggest that a diminished PPTG response of rats may also be transmitted to their progeny.

This seemingly inherited abnormal response to ingestion of exogenous fat does not, however, seem to be the result of any change in the metabolism of endogenous fat, for not only is the fasting plasma TG unaltered in both the hyperlipaemic and hypolipaemic reactors but also the hepatic discharge of endogenously produced fat as determined by the tyloxapol technique[5] is normal.

These observations also make it clear that the intestinal absorption of fat in the hyperlipaemic rat is the same as that in the hypolipaemic rat although the plasma and liver of the former contain far more fat derived from a single ingestion of oil than the comparable tissues of the latter type of rat. Seemingly, the hyperlipaemic rat fails to metabolize the same quantity of absorbed fat as rapidly as the hypolipaemic animal.

We have already reported[2] that external environmental factors are very important in the rat's ability to handle a given amount of fat—an indication that the central nervous system is capable of playing a very significant part in an animal's handling of exogenously derived fat. This is emphasized here because we suspect that the offspring of the original hyperlipaemic and hypolipaemic parent rats have not inherited any fixed or permanent defect either in their metabolism of endogenous fat or in their disposition of exogenously derived fat, but rather a neurogenic or psychogenic propensity to over-react or under-react to aspects of the external environment which influence the concentration of plasma lipid. We believe this to be the possible mechanism because there was no indication of any intrinsic derangement of fat metabolism and especially because the exaggerated concentration of PPTG in the hyperlipaemic offspring almost disappeared when they were isolated in the sound-proof room. Then, too, these same rats seemed in general to be more excitable and to have a higher blood pressure than the hypolipaemic offspring.

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[1] Friedman, M., and Byers, S. O., *Amer. J. Physiol.* (in the press).

[2] Friedman, M., and Byers, S. O., *Amer. J. Physiol.* (in the press).

[3] Van Handel, E., and Zilversmit, D. B., *J. Lab. Clin. Med.*, **50**, 152 (1957).

[4] Saifer, A., and Kammerer, O. F., *J. Biol. Chem.*, **164**, 657.

[5] Byers, S. O., and Friedman, M., *Amer. J. Physiol.*, **198**, 629 (1960).

RNA Changes in Goldfish Brain during Learning

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Acquisition of new swimming skills by goldfish during learning experiments is accompanied by changes in the base ratio of RNA Several hypotheses are put forward to explain these changes

SEVERAL studies[1-13] have correlated RNA and protein synthesis in the brain with the acquisition and retention of learned behaviour. Hyden and Egyhazi[1-4] were the first to show synthesis of an RNA with a unique base composition during learning Flexner, Flexner and Stellar[7], in studies of learning in mice, noted that inhibitors of protein synthesis could prevent the fixation of learned behaviour. Similar effects have been observed by Agranoff, Davis and Brink[10] in studies of learning in goldfish Implicit in these experiments was the hypothesis that the establishment of memory is at least a two step process the first is the acquisition phase which does not seem to require protein synthesis in mice[11] and goldfish[13], and the second is a consolidation step which can be inhibited by drugs which prevent protein synthesis

The results of these studies imply that RNA is involved during learning In particular, it is important to find out whether the RNA base ratio changes which can be observed are biochemical correlates of learning or if they are caused by some non-specific factors such as stress or use This article attempts to correlate the acquisition and storage of a new swimming skill with the composition of newly synthesized RNA in goldfish brain

By using orotic acid labelled with tritium as the precursor of uridine and cytidine, the RNA which was formed during learning was isolated and its base composition was determined in order to give the ratios of uridine to cytidine The task for this study was chosen so that it gave a large challenge to the goldfish brain to produce maximal changes in RNA synthesis, thus any unique base composition could be detected in analyses of whole brain extracts

Goldfish ('Comet' variety) measuring 7–8 cm in length and weighing 7–8 g were used throughout this study The animals were fed 24 h before each experiment Groups of seven experimental and seven control fish were placed in separate 5 gallon tanks each of which was filled with 3 gallons of water Orotic acid (10 µc, specific activity 250 mc /mmole) dissolved in 10 µl of 0 15 normal sodium chloride was injected into the ventricle below the region of the optic tectum After injection of the label the goldfish were trained to swim in a new pattern by attaching a foamed polystyrene float to a suture at the ventral surface of the fish, 1 mm caudal to the base of the first pair of lateral fins A 0 7 cm cube was used for a 7 g fish. The sequence of adaptation of a goldfish to the challenge which was produced by the float is shown in Fig 1 The animal acquired new swimming skills during the challenge In stage I the animal is pulled up to the surface in either of two configurations, one with the head out of the water and the other in an upside down position This is a very noxious stimulus and the fish try to escape to the bottom of the tank, but the float pulls them up Thus stage I is characterized by considerable struggling Stage II is usually attained within 1 h Here the animals assume a 45° angle and swim constantly to avoid being turned upside down by the float At stage III, the goldfish have become sufficiently skilful to swim horizontally with the float in a ventral position Frequently, the tails are bent to one side to compensate for the lateral torque imposed by the float The fish constantly swim in this position One can readily observe that the pattern of motion of the lateral fins is quite different from that of controls In each experiment, the controls received a suture at the same position as the experimental groups but no float In considering the various aspects of the adaptation of the fish to the task, one can postulate influences on many parts of the brain, including motor areas, cerebellum, optic tectum, vestibular regions (Mauthner cell) and certainly controls of visceral functions especially the modulation of the air content of the swim bladder

The use of orotic acid in these experiments resulted in the formation of labelled UTP and CTP as the precursors for RNA synthesis[14] In the conditions of the experiment only about 10,000 c p m of the label became associ-

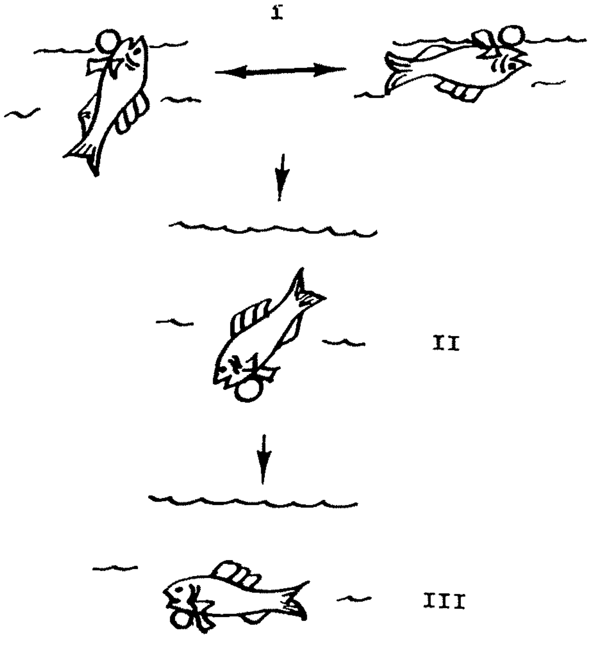

Fig 1 Stages in the adaptation of a goldfish to the float

ated with each animal brain Of this, on an average, 1,000 c p m of label was incorporated into phenol extractable RNA After completion of a 4 h training period the animals were decapitated, the brains were washed with distilled water and homogenized with a 'Dounce' homogenizer in 0 02 molar phosphate buffer (*p*H 7 2) containing 0 5 per cent sodium lauryl sulphate The pooled brain homogenates from seven animals were extracted with hot phenol (60° C) in order to isolate the nuclear RNA according to the methods of Scherrer and Darnell[15] and Perry[16] The product was purified by alcohol precipitation to give an RNA with a sucrose density gradient pattern which was characteristic of nuclear RNA[20] The purified RNA was analysed for total radioactivity as trichloroacetic acid precipitable counts, for total quantity of RNA as absorption at 260 mμ and for base composition after alkaline hydrolysis (0 3 molar potassium hydroxide for 17 h at 37° C) using paper electrophoresis[17]

Fig 2*A* shows that the task used in this paper has a learning component which is remembered by the goldfish after 22 h The results for experiment *A* were obtained for a group of ten animals each with a different coloured float and suture so that each animal could be identified and rechallenged with the same float on the second trial The animals were observed at fixed times during the 4 h training period and those at stage I, stage II and stage III (Fig 1) were arbitrarily scored at 0 per cent, 50 per cent and 100 per cent trained At each test time the group score was obtained and plotted to give the learning curve for trial I in Fig 2*A* After 4 h the floats were removed from the fish, and 22 h later the fish were tested again with the same floats to give curve II in Fig 2*A* The faster rate of learning on the second trial clearly shows retention, that is, the mean time for trial II was 21 ± 4 min as compared with 156 ± 13 min in trial I ($P <$ 0 01 in a *t* test)

Fig 2*B* is a repeat of these training and testing experiments using a separate group of animals each of which received a dose of 120 μg of puromycin 1 5 h before trial I The results show that the rate of acquisition of the task is not significantly altered from that of the group in trial I (Fig 2*A*) and that there is no significant evidence of remembering the task 22 h later, that is, a score in trial II of 100 ± 20 min is not statistically different ($P >$ 0 05) from the score in trial I of 136 ± 13 min The dose of puromycin in this experiment has been previously reported by Brink, Davis and Agranoff[18] to inhibit 80 per cent of the protein synthesis in goldfish brain within 1 5 h Thus inhibition of protein synthesis in the behavioural task of this experiment resulted in no significant retention of the training after 22 h

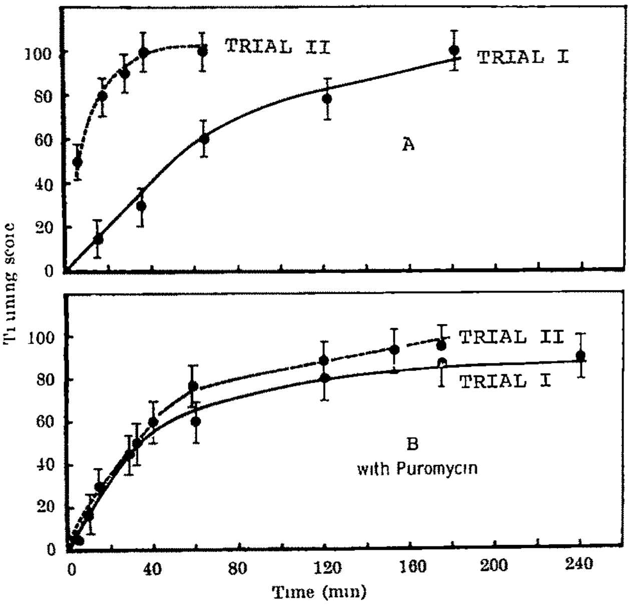

Fig 2 Learning (trial I) and retention (trial II) curves for the goldfish Each curve was obtained on a group of ten animals Trial II was 22 h after trial I (see text) *A*, Experiment *A* with no drugs, *B* experiment *B* group received 120 μg of puromycin (intracranial) 1 5 h before the beginning of trial I

Table 1 summarizes the RNA base ratio measurements for seventy animals, thirty-five experimental and thirty-five control The results show a consistent pattern of increase in the uridine cytidine $(U/C)_E$ values for the experimental group over the $(U/C)_C$ control group values The average values for $(U/C)_E$ minus $(U/C)_C$ are 3 3 with a corresponding mean increase of 96 per cent These base ratio changes, however, do not occur when the animals are first injected with puromycin (see Table 2) The data suggest that the new RNA synthesis accompanying the task has an increased uridine (by about a factor of 3) over its cytidine content

Table 1 SUMMARY BASE RATIO MEASUREMENTS

No	c p m Up	Cp	U/C	$(U/C)_E-$ $(U/C)_C$	% Change
1E	336	44	7 6		
1C	415	112	3 7	3 9	+108
2E	446	69	6 2		
2C	116	36	3 2	3 0	+ 94
3E	501	93	5 4		
3C	726	308	2 4	3 0	+128
4E	560	72	7 8		
4C	618	135	4 6	3 2	+ 70
5E	490	65	7 6		
5C	286	67	4 3	3 4	+ 78

Average values

No of animals	$(U/C)_E$	$(U/C)_C$	$(U/C)_E-(U/C)_C$	% Change
70	6 9 ± 0 47	3 6 ± 0 20	3 3 ± 0 29	+96

The animals were last fed 24 h before each experiment E and C values represent data from seven pooled brains for each experimental and control group, respectively

Table 2 EFFECT OF PUROMYCIN ON RNA BASE RATIOS

No	$(U/C)_E-(U/C)_C$*	% Change
6	0 25	+10
7	0 06	+ 2
8	0 41	+ 8
Average	0 24	+ 7

Puromycin dose = 170 μg (intercranial) 1 5 h before training Fourteen animals were used for each datum point

* $(U/C)_E-(U/C)_C$ represents the difference between the uridine to cytidine ratios in experimental and control groups

In this behavioural experiment, the experimental group of goldfish was under constant stress They also performed much work in addition to learning a new swimming skill Thus each of the variables—stress, work, learning or all three—could be responsible for promoting the RNA base ratio changes which were observed Because the animals which were under the influence of puromycin seemed to acquire the swimming skill at about the same rate as those without the drug, they must necessarily have been exposed to approximately the same level of stress and work Moreover, when tested 22 h later, these animals showed no significant evidence of remembering the task In addition the puromycin group showed no significant base ratio changes (Table 2) after the acquisition of the new swimming skill It follows therefore that the changes in RNA base ratio are probably the result of the learning component of this behavioural experiment and not caused by general factors such as work or stress

These data suggest that RNA base ratio changes accompany the acquisition stage of learning and imply that protein synthesis is required for the establishment of these changes Recent observations[19], however, that puromycin produces hippocampal seizures in rat brain, in addition to inhibition of protein synthesis, indicate caution on this point The RNA results are similar to the biochemical findings of Hyden and Egyhazi[3] concerning the experiment on transfer of handedness in rat brain

There are a number of possibilities for explaining the RNA base ratio changes which are observed as the concomitant biochemical change with the acquisition of new swimming skills by the goldfish For example, the behavioural task could merely lead to changes in the metabolic pools of CTP and UTP to produce the base ratio changes in the RNA This, however, is ruled out by direct measurements of the UTP and CTP contents of the metabolic pools (my unpublished work)

Another possibility is that learning causes specific instructional changes in the newly synthesized RNA A more attractive hypothesis is that the brain operates in many functional states such as sleep, wakefulness, learning, and so on, each of which is biochemically distinct Thus the biochemical consequences of learning could result in an induction at the genome level of a special RNA which is required for promoting such processes as neuronal growth which could occur during the consolidation step of learning Only further experimental evidence can distinguish which of these mechanisms or alternative processes are the correct biochemical correlates of learning

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[1] Hyden, H V , and Egyhazi, E , *Proc US Nat Acad Sci* , **48**, 1366 (1962)
[2] Hvden, H V , and Egyhazi, E , *Proc US Nat Acad Sci* , **49**, 618 (1963)
[3] Hvden, H V , and Egyhazi, E , *Proc US Nat Acad Sci* , **52**, 1030 (1964)
[4] Hyden, H V , and Lange, A W , *Proc US Nat Acad Sci* , **53**, 948 (1965)
[5] Dingman, W , and Sporn, M B , *Biochim Biophys Acta*, **61** 164 (1962)
[6] Dingman, W , and Sporn, M B , *Science*, **144**, 26 (1964)
[7] Flexner, J B , Flexner, L B , and Stellar, E , *Science*, **141**, 57 (1963)
[8] Flexner, J B , and Flexner, L B , *Proc US Nat Acad Sci* , **55**, 369 (1966)
[9] Flexner L B , Flexner, J B , and Roberts, R B , *Proc US Nat Acad Sci* , **56**, 730 (1966)
[10] Agranoff, B W , Davis, R E , and Brink, J J , *Proc US Nat Acad Sci* , **54**, 788 (1965)
[11] Barondes, S H , and Cohen, H D , *Science*, **151**, 594 (1966)
[12] Zemp, J W , Wilson, J E , Schlesinger, K , Boggan, W O and Glassman, E , *Proc US Nat Acad Sci* , **55**, 1423 (1966)
[13] Davis, R E , and Agranoff, B W , *Proc US Nat Acad Sci* , **55**, 555 (1966)
[14] Potter, V R , *Nucleic Acid Outlines*, **1** (Burgess Publishing Co , Minneapolis, Minnesota 1960)
[15] Scherrer, K , and Darnell, J , *B B R C* , **7**, 486 (1962)
[16] Perry, R P , *Proc US Nat Acad Sci* **48**, 2179 (1962)
[17] Wagner, E K , and Ingram, V M , *Biochemistry*, **5**, 3019 (1966)
[18] Brink, J J , Davis, R E , and Agranoff, B W , *J Neurochem* , **13**, 889 (1966)
[19] Cohen, H D , and Barondes, S H *Science* **157**, 333 (1967)

Evolution of the MG

by
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Evolutionary principles can be applied to the development of the sportscar of the genus MG after its migration to the United States

THIS article aims to examine the development of the MG sportscar in the light of modern evolutionary thought It is obvious that the application of biological principles to a man-made, profit-oriented item will require a degree of extrapolation It seems worthwhile, however, to stretch a principle to a seemingly inapplicable situation if it allows us to examine more clearly the principle itself

The name MG derives from the initials of the Morris Garages, where the first cars were fabricated in 1923, based on parts from Morris automobiles. The company continues this semi-independent role, combining parts of its own manufacture with those available from other producers

At various times, the company has produced small sedans in addition to the sportscars Their production is not the chief endeavour of the company, but rather these sedans represent the attachment of the MG name with connotations of sporting flavour to cars similar to others being produced concurrently This situation is analogous to a perverted form of Batesian mimicry where a relatively distasteful form, the sedan, seeks to imitate a more tasteful form, the sportscar, to increase predation—that is, buying pressure—on itself

Basic to our discussion is the establishment of the breed of MGs relative to some taxonomic level Although obviously asexual, their phylogenetic pattern and progressive development prompt their consideration as a sexual organism The development of a working hypothesis treating the entire MG lineage as a genus with each model representing a species may allow us the flexibility to develop meaningful analogies

The phylogenetic tree (ref 1, Fig 1), reproduced here with recent additions, will be useful in reviewing the history of production As with the palaeontological record, however, recent forms provide the most information about competition, habitats and development Consequently, the succeeding illustrations will involve the models produced since the Second World War—the MG-TC (Fig 2*a*)—and its derivations

The first MG-TC migrated to the United States in the hands of private owners, these founders survived in this large, competition-free habitat These cars were followed by others imported through an embryonic dealership system Priced at less than $2,000 in the period 1947 to 1949, they sold readily, particularly in the eastern and southwestern parts of the country This model was not perfectly pre-adapted to the new and prosperous habitat it found in America All MG-TCs were produced for home use only and consequently were equipped with right-hand drive (Fig 2*a*) In the United States there must have been strong selection against this factor To pass another car, the driver had to swing far left in order to have a full view of the oncoming traffic Information regarding the head-on collisions which frequently resulted was relayed to the factory

The company, to increase its profits, would have to adapt itself to extend the product to the limits of its niche This exploitation is analogous to the tendency of any group of animals to spread themselves throughout the resource area

The factory which contains the component parts for production of species is analogous to a gene pool The influx of profits from American sales took the form of information which was combined with the efforts of the engineers (egg-heads) at the factory to produce a new species This species—the MG-TD—was more streamlined and would be offered to American buyers with left-hand drive (Fig 2*b*)

These events are comparable with the Baldwin effect, in which an organism is able to stay in a favourable environment until genetic fixation achieves suitable modification of the phenotype

During the last years of TD production the TD Mark II model with increased horsepower was offered as well as the standard model This increase with additional streamlining and more comfort for the passenger was incorporated into the model TF (Fig 2*c*) This speciation was not based on geographic factors as in the TC–TD situation, but was adaptive in nature The TF model continued until 1955 when the effects of the American horsepower race and increased competition from Triumph sportscars indicated that adaptation would be necessary if the breed was to survive

Although the T-series cars had undergone progressive increases in power and slight changes of streamlining and styling, the overall body shape of clamshell fenders, running boards and the absence of luggage space was strongly characteristic of pre-war automobiles The modern envelope body style had been experimented with as early as 1951 Test data indicated that continued use of TF engines and chassis with simple substitution of a streamlined body would allow a 12 mile/h gain in top speed (Fig 3, from Thornley, ref 1) with an appropriate gain in acceleration times This model which consisted of a new body shell over unchanged mechanical components was designated as the MGA (Fig 2*d*) This speciation can be compared with a problem in palaeontology where a slight difference in total genetic make-up produced an

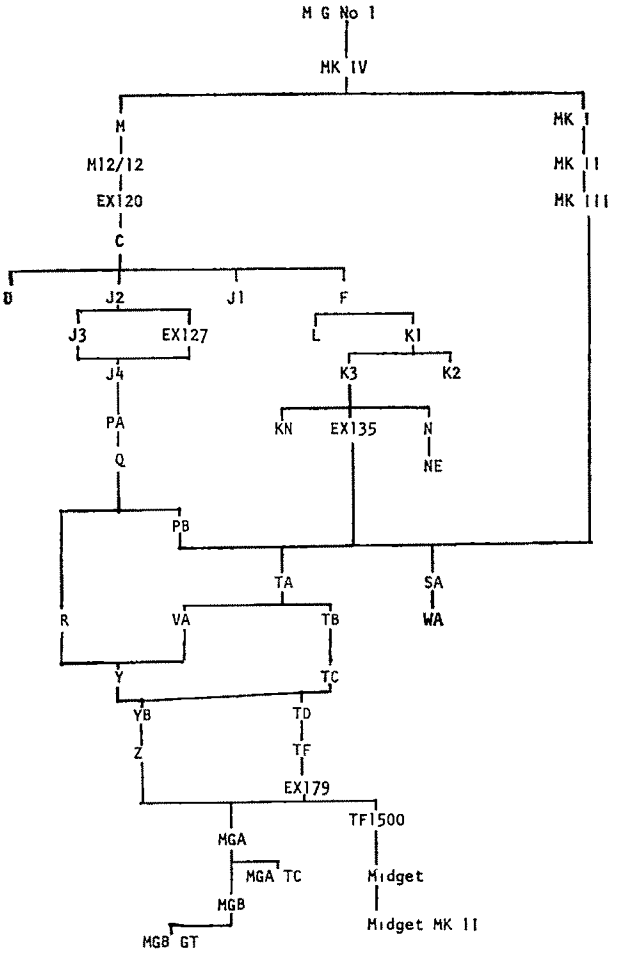

Fig 1 Phylogenetic pattern of the MG automobiles (after Thornley, ref 1)

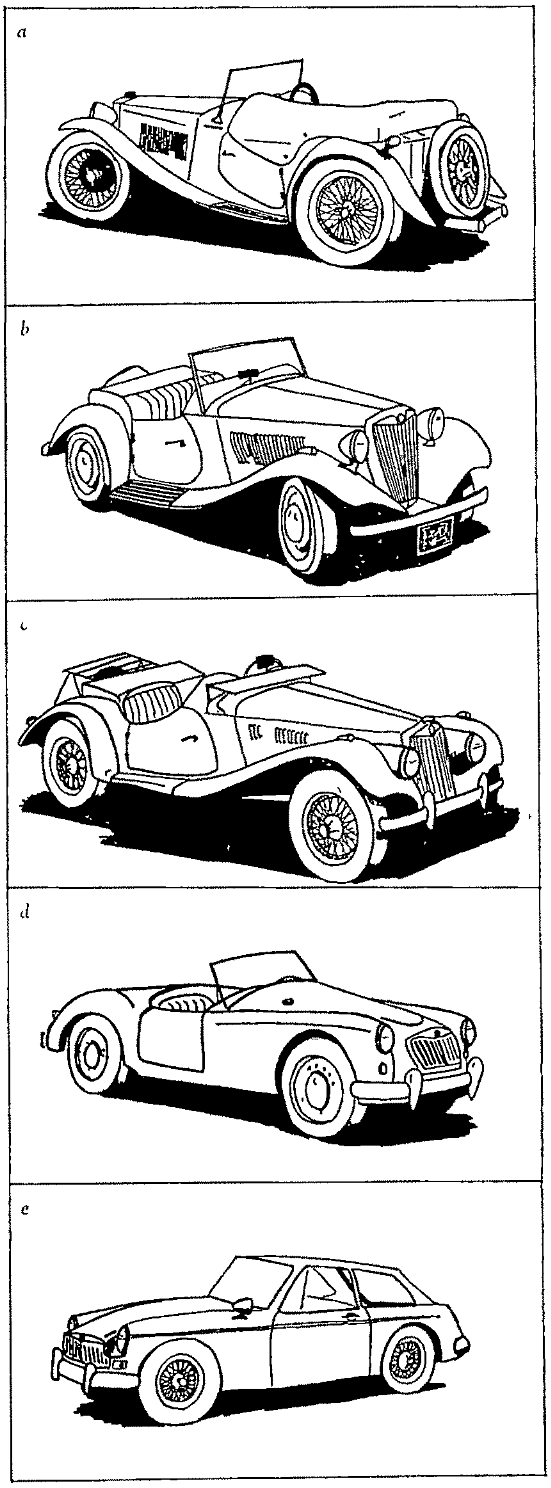

Fig 2 MG models, *a*, MG-TC, *b*, MG-TD, *c*, MG-TF, *d*, MGA, *e*, MGB-GT

entirely changed shell which, if found without the internal organs, would show little affinity to its predecessors

The basic MGA body design remained in production for more than 8 yr During the production, slight changes in the genetic make-up were enacted The power of the engine was increased and there were small phenotypic changes in the design of the grille and the placement of the tail-light The duration of this body style suggests that there was a selective premium for the maintenance of the phenotype and that the variations that developed during the 8 yr period are analogous to sibling species

The MGA Twin-Cam, essentially an MGA with a highly developed twin-overhead camshaft engine and racing-type suspension, was produced from 1958 to 1961 The price of these cars was in excess of $4,000, nearly twice the price of the standard model MGs, since their introduction, had been the basis of the low-priced sportscar market, although the MGA was at this time costing $600–$800 more than the first TC models The MGA-TC was not in the low-priced category and found itself in direct competition with the Jaguar models with which it had sympatrically existed since the early 1950s The direct competition with another genus highly adapted to its niche resulted in extinction of this species The gene pool did not continue to produce a model obviously incapable of surviving the selection pressures Finding that the high-priced niches could not be invaded, the factory turned to lower-priced niches for additional exploitation

In 1962 the MG Midget was introduced The MGA was not referred to as a midget series car, possibly because the price, in dollars, and the weight, in pounds, both exceeded 2,000, unlike the earlier TC–TD–TF models The reintroduction of the Midget, the price and weight of which were less than 2,000, met with less competition and flourished Some of this success is in part analogous to the Batesian-type mimicry presented earlier in the discussion of sedan production

Production of MGAs continued until 1963 During the 8 yr of its production both the quantity and quality of competition improved These factors brought about the necessity for more adaptation if the marque was to survive The economic pressure to maintain a mid-$2,000 price was relieved slightly by the concurrently produced Midget model, consequently, the new species could represent a departure from the standard price category The species developed was the MGB (Fig 2e), produced according to styling trends established by Jaguar's successful XK–E model, offered with a slight increase of horsepower over the MGA and with roll-up windows The current price of this car is about $3,000, well below Jaguar's niche and there is no direct competition between these two genera

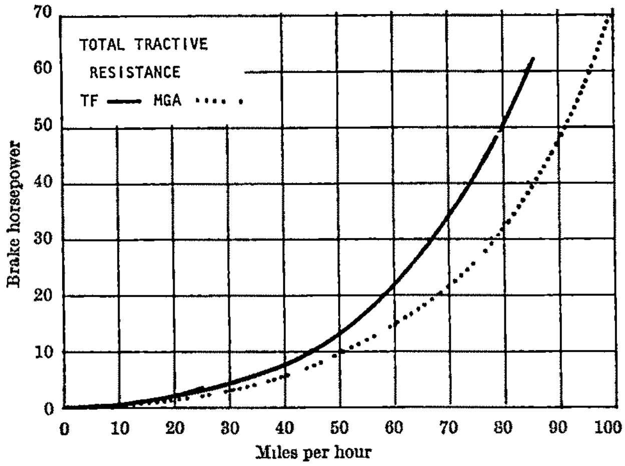

Fig 3 Total tractive resistance curves At 50 horsepower the MGA body style allowed an increase of 10 miles/h over the TF model (from ref 1)

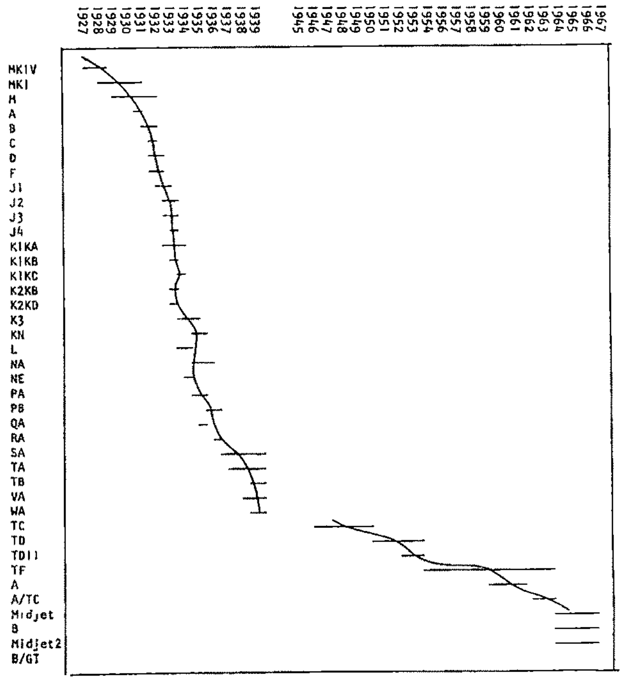

Fig 4 Life span chart of models produced (after Thornley, ref 1)

In 1966 an additional form of the MGB was introduced, the MGB-GT with non-removable steel top (Fig 2e) It remains to be seen if this more protected model, an ecological polymorph, gains popularity in areas where the climate is not compatible with the soft-top varieties

This topic is an obvious stretching of scientific investigation The basic weakness in this proposed analogy is the lack of concrete, supportable data Thornley's book did provide some data and graphs which are included here, but it did not contain the actual production figures which would have allowed insight into population characteristics of the breed

Individual instances analogous to Batesian mimicry, palaeospecies, genetic structure, the Founder principle, pre-adaptation, gene pools, the Baldwin effect, sibling species, species extinction and ecological polymorphism were recognized

The overall structure of the various species is presented (Fig 4). A curve approximating the centre points of the life span of each species indicates that the more recently produced species have had longer life spans and that the rate of evolution before the Second World War is not necessary for the genus to survive As long as the output of the gene pool is successful in finding and holding a niche the stability continues The sale of automobiles to the public is the stabilizing factor and the rate at which each car produced is sold relates information to the gene pool Information flow back to the gene pool in the form of money indicates a successful product If the breeding rate decreases, the gene pool attempts to reorganize itself to produce a new species which, if successful, will carry on the lineage

Evolution is a real process and, as can be seen from this article, similar processes can be shown to exist in man-made products To what degree does this simulated evolution affect the thinking of evolutionary workers who knowingly or unknowingly live in an environment where evolution is fallaciously applied ?

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[1] Thornley John, *Maintaining the Breed*, 187 (Motor Racing Publications, London, 1956)

[2] Mayr, Ernst, *Animal Species and Evolution*, 797 (Belknap Press, Cambridge, Massachusetts, 1965)

LETTERS TO THE EDITOR

PLANETARY SCIENCE

Metals on the Lunar Surface

IN a recent paper[1] written before the flight of Surveyor V, I suggested that metallic iron, aluminium and magnesium may exist on the lunar surface as a result of the chemical reduction of the surface by atomic hydrogen accumulated from long bombardment by solar wind protons. The large number of hydrogen atoms available and the continuous turnover of the lunar surface layer throughout the ages led me to suggest that there is a layer a few feet thick which contains metals.

Assuming an arbitrarily chosen lunar composition of $5FeO\ 2Al_2O_3\ 5MgO\ 20SiO_2$, I estimated the range of 1 to 2 keV protons in this material to be about 150 Å. The total number of oxygen atoms, which is rather insensitive to composition, is about 7×10^{16} cm^{-2}. Assuming a solar wind flux of 3×10^{8} protons cm^{-2} sec^{-1}, the number of hydrogen atoms available in a layer of 150 Å material during a period of 15 yr will be twice the number of the combined oxygen atoms.

Thermodynamically, the reduction of oxides by atomic hydrogen to form metals or elements and water vapour is energetically favourable. For example, the values of free energy for the reduction (by atomic hydrogen) of the oxides, magnesium oxide, calcium oxide, ferrous oxide, aluminium oxide and silicon dioxide are −14·9, −6·3, −91·3, −74·3 and −96·4 kcal/mole, respectively, at 400° K. The free energy values for silicates or combined oxides at 400° K as well as 100° K and 298° K are not materially different from those of single oxides. Atomic hydrogen is known to be very active chemically and there were indications in the laboratory that iron oxides can be reduced to lower oxidation states or to metal by protons of a few keV energy[2]. It is therefore reasonable to suggest that the oxides or silicates on the lunar surface are reduced to elements by hydrogen from the Sun, and that the water produced diffuses out to the surface through a layer less than 150 Å thick and escapes to the vacuum above. The reverse reaction of water on aluminium and magnesium to form molecular hydrogen is thermodynamically favourable. Because, however, atomic hydrogen is continuously supplied and water, under vacuum, escapes easily and continuously, the formation of metallic elements should be favoured in the long bombardment. It may be noted that the proton bombardment also causes sputtering. This effect, however, is relatively insignificant.

If we arbitrarily quintuple the time of reduction or the amount of hydrogen atoms required for reduction and also assume that the Moon has been bombarded by solar wind for $4·5\times 10^{9}$ yr, the incident flux of hydrogen is capable of reducing a layer of silicates as thick as 1 m. If the lunar surface layer is stagnant, the reduction reaction cannot occur much deeper than the range of protons. Most selenologists, however, believe that there is a continuous flow or turnover of the surface material because of meteorite bombardment or volcanic action. The thickness of lunar "dust" is not known, but the best estimate by Newell[3] is about 1 m. If it is assumed that a layer 1 m thick was turned over once completely and uniformly during the life of the Moon, it is likely that a fraction of the material exists in an elemental or metallic form, or in some kind of reduced state.

Magnesium and aluminium, if present, are valuable because of their high energy content and structural value. These metals, after collection, could be heated with the readily available silicates on the Moon. The silicon dioxide component in the silicates could serve as an oxidizing agent and when the reactions occur a large amount of energy would be liberated. In addition to metallic magnesium and aluminium, metallic iron would certainly be present. This iron powder can easily be separated magnetically and may be used as structural material. Whether elemental silicon is present is a difficult matter to determine because silicon may form volatile silanes when reacting with hydrogen.

Another aspect of the possible presence of these reduced materials concerns the safety of the astronauts. The metals, if present, must be quite loose in structure and have large surface area. If astronauts should carry some of these loose materials into a space cabin, these materials may burn spontaneously in an oxygen atmosphere.

The recent results of Surveyor V (ref 4) seem to substantiate in part this hypothesis. From the magnet experiment, Wys[4] estimated that lunar surface material may contain 1 per cent by volume of metallic iron. If the lunar surface material has a chemical composition similar to that of terrestrial basalts, this is equivalent to more than 3 per cent by weight and the metallic form constitutes more than 30 per cent of the total iron—a surprisingly high percentage. Because some meteorites are known to contain metallic iron, its high content in the lunar surface could not be attributed entirely to the hydrogen reduction suggestion. Furthermore, the magnetic material present on the lunar surface may be also attributed to magnetite instead of metallic iron. An examination of the analysis of the lunar surface by Turkevich *et al*[4], however, also seems to give evidence that the lunar surface material is deficient in oxygen or rich in metal.

Turkevich *et al*[4] gave the chemical composition, in atomic per cent, of the lunar surface at the Surveyor V site as follows: carbon, <3, sodium, <2, magnesium, 3±3, aluminium, 6·5±2, silicon, 18·5±3, 28 <A <65, 13±3, A >65, <0·5, and O, 58±5, where A represents any element with its mass number as indicated. The composition compares favourably with that of terrestrial basalts, and Gault *et al*[4] stated that this finding "is consistent with a widely accepted hypothesis that extensive volcanic flows have been responsible for flooding and filling of the mare basins".

Although the errors of the chemical composition are large, some confidence may be placed on the experimentally assigned values because they seem to match well with that of basalts. If these figures are taken as close to the true values, the amount of oxygen required to satisfy the valency of the electropositive elements in the lunar analysis can be estimated. In such an estimation, I conservatively assume that carbon does not take up any combined oxygen because it may form carbides instead of carbonates. On the other hand, because the terrestrial basalts contain about 2 per cent of sodium, we allocate 1 per cent oxygen for sodium in our estimate. A study of the terrestrial basalts indicates that for all elements with mass number above 28, the average oxygen requirement is more than unity. This is particularly true because basalt contains some ferric oxide and very little sulphur and potassium. We conservatively assign one oxygen atom to each atom of elements above mass 28. The total oxygen required to balance all the electropositive elements was calculated to be 64·3 "atomic per cent" as compared with the experimentally measured value of 58. The Surveyor V experiment could not measure the hydrogen content and the terrestrial basalts do contain about 4 atomic per cent of hydrogen equivalent to 2 atomic per cent of oxygen. Because there is indication that the lunar surface is volcanic in origin, it is reasonable to assume that the lunar surface material contains little or no hydrogen.

If the electropositive elements are partially reduced, the extent of the reduction can be estimated by matching the oxygen required to the experimentally measured oxygen content. A simple calculation indicates that 10 per cent to 20 per cent of the elements are in an elemental form, depending on whether one assumes that silicon is also reduced to elemental form or escapes into space as silanes. While it is not certain that aluminium and magnesium may exist as metals, this argument is certainly not inconsistent with the information at present available.

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[1] Sun, K. H., *Westinghouse Res. Lab. Sci. Paper 67-1C2-APOLO-P1* (1967).

[2] Salisbury, J. W., and Glaser, P. E., in *The Lunar Surface Layer* (Academic Press, New York, 1964).

[3] Newell, H. E., *Nat. Geog. Mag.*, 130, 578 (1966).

[4] Jaffe, L. D., *et al.*, de Wys, J. N., Turkevich, A. L., *et al.*, and Gault, D. E., *et al.*, see *Science*, 158, 631 (1967).

General Relationship between Suspended Sediment Concentration and Water Discharge in the Alpenrhein and some other Rivers

IN order to make a long-term prediction of sedimentation in Lake Constance (Central Europe), about five hundred measurements of the suspended load in the Rhine ("Alpenrhein") at Lustenau, Austria (Fig. 1), have been carried out[1,2] at different water levels.

The Alpenrhein drains a total area of 6,122 km², a chiefly mountainous region in the Northern Alps of Switzerland and Austria. According to Waibel[3], between 1931 and 1960 the average water flow was 223.6 m³/sec and the average suspended sediment load was 454.1 mg/l. Thus the river supplied an average annual suspended sediment load of 3.334×10^6 tons, the bulk of which is deposited in the Obersee of Lake Constance[4]. As in most other rivers in humid climates, the bed load carried into the lake can be ignored because it only amounts to about 1 per cent of the total sediment load.

The concentration of suspended sediment load, C_s (mg/l), in the Alpenrhein is strongly related to the water discharge, Q (m³/sec): with increasing water discharge, and increasing flow velocity, the suspended sediment load increases rapidly (Fig. 2).

On a double logarithmic scale this relation can be expressed by a straight line. Its equation is

$$C_s \text{ (Alpenrhein at Lustenau)} = 0.004 \quad Q^{2.2}$$

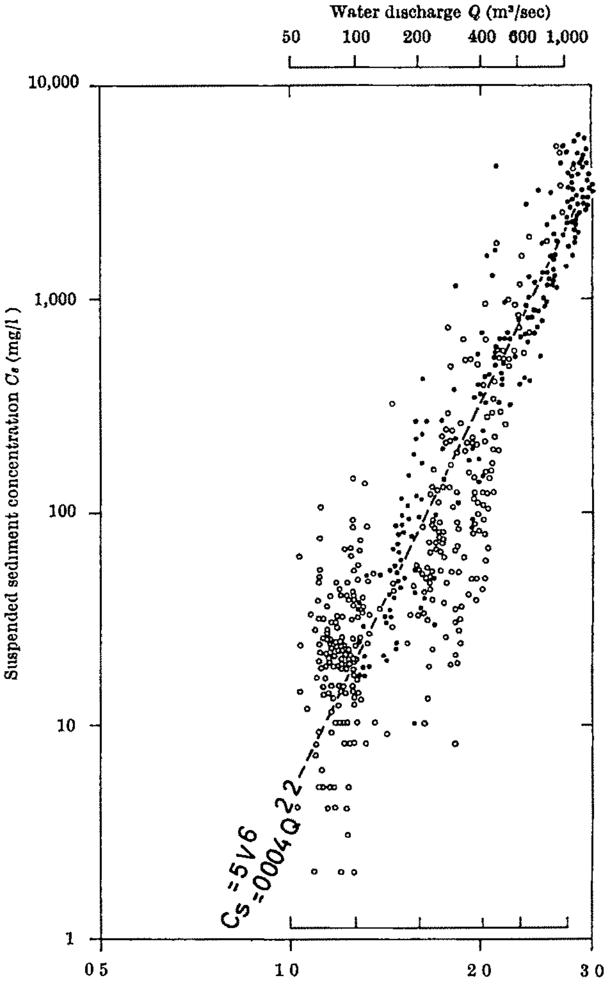

Fig. 2. Suspended sediment concentration versus flow velocity and water discharge in the Alpenrhein at Lustenau (Austria). ●, This communication; ○, Muller, 1965.

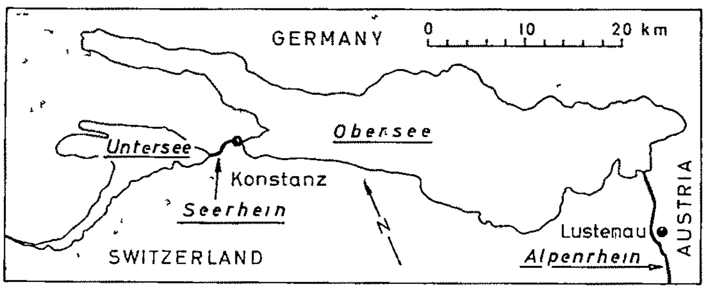

Fig. 1. Lake Constance with Alpenrhein and Seerhein. Sampling stations Lustenau (Austria) and Konstanz (Germany).

The Seerhein is the natural effluent of the Alpenrhein (and of other smaller rivers flowing into the Obersee); it connects the Obersee with the Untersee of Lake Constance (Fig. 1). Because all sand and silt sized material as well as most of the clay sized material has been deposited in the Obersee, the concentration of sediment load in the Seerhein is extremely low. About three hundred measurements of the suspended sediment load in the Seerhein[1] resulted in the equation

$$C_s \text{ (Seerhein at Konstanz)} = 0.20 \quad Q^{0}$$

Data for other rivers were collected from the literature[5-9] and evaluated similarly (Fig. 3, Table 1). In all cases where sufficient measurements had been made over a long period of time, similar relations could be obtained. They can all be covered by a general empirical equation

$$C_s = a \quad Q^b$$

In the rivers concerned, the factor a ranges between 0.004 in the Alpenrhein at Lustenau and 80,000 in the Rio Puerco at Bernardo. The exponent b varies between 1.1 in the Seerhein at Konstanz to 2.5 in the White River in the Mt. Rainier area.

Because the water discharge of a river at a defined cross-section is a function of its flow velocity (Manning equation), a relationship also exists between the suspended sediment load and the flow velocity, V (m/sec),

$$C_s = x \quad V^y$$

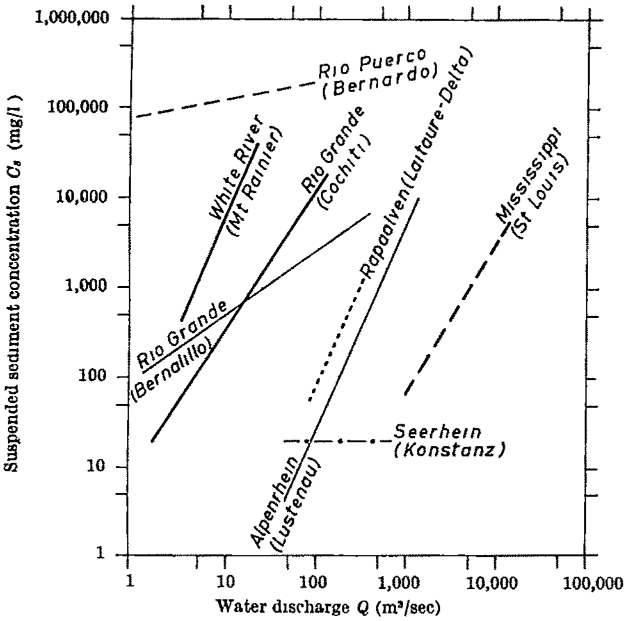

Fig. 3. Suspended sediment concentration versus water discharge of different rivers at single localities.

In this equation, the rivers shown in Table 1 are in the range 5 to 80,000 for x and 0 to 8 for y.

In Table 1 the rivers are arranged in order of increasing exponents b and y, respectively, which are measures of the slope of the curves in Fig. 2. Usually these exponents seem to express the intensity of the erosional forces which are determined chiefly by the gradient and the cross-section of a river channel. In the Seerhein at Konstanz, no erosion at all is effective (b and $y = 0$), while Rapaalven, the Alpenrhein and the White River are typical mountain rivers with high relief energy ($b = 2.2$ to 2.5, $y = 4.5$ to 8).

Table 1. NUMERICAL VALUES FOR a, b, x AND y FOR DIFFERENT RIVERS AT SINGLE LOCALITIES

	$C_s = a\ Q^b$		$C_s = x\ V^y$		
River	a	b	x	y	References
Seerhein (Konstanz)	20	0.0	20	0.0	1
Rio Puerco (Bernardo, N. Mex.)	80,000	0.2	80,000	0.8	5
Rio Grande (Bernalillo)	100	0.7	2,000	1.0	6
Rio Grande (Cochiti, N. Mex.)	10	1.5	200	4.0	6
Mississippi (St. Louis)	0.01	1.6	300	4.0	7
Rapaalven (Laitaure-Delta)	0.04	2.2	700	4.5	8
Alpenrhein (Lustenau)	0.004	2.2	5	6.0	1 and unpublished work of G. Muller and U. Forstner
White River (Mt. Rainier, Wash.)	40	2.5	40	8.0	9

Factor a (or x) is large in rivers which drain areas with a high intensity of weathering or a high erodibility of the outcropping rocks or rapid changes in the channel geometry or any combination of these three. An extreme case in which all these factors coincide is the Rio Puerco at Bernardo, an ephemeral stream in which rainstorms cause large mudflows after long dry periods. In the hinterland of the Alpenrhein and the White River, weathering caused by the alpine climate and vegetation is low, channel changes do not occur and rock erodibility is generally low.

It is to be expected that relationships between suspended sediment load and water discharge are easy to obtain from any river at any cross-section. If the relationships are such that they can be expressed by a simple function, prediction of a long-term sediment transport can be readily established.

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[1] Müller, G., *Fort. Wasserchemie*, 2, 33 (1965).
[2] Muller, G., in *Deltas in their Geologic Framework* (edit. by Shirley, L.), 107 (Houston Geol. Soc., Houston, 1966).
[3] Waibel, F., in *Das Rheindelta im Bodensee*, 10 (Österr. Rheinbauleitung, Bregenz, 1962).
[4] Muller, G., *Naturwissenschaften*, 53, 237 (1966).
[5] Nordin, C. F., *US Geol. Surv., Prof. Paper*, 462-*C*, 21 (1963).
[6] Nordin, C. E., and Beverage, J. P., *US Geol. Surv., Prof. Paper*, 462-*F*, 35 (1965).
[7] Jordan, P. R., *US Geol. Surv., Water Supply Paper*, 1802, 89 (1965).
[8] Axelssohn, V., *Geograf. Ann.*, A, 49, 127 (1967).
[9] Fahnestock, R. K., *US Geol. Surv., Prof. Paper*, 422-*A*, 69 (1963).

Depth Habitats of Pelagic Foraminifera during the Pleistocene

PLANKTONIC foraminifera, numbering about thirty modern species, have been found alive not only near the surface but as deep as 2,000 m (ref. 1). Emiliani[2] analysed the O^{18}/O^{16} composition of the shells of several species and concluded that different species occupy different depth habitats, with *Globigerinoides ruber* and *G. sacculifera* living very close to the ocean surface, *Globigerina* spp. living somewhat deeper and *Globorotalia* spp. occupying the deepest habitats down to about 200 m for population averages. Whereas O^{18}/O^{16} analysis of the carbonate shells can only establish the depth at which average shell deposition takes place, not only for individual shells but for entire populations, recent work by Jones[3], using opening and closing plankton nets, has shown that the conclusions reached by Emiliani[2] are essentially correct. It therefore seems that the average depths of shell deposition are also the average depths at which the different species live.

In the present study, the work initiated by Emiliani[2] has been extended through different glacial and interglacial intervals as shown by the deep-sea core *P*6304–8 from the central Caribbean (14° 59′ N., 69° 20′ W., 3,927 m deep, 1,054 cm long). This core has been analysed both isotopically[4] and micropalaeontologically[5] and shown to represent a continuous and undisturbed section of pelagic sediments extending in time back to 275,000 yr ago. Four complete temperature cycles, plus the post-glacial, are represented in this core. All planktonic foraminiferal species present in sufficient abundance were separated from each one of the warm (10, 300, 550, 800 and 1,010 cm below top) and cold (70, 370, 690 and 890 cm below top) stages of the core as indicated by the oxygen isotopic analysis of Emiliani[4]. In addition, a sample showing intermediate temperatures (150 cm below top) was also studied. A total of fourteen species was separated from the samples.

The monospecific samples were crushed, washed in distilled water and directly reacted with 100 per cent H_3PO_4 acid (compare ref. 4). The carbon dioxide gas thus obtained was analysed with a high-precision Nier–Urey mass spectrometer. The results are shown in Tables 1 and 2. The different species have been arranged in order of decreasing temperatures for both the 10 cm level (post-glacial) (Table 3) and for the averages of all warm and cold stages (Table 4). The sequence is usually the same. Corresponding water depths have been calculated using the Atlas published by Fuglister[6].

The following conclusions can be drawn from Table 3: the four species and subspecies of *Globigerinoides* occupy

Table 1 OXYGEN ISOTOPIC RATIOS (δ/ML WITH RESPECT TO THE CHICAGO STANDARD *PDB*-1) FROM SELECTED LEVELS OF CARIBBEAN CORE *P*6304–8

Species	W 10	C 70	I 150	W 300	C 370	W 550	C 690	W 800	C 890	W 1,010
Globigerinoides ruber	−1 76	+0 03	+0 16	−0 99	+0 04	−0 69	−0 03	−1 25	−0 12	−0 93
G trilobus sacculifera	−1 69	+0 43	+0 05	−1 32	+0 73	−0 76	+0 47	−0 96	+0 40	−1 00
G conglobatus	−1 45	+0 71	+0 89	−0 45	+0 53	−0 12	+0 90	−0 92	+0 69	−0 57
G trilobus trilobus	−1 43	+0 32	+0 72	−1 20	+0 86	−0 07	+0 83	−0 66	+0 48	−0 68
Orbulina universa	−1 04	+1 23	+0 41	−0 43	+0 49	−0 98	+0 86	−0 65	+0 70	−0 96
Pulleniatina obliquiloculata	−0 99	—	+0 71	+0 05	—	−0 10	—	−0 43	—	−0 22
Hastigerina pelagica	−0 94	+1 09	−0 22	−0 57	+1 00	−0 59	+0 75	—	+0 96	−0 71
Sphaeroidinella dehiscens	−0 65	—	—	−0 66	—	+0 42	—	+0 05	—	−0 01
Globoquadrina dutertrei	−0 37	+0 94	+0 69	−0 33	+1 22	−0 30	+0 87	−0 08	+0 96	−0 80
Globorotalia menardii tumida	−0 14	—	—	−0 07	—	+0 30	+1 14	+0 11	+0 82	+0 20
G menardii menardii	0 00	—	—	−0 56	—	−0 70	—	+0 37	+1 42	+0 16
G menardii flexuosa	+0 41	—	—	−0 13	—	+0 02	—	−0 11	—	+0 01
G truncatulinoides	+1 25	+1 88	+1 76	+1 29	+2 15	+0 94	+2 38	+1 44	+2 18	—
G crassaformis	—	+2 35	—	+0 57	+3 23	+2 04	+1 48	+2 01	—	+0 86

W, Warm, I, intermediate, C, cold

Table 2 OXYGEN ISOTOPIC TEMPERATURES (°C) BASED ON DATA FROM TABLE 1

Species	Depth below top of core (cm) W 10	C 70	I 150	W 300	C 370	W 550	C 690	W 800	C 890	W 1,010
Globigerinoides ruber	29 1	20 4	19 9	25 2	20 4	23 8	20 7	26 5	21 1	24 9
G trilobus sacculifera	28 7	18 7	20 4	26 9	17 4	24 1	18 5	25 1	18 8	25 3
G conglobatus	27 5	17 4	16 7	22 6	18 3	21 1	16 6	24 9	17 5	23 2
G trilobus trilobus	27 4	19 1	17 4	26 3	16 8	20 9	16 9	23 6	18 5	23 7
Orbulina universa	25 5	15 2	18 8	22 6	18 4	25 2	16 8	23 6	17 5	25 1
Pulleniatina obliquiloculata	25 2	—	17 4	20 4	—	21 0	—	22 6	—	21 5
Hastigerina pelagica	25 0	15 8	21 5	23 2	16 2	23 3	17 3	—	16 4	23 9
Sphaeroidinella dehiscens	23 6	—	—	23 6	—	18 7	—	20 4	—	20 6
Globoquadrina dutertrei	22 2	16 5	17 5	22 1	15 3	21 9	16 8	21 0	16 4	24 3
Globorotalia menardii tumida	21 2	—	—	20 9	—	19 2	15 6	20 1	17 0	19 7
G menardii menardii	20 6	—	—	23 2	—	23 8	—	18 9	14 4	19 9
G menardii flexuosa	18 8	—	—	21 2	—	20 5	—	21 1	—	20 5
G truncatulinoides	18 0	12 4	13 0	15 0	11 4	16 5	10 5	14 3	11 3	—
G crassaformis	—	10 6	—	18 1	7 3	11 8	14 1	11 9	—	16 8

Correction for the isotopic composition of sea water 0 92 °/oo

Table 3 ISOTOPIC TEMPERATURES AND CALCULATED DEPTH HABITATS OBTAINED FROM THE RECENT SAMPLE 10 CM BELOW TOP

Species	Isotopic temperature (°C)	Depth of water column (m)
Globigerinoides ruber	29 1	20
G trilobus sacculifera	28 7	20
G conglobatus	27 5	16–30
G trilobus trilobus	27 4	8–40
Orbulina universa	25 5	81
Pulleniatina obliquiloculata	25 2	85
Hastigerina pelagica	25 0	88
—	—	—
Sphaeroidinella dehiscens	23 6	106
Globoquadrina dutertrei	22 2	121
Globorotalia menardii tumida	21 2	132
G menardii menardii	20 6	139
—	—	—
G menardii flexuosa	18 8	166
G truncatulinoides	18 0	181
G crassaformis	No value	

Table 4 ISOTOPIC TEMPERATURES AND INFERRED DEPTH HABITATS OBTAINED FROM THE AVERAGE OF ALL WARM SAMPLES, WITH MEAN ISOTOPIC TEMPERATURES OF THE COLD STAGES

Species	Mean warm water isotopic temperature (°C)	Calculated depth in water column (m)	Mean cold water isotopic temperature (°C)
Globigerinoides trilobus sacculifera	26 0	74	18 3
G ruber	25 9	76	20 6
Orbulina universa	24 4	96	16 9
Globigerinoides trilobus trilobus	24 3	97	17 8
G conglobatus	23 8	104	17 4
Hastigerina pelagica	23 8	104	16 4
—	—	—	—
Globoquadrina dutertrei	22 3	121	16 2
Pulleniatina obliquiloculata	22 2	122	—
Sphaeroidinella dehiscens	21 3	132	—
Globorotalia menardii menardii	21 2	134	14 4
G menardii flexuosa	20 4	144	—
G menardii tumida	20 2	147	16 3
—	—	—	—
G truncatulinoides	15 9	228	11 4
G crassaformis	14 6	265	10 6

the shallowest habitats (top 40 m), the six species and subspecies of *Globorotalia* occupy the deeper habitats (120 to about 200 m), and *Orbulina universa, Pulleniatina obliquiloculata, Hastigerina pelagica* and *Sphaeroidinella dehiscens* occupy intermediate depths (80 to 110 m)

The temperature–depth distribution obtained from the sample at 10 cm in core *P*6304–8 is compared with the observed temperature and depth distribution obtained by Jones[3] for a station in the equatorial Atlantic (0° 2 5′ S, 7° 55′ W) This comparison (Table 5) shows general agreement, except for *Hastigerina pelagica* Jones noted, however, that the depth distribution of *H pelagica* was unexpected and contrary to most previously reported occurrences, which usually indicate a warm, upper water habitat

Emiliani[4,7] stated that a portion of the amplitude of the isotopic ratios in pelagic foraminiferal shells from warm and cold stages of Pleistocene cores is a result of variations in the oxygen isotopic composition of the sea water He estimated this portion to be about 30 per cent[4]. Olausson[8] and Shackleton[9], on the other hand, claim that the entire measured amplitude is caused by variations in the isotopic composition of the sea water In Fig 1 is a plot of the warm/cold amplitude of the isotopic variations for the different species against mean warm stage isotopic composition A gross relationship is shown, with the deeper species (*Globorotalia tumida, G truncatulinoides* and *G crassaformis*) exhibiting the smallest amplitude This amplitude is about 50 per cent of that given by the shallower species, and so we conclude that variations in the isotopic composition of the sea water cannot account for more than half of the isotopic variations measured in the foraminiferal shells

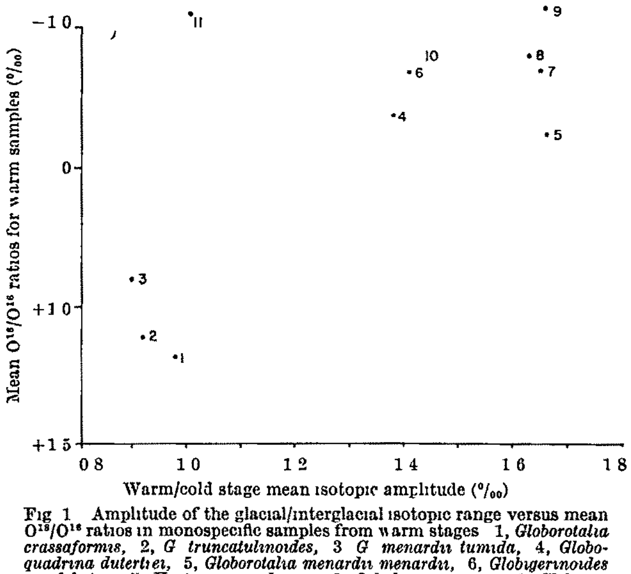

Fig 1 Amplitude of the glacial/interglacial isotopic range versus mean O^{18}/O^{16} ratios in monospecific samples from warm stages 1, *Globorotalia crassaformis*, 2, *G truncatulinoides*, 3 *G menardii tumida*, 4, *Globoquadrina dutertrei*, 5, *Globorotalia menardii menardii*, 6, *Globigerinoides conglobatus*, 7, *Hastigerina pelagica*, 8, *Orbulina universa*, 9, *Globigerinoides trilobus sacculifera*, 10, *G trilobus trilobus*, 11, *G ruber*

Table 5 COMPARISON OF THE ISOTOPIC TEMPERATURES AND DEPTH HABITATS OBTAINED FROM CORE *P*6304–8 (10 CM LEVEL) WITH THE TEMPERATURES AND DEPTH HABITATS OBSERVED BY JONES (1967) IN THE EQUATORIAL ATLANTIC

Species	Temperature (°C) Isotopic *P*6304–8 (10 cm)	Temperature (°C) Observed Atlantic	Depth in water column (m) Calculated *P*6304–8 (10 cm)	Depth in water column (m) Observed Atlantic
Globigerinoides ruber	29 1	14–29	20	0–100
G trilobus trilobus	27 4	25–29	8–40	0–75
Orbulina universa	25 5	25–27	81	25–75
Pulleniatina obliquiloculata	25 2	25	85	25–50
Hastigerina pelagica	25 0	7–10	88	300–400
Globoquadrina dutertrei	22 2	24–27	121	25–50
Globorotalia truncatulinoides	18 0	14–17	181	100–200
G crassaformis	——	14–17	——	75–200

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[1] Phleger, F B , *Ecology and Distribution of Recent Foraminifera* (The Johns Hopkins Press, 1960)

[2] Emiliani, C , *Amer J Sci* , **252**, 149 (1954)

[3] Jones, J , *Micropaleontology*, **13**, 489 (1967)

[4] Emiliani, C , *J Geol* , **74**, 109 (1966)

[5] Lidz, L , *Science*, **154**, 1448 (1966)

[6] Fuglister, F C , *Atlantic Ocean Atlas—Temperature and Salinity Profiles and Data from the International Geophysical Year of 1957–1958* (Woods Hole, Mass , 1960)

[7] Emiliani, C , *J Geol* , **63**, 538 (1955)

[8] Olausson, E , *Progress in Oceanography*, **3**, 221 (1965)

[9] Shackleton, N , *Nature*, **215**, 15 (1967)

MOLECULAR STRUCTURE

Further X-ray Evidence of Regularly Distributed Lysine in α-Keratin

IT has recently been shown that in mohair fibres the fifth order of the 198 Å pseudo repeat (39 Å reflexion) is intensified when the ε-amino groups of lysine residues are acylated with 3,4,5-triiodobenzoic acid *p*-nitrophenylester[1] Particularly high yields of acylated ε-amino groups were obtained when dimethylsulphoxide was used as reaction medium A sample of mohair which had been treated for 24 h at 40° C in this solvent, however, showed a somewhat diffuse X-ray pattern We therefore looked for a reaction medium which did not cause structural distortion of the α-keratin and which gave high yields of acylated ε-amino groups

Because dimethylformamide (DMF) is a suitable solvent for coupling reactions in peptide chemistry, mohair was treated with 3,4,5-triiodobenzoic acid *p*-nitrophenylester in DMF for 120 h at 50° C In these conditions, 80 per cent of the ε-amino groups were acylated (DNP analysis) The meridional photometer curve of the X-ray pattern of this sample is shown in Fig 1*b* There is a relatively strong intensification of the 39 Å reflexion in comparison with the unstained sample (Fig 1*a*)

In addition to the ester which was labelled with iodine we also used one which was labelled with mercury, as mercury has a smaller absorption factor and a higher atomic scattering power than iodine *p*-(carboxymethylthiomercury)benzoic acid *p*-nitrophenylester was synthesized according to Matyash and Stepanov[2] When the ε-amino groups were acylated with the mercury compound a partial decomposition of the mercury organic compound occurred

The best method of acylation which we have found consists of treating mohair with *p*-(carboxymethylthiomercury)benzoic acid *p*-nitrophenylester in DMF for 24 h at 60° C In these conditions only 65 per cent of the ε-amino groups are acylated (DNP analysis) but no decomposition is observed In the X-ray pattern of such a sample (meridional photometer curve in Fig 1*c*) there is also an intensification of the 39 Å reflexion, but the representation of this pattern in other reflexions is more comparable with that of the unstained sample Molybdenum disulphide powder was used as an internal standard (6 15 Å reflexion)

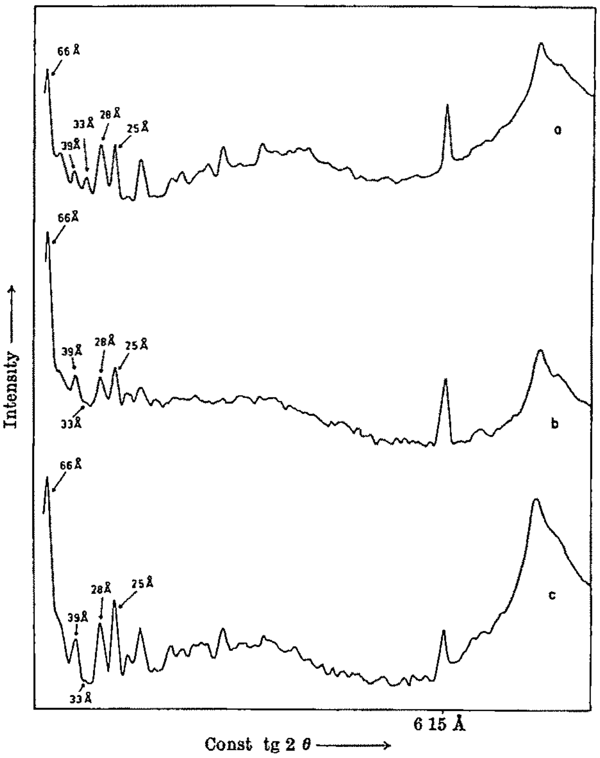

Fig 1 Meridional photometer curves of the low angle X-ray diffraction patterns taken at room temperature with nickel-filtered CuKα radiation *a*, Unstained mohair CSFH , *b*, mohair CSFH stained with 3,4,5-triiodobenzoic acid *p*-nitrophenylester in DMF for 120 h at 50° C, *c*, mohair CSFH stained with *p*-(carboxymethylthiomercury)benzoic acid *p*-nitrophenylester in DMF for 24 h at 60° C

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[1] Heidemann, G , and Halboth, H , *Nature*, **213**, 71 (1967)

[2] Matyash, L P , and Stepanov, V M , *Izv Akad Nauk SSR*, Ser Khim 111 (1964)

CHEMISTRY

Novel Facilitation of Peptide Synthesis

THE "solid phase" method of synthesizing peptides[1], in which the carbon-terminal residue is first esterified to a hydroxymethylpolystyrene resin, has been used successfully for the rapid synthesis in high yield of peptides which can be purified after removal from the resin[2] If such a procedure, which omits the purification of intermediates, is to yield a homogeneous product without

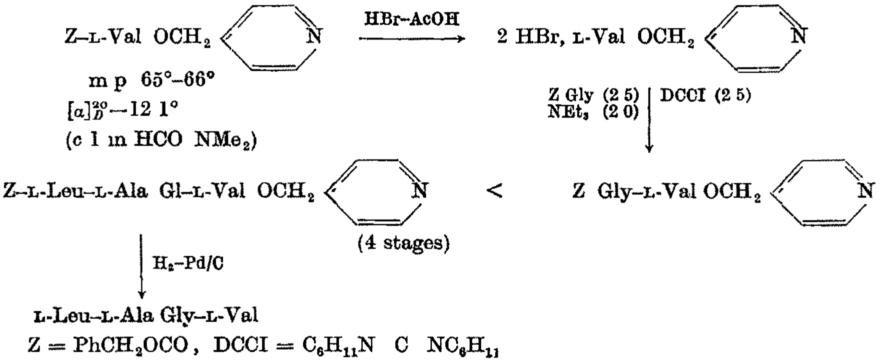

Fig 1

further purification, every stage must proceed to completion For example, if 0 5 per cent of the amino-groups remain unacylated after each coupling, then after one hundred stages the product will contain only 61 per cent of the required peptide, the remainder being peptides with closely similar but defective sequences It is a serious disadvantage of the solid phase method that one of the reactants is polymeric, making it difficult to force reactions to completion

We are examining a fundamentally different procedure in which reactions are carried out in solution and the protected peptide is then adsorbed on an ion-exchanger while the excess of acylating agent and the co-products are washed away For this purpose, a weakly basic group has been incorporated in the carbon-terminal residue by using its 4-picolyl ester (prepared from the reaction of the benzyloxycarbonylamino-acid triethylammonium salt with 4-picolyl chloride in dimethylformamide), these esters are stable to hydrogen bromide in acetic acid at room temperature but are cleaved by catalytic hydrogenation The method[3] is illustrated by the synthesis of L-leucyl-L-alanyl-glycyl-L-valine, as outlined in Fig 1 In the coupling reactions, 2 5 molar proportions of benzyloxycarbonylamino-acid and of dicyclohexylcarbodi-imide (relative to the amino-component) were used in tetrahydrofuran solution After filtration to remove dicyclohexylurea, the coupling product was taken up on moist sulphoethyl 'Sephadex' (*C*-25, H$^+$ form), which was then washed thoroughly with tetrahydrofuran. The benzyloxycarbonylpeptide 4-picolyl ester was next eluted with 2 per cent triethylamine in aqueous tetrahydrofuran, and after evaporation of the solvent the benzyloxycarbonyl group was removed by treatment of the residue with 3 5 normal hydrogen bromide in acetic acid. The peptide ester dihydrobromide was precipitated with ether, and again from ethanol by ether, and the cycle was repeated After the final hydrogenation in 80 per cent acetic acid, the solution was evaporated and the residue was dissolved in ethanol and precipitated by ether Without further purification, the crude tetrapeptide monohydrate (60 per cent yield overall, from L-valine 4-picolyl ester dihydrobromide) was analytically and chromatographically pure, no alanylglycylvaline or glycylvaline could be detected by thin-layer chromatography which (in control experiments) could detect 0 2 per cent of these impurities Amino-acid analysis of an acid hydrolysate gave the ratios· Leu, 0 96, Ala, 0 97; Gly, 1 00; Val, 1 00 A more sensitive test of the completion of each coupling stage was obtained by thin-layer chromatography of the intermediate benzyloxycarbonylpeptide 4-picolyl esters (a check not available in the solid-phase procedure), in every case, in conditions which will detect 0 1 per cent of the corresponding amino-component, none was found The same tetrapeptide was obtained using *p*-nitrophenyl esters (4 molar proportions) for coupling (65 per cent overall yield of protected tetrapeptide) The specific rotations of the final products, $[\alpha]_D^{20}$ + 22 5° (*c* 1–2 in ethanol) (dicyclohexylcarbodi-imide method), + 23 7° (*p*-nitrophenyl ester method), are higher than those reported previously[1], $[\alpha]_D^{21}$ + 17 5°, + 18 0°, but the tetrapeptide (dicyclohexylcarbodi-imide method) was completely hydrolysed by leucineaminopeptidase (Leu, 1 00, Ala, 0 88, Gly, 0 98, Val, 1 04), and hydrogenation of the intermediate benzyloxycarbonylglycyl-L-valine 4-picolyl ester gave fully active glycyl-L-valine A trace of a ninhydrin-positive contaminant, apparently arising from the 4-picolyl residues, always appeared in the products of hydrogenation of the picolyl esters, it is soluble in organic solvents and is therefore readily removed from normal peptides This tetrapeptide is, however, unusually soluble in these solvents, and the contaminant was removed during drying at 100° C and 1 mm mercury The protected tetrapeptide 4-picolyl ester was converted into the crystalline hydrazide by means of hydrazine hydrate, and this route should enable components which have been synthesized independently to be united through the acid azide As a further variant, picolyl esters may be converted into amides in the usual way, and this should provide convenient syntheses of peptides having a carbon-terminal amide group Such possibilities are now being examined

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[1] Merrifield, R B, *J Amer Chem Soc*, 85, 2149 (1963)

[2] Merrifield, R B, *J Amer Chem Soc*, 86, 304 (1964) Marglin, A, and Merrifield, R B, *J Amer Chem Soc*, 88, 5051 (1966)

[3] UK Patent Application 40286/67, assigned to the National Research Development Corporation

Ultraviolet Photolysis of Sodium Nitrate Solutions in the Laboratory and by Sunlight

CHEMICAL processes, induced by ultraviolet light in some aqueous inorganic nitrates, are of substantial interest in the chemistry of atmospheric aerosols Nitrates form a large fraction of the inorganic natural aerosol[1] and, although they might be expected to decompose under sunlight[2], no systematic work on such photolysis has, as yet, been reported.

Earlier work on ultraviolet reduction of nitrate to nitrite was done by Villars[3] and Cultrera and Ferrari[4-6] The effect was confirmed by others[7-10] and also in this laboratory, where it was found to be accompanied by the formation of highly oxidizing intermediates[11], and it was also found that the formation of nitrite is caused by primary steps in which higher oxides of nitrogen are involved[12-15] The process of nitrite formation during photolysis of nitrates is reproducible as long as the solution is properly stirred[11], because stirring ensures the removal of gaseous reaction products from the zone below the interphase where penetration by ultraviolet light is possible. Our quartz cell (Fig 1) had an overall capacity of 140 ml and was rigidly supported on a rack which oscillated mechanically thirteen times per min about its horizontal position Because the cell initially contained 110·0 ml of nitrate solution, a bubble of air helped to mix the solution and remove the gas The rack supported another identical parallel tube filled with 110 0 ml of an actinometric solution, with 1 90 g/l and 1 124 g/l of oxalic acid and uranyl oxalate, respectively[16], which, in our conditions, absorbs in the near ultraviolet in a similar way to nitrates[17] Equal samples (5 00 ml) were periodically taken from both tubes and analysed for nitrite using the Griess reagent[18] and, by titrating with permanganate, the samples were analysed for the amounts of near ultraviolet light absorbed, using a quantum efficiency of 0 56 and assuming full absorption of light[16] Merck's reagent, very pure chemicals and distilled water were used, the results are expressed in terms of original volumes of solutions photolysed

A method was developed for studying the photolysis by sunlight of some aqueous compounds known to exist as natural aerosols Preliminary work involved testing the system sodium nitrate–sodium chloride, on irradiation with a 550 W stabilized high-pressure mercury Hanovia ultraviolet lamp (No 673-*A*) At constant radiation doses of about $2\ 3 \times 10^{21}$ UV quanta per h, the rates of nitrite formation, α, were independent of nitrate concentration in the range of 0 6 to 7 1 mole per l and at *p*H values higher than 5 (ref 18) Nitrite formation increased according to a competitive process

$$NO_2^- = \frac{\alpha R}{1+\beta t} \qquad (1)$$

where R is the radiation absorbed up to time t, and α and β are constant of the experimental run. The term β was found to be dependent on *p*H and stirring and thus on the release of nitrous anhydride from solutions

We have also found (results to be published) that the formation of nitrite is enhanced by the chloride ion, probably because of a stabilization of the nitrogen oxides evolved, by NOCl intermediates In order to test the possibility of occurrence in nature of such photolytic processes, experiments were carried out last summer at different altitudes and on cloudless days Table 1 shows the locations, Fig 2 shows the averaged results Each point represents an average of about eight and five experimental values for nitrate and nitrate + chloride systems, respectively, taken between 8 a m and 4 p m Linear correlation coefficients between $\frac{R}{NO_2^-}$ and t (equation (1)), based on about five daily readings and ten and six kinetic experiments spread over different altitudes, yielded root mean square averages of 0 99±0 01 and 0 97±0 03 for sodium nitrate and sodium nitrate–sodium chloride solutions, respectively Because parallel measurements also indicated a good linear approximation between R and t (root mean square correlation coefficient from sixteen values, $r = 0\ 998 \pm 0\ 002$), the right hand side of equation (1) was expressed in terms of parameter R alone

$$NO_3^- = \frac{\alpha' R}{1+\beta' R} \qquad (2)$$

as shown in Fig 2 Values of α′ and β′, corresponding to the plots illustrated, are given in Table 2, "errors" are largely caused by systematic changes of α′ and β′ with temperature (results to be published) Blank tests failed to reveal detectable absorption of NO_X from the air

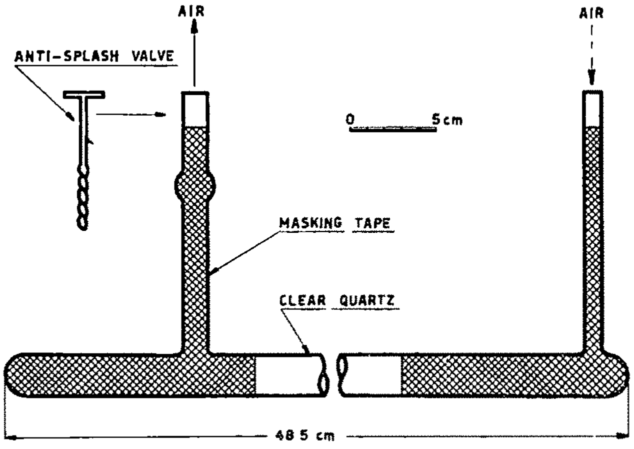

Fig 1 Ventilated quartz cell for photolysis by sunlight

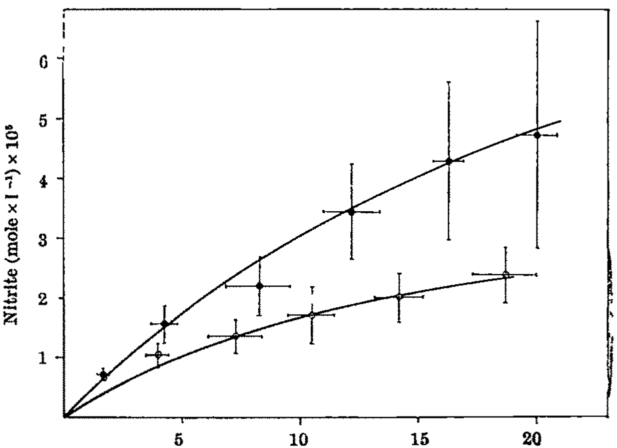

Fig 2 Photolysis of 30 per cent sodium nitrate solutions with 18 per cent (below) and without sodium chloride additive (above) Solid lines were drawn according to equation (2) and constants given in Table 2

Table 1 LOCATIONS FOR EXPERIMENTATION DURING PERIODS OF TIME INDICATED TEMPERATURES WERE TAKEN IN SHADOW, AT 8 A M L S T

Area	Location	Altitude	Week and month	Temperature
Naples Bay	Faito Ridge	1,040 m	2–May	16 8° C
Sea shore near Rome	Fregene	0 m	4–May, 1–June	20 0° C
Matterhorn	Cervinia	2,040 m	4–June	13 0° C
Matterhorn	Plan Maison	2,660 m	4–June, 1–July	7 0 °C
Matterhorn	Plateau Rosa	3,500 m	2–July	0 5° C

Table 2 CONSTANTS, OBTAINED BY LEAST SQUARES ACCORDING TO EQUATION (2), OF CURVES GIVEN IN FIG 2

Solution	α′ Mole l⁻¹ cal⁻¹	β′ Cal⁻¹
$NaNO_3$	$2\ 70\ 10^{-4}$	6 27
$NaNO_3$ + NaCl	$4\ 10\ 10^{-4}$	3 47

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[1] Junge, C E, in *Air Chemistry and Radioactivity* (Academic Press Inc, New York, 1963)

[2] Cultrera, R, *Gazz Chim Ital*, **66**, 440 (1936)

[3] Villars, D S, *J Amer Chem Soc*, **49**, 326 (1927)

[4] Cultrera, R, *Gazz Chim Ital*, **76**, 187 (1946)

[5] Cultrera, R, and Ferrari, G, *Ann Chim*, **47**, 1321 (1957)

[6] Cultrera, R, and Ferrari, G, *Ann Chim*, **47**, 1331 (1957)

[7] Geissler, G, *Naturwissenschaften*, **50**, 303 (1963)

[8] Geissler, G, *Strahlentherapie*, **129**, 472 (1966)

[9] Huckstedt, G, *Naturwissenschaften*, **51**, 382 (1964)

[10] Hyder, M L, *J Phys Chem*, **69**, 1858 (1965)

[11] Papée, H M, and Petriconi, G L, *Nature*, **204**, 142 (1964)

[12] Petriconi, G L, and Papée, H M, *Canad J Chem*, **44**, 1977 (1966)

[13] Petriconi, G L, and Papée, H M (in the press)

[14] Jaffe, S, and Ford, H W, *J Phys Chem*, **71**, 1832 (1967)

[15] Benson, S W, in *The Foundations of Chemical Kinetics*, 408 (McGraw-Hill, New York, 1960)

[16] Bowen, E G, in *The Chemical Aspects of Light* (Clarendon Press, Oxford, 1949)

[17] Kortüm, G, and Finckh, B, *Phys Chem*, **48B**, 32 (1940)

[18] Charlot, G, in *Les Méthodes de la Chimie Analytique* (Masson et Cie, Paris 1961)

BIOCHEMISTRY

Sites of Inhibition of DNA Synthesis by Kethoxal *bis*(Thiosemicarbazone)

THE growth of several neoplastic cell lines is inhibited by 2-keto-3-ethoxybutyraldehyde *bis*(thiosemicarbazone) [kethoxal *bis*(thiosemicarbazone), KTS][1-9], an agent which forms tetradentate chelates with metals of the first, second and third transition series In the presence of either copper or zinc ions the antineoplastic potency of KTS is markedly increased, particularly with the cupric chelate [Cu(II)KTS][8-12]

KTS and its cupric chelate primarily inhibit the biosynthesis of DNA The rate of formation of RNA and protein is also depressed by these agents, but inhibition of these latter processes is less pronounced[11,13-15] Although both KTS and Cu(II)KTS decrease the rate of incorporation of several isotopic precursors into DNA, the patterns of inhibition produced by these agents are distinctly different[15] The effects which are induced by Cu(II)KTS in sarcoma 180 ascites cells are characterized by a pronounced decrease in the rate of incorporation of ^{3}H-thymidine into the thymine of DNA and lesser inhibitions of the utilization of ^{14}C-formate, ^{3}H-deoxycytidine and ^{3}H-5,6-uridine for the formation of the pyrimidine nucleotides of DNA The site which is most sensitive to the inhibitory activity of Cu(II)KTS seems to be the enzyme thymidine kinase In contrast, the ligand KTS does not decrease the rate of incorporation of ^{3}H-thymidine into the DNA of sarcoma 180, but causes marked inhibition of the utilization of radioactive formate, deoxycytidine and uridine for the formation of DNA[15] The cellular toxicity of Cu(II)KTS is essentially caused by copper The relatively lipid-soluble Cu(II)KTS complex effectively serves to transport and deposit copper at sensitive sites within cells Thus dissociation of the Cu(II)KTS occurs within neoplastic cells treated with the cupric chelate, this phenomenon is followed by the cellular elimination of the ligand portion of the molecule and the much slower expulsion of copper[15]. Because only small doses of Cu(II) KTS can be administered to animals with tumours, only relatively small amounts of KTS released by dissociation of the cupric chelate are present, thus differences are observed in the biochemical lesions which are induced by Cu(II)KTS and KTS in whole cells The data in this communication describe the inhibition of several enzyme reactions which could account for the inhibition of the synthesis of DNA induced by KTS

Experiments were performed on 9–11 week old female *CD*-1 mice which had been inoculated intraperitoneally 5–6 days previously with 4 × 10^{6} sarcoma 180 ascites cells The activities of dihydrofolate reductase[16], 10-formyltetrahydrofolate synthetase[17], 5,10-methylenetetrahydrofolate dehydrogenase[18] and thymidylate synthetase[19] were measured as previously described Protein was determined by the biuret method Enzyme extracts were prepared from sarcoma 180 ascites cells which were collected through an incision in the peritoneal wall All subsequent procedures were performed at 4° C unless otherwise stated The suspensions were centrifuged at 1,600*g* and the ascitic fluid was discarded The cell pack was washed with five volumes of isotonic saline and was collected by centrifugation at 1,600*g*, contaminating erythrocytes were lysed by the addition of five volumes of 0 2 per cent saline and mixing for 30 sec Five volumes of 1 6 per cent saline were added to restore isotonicity and the suspension was centrifuged at 1,600*g* for 2 min The cell-free extract which was used for the preparation of dihydrofolate reductase was prepared by washing each cell pack with isotonic saline and suspending each in five volumes of 0 5 molar *tris* buffer (*p*H 7 5), cells were lysed by three cycles of alternate freezing and thawing The supernatant solution which was obtained after centrifugation of the lysate at 10,000*g* for 10 min was dialysed for 20 h against two hundred volumes of distilled water 10-Formyltetrahydrofolate synthetase and 5,10-methylenetetrahydrofolate dehydrogenase were prepared from the cell pack which had been freed from contaminating erythrocytes by washing the cell pack) once with four volumes of 0 05 molar *tris* buffer (*p*H 7 5- containing 0 05 molar EDTA The cell pack was suspended in four volumes of the *tris*-EDTA buffer and was subjected to three cyles of alternate freezing and thawing, the supernatant solution obtained after centrifugation of the lysate at 12,000*g* was further fractionated by the addition of ammonium sulphate The fraction which precipitated between 30 and 45 per cent saturation with ammonium sulphate was passed through a column of 'Sephadex *G*-25' to remove the EDTA, using water as the eluant Thymidylate synthetase was prepared from the erythrocyte-freed ascites cells by washing the cell pack once with five volumes of isotonic saline, the resulting cell pack was suspended in two volumes of 0 05 molar potassium phosphate buffer (*p*H 6 7) Cells were lysed by sonication with a Branson sonifier using two 30 sec bursts at an energy setting of 8 amp with a 20 sec period between each burst The sonicate was centrifuged for 1 h at 104,000*g* and the clear supernatant was filtered through glass wool

The incorporation of ^{14}C-formate into the thymine of DNA, a process which is inhibited by KTS, requires the participation of a series of enzyme reactions (Fig 1). The effects of KTS on several of the enzymes which are involved in this pathway are shown in Table 1 Thymidylate synthetase seemed to be the enzyme most sensitive to this agent, a concentration of 0 02 mmolar of KTS caused 66 per cent inhibition, employing the substrate deoxyuridine 5′-phosphate, tetrahydrofolate and formaldehyde at concentrations of 50 mmolar, 0 2 mmolar and 5 mmolar, respectively 5,10-Methylenetetrahydrofolate dehydrogenase was also inhibited by KTS With 1 0 mmolar tetrahydrofolate and 5 mmolar formaldehyde, 0 04 mmolar KTS caused 68 per cent inhibition Dihydrofolate reductase was also sensitive to this agent and a comparable degree of inhibition was attained using 0 05 to 0 06 mmolar KTS in the presence of 0 05 mmolar dihydrofolate and 0 04 mmolar NADPH Preincubation of the cell-free extract with KTS for periods of up to 20 min did not increase the degree of inhibition of dihydrofolate reductase Dialysis against water of the enzyme

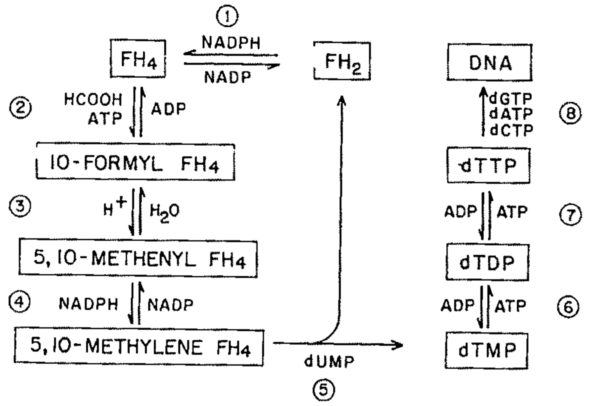

Fig 1 FH_2, Dihydrofolate, FH_4, 5,6,7,8-tetrahydrofolate, 10-formyl FH_4, 10-formyltetrahydrofolate, 5,10-methenyl FH_4, 5,10-methenyltetrahydrofolate, 5,10-methylene FH_4, 5,10-methylenetetrahydrofolate, NADP, nicotinamide adenine dinucleotide phosphate, NADPH, reduced nicotinamide adenine dinucleotide phosphate, ATP, adenosine 5′-triphosphate, ADP, adenosine 5′-diphosphate, dUMP, deoxyuridine 5′-phosphate, dTMP, deoxythymidine 5′-phosphate, dTDP, deoxythymidine 5′-diphosphate, dTTP, deoxythymidine 5′-triphosphate, dGTP, deoxyguanosine 5′-triphosphate, dATP, deoxyadenosine 5′-triphosphate, dCTP, deoxycytosine 5′-triphosphate, DNA, deoxyribonucleic acid The enzyme reactions are depicted (1) dihydrofolate reductase, (2) 10-formyltetrahydrofolate synthetase, (3) 5,10-methenyltetrahydrofolate cyclohydrolase, (4) 5,10-methylene tetrahydrofolate dehydrogenase, (5) thymidylate synthetase, (6) deoxythymidine monophosphate kinase, (7) deoxythymidine diphosphate kinase, (8) DNA nucleotidyltransferase

Table 1 EFFECT OF KETHOXAL *bis*(THIOSEMICARBAZONE) ON DIHYDROFOLATE REDUCTASE, 10-FORMYLTETRAHYDROFOLATE SYNTHETASE, 5,10-METHYLENETETRAHYDROFOLATE DEHYDROGENASE AND THYMIDYLATE SYNTHETASE OF SARCOMA 180

Conc of KTS (mmolar)	% Inhibition			
	Dihydrofolate reductase*	10-Formyltetrahydrofolate synthetase †	5,10-Methylenetetrahydrofolate dehydrogenase ‡	Thymidylate synthetase §
0 01	0	—‖	—	21
0 02	11	—	34	66
0 04	37	—	68	70
0 05	48	—	85	—
0 06	70	—	—	—
0 10	86	32	—	—

KTS was dissolved with the aid of ethanol the final concentration of ethanol in the enzyme assays was 1 per cent which was not inhibitory to the activities of these enzymes

* The complete system contained, in a volume of 1 0 ml 100 µmoles of *tris* buffer (pH 7 5), 0 04 µmoles of NADPH, 0 1 ml of enzyme, 0 5 mg of protein, 150 µmoles of potassium chloride, and 0 05 µmole of dihydrofolate, containing 10 µmoles of 2-mercaptoethanol The dihydrofolate was added last to initiate the reaction The decrease in absorbancy at 340 mµ caused by the oxidation of NADPH and the reduction of dihydrofolate was followed at intervals of 15 sec Control activity, expressed as Δ340 mµ/4 min, was 0 21

† The complete system contained, in a volume of 1 0 ml 100 µmoles of *tris* buffer (pH 7 5), 10 µmoles of ATP, 0 1 ml of enzyme (1 0 mg of protein), 20 µmoles of magnesium chloride, 1 0 µmole of tetrahydrofolate containing 10 µmoles of 2-mercaptoethanol and 50 µmoles of ammonium formate The ammonium formate was added last to initiate the reaction The components were incubated at 37° C for 3 min, the reaction was terminated by the addition of perchloric acid and the increase in absorbancy at 355 mµ caused by the formation of 5,10-methylenetetrahydrofolate was determined by comparing the complete system with a reaction mixture without ammonium formate Control activity, expressed as Δ355 mµ/3 min, was 0 79

‡ The complete system contained, in a volume of 1 0 ml 100 µmoles of *tris* buffer (pH 7 0), 1 0 µmole of tetrahydrofolate containing 10 µmoles of 2-mercaptoethanol, 5 µmoles of formaldehyde, 0 1 ml of enzyme (1 mg of protein), and 0 4 µmole of NADP The NADP was added last to initiate the reaction The increase in absorbancy at 340 mµ caused by the formation of NADPH was followed Control activity, expressed as Δ340 mµ/10 min, was 0 13

§ The complete system contained, in a volume of 1 0 ml 500 µmoles of potassium phosphate buffer (pH 6 7), 50 µmoles of deoxyuridine 5′-phosphate (dUMP), 5 µmoles of formaldehyde, 5 µmoles of magnesium chloride, 0 2 µmole of tetrahydrofolate, 30 µmoles of 2-mercaptoethanol, and 0 3 ml of enzyme (3 9 mg of protein) The complete mixture minus dUMP was preincubated at 37° C for 5 min, dUMP was added last to initiate the reaction and the increase in absorbancy at 340 mµ was measured Control activity, expressed as Δ340 mµ/10 min, was 0 05

‖ Not determined at indicated concentrations

which had been treated with KTS completely reversed the drug-imposed inhibition, but neither dihydrofolate nor NADPH afforded protection In contrast to these enzymes, 10-formyltetrahydrofolate synthetase was only slightly inhibited by a concentration of KTS of 0 1 mmolar, when the substrates tetrahydrofolate, formate and ATP were used at levels of 1 0 mmolar, 50 mmolar and 10 mmolar, respectively The findings that the enzymes deoxythymidine mono- and di-phosphate kinases and DNA nucleotidyltransferase were not inhibited by KTS suggest that the blockade of the incorporation of ^{14}C-formate into the thymine of DNA induced by KTS in intact sarcoma 180 ascites cells[15] can be accounted for by inhibition of thymidylate synthetase, 5,10-methylenetetrahydrofolate dehydrogenase and/or dihydrofolate reductase

To determine whether intracellular concentrations of KTS were large enough to inhibit these enzyme reactions, sarcoma 180 tumour-bearing mice were exposed to ^{14}C-KTS (36 6 × 10^3 c p m /µmole) at a level of 200 mg/kg body weight The cellular concentrations of KTS were determined by measuring the radioactivity which was associated with cells exposed to ^{14}C-KTS, the radioactive carbon was present in the thiosemicarbazide portions of the molecule KTS-^{14}C was suspended by homogenization in ethanolic sodium chloride containing 'Tween 80' (ref 15) and was administered to each animal by intraperitoneal injection At selected intervals of time thereafter, ascites fluid was removed from each mouse and the cells were collected by centrifugation for 2 min (1,600g) The fluid was decanted and the packed cells were diluted with four volumes of distilled water, 1 ml of this suspension was solubilized by the addition of a concentrated solution of sodium hydroxide (final concentration, 0 2 normal) A sample was taken for measurement of radioactivity in a Packard 'Tri-carb' liquid scintillation spectrometer using a phosphor solution of p-*bis*-[2-(5-phenyloxazolyl)] benzene and 2,5-diphenyloxazole dissolved in toluene and ethanol[15] Radioactivity equivalent to approximately 2 0 µmoles of KTS was present in each g of cells 2 h after administration of the drug Relatively rapid loss of KTS occurred in these experiments, thus, by 12 h after exposure of the cells to radioactive KTS, about 0 58 µmole of KTS/g remained, and by 24 h each g of cells contained only 0 16 µmole of KTS If it is assumed that the radioactivity present in the cells represents unaltered KTS, that the drug distributes itself uniformly throughout the cell, and that water comprises 75 per cent of the cell volume, the cellular concentration of KTS would be about 2 5 mmolar, 0 7 mmolar and 0 2 mmolar at 2, 12 and 24 h, respectively, after the injection of KTS Thus intracellular concentrations of KTS seem to be achieved which are inhibitory to thymidylate synthetase, 5,10-methylenetetrahydrofolate dehydrogenase, and dihydrofolate reductase The blockade of these enzyme reactions requires the inhibition of the incorporation of both deoxycytidine-^{3}H and ^{3}H-uridine into DNA in cells which were exposed to KTS That such inhibition does indeed occur was shown in an earlier report from this laboratory[15]

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[1] French, F A , and Freedlander, B L , *Cancer Res* ,**20**, Part 2, 505 (1960)
[2] Petering, H G , and Buskirk, H H , *Fed Proc* ,**21**, 163 (1962)
[3] Mihich, E , and Nichol, C A , *Proc Amer Assoc Cancer Res* , **4**, 44 (1963)
[4] Petering, H G , Buskirk, H H , and Underwood, G E , *Cancer Res* , **24**, 367 (1964)
[5] Mihich, E , and Mulhern, A I , *Fed Proc* , **24**, 454 (1965)
[6] Mihich, E , and Nichol, C A , *Cancer Res* , **25**, 1410 (1965)
[7] Crim, J A , Buskirk, H H , and Petering, H G , *Cancer Res* , **27**, 1109 (1967)
[8] Petering, H G , Buskirk, H H , and Crim, J A , *Cancer Res* , **27**, 1115 (1967)
[9] Crim, J A , and Petering, H G , *Cancer Res* , **27**, 1278 (1967)
[10] Petering, H G , Buskirk, H H , Crim, J A , and Van Giessen, G J , *Pharmacologist*, **5**, 271 (1963)
[11] Booth, B A , and Sartorelli, A C , *Nature*, **210**, 104 (1966)
[12] Sartorelli, A C , and Booth, B A , *Proc Amer Assoc Cancer Res* , **8**, 58 (1967)
[13] Sartorelli, A C , Welch, A D , and Booth, B A , *Fed Proc* , **24**, 454 (1965)
[14] Sartorelli, A C , and Booth, B A , *Proc Amer Assoc Cancer Res* , **7**, 62 (1966)
[15] Booth, B A , and Sartorelli, A C , *Mol Pharmacol* , **3**, 290 (1967)
[16] Osborn, M J , and Huennekens, F M , *J Biol Chem* , **233**, 969 (1958)
[17] Bertino, J F , and Fischer, G A , *Meth Med Res* ,**10**, 297 (1964)
[18] Bertino, J R , Silber, R , Freeman, M , Alenty, A , Albrecht, M , Gabrio, B W , and Huennekens, F M , *J Clin Invest* , **42**, 1899 (1963)
[19] Reyes, P , and Heidelberger, C , *Mol Pharmacol* ,**1**, 14 (1965)

Differences in Isozyme Patterns of Nucleic and Cytoplasmic Lactate Dehydrogenase of Rat Liver Cells

THE resistance of lactate dehydrogenase (LDH) activity to the denaturating action of 4 molar urea in the nuclei of rat liver cells is considerably greater than that in the cytoplasm[1] To investigate these differences we have examined the resistance of LDH to high temperatures and the relationship of temperature to substrate (pyruvate) concentration We have also determined LDH isozymes in starch gel after electrophoresis

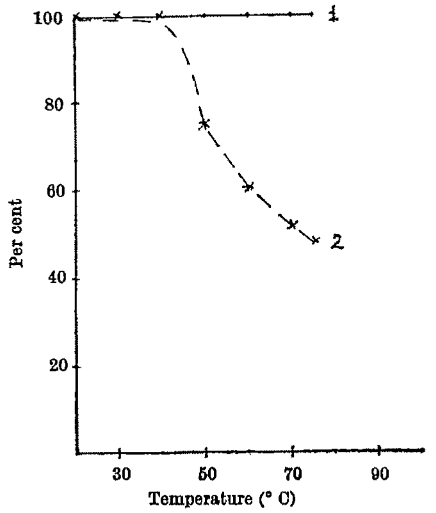

Fig. 1. Effects of heating on LDH activities of nuclear extract and soluble cytoplasmic fraction of rat liver cells. The abscissa gives the temperature (° C), the ordinate gives the per cent of inhibition of LDH activities. 1, Nuclear extract; 2, soluble cytoplasmic fraction.

We used rats weighing between 80 and 140 g. Nuclei and cytoplasm were isolated from perfused rat livers by differential centrifugation in medium containing 0·25 molar sucrose and 0·003 molar calcium chloride. The nuclei were isolated by the technique of Stirpe and Aldridge[2] with a slight modification[3].

The livers from three rats were perfused *in situ* with about 150 ml. of ice cold 0·85 per cent sodium chloride solution, minced finely with scissors and homogenized in a mixture of 0·25 molar sucrose and 1·8 mmolar calcium chloride (one part liver, three parts medium) in a Potter-Elvehjem type homogenizer at a speed of 1,800 r.p.m. The resulting homogenate was diluted to a volume in ml. equal to ten times the weight of the liver in grams with the homogenizing medium and then filtered through two layers of flannelette. The filtered homogenate was then centrifuged for 10 min at 600*g*. The supernatant was pipetted from the sedimented material and suspended in a mixture of 0·25 molar sucrose and 1·8 mmolar calcium chloride. A portion (10 ml.) of the suspension was carefully layered over 20 ml. of a mixture of 0·34 molar sucrose and 1·8 mmolar calcium chloride and centrifuged for 10 min at 600*g*. The supernatant was removed and the sedimented material was resuspended in 5 ml. of 0·25 molar sucrose and 1·8 mmolar calcium chloride. Then 10 ml. of a mixture of 0·34 molar sucrose and 1·8 mmolar calcium chloride was layered under this suspension and centrifuged as before. The final sediment was resuspended in 0·25 molar sucrose and 3 mmolar calcium chloride. All stages were carried out at 4° C.

We did not use 2·2 molar sucrose, although in this medium the nuclei were free from cytoplasmic contamination, because they lost much of the activity of the enzymes associated with the soluble fraction of the nuclei.

In every preparation the nuclei were examined microscopically as smears stained by fast green-aceto-orcein. The nuclei resembled those in the living cells. The preparations were contaminated by a few unbroken cells and nuclear fragments. The nuclei formed quickly into a transparent viscous gel when the *p*H was brought to 9.

The contamination of the nuclear fraction by cytoplasm and whole liver cells was slight: not more than 10 per cent of glucose-6-phosphatase activity of the homogenate was found in the nuclei. The nuclei do not contain glucose-6-phosphatase dehydrogenase as one of the cytoplasmic enzyme markers.

To compare LDH activity in the nucleus and cytoplasm we used a nucleic extract and a soluble cytoplasmic fraction. The nucleic extract was prepared by homogenizing the nuclei in 0·25 molar *tris*-buffer while the soluble cytoplasmic fraction was obtained by centrifuging the remaining supernatant fluid, after the precipitation of nuclei, at 30,000*g* for 40 min. Assays of LDH activity in enzyme preparations were made in an '*SF*-4' spectrophotometer using pyruvate as substrate by decreasing the NADH concentration in solution (340 mμ). The buffer solution used was KH_2PO_4 and K_2HPO_4, 0·05 molar at *p*H 7·5. The assay mixture contained $3·1 \times 10^{-4}$ molar sodium pyruvate, made up in 2·7 ml. of the buffer. $NADH_2$ (8×10^{-3}) molar was made up in 0·5 ml. of 1 per cent sodium bicarbonate and 0·1 ml. of extract and placed in the spectrophotometer cuvette. The change in absorbancy was read after exactly 2 and 4 min at room temperature[4]. To test the effect of substrate concentration on LDH activity the same reaction medium was used except that the substrate concentration was varied within the range $2·5 \times 10^{-4}$—$2·5 \times 10^{-2}$ molar. The extracts were subjected to temperatures of 30°, 40°, 50°, 60°, 70° and 75° C for 15 min.

The technique of vertical electrophoresis in starch gel developed by Smithies[5] was used to resolve the isozymes of LDH. A sample (0·07 ml.) of the extract (30 to 50 mg protein/ml.) was placed on the gel. Electrophoresis was carried out for 15–17 h at 4° C with a voltage gradient of 10–15 V/cm. Usually a borate buffer at *p*H 8·6 and 0·006 ionic strength was used. After electrophoresis the location of LDH activity on the starch gel was visualized by formazan formation[6].

The nuclear LDH activity was more resistant to heating than the enzyme from the cytoplasm. This difference was seen very clearly using the livers of young rats weighing about 100 g. There was a 50 per cent inactivation of the cytoplasmic LDH at a temperature of 70° C, while the activity of the nucleic enzyme remained unchanged at 75° C (Fig. 1). The data on the inhibition of LDH activity by heat and also by the action of urea show that the LDH of the nucleus has a greater resistance than the LDH of the cytoplasm. One of the possible reasons for this is a different isozyme content in the nucleus and cytoplasm. It has been shown that there is a difference in the sensitivity of LDH isozyme to the deleterious effects of low and high temperatures[7-10]. The LDH sensitivity to some factors increases from the first to the fifth isozyme. To support this suggestion about the different LDH isozyme content of the nucleus and cytoplasm the relationship between the enzyme activity and the substrate (pyruvate) concentration was studied. Kaplan *et al.*[11] have shown that LDH isozymes have different substrate optima, and that LDH-1 is more sensitive to large pyruvate concentrations than is LDH-5. Fig. 2 shows that the inhibition of LDH activity in the

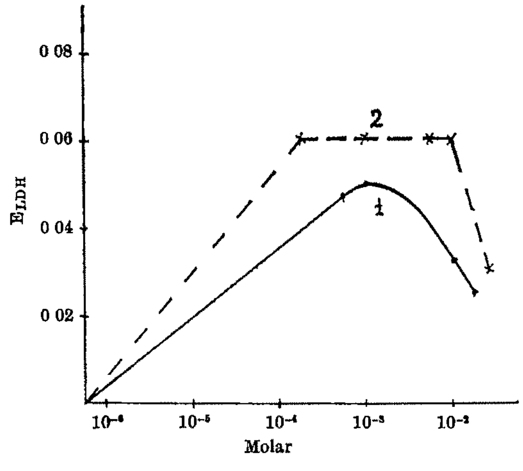

Fig. 2. Effects of different concentrations of sodium pyruvate on LDH activities of nucleic extract and cytoplasmic soluble fraction of rat liver cells. The abscissa gives the sodium pyruvate concentration, the ordinate gives the activity in units. 1, Nuclear extract; 2, cytoplasmic soluble fraction.

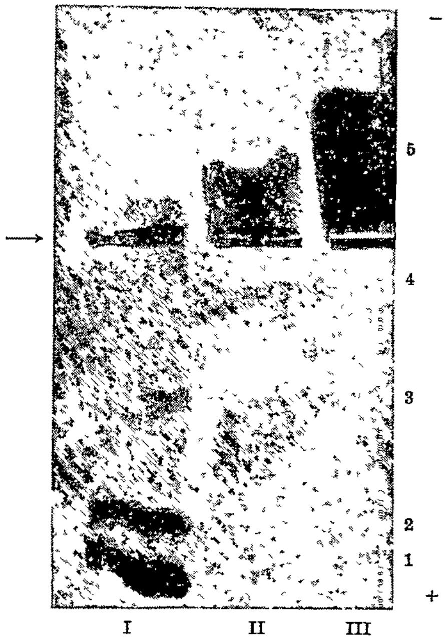

Fig 3 Separation of LDH isozymes in starch gel I, Homogenate of rat heart, II, nuclear extract from rat liver, III, cytoplasmic soluble fraction from rat liver, 1–5, LDH isozymes The arrow indicates the starting line

cytoplasm starts at a higher pyruvate concentration (1×10^{-2} molar) than in the nuclei (1×10^{-3} molar). This is indirect evidence that there is more LDH-5 in the cytoplasm than in the nucleus

Direct evidence of this difference was obtained by subjecting the solutions to electrophoresis in starch gel As the LDH zymograms show (Fig 3), both the nucleus and cytoplasm of the rat liver cells are represented by isozymes 3, 4 and 5, with distinct, though quantitative, differences between the nucleus and cytoplasm These differences mostly concern LDH-5 which can be found in larger amounts in the cytoplasm than in the nucleus. A quantitative evaluation of these differences was obtained by spectrophotometric measurements of LDH activities in corresponding sites of the electropherogram after freezing and thawing It has been established that the activity of LDH-5 expressed in units of the enzymes is 4 to 5 times greater in the cytoplasm than in the nucleus As for LDH-3 and 4, there are only traces of them in the cytoplasm, while their activity in the nucleus is about 50 per cent of the activity of LDH-5.

It should be pointed out that the differences in the LDH activity of the nucleus and cytoplasm can only be shown by using electrophoresis in starch gel When electrophoresis was carried out using paper the LDH activity in the nucleus and cytoplasm of rat liver cells had only one corresponding band[12].

Thus LDH of the nucleus and cytoplasm of rat liver cells show different isozyme contents This agrees with the suggestion that glycolytic enzymes in the cell are bound to structures and are not capable of moving freely from one part of the cell to another[13–15] Recently it has been suggested that LDH is synthesized in both the nucleus and the cytoplasm[16] These data may explain differences in the isozyme content of LDH of the cell There may be evidence for the isozymes playing a physiological part in the regulatory processes of the cell The LDH isozymes that show different kinetic properties may regulate the ratio of $NADH_2$ to NAD in the transformation of pyruvate into lactate which is important for controlling many reaction rates in the cell[6] Furthermore, the suggestion that LDH isozymes show allosteric regulatory ability offers a method for regulating their relative contribution to the total LDH activity According to Kaplan[18], whose hypothesis is based on the fact that LDH-1 is inhibited by pyruvate concentrations to which LDH-5 is resistant, LDH-1 must prevail in tissues with aerobic metabolism, while LDH-5 prevails in tissues with anaerobic metabolism In this connexion, an increased amount of LDH in the cytoplasm compared with the nucleus seems to indicate that the soluble fraction of the cytoplasm plays a principal part in the anaerobic method of energy supply in the cell

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[1] Nemchinskaya, V L , Ganelina, L Sh , and Braun, A D , *Cytology (USSR)*, **9**, 307 (1967)
[2] Stirpe, F , and Aldridge, W N , *Biochem J* , **80**, 481 (1961)
[3] Ganelina, L Sh , Nemchinskaya, V. L , and Braun, A D , *Cytology (USSR)*, **8**, 526 (1966)
[4] Hardy, S M , *Nature*, **206**, 933 (1965)
[5] Smithies, O , *Biochem J* , **61**, 629 (1955)
[6] Markert, C L , and Ursprung, H , *Devel Biol* , **5**, 363 (1962)
[7] Plagemann, P G , Gregory, K , and Wróblewski, F , *Biochem Z* , **334**, 37 (1961)
[8] Plumer, D T , Wilkinson, J H , and Withycombe, W. A , *Biochem J* , **89**, 48 P (1963)
[9] Zondag, H , *Science*, **142**, 965 (1963)
[10] Konttinen, A , and Lindy, S , *Nature*, **208**, 782 (1965)
[11] Cahn, R D , Kaplan, N O , Levine, L , and Zwilling, E , *Science*, **136**, 962 (1962)
[12] Siebert, G , and Hannover, R , *Biochem Z* , **339**, 162 (1963)
[13] Amberson, W R , Roisen, F J , and Bauer, A , *J Cell Comp Physiol* , **66**, 71 (1965)
[14] Green, D E , Murer, E , Hulton, H O , Richardson, S H , Salmon, B , Brierleg, G P , and Baum, H , *Arch Biochem Biophys* , **112**, 365 (1965)
[15] Ganelina, L Sh , Nemchinskaya, V L , and Braun, A D , *Cytology (USSR)*, **8**, 526 (1966)
[16] Le Roy Kuehl, *Fed Proc* , **26**, 821 (1967)
[17] Fritz, P J , *Science*, **150**, 364 (1965)
[18] Kaplan, N O , and Goodfriend, T L , *Adv Enzyme Reg* , **2**, 203 (1964)

Urokinase Inhibitor in Human Placenta

Fibrinolytic activity in the blood markedly decreases in the later months of pregnancy and in labour, but rapidly returns to normal after delivery[1–3] Brakman and Astrup[4] observed a significant increase in the selective inhibition of fibrinolysis induced by urokinase in plasma and serum of pregnant women, and their findings suggested that an inhibitory mechanism was involved in depressing fibrinolysis during pregnancy The same characteristics of fibrinolytic inhibition in saline extract of human placenta have been reported by one of us[5], that is placental extract inhibited only urokinase but not streptokinase or glycerol-activated plasmin Because the placental extract contained blood components, however, it was uncertain whether the inhibition was derived from pregnancy blood or from placenta itself

The purpose of this communication is to report that the selective inhibition of placental extract by urokinase is chiefly derived from the placenta itself Two hundred human placentae, frozen immediately after parturition, were minced, mixed with 200 l of 0 9 per cent saline and stirred for 30 min Placental extract was obtained as the supernatant after centrifugation The protein concentration was about 2 0 per cent, of which haemoglobin constituted 50 to 70 per cent The concentration of the other proteins (chiefly plasma proteins) was therefore 0 6–1 0 per cent

Usually 1,200 ml of saline extract was obtained from one placenta Samples of blood were obtained from women in the third trimester of pregnancy and allowed to clot by storing in a refrigerator overnight Serum was then separated by centrifugation The normal serum used here was prepared from citrated, pooled plasma from 100 donors by adding 2 NIH units/ml of human thrombin and removing any clots after retraction

Inhibitory activity against urokinase was determined on normal fibrin plates, which were prepared by the method of Astrup and Mullertz[6], as modified by Alkjaersig, Fletcher and Sherry[7] Human fibrinogen and human thrombin were used instead of bovine products as in the original papers

Urokinase solution was prepared by dissolving a urokinase preparation (Green Cross Corp , Osaka) in 0 9 per cent saline to give a concentration of 30 Ploug units (ref 8) of urokinase/ml and this was mixed with an equal volume of saline The mixture was then diluted two-fold with saline The former mixture was taken as 100 per cent activity and the latter as the control at 50 per cent activity

Samples were diluted with saline in two-fold dilution series and each 0 5 ml of the diluted samples was mixed with 0 5 ml of the urokinase solution A sample of each mixture (0 02 ml) was put on a fibrin plate in duplicate After incubation at 37° C for 20 h, the lysis areas were measured and plotted against sample concentrations on log-log paper

A sample concentration giving 50 per cent inhibition was determined from the lysis area equivalent to the 50 per cent activity described One inhibitory unit was defined as the activity necessary to inhibit 1 Ploug unit of urokinase, and so the number of inhibitory units/ml in an original sample was calculated by multiplying the dilution factor of the sample giving 50 per cent inhibition by 15

Inhibitory activity against trypsin was determined on heated fibrin plates[9] as for urokinase Trypsin solution was prepared by dissolving twice crystallized trypsin to give 23 4 γ/ml of 0 025 molar calcium chloride solution One inhibitory unit was defined as the activity which inhibited 1γ of trypsin and therefore the number of inhibitory units/ml in an original sample was calculated by multiplying the dilution factor of the sample giving 50 per cent inhibition by 11 7

Results are shown in Table 1 Pregnancy sera showed stronger inhibition of both urokinase and trypsin than did normal sera The increase of urokinase inhibitor[4] and trypsin inhibitor[10] in pregnancy blood was confirmed The values for inhibitory activity against trypsin in both pregnancy serum and normal serum were greater in our results than in those of Faarvang and Lauritsen[10], but the reason for the difference was not clear

Compared with pregnancy sera, placental extracts showed a much stronger inhibition of urokinase and much weaker inhibition of trypsin The concentration of plasma proteins in placental extracts was calculated to be about one tenth of the sera and the average ratio of trypsin inhibition by placental extracts to trypsin inhibition by pregnancy sera corresponded to this value, so that the trypsin inhibitor in placental extract is probably derived from pregnancy blood alone On the other hand, there was on average 8 4 times as much urokinase inhibitor in placental extract as in pregnancy serum The selective urokinase inhibitor, which has been found in placental extract[5], must therefore be chiefly derived from the placenta itself

Table 1. INHIBITORY UNITS/ML OF PLACENTAL EXTRACT, PREGNANCY SERUM AND NORMAL SERUM

	Against urokinase	Against trypsin
Placental extract	1,420	378
	1,970	337
	1,810	359
(Average)	(1,730)	(358)
Pregnancy serum	109	3,630
	180	2,755
	297	4,970
	218	4,310
	237	3,000
	220	4,670
	175	3 355
(Average)	(205)	(3,813)
Normal serum	48	1,855
	30	2,350
	30	1,470
	32	1,585
(Average)	(35)	(1,815)

As much as about 2×10^6 units of urokinase inhibitor can be extracted from a placenta This is comparable with 10 l. of pregnancy serum, and this fact suggests a reason for the abrupt return to normal of the time of euglobulin lysis after placental separation[3]

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[1] Niesert, H W , *Arch Gynakol* ,187, 144 (1955)
[2] Biezenski, J J , and Moore, H C , *J Clin Pathol* , 11, 306 (1958)
[3] Shaper, A G , Macintosh, D M , and Kyobe, J , *Lancet*, ii, 874 (1966)
[4] Brakman, P , and Astrup, T , *Scand J Clin Lab Invest* ,15, 603 (1963)
[5] Kawano, T , *J Juzen Med Soc* (in Japanese), 74, 485 (1966)
[6] Astrup, T , and Mullertz, S , *Arch Biochem Biophys* , 40, 346 (1952)
[7] Alkjaersig, N , Fletcher, A P , and Sherry, S , *J Biol Chem* , 234, 832 (1959)
[8] Ploug, J , and Kjeldgaard, N O , *Biochim Biophys Acta*, 24, 278 (1957)
[9] Lassen , M , *Acta Physiol Scand* ,27, 371 (1952)
[10] Faarvang, H J , and Lauritsen, O S , *Nature*, 199, 290 (1963)

Evidence for a Genetic Relationship between Avidins and Lysozymes

PROTEINS which bind biotin do not seem to be widely distributed in nature, possibly because their function seems to be restricted to that of a somewhat inefficient antibacterial agent Until recently, avidin had been detected only in the egg-white of birds and in the homologous egg jelly of frogs[1] In 1963 an avidin-like protein was discovered as part of an antibiotic system in culture filtrates of several species of *Streptomyces*[2]. This protein, called streptavidin, was purified, crystallized and characterized by Chaiet and Wolf[3], who found that it resembled egg-white avidin both in possessing four biotin binding sites (one/15,000*g*) with great affinity for biotin and in some aspects of its amino-acid composition (high tryptophan and threonine, low methionine, proline and histidine) Many of the characteristics of the biotin binding mechanism were also similar There was a red-shift of the tryptophan absorption band accompanying the binding of biotin, and at the same time the biotin protected four tryptophan residues in each sub-unit against oxidation by *N*-bromosuccinimide (ref 4 and my unpublished work) Like avidin, streptavidin also bound the dye 4-hydroxyazobenzene-2′-carboxylic acid giving a new absorption band at 500 mμ which disappeared when the dye was displaced by biotin[5] Some of the physico-chemical characteristics of these two proteins and of hen egg-white lysozyme are summarized in Table 1

Table 1 PHYSICO-CHEMICAL CHARACTERISTICS OF AVIDINS AND LYSOZYME

	Avidin	Streptavidin	Lysozyme
Molecular weight[3 8 10]	70,000	60,000	14,300
Chain length of subunit	148	~135	129
ORD[7 11] per cent helix from $[M']_{233}$	0	>0	~25
CD[7 11 12] λ_{max}	275	275	288
θ_{max} (degrees/cm²/decimole)	350	370	100
Binding characteristics of			
Biotin			
Difference spectrum $\Delta\varepsilon_{293}$[4]	2,200	2,300*	1,300*
Dissociation constant (M)[6]	$\sim10^{-15}$	—	4×10^{-2}*
Heat of binding (kcal/mole)[13]	21	23	—
N-Acetyl glucosamine[9 15 16]			
Difference spectrum $\Delta\varepsilon_{293}$	0*	—	1,300
Dissociation constant (M)	—	—	4×10^{-2}
4-Hydroxyazobenzene-2′-carboxylate[5]			
ε_{500} of bound dye	34,000	17,000*	0*
Dissociation constant (M)	6×10^{-6}	5×10^{-5}*	—

* Results published here for the first time using methods described in refs 4 and 5

Although the probable biological roles of avidin and lysozyme are similar, the biochemical bases of their performance are quite unrelated While lysozyme acts as a muramidase on many types of bacterial cell wall, avidin holds biotin in a firm non-covalent grasp and exerts no

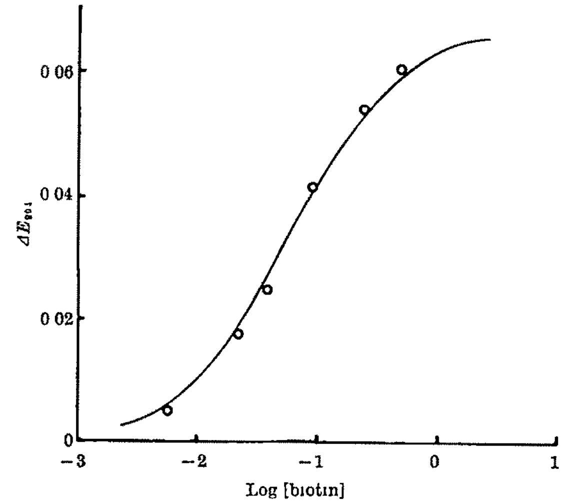

Fig 1 Effect of increasing biotin concentration on the spectrum of lysozyme A series of difference spectra were run of lysozyme ($E_{280} = 2\ 2$) + biotin against lysozyme, in cuvettes of path length 0 1, 0 5, 1 0 and 4 0 cm The solvent was 0 05 molar sodium acetate buffer, *p*H 6 0 at 25° C The difference spectrum showed a double maximum (286, 294 mμ) typical of a red-shifted tryptophan spectrum Both the spectrum and its concentration dependence resembled that given by *N*-acetyl glucosamine with lysozyme[9,16] The change in absorbance at 294 mμ was corrected for the small contribution from free biotin The continuous curve was calculated from the law of mass action for binding of a single biotin molecule with $K = 0\ 04$ molar

apparent catalytic action Moreover, lysozyme has no sub-units, while avidin has four[8] The only functional similarities are the red-shifts of the tryptophan spectra, observed when both proteins combine with their respective substrates or analogues, and the loss of activity when one or two of the tryptophan residues are oxidized by *N*-bromo-succinimide[4,9]

It was therefore with some surprise that I noted a few years ago a marked resemblance in the amino-acid compositions of these two proteins[10,14] Unsuccessful attempts were made to show inhibition by biotin of the action of lysozyme on the cell walls of *B subtilis* No effects were detectable, even with biotin concentrations of 0 1 molar Recently, however, the formation of a biotin-lysozyme complex has been detected in the absence of substrate, using the red-shift of the tryptophan spectrum to measure complex formation From the effect of biotin concentration on the difference spectrum (Fig 1) the dissociation constant was found to be 0 04 molar It is difficult to evaluate the significance of such a weak interaction, although it is no weaker than that between lysozyme and *N*-acetyl glucosamine[15,16] It was not merely caused by hydrogen bonding of the ureido ring of biotin, for hydrolysis of this ring to give a diamine had little effect on the binding Although one cannot give much weight to this evidence in isolation, it is of some significance in the context of the other similarities discussed here The reciprocal system of avidin with *N*-acetyl glucosamine (0 15 molar) showed no spectral shifts

It is now interesting to ask whether there is any comparable relation between streptavidin and lysozymes of *Streptomyces* Fortunately, Ward and Perkins have recently purified two similar lysozymes from *S griseus*, which they showed to be muramidases[17], and have determined their amino-acid composition[18] The analysis for the better characterized lysozyme 2 is shown in Fig 2 It provides further support for the avidin-lysozyme relationship

Before discussing these amino-acid analyses in more detail, I would like to introduce one further member of the lysozyme family This is the protein α-lactalbumin, whose amino-acid sequence was recently determined[19], after the observation of a resemblance between its molecular weight and composition and that of lysozyme[20] After allowing for a few small deletions it was found that forty out of the 123 amino-acid residues were identically placed and a further twenty-seven showed conservative changes The composition of bovine α-lactalbumin is therefore included in Fig 2 for comparative purposes

In spite of the strikingly homologous sequence, the composition of lactalbumin is no closer to that of lysozyme than is that of avidin Each avidin resembles its corresponding lysozyme in its content of about ten of the eighteen amino-acids. The totals of basic amino-acids and of acidic amino-acids are also similar The resemblance in composition is closer than that between trypsinogen and chymotrypsinogen[21] or haemoglobin and myoglobin[22], where the genetic relationships are firmly based on sequence identities By analogy with these established cases, it is likely that the similarities of composition imply that avidins and lysozymes have an appreciable proportion of their sequences in common and that they have originated by differentiation from a common ancestral protein Although argument from composition is not the best way of establishing relationship between proteins, it is here particularly convincing because of the unusual composition and low molecular weight of lysozyme

There are also some considerable differences in composition between avidins and lysozymes, as would be expected for such functionally different proteins The most interesting of these differences are common to both pairs of proteins (from hen egg-white and from *Streptomyces*) The avidins have a much higher content of threonine—it is

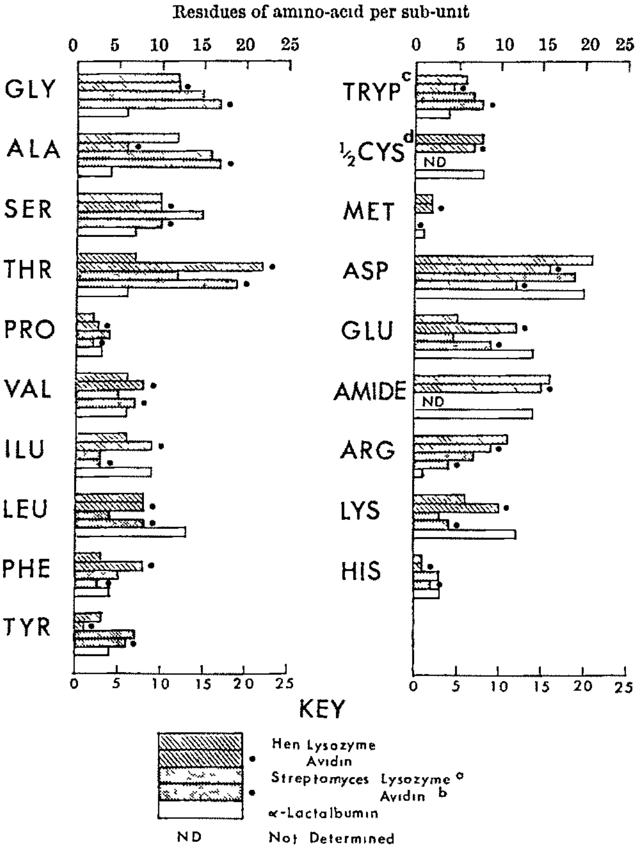

Fig 2 *a*, The analytic results for the *Streptomyces* proteins lack figures for cystine and amide groups The numbers of residues given were calculated on the basis of a molecular weight of 14,000 for the lysozyme and 15,000 for streptavidin All figures are given to the nearest residue or, if there are fewer than ten residues, the nearest half residue No corrections were applied for losses of serine and threonine during the 24 h hydrolysis *b*, The streptavidin analysis was carried out on a sample hydrolysed for 24 h in 6 molar HCl at 110° C *in vacuo* The results agreed well with those of Chaiet and Wolf[3], except that they found only traces of arginine and made no mention of proline They also found six residues of isoleucine and some "α-aminobutyric acid" *c*, Spectrophotometric determination[14] *d*, There are no free sulphydryl groups on avidin, and so there are probably four cystines per sub-unit, or perhaps two sub-units with three and two with four cystines higher than that of any other protein for which figures are available—and they contain more glutamic and less aspartic acid than do the lysozymes In both cases, the avidin has a higher molecular weight, accounted for by an extra ten to twenty amino-acid residues, about half of which appear to be threonine There are also individual differences, such as the presence of mannose and *N*-acetyl glucosamine[14] and the low content of alanine in avidin

One may next enquire whether the similarities of composition and probably of sequence give rise to any common pattern of tertiary structure Both the egg-white proteins (and lactalbumin) have four disulphide bonds and are stable over a wide range of *p*H and temperature The *Streptomyces* proteins are also rather stable, although there is no information concerning their disulphide bonds Both avidin and streptavidin retain their activity in 3–4 molar guanidinium chloride[23] The ORD and CD spectra (Table 1) of both egg-white proteins show positive Cotton effects at 280 mμ arising from the absorption of the aromatic amino-acids There is, however, no evidence from the shorter wavelength region for any α-helix in avidin, while lysozyme contains about 20–30 per cent of its residues in this conformation It is impossible to say how big a difference in sequence is necessary to produce this transformation of tertiary structure It need not necessarily be large, for a few strategically placed charged residues could disrupt a considerable length of α-helix Admittedly, the haemoglobin-myoglobin family has maintained an almost invariant tertiary structure in the face of considerable variation in sequence It should be remembered, however, that these proteins have been selected for a common haem and oxygen binding function, whereas avidin and lysozyme have been selected with different ends in view.

It is implicit in current ideas about the origin of life that the enormous diversity of enzymes and proteins now existing have descended from a much more restricted number of parent proteins The common ancestry of many functionally unrelated proteins should eventually become clear when more amino-acid sequences are known So far, sequence work has been centred on functionally related proteins[24], the cytochromes, haemoglobins and myoglobins, and on the active centres of proteases[25] and dehydrogenases[24] The first clear example of two more distantly related proteins with a common basic sequence is that of lysozyme and α-lactalbumin mentioned above It now seems that we can add avidin and streptavidin to this family, whose further relatives may be recognizable by their somewhat unusual amino-acid composition

So far lysozyme and avidin have been compared within a species If the two hen egg-white proteins are compared with their homologues from *Streptomyces* some further interesting correlations can be made It will be seen that glycine. alanine, isoleucine, tyrosine, methionine, arginine and lysine all change in parallel on going from one species to the other Similar parallels have been observed when the haemoglobins and myoglobins of primates were compared with those of fishes[26] If these changes are significant, they could be explained, either by postulating a primitive gene, say for lysozyme, which has been duplicated on at least two separate occasions, followed by parallel differentiation of the duplicates to give biotin binding proteins, or by postulating a single early duplication followed by parallel changes in both lysozyme and avidin genes during the evolution of birds from the precursors of actinomycetes Both interpretations seem to require improbable coincidences and it is profitless to argue the balance of improbabilities until more information is available If the avidins diverged from the lysozymes after a single early duplication, the avidin structural gene and possibly avidin itself would be expected to be present in many species more primitive than birds and amphibia If both avidin and lysozyme perform an antibacterial function, it is possible that both proteins will be produced together in situations where the organism requires such protection A search conducted with this in mind might show avidin to be more widespread than has hitherto seemed likely

It is interesting to recall that in 1942 Meyer[27] proposed that avidin and lysozyme were related, basing his suggestion largely on the difficulty of separating the two proteins from each other Further experiments failed to substantiate this claim, but it now seems that Meyer's original suggestion may not have been so wide of the mark as it seemed to be at that time It is to be hoped that the present proposals are more firmly based and that fingerprinting and sequence studies will eventually confirm them

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[1] Sebrell, W H , and Harris, R S , *The Vitamins*, 1, 555 (Academic Press New York, 1954)

[2] Stapley, E O , Mata, J. M , Miller, I M , Demny, T C , and Woodruff, H B , *Antimicrobial Agents and Chemotherapy*, 20 (American Society for Microbiology, 1963)

[3] Chaiet, L , and Wolf, F J , *Arch Biochem Biophys* ,**106**, 1 (1964)

[4] Green, N M , *Biochem J* , **89**, 599 (1963)

[5] Green, N M , *Biochem J* , **94**, 23C (1965)

[6] Green, N M , *Biochem J* , **89**, 585 (1963)

[7] Green, N M , and Melamed, M D , *Biochem J* , **100**, 614 (1966)

[8] Green, N M , *Biochem J* , **92**, 16C (1964)

[9] Hayashi, K , Imoto, T , and Funatsu, M , *J Biochem* , Tokyo, **54**, 381 (1963)

[10] Canfield, R E , *J Biol Chem* ,**238**, 2698 (1963).

[11] Ikeda, K , Hamaguchi, K , Imanishi, M , and Amano, T , *J. Biochem* , Tokyo, **62**, 315 (1967)

[12] Glazer, A N , and Simmons, N S . *J Amer Chem Soc* , **88**, 2335 (1966)

[13] Green, N M , *Biochem J* ,**101**, 774 (1966)

[14] Melamed, M D , and Green, N M , *Biochem J* , **89**, 591 (1963).

[15] Rupley J A , Butler, L , Geming, M , Hartdegen, F J , and Pecoraro, R , *Proc US Nat Acad Sci* , **57**, 1088 (1967)

[16] Dahlquist, F W , Jao, L , and Raftery, M , *Proc US Nat. Acad Sci* , **56**, 26 (1966)

[17] Ward, J B , and Perkins, H R , *Biochem J* (in the press).

[18] Ward, J B , thesis, Bath Univ of Technology (1967)

[19] Brew, K , and Campbell, P N , *Biochem J* ,**102**, 258 (1967)

[20] Brew, K , Vanaman, T. C , and Hill, R L , *J. Biol Chem* ,**242**, 3747 (1967).

[21] Walsh, K A , and Neurath, H , *Proc US Nat Acad Sci* , **52**, 884 (1964)

[22] Braunitzer, G , Hilse, K., Rudloff, V , and Hilschmann, N , *Adv. Prot. Chem* , **19**, 1 (1964)

[23] Green, N M , *Biochem J* , **89**, 609 (1963)

[24] Bryson, V , and Vogel, H J , *Evolving Genes and Proteins* (Academic Press, New York and London, 1965)

[25] Hartley, B S , Brown, J R , Kauffman, D L , and Smillie, L B , *Nature*, **207**, 1157 (1965)

[26] Atassi, M Z , *J Theor Biol* , **11**, 227 (1966)

[27] Meyer, K , *Science*, **99**, 391 (1944)

Electrophoretic Study of Glutathione Reductase in Human Erythrocytes and Leucocytes

THE question of whether the activities of NADPH—or NADH—glutathione reductase are caused by a single enzyme or by two different enzymes has never been satisfactorily resolved[1–4] We have devised a new method for detecting glutathione reductase (GSSGR) activity after starch gel electrophoresis The study of subjects with an electrophoretically fast mutant glutathione reductase has enabled us to present findings which strongly favour the suggestion that glutathione reductase activities with respect to both pyridine nucleotide coenzymes reside in the same molecule Some observations have also been made in glutathione reductase deficient and NADH-diaphorase (NADH methaemoglobin oxido-reductase) deficient subjects

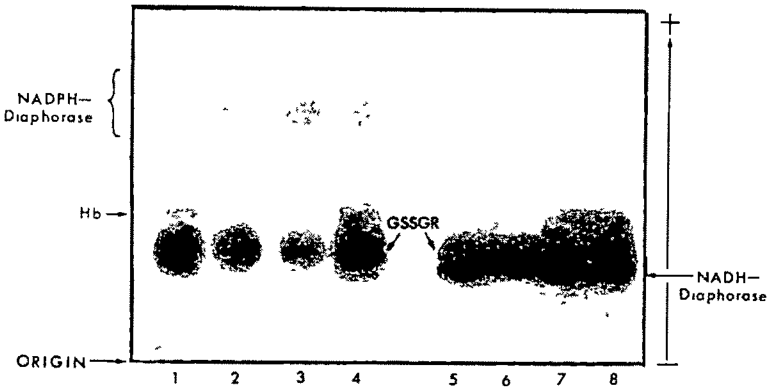

Fig 1 Gel stained for NADPH-glutathione reductase (1–4) and NADH-glutathione reductase (5–8) 1 and 5, Haemolysate from a normal subject No 1, 2 and 6, haemolysate from normal subject No 2, 3 and 7, haemolysate from patient No 1 with partial deficiency in glutathione reductase (activity, 30 per cent of normal), 4 and 8, haemolysate from patient No 2 with partial deficiency in glutathione reductase (activity adjusted to normal) 1–4, With GSSG and NADPH as substrates (see text), 5–8, with GSSG and NADH as substrates (see text) Hb designates the red haemoglobin band

Glutathione reductase catalyses the reaction

$$GSSG + NADPH \text{ (or NADH)} + H^+ \rightarrow 2 \; GSH + NADP \text{ (or NAD)}$$

Our method for detecting glutathione reductase activity is based on the fact that reduced glutathione (GSH), a product of the reaction, rapidly reduces 2,6-dichlorophenol indophenol (DCIP) The reduced DCIP reacts with a tetrazolium salt (3 (4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium) (MTT)[5]—a reaction which is not catalysed by enzymes—and the resulting dark blue formazan precipitate permits the localization of glutathione reductase activity on the gel This method differs from the only other technique[6], not only in a more rapid staining of the gel, but in the capacity to clearly detect the NADH activity as well as the NADPH activity in a positive stain

Haemolysates were prepared by adding two volumes of cold distilled water to the washed red cells and freezing and thawing three times The stroma was discarded after centrifugation at 31,000*g* for 30 min Leucolysates were prepared by adding one volume of cold distilled water to one volume of packed leucocytes which had been substantially freed of erythrocytes by osmotic shock lysis The suspension was frozen and thawed three times and centrifuged at 1,600*g* for 15 min Horizontal starch gel electrophoresis was carried out with an average gradient of 20 to 25 V/cm for 4 h at 4° C A 0·1 molar *tris*-hydrochloric acid buffer, *p*H 9 6 (4° C), containing 4 5 mmolar ethylenediamine tetra-acetic acid (EDTA) was used for the electrode compartments, a dilution 1 10 of this buffer was used for the starch gel The sliced gel was stained in the dark for 2 h, at room temperature, with a freshly prepared developing mixture of 3 4 mmolar oxidized glutathione (GSSG), 1 3 mmolar NADPH, 1 2 mmolar MTT, and 0 04 mmolar DCIP in a 0 25 molar *tris*-hydrochloric acid buffer *p*H 8·4 To detect NADH-glutathione reductase activity, 1 3 mmolar NADH was substituted for NADPH

This reagent mixture detects the activity of diaphorase as well as glutathione reductase, because the diaphorase will transfer an electron directly from the reduced pyridine nucleotide to DCIP, and thus reduce MTT[5] One side of the gel was therefore always stained with a reaction mixture from which GSSG had been omitted, in order to identify clearly the position of the diaphorase bands

Using NADPH, a distinct pattern of glutathione reductase activity was obtained, consisting of a single band located between Hb A and Hb A$_2$ in the haemolysates In the leucolysates a band with an identical mobility was observed There was no interference from the NADPH-diaphorase because this enzyme migrates far ahead of haemoglobin[5]

Accurate localization of erythrocyte glutathione reductase activity using NADH as an electron donor poses technical problems because the NADH-diaphorase, which is visualized at the same time, has almost the same mobility, being only slightly slower Because of this, and because of the relatively low activity of the NADH-glutathione reductase, clear cut patterns are not seen in haemolysates (Fig 1). In leucolysates, however, there is no such interference because leucocyte NADH-diaphorase is usually not released into the supernatant without special treatment (our unpublished data) The NADH-glutathione reductase can thus be seen clearly in leucocyte extracts (Fig 2). Its mobility was found to be identical to the NADPH-glutathione reductase of erythrocytes and leucocytes

An electrophoretically fast variant of NADPH-glutathione reductase, probably identical to the type described recently by Long[6], has been found among normal Negroes Some subjects, apparently homozygotes, have a single fast band, others, apparently heterozygotes, have a wide band representing the juxtaposition of the normal and the variant band This was observed in both erythrocytes and leucocytes, and this finding is consistent with the assumption that the enzyme is under the same genetic control in both cell types In a subject homozygous for the fast variant of glutathione reductase, the fast band observed with NADPH was also obtained with NADH at the same position This was seen most clearly in leucolysates (Fig 2), but was also evident in haemolysates

Haemolysates from two subjects with partial glutathione deficiency have been studied Their glutathione reductase had a mobility identical to the common normal glutathione reductase, both with NADPH and NADH

Subjects with congenital methaemoglobinaemia with erythrocyte NADH-diaphorase deficiency have been found to show a decrease in glutathione reductase activity of about 20 per cent[7] The possibility that these two enzymes share a common sub-unit seemed to warrant consideration Accordingly we have studied three subjects homozygous for NADH-diaphorase deficiency, one of whom had an electrophoretic abnormality of the enzyme (the California variant)[5] Although they had a moderate decrease of erythrocyte glutathione reductase activity, no electrophoretic abnormality was found Furthermore, in haemolysates from these subjects, a well defined band of NADH-glutathione reductase activity could be obtained, without interference from the completely or almost completely missing NADH-diaphorase band The position

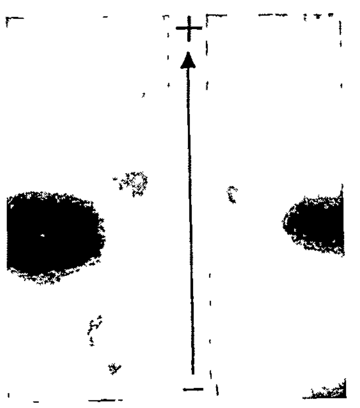

Fig 2 1 and 4, Leucolysate from a normal control, 2 and 3, leucolysate from a homozygous subject for the fast variant of glutathione reductase Gel stained for NADPH- (1–2) and NADH-glutathione reductase (3–4) of NADH-glutathione reductase band confirmed the identical mobility of NADH- and NADPH-glutathione reductase band in erythrocytes

We find that glutathione reductase activity of normal leucolysates and haemolysates moves with identical electrophoretic mobility regardless of whether NADH or NADPH is employed as hydrogen donor This, and the further finding that identical mobilities are also observed when haemolysates and leucolysates are examined from subjects with an electrophoretically detectable mutant enzyme, strongly supports the idea that activities with the two hydrogen donors are the properties of the same enzyme molecule The less likely possibility, however, that there are two different enzymes sharing a common sub-unit which is affected by the electrophoretic mutation cannot be altogether eliminated by the studies reported The glutathione reductase from two subjects with quantitative diminution of glutathione reductase activity has been found to be electrophoretically normal The technique which we have reported for identifying the position of glutathione reductase in starch gel should make it possible to study other families already reported[4,8-10], in order to determine whether further electrophoretic polymorphisms involving the enzyme exist

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[1] Beutler, E , and Yek, M K Y , *Blood*, 21, 573 (1963)

[2] Scott, E M , Duncan, J W , and Ekstrand, V , *J Biol Chem* , 238, 3928 (1963)

[3] Eifler, R , and Wagenknech, C , *Acta Biol Med German* , 13, 674 (1964)

[4] Waller, H D , *City of Hope Symp Ser* , 1, *Genetically Determined Abnormalities of Red Cell Metabolism* (Grune and Stratton) (in the press)

[5] Kaplan, J C , and Beutler, E , *Biochem Biophys Res Commun* (in the press)

[6] Long, W K , *Science*, 155, 712 (1967)

[7] Jaffé, E R , *Amer J Med* , 41, 786 (1966)

[8] Carson, P. E , Brewer, G J , and Ickes, C , *J Biol Clin Med* , 58, 804 (1961)

[9] Lohr, G W , and Waller, H D , *Med Klin* , 57, 1521 (1962)

[10] Waller, H D Lohr, G W , Zysno, E , Gerok, W , Voss, D , and Strauss, G , *Klin Wochen* , 43, 413 (1965)

Electrophoresis of Ferritins

ELECTROPHORETIC studies of ferritins—iron containing proteins—from horse, man and HeLa cells have already been recorded[1,2] This communication describes the electrophoretic patterns of a plant ferritin known as phytoferritin[3] and of ferritins from pig, ox and guinea-pig, compared with human and horse ferritin

The ferritins other than guinea-pig and phytoferritin were extracted by the method of Granick which, briefly, consists of homogenizing the tissue (liver or spleen) in distilled water and heating to 80° C, after which the supernatant is half saturated with ammonium sulphate, and the precipitate is dissolved in distilled water The ferritin present in the solution is crystallized with cadmium sulphate, and finally purified by recrystallization When processed in this way bovine and porcine ferritins fail to crystallize

To provide an adequate ferritin yield old stock guinea-pigs were given three intraperitoneal injections of 2 ml of 1 per cent iron ammonium citrate every other day and the animals were killed on the day after the last injection Extraction of the liver and spleen was carried out using Friedberg's modification of Granick's method[4]

Phytoferritin was extracted[3] from the primary and secondary leaves of the French bean, *Phaseolus vulgaris*, grown in an iron enriched soil

Human ferritin was extracted from the liver and spleen of a patient with myeloid metaplasia Horse ferritin, the easiest of all ferritins to prepare, was extracted from the spleen of an old animal

The pattern of bands shown by the ferritins was compared in polyacrylamide gel electrophoresis using 5 per cent acrylamide gel, *p*H 7 2, run for 75 min at 5 m amp/tube The six ferritins examined differed in their mobility (Fig 1), the slowest moving being bovine, with porcine, human, horse, guinea-pig and the phytoferritin forming a series of increasing speed of mobility

Polyacrylamide gel acts like a molecular sieve, and so it is reasonable to suppose that the rate of movement of the ferritin is a function of their size Unfortunately electron microscopy, using the phosphotungstic acid negative contrast technique, and ultracentrifuge studies failed to demonstrate any appreciable difference in size between the human and horse ferritins, although in the polyacrylamide gel electrophoresis the former moved more slowly

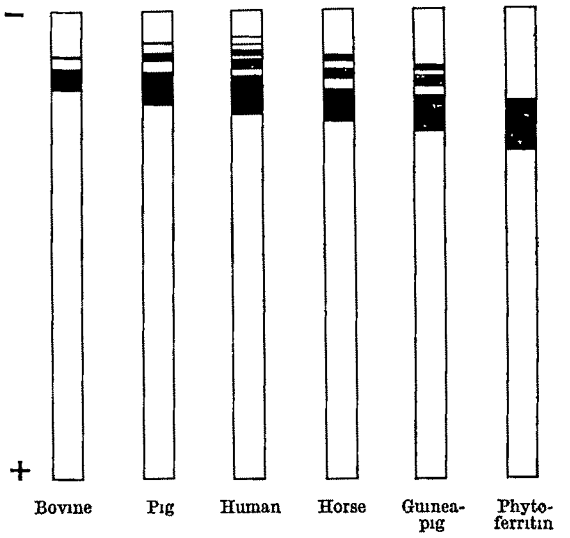

Fig 1 Patterns shown by ferritins from various species when examined in standard conditions by polyacrylamide disc electrophoresis

The number of bands formed by the ferritins in standard conditions of 500 μg/tube varied, human ferritin formed five bands, guinea-pig, horse and porcine three bands, bovine two and phytoferritin remained as one broad band

Hyde[3] reported that the electron microscopic, centrifugal and spectrophotometric properties of phytoferritin were very similar to those of animal ferritins In the samples we examined, phytoferritin did not show band formation This may be a consequence of the low concentration of the material in the plant, or in the extract, for in other ferritins the minor bands are not demonstrable when the concentration of ferritin is very low.

It is believed that at high concentrations the molecules of ferritin aggregate to form polymers When each of the monomer, dimer and trimer bands of horse ferritin were eluted and examined with the electron microscope they showed a proponderance of single, double and treble molecules respectively[5] We have been able to demonstrate similar aggregates in samples eluted from the bands of human ferritin prepared by electrophoresis in polyacrylamide gels

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[1] Fine, J M, and Harris, G, *Clin Chem Acta*, **8**, 794 (1963)
[2] Richter, G W, *Nature*, **207**, 616 (1965)
[3] Hyde, B B, Hodge, A M, Kahn, A, and Birnstiel, M L, *J Ultrastruct Res*, **9**, 248 (1963)
[4] Granick, S, *Chem Rev*, **38**, 379 (1946)
[5] Williams, M A, Gregory, D W, and Harrison, M P, *Biochem J*, **99**, No 1, 10*P* (1966)

IMMUNOLOGY

Absence of β-Globin Synthesis and Excess of α-Globin Synthesis in Homozygous β-Thalassaemic Subjects from the Ferrara Region

β-Thalassaemia is a genetically determined anaemia of man, characterized by a decrease in production[1-6] or the absence[7] of β-globin, and by an excess of α-globin synthesis[4,6-8], which results in the $\alpha/(\beta+\gamma+\delta)$ globin synthesis ratio largely exceeding the normal value of 1 0

The high frequency of occurrence of the β-thalassaemic gene in the area of Ferrara, Northern Italy[9], has provided us with the opportunity to examine a relatively large number of homozygous and heterozygous β-thalassaemic subjects The investigations were based on the following determinations (*a*) globin chain synthesis in peripheral blood and bone marrow erythroid cells, and (*b*) characterization of the β-thalassaemic haemoglobin types by starch gel electrophoresis and by column chromatography on 'Amberlite *IRC* 50'

A typical separation of labelled thalassaemic globin chains is shown in Fig 1 This shows the absence of β-globin and the excess of α-globin synthesis in reticulocytes from homozygous β-thalassaemic patients, confirming our previous findings[7] Erythroid cells from bone marrow have an identical chromatographic pattern, except for the amino-acid incorporation, which is greater

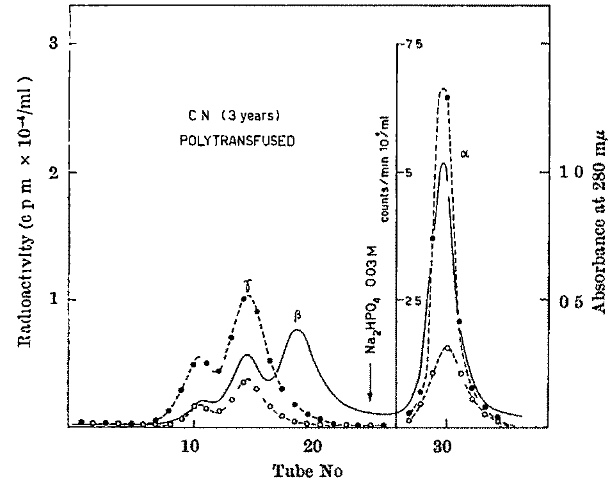

Fig 1 Column chromatography on CM-cellulose of the labelled globin chains from a homozygous β-thalassaemic subject The erythroid cells from the peripheral blood and from the bone marrow of the subject were incubated with ^{3}H amino-acids[7,10] The chromatographic separation was performed according to the procedure of Clegg *et al*[11] Each tube corresponds to 2 5 ml ———, *OD* pattern, ○ - - - ○, peripheral blood radioactivity, ● - - - ●, bone marrow radioactivity

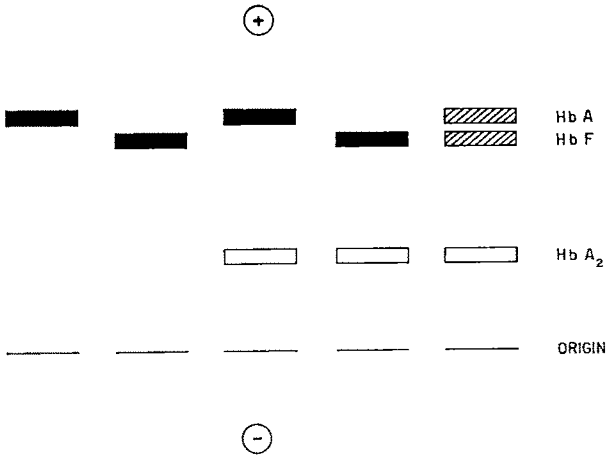

Fig 2 Starch gel electrophoresis at *p*H 8 6 (tris-EDTA-borate) of the haemoglobin types present in the various subjects examined The diagram refers, from left to right, to purified haemoglobin A, purified haemoglobin F, haemoglobins from the peripheral blood and from the bone marrow of homozygous β-thalassaemic subjects who had never been transfused, haemoglobins from the peripheral blood and from the bone marrow of poly-transfused homozygous β-thalassaemic patients

The excess of α-globin synthesis, as calculated from the chromatographic analyses, on *CM*-cellulose, of the β-thalassaemic subjects examined, is shown by Table 1 The ratio of α-globin synthesis to counterpart globin synthesis, observed in reticulocytes, is comparable with that in erythroid bone marrow cells, this ratio, equal to 3 0 in the homozygous state, becomes 1 7 in the heterozygous state The ratio of the control subjects is 1 0 (Table 2)

Starch gel electrophoresis of the haemoglobin types of the subjects examined is shown in Fig 2 The patterns reveal the absence of the band corresponding to haemoglobin A in the subjects who have never been transfused The presence of the haemoglobin A band in the polytransfused patients is derived from the transfused normal haemoglobin In fact (see Fig 3) the intensity of the adult haemoglobin band is related to the time between transfusion and chromatographic analysis

Table 1 EXCESS OF α-GLOBIN SYNTHESIS IN HOMOZYGOUS β-THALASSAEMIA
Ratio ^{3}Hα/^{3}H (γ + δ)

Patient	Age	Peripheral blood	Bone marrow	Patient	Age	Peripheral blood	Bone marrow
1 (a)	6 months	2 5	—	17 (b)	4 years	3 0	—
2 (a)	6 months	2 4	—	18 (b)	4 years	6 6	—
3 (a)	9 months	3 1	—	19 (b)	5 years	4 0	—
4 (a)	10 months	3 8	—	20 (b)	5 years	2 1	2 2
5 (a)	1 year	3 0	3 1	21 (c)	6 years	3 0	—
6 (a)	1 year	3 2	3 5	22 (c)	7 years	3 4	—
7 (a)	2 years	2 7	—	23 (b)	7 years	3 1	3 1
8 (b)	2 years	3 6	—	24 (b)	7 years	2 9	—
9 (b)	2 years	3 0	3 1	25 (b)	7 years	2 6	2 4
10 (a)	2 years	2 7	2 6	26 (c)	8 years	2 9	—
11 (b)	3 years	2 7	—	27 (b)	8 years	3 5	—
12 (a)	3 years	2 8	2 6	28 (c)	9 years	2 4	—
13 (b)	3 years	3 6	—	29 (c)	10 years	2 5	—
14 (b)	3 years	2 6	—	30 (c)	10 years	2 6	—
15 (b)	4 years	3 5	3 6	31 (c)	11 years	2 8	—
16 (b)	4 years	2 5	—	32 (c)	12 years	2 7	—
					Averages	3 0	3 0

(a) Never transfused, (b) polytransfused, (c) polytransfused and splenectomized

Table 2 RATIO OF α-GLOBIN SYNTHESIS VERSUS COUNTERPART GLOBIN SYNTHESIS IN HETEROZYGOUS β-THALASSAEMIC PATIENTS AND IN CONTROL SUBJECTS

Heterozygous β-thalassaemia

Patient	Age	Ratio ^{3}Hα/^{3}H (β+γ+δ) Peripheral blood
1	27 years	1 74
2	31 years	1 90
3	31 years	1 77
4	32 years	1 44
5	34 years	1 83
6	35 years	1 42
7	41 years	1 87
8	45 years	1 75
9	50 years	1 57
	Average	1 69

Control subjects

Patient	Age	Ratio ^{3}Hα/^{3}H (β + δ) Peripheral blood
1	23 years	1 00
2	31 years	1 05
	Average	1 0

A typical chromatographic pattern on 'Amberlite *IRC* 50' of the haemoglobins from β-thalassaemic reticulocytes and from erythroid cells of the bone marrow, after amino-acid incorporation, is shown in Fig. 4 The elution profile reveals the presence of three proteins corresponding to haemoglobin F, haemoglobin A and haemoglobin A_2 The first and the third peaks are radioactive, while the second contains no detectable radioactivity This pattern is consistent with the findings obtained with the other techniques and confirms the absence of β-globin synthesis in homozygous β-thalassaemia. The sensitivity of this technique in detecting the various haemoglobin fractions has been reported to be 0 1 per cent[13]

These findings provide further evidence that the gene for β-globin, in homozygous β-thalassaemic subjects from the Ferrara region, is not expressed This result is substantiated by the many cases so far examined (forty-two) and by the evidence provided by several techniques (Of the forty-two patients so far examined, four were originally from other areas of thalassaemia in Italy. In four cases, not described in this report, β-chain synthesis was suggested by the chromatographic pattern of the globin, further studies are in progress to characterize these subjects) The absence of β-globin in our population, compared with the decreased synthesis observed in other areas, suggests that Cooley's disease is caused by a heterogeneous group of mutation of the β-gene, leading to the synthesis of different amounts of β-globin in different thalassaemic populations

The identical behaviour of reticulocytes and bone marrow erythroid cells excludes a selective retention in the bone marrow of cells which synthesize β-globin, and the presence of functional messenger RNA for β-globin during the different stages of maturation of red blood cells

The absence of β-globin is known to be associated with an excess of α-globin synthesis· this can be interpreted as a consequence of the defect in β-globin synthesis, because it is higher in the homozygous than in the heterozygous state

The free α-chain does not tend to assume a stable tetrameric form[14,15] and, as reported elsewhere[10], is rapidly removed from the cytoplasm, becoming associated with the stromal material This may be the reason why only small amounts, if any, of free α-chain are detectable in the β-thalassaemic haemolysates by either chromatographic or electrophoretic techniques[16,17], while β_4-tetramers (HbH) or γ_4-tetramers (Hb Bart), because of their higher stability, are detectable in α-thalassaemic haemolysates

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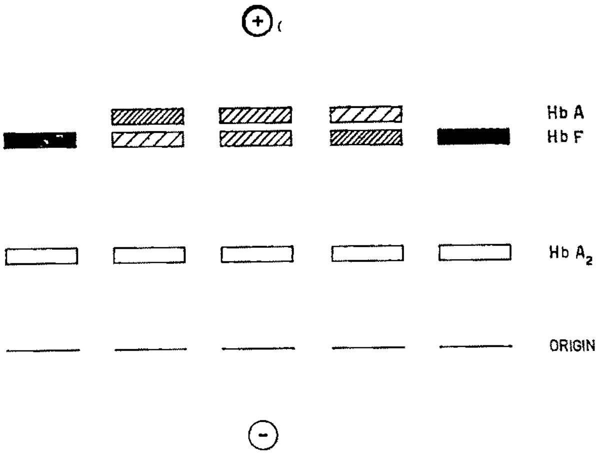

Fig 3 Starch gel electrophoresis at *p*H 8 6 (*tris*-EDTA-borate) of the haemoglobin types of a homozygous β-thalassaemic subject who had never been transfused The diagram represents from left to right the haemoglobin composition immediately before transfusion, and 1, 30, 60 and 90 days after transfusion

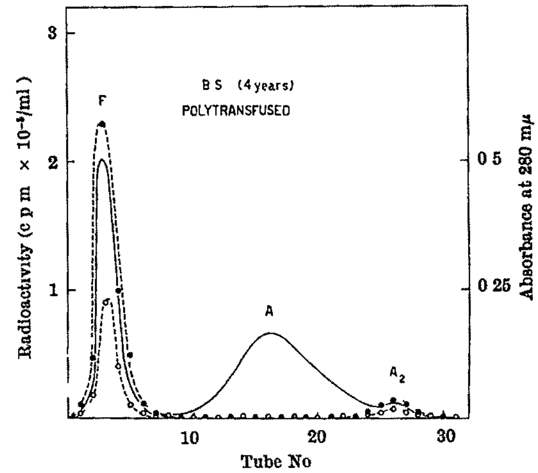

Fig 4 Column chromatography on 'Amberlite *IRC* 50' of the labelled haemoglobins present in the peripheral blood and in the bone marrow erythroid cells of a homozygous β-thalassaemic subject after incubation with ^{3}H-amino-acids The chromatographic separation of the haemoglobin types was performed according to the procedure described by Allen *et al*[12] ——, *OD* pattern, ○ - - - ○, peripheral blood radioactivity, ● - - - ●, bone marrow radioactivity

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[1] Gabudza, T G , Nathan, D G , and Gardner, F H , *J Clin Invest* , 42, 1678 (1963)

[2] Guidotti, G , in *Genetics and Evolution* (edit by Ingram, V M), 116 (Columbia Univ Press, New York, 1963)

[3] Marks, P A , and Burka, E R , *Science*, 144, 522 (1964)

[4] Heywood, J D , Karon, M , and Weismann, S , *Science*, 144, 530 (1964)

[5] Motulsky, A G , *Cold Spring Harbor Symp Quant Biol* , 29, 339 (1964)

[6] Bank, A , and Marks, P A , *Nature*, 212, 1198 (1966)

[7] Bargellesi, A , Pontremoli, S , and Conconi, F , *Europ J Biochem* , 1, 73 (1967)

[8] Weatherall, D J , Clegg, J B , and Naughton, M A , *Nature*, 208, 867 (1965)

[9] Silvestroni, E , and Bianco, I , *G Stud Prob Soc Microcit morbo Cooley*, Istituto Italiano di Medicina Sociale, Rome (1961)

[10] Bargellesi, A , Pontremoli, S , Menini, C , and Conconi, F , *Europ J Biochem* (in the press)

[11] Clegg, J B , Naughton, M A , and Weatherall, D J , *Nature*, 207, 945 (1965)

[12] Allen, D A , Schroeder, W A , and Balog, J , *J Amer Chem Soc* , 80, 1628 (1958)

[13] Brancati, C , and Baglioni, C , *Nature*, 212, 262 (1966)

[14] Huehns, E R , Dance, N , Shooter, E M , and Beaven, G H , *J Mol Biol* , 5, 511 (1962)

[15] Huehns, E R , and Shooter, E M , *Biochem J* , 101, 843 (1966)

[16] Fessas, F , *Intern Symp Comp Hemoglobin Structure, Thessaloniki*, 51 (1966)

[17] Huehns, E R , *Intern Symp Comp Hemoglobin Structure, Thessaloniki*, 58 (1966)

Effect of F(ab')₂ from Rabbit Anti-mouse Lymphocyte IgG on the Graft versus Host Reaction in F_1 Hybrid Mice

HETEROLOGOUS antisera raised against lymphoid cells (ALS) have been found to suppress a large variety of immune responses (see review by James[1]) In particular, lymphoid cells from mice which have been treated with

ALS exhibit a diminished ability to produce graft versus host (GVH) reactions[2-5]. Such responses result from immunological interaction between lymphoid graft and the histocompatibility antigens of the host. One such reaction occurs when parental spleen cells are injected into adult F_1 hybrid mice, and its occurrence and severity can be assayed by measurement of the resulting increase in size of spleen and liver[6]. As anti-lymphocytic antibody fragments seem incapable of suppressing humoral antibody formation[7-10] and inhibiting allograft rejection[11], we have investigated the effect of such preparations on the following GVH system.

We have compared the ability of spleen cells from *C57Bl* mice treated with intact IgG and fragments (antibody and normal) to produce a GVH-reaction on subsequent injection into normal $(C57Bl \times CBA)F_1$ hybrid recipients. The mice used were derived from inbred strains maintained in this department; the *C57Bl* donors were males and females weighing 25–30 g and the recipient F_1 hybrids were males between 30 and 35 g.

ALS was raised by intravenous injection of New Zealand white rabbits with about 10^9 *CBA* mouse thymus cells on each of three occasions during a 4 week period. The rabbits were bled 1 week after the last injection. The ALS obtained was inactivated (56° C for 30 min) and absorbed with mouse erythrocytes to remove agglutinins.

Small amounts of this antiserum prolonged the survival (three times) of A strain skin grafts on *CBA* male mice and produced a marked lymphopenia in *C57Bl* mice. The normal rabbit serum (NRS) IgG and rabbit anti-mouse lymphocyte IgG preparations used in these studies were prepared by batch chromatography[12] on Whatman *DE*52 ion exchange cellulose (degree of substitution = 1.0 m equiv./g). Immunoelectrophoresis using a sheep anti-whole rabbit serum indicated that the products contained only trace amounts of contaminating proteins. The non-cytotoxic $F(ab')_2$ portion of the antibody molecule (which contains both antibody combining sites) was obtained by pepsin digestion[13] at 37° C for 24 h in *p*H 4.0, 0.1 molar acetate buffer. For each 100 mg of protein 2 mg of enzyme was used. Degradation of the IgG to the $F(ab')_2$ product was shown to be complete by immunodiffusion and cytotoxic analyses. The products were concentrated to 1 g per cent by ultrafiltration and dialysed against 0.15 molar sodium chloride before use.

C57Bl donor animals were given a total of 1 ml of the appropriate 1 g per cent preparation intraperitoneally in doses of 0.25 ml on days 4, 3, 2 and 1 prior to cell transfer.

Spleen cell suspensions were prepared from the pooled spleens of four or five treated animals by gently breaking them up in a ground glass homogenizer containing balanced salt solution plus 0.1 per cent normal mouse serum. The suspensions were passed through a fine meshed stainless steel sieve, allowed to stand for 10 min at 4° C, then the cells in the supernatant were washed twice and re-suspended to give a concentration of about 200×10^6/ml. The viability of the cells was assessed by their ability to exclude 0.05 per cent trypan blue, and was never less than 86 per cent. The volume necessary to give a dose of 100×10^6 viable cells was calculated, and this volume of suspension was injected into the tail vein of the appropriate animal.

The $(C57Bl \times CBA)F_1$ animals were arranged in six groups, as shown in Table 1. Nine days after cell transfer the animals were weighed, killed by cervical dislocation, and the weights of their spleens and livers determined.

As Table 1 shows, ALS IgG alone inhibited the production of splenomegaly and hepatomegaly while ALS $F(ab')_2$ and the normal IgG preparations were ineffective. This result is in agreement with other findings that anti-lymphocytic fragments seem incapable of suppressing humoral antibody formation[7-10] or inhibiting homograft rejection[11], and suggest that the immunosuppressive properties of ALS are dependent on the possession of an intact Fc (complement binding) portion. It is possible, however, that other properties associated with this region of the molecule are involved (that is, membrane attachment, placental transfer, immunogenicity).

Table 1. MEAN RELATIVE SPLEEN AND LIVER WEIGHTS OF $(C57Bl \times CBA)F_1$ HYBRID MICE, 9 DAYS AFTER INTRAVENOUS INJECTION OF 100×10^6 SPLEEN CELLS FROM *C57Bl* MICE OR SALINE

Group	No.	Relative spleen weights* mean (±1 *S.D.*)	Relative liver weights* mean (±1 *S.D.*)
I Cells from donors treated with ALS IgG	6	42.5 (±7.7)	568.7 (±47.1)
II Cells from donors treated with ALS $F(ab')_2$	5	128.5 (±23.9)	752.4 (±55.4)
III Cells from donors treated with NRS IgG	5	108.0 (±8.3)	698.8 (±54.6)
IV Cells from donors treated with NRS $F(ab')_2$	5	120.3 (±8.1)	637.6 (±49.9)
V Cells from donors treated with saline	5	120.9 (±7.3)	746.1 (±57.3)
VI Saline only	7	43.1 (±19.2)	585.7 (±31.9)

* Relative organ wt. $= \frac{\text{Organ weight (mg)} \times 10}{\text{Body weight (g)}}$. Group I against group V in respect of spleen weight $P < 0.01$. Group I against group V in respect of liver weight $P < 0.01$. Group II against group V in respect of spleen weight or liver weight, not significant.

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[1] James, K., *Clin. Exp. Immunol.*, 2, 615 (1967).
[2] Boak, J. L., Fox, M., and Wilson, R. E., *Lancet*, i, 750 (1967).
[3] Monaco, A. P., Wood, M. L., van der Werf, B. A., and Russell, P. S., in *Ciba Foundation Study Group on Antilymphocytic Serum* (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 111 (Churchill, London, 1967).
[4] van Bekkum, D. W., Ledney, G. D., Balner, H., van Putten, L. M., and de Vries, M. J., in *Ciba Foundation Study Group on Antilymphocytic Serum* (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 97 (Churchill, London, 1967).
[5] Levey, R. H., and Medawar, P. B., *Proc. US Nat. Acad. Sci.*, 58, 470 (1967).
[6] Simonsen, M., *Prog. Allergy*, 6, 349 (1962).
[7] Harris, S., and Harris, T. N., *J. Immunol.*, 96, 478 (1966).
[8] James, K., and Anderson, N. F., *Nature*, 213, 1195 (1967).
[9] James, K., *Clin. Exp. Immunol.*, 2, 685 (1967).
[10] Ogburn, C. A., Harris, T. N., and Harris, S., *J. Immunol.*, 99, 713 (1967).
[11] Anderson, N. F., James, K., and Woodruff, M. F. A., *Lancet*, i, 1126 (1967).
[12] Stanworth, D. R., *Nature*, 188, 156 (1960).
[13] Nisonoff, A., Wissler, F. C., Lipman, L. N., and Woernly, D. L., *Arch. Biochem.*, 89, 230 (1960).

In vivo and *in vitro* Effect of Anti-lymphocytic IgG on Sensitized Spleen Cells

It has been shown that treatment of a number of animals with species specific anti-lymphocytic antibody suppresses their primary immune response to a variety of antigens[1]. Cell transfer experiments have also shown that "non-sensitized" lymphoid tissue from mice treated with anti-lymphocytic antibody can exhibit a reduced immunological potential when transferred to suitably prepared recipients[2-5]. Furthermore, suppression of suspensions of sensitized mouse and rabbit lymphoid cells can also be achieved by incubating them *in vitro* with anti-lymphocytic antibody before transferring them to test recipients[6,7]. In order to confirm and extend these previous cell transfer results, preliminary experiments have been undertaken to determine the effect of anti-rat lymphocyte antibody treatment (*in vivo* and *in vitro*) on the humoral antibody producing potential of sensitized rat spleen cells on subsequent transfer to irradiated recipients.

The suspensions of sensitized spleen cells were prepared from female rats of a hooded strain which had received two intraperitoneal injections of 5 mg of alum precipitated bovine serum albumin (alum BSA), 6 weeks and 2 weeks before the spleen was removed. The preparation and

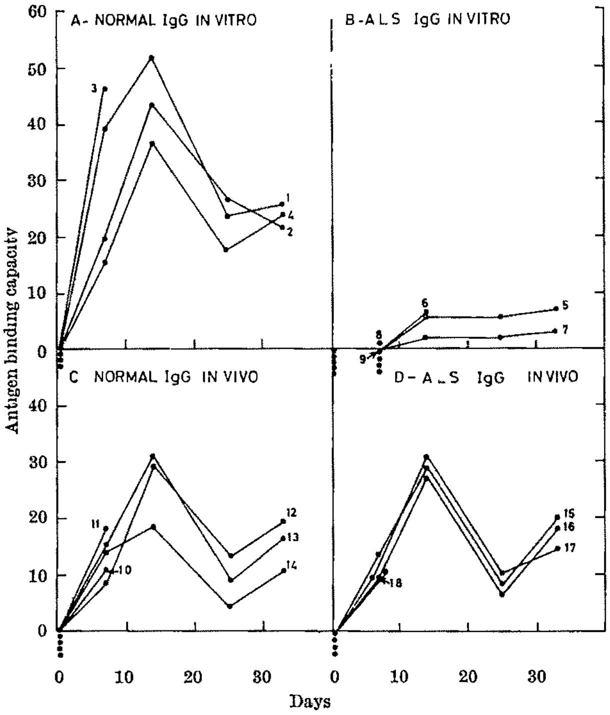

Fig 1 The effect of *in vivo* and *in vitro* anti-lymphocytic antibody treatment of sensitized spleen cells on their subsequent response to sensitizing antigen (BSA) following transfer to irradiated recipients For further details of the treatment of the sensitized spleen cells see the text Note that several animals died during the course of the experiment

properties of the horse anti-rat lymphocyte IgG and normal horse IgG have been reported elsewhere[8–10]

The *in vitro* treatment of suspensions of the sensitized spleen cells consisted of incubating them for 1 h at 37° C in sterile 0 15 molar solutions of sodium chloride containing normal horse or anti-lymphocytic IgG (1 mg of protein/ml) No extraneous complement was added to the mixture[6] Each incubation mixture (total volume 20 ml) contained cells from six spleens The *in vivo* treatment consisted of injecting (intraperitoneally) the sensitized animals daily with 2 ml of a 1 g per cent solution of the normal or the anti-lymphocytic IgG on the 3 days before the spleen was removed These cells were incubated in sterile saline alone All suspensions were washed twice following incubation and were made up to a volume of 7 ml (equivalent to six spleens)

Table 1 DETAILS OF THE TRANSFERRED SPLEEN CELL SUSPENSIONS

Experimental group	Treatment of sensitized spleen cells	Number of cells transferred	Percentage viable
A	Normal horse IgG *in vitro*	200×10^6	50
B	Horse anti-rat lymphocyte IgG *in vitro*	150×10^6	40
C	Normal horse IgG *in vivo*	200×10^6	80
D	Horse anti-rat lymphocyte IgG *in vivo*	150×10^6	54

The recipients of the various suspensions of sensitized spleen cells were 3–6 month old male rats of the hooded strain weighing from 200–390 g They all received terramycin in their drinking water (1 mg/100 ml) commencing 2 days before receiving 600 rads of whole body X-irradiation This dose of radiation had previously been shown to suppress completely the primary response of hooded male rats to alum BSA Twenty-four hours after irradiation the rats received an intraperitoneal injection of 1 ml of one of the suspensions of spleen cells (see Table 1) A further 24 h after cell transfer the animals were injected intraperitoneally with 5 mg of alum BSA The animals were bled 7, 14, 25 and 33 days after injection of antigen and the serum antibodies were determined by the Farr procedure[11] using an iodine-131 labelled BSA preparation containing 0 2 μg of nitrogen in each test Further details on the preparation of labelled antigen[12] and the Farr technique are recorded elsewhere[13]

It is apparent from Fig 1*B* that the treatment of sensitized spleen cells *in vitro* with anti-lymphocytic IgG markedly inhibits their subsequent response to alum BSA following transfer to X-irradiated recipients In contrast, the animals (Fig 1*A*, *C* and *D*) which received the other suspensions of spleen cells showed a characteristic secondary response to the bovine serum albumin[9]

The suppression of the humoral antibody response of sensitized lymphoid cells by *in vitro* treatment with anti-lymphocytic antibody confirms previous results in other species[6,7] These observations suggest that this form of treatment could be capable of erasing immunological memory, presumably as a result of direct interaction between memory cells and antibody It is appreciated, however, that alternative explanations exist including the rapid destruction of transferred antibody coated cells, their poor "seeding" and reduced proliferation Furthermore, one also has to take into consideration the fact that the animals in group *B* received fewer viable cells than those in group *A* although a comparison of the results between groups *C* and *D* would suggest that this was not a principal factor

The *in vivo* results support previous observations in rats[9,10] where a similar dose of anti-lymphocytic antibody did not have a marked effect on the secondary response of rats to alum BSA (ref 9) and also failed to suppress the primary response if given a short while after the antigen[10] It has been suggested that *in vivo* the anti-lymphocytic IgG has limited access to central lymphoid tissue such as the spleen and lymph nodes[7] and that its effect is primarily on peripheral lymphocytes[5] Thus the antibody is unable to inactivate memory cells or prevent plasma cell proliferation in these sites In contrast, however, it should be emphasized that studies on skin allograft rejection[5] and the graft versus host phenomenon[2–4] indicate that *in vivo* treatment with antilymphocytic antibody can significantly modify the subsequent immunological response of non-sensitized lymphoid tissue

Further experiments are now under way to confirm these preliminary observations using larger numbers of animals and transferring identical numbers of viable cells

I thank Dr N F Anderson for irradiating the rats, Mrs V James, Miss E Nelson and Mr A Wood for technical assistance, and Mr C Shepley for preparing the diagram I also thank Professor M F A Woodruff and Dr R N Pinckard for their advice and interest in this work

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[1] James, K , *J Clin Exp Immunol* ,2, 615 (1967)

[2] Boak, J L , Fox, M , and Wilson, R E , *Lancet*, i, 750 (1967)

[3] Van Bekkum, D W , Ledney, G D , Balner, H , van Putten, L M , and de Vries, M J , *Ciba Foundation Study Group on Antilymphocytic Serum* (edit by Wolstenholme, G E W , and O'Connor, M), 97 (Churchill, London, 1967)

[4] Monaco, A P , Wood, M L , van der Werf, B A , and Russell, P S , *Ciba Foundation Study Group on Antilymphocytic Serum* (edit by Wolstenholme, G E W , and O'Connor, M), 111 (Churchill, London, 1967)

[5] Levey, R H , and Medawar, P B , *Proc US Nat Acad Sci* , 58, 470 (1967)

[6] Harris, S , and Harris, T N , *J Immunol* , 96, 478 (1966)

[7] Denman, A M , Denman, E J , and Holborow, E J , *Lancet*, i, 1084 (1967)

[8] James, K , and Anderson, N F , *Nature*, 213, 1195 (1967)

[9] James, K , and Jubb, V S , *Nature*, 215, 367 (1967)

[10] James, K , *J Clin Exp Immunol* ,2, 685 (1967)

[11] Farr, R S , *J Infect Dis* ,103, 239 (1958)

[12] Hunter, W M , and Greenwood, F C , *Nature*, 194, 495 (1962)

[13] Pinckard, R N , Weir, D M , and McBride, W M , *J Clin Exp Immunol* , 2, 331 (1967)

Proliferation of Mast Cells after Antigenic Stimulation in Adult Rats

IN the course of a series of experiments using tritiated thymidine to study long-lived lymphocytes and plasma cells in rat lymph nodes[1-3] we have frequently found a high percentage of labelled mast cells Because these findings seem inconsistent with reports that mast cells are stable and only divide slowly[4-6], we have re-examined our earlier experiments and have found evidence which shows that mast cells are actively produced after antigenic stimuli

The details of all the experiments to be considered have been previously reported In one group of experiments tritiated thymidine was injected twice daily for 2 days into the hind foot pads of 4–6 month old unstimulated rats or into similar rats on the third and fourth days after primary or secondary injections of 50 μg of *Salmonella adelaide* flagella[1] In subsequent experiments 2–3 month old rats were similarly injected with tritiated thymidine for the first 4 days after secondary stimuli with 0 10 or 0 25 ml of typhoid–paratyphoid A and B vaccine (TAB vaccine)[2,3] Thirty or 31 days after the last dose of tritiated thymidine had been given, rats of the latter group were exposed to 850 rads of X-irradiation[2], or to progressively increasing daily doses of prednisone, cyclophosphamide, 6-mercaptopurine or actinomycin *D* (ref 3) Pairs of rats were killed at various times after labelling with tritiated thymidine, 3 days after X-irradiation, and at intervals during drug administration Autoradiographs of smears of cells which had been teased from each popliteal node were prepared using Kodak *AR* 10 stripping film and Giemsa stain

For the studies which are reported here mast cells were identified on each autoradiograph by the unique colour of the cytoplasmic granules The labelling index for mast cells—that is, the percentage of mast cells with five or more grains/nucleus—was determined by counting fifty or one hundred cells on each smear Individual grain counts of thirty labelled mast cells/smear, or as many as could be found, occasionally less than ten, were used to determine collectively the median grain count for each identically treated group of rats For statistical analysis, the data were expressed as the ratio of labelled cells unlabelled cells, and the value of χ^2 was calculated from these ratios

Fig 1 shows the ranges and mean values for the labelling index of mast cells in popliteal nodes at various times after labelling in two different experiments When tritiated thymidine was given on the third and fourth days after a secondary stimulus of *Salmonella* flagella, the initial labelling index of the mast cells did not exceed 2 per cent Twenty-one days after the last dose of tritiated thymidine was administered, however, the labelling index increased to values as high as 22 per cent When tritiated thymidine was injected on days 1–4 after a secondary stimulus of TAB vaccine, the initial labelling indices were the highest which were found and exceeded any of those of the first experiment In both experiments, labelled mast cells were found months after the last dose of tritiated thymidine had been given, and in each case, after the initial 2 weeks, the median grain counts of the labelled mast cells decreased only slightly, indicating a stable, non-dividing population

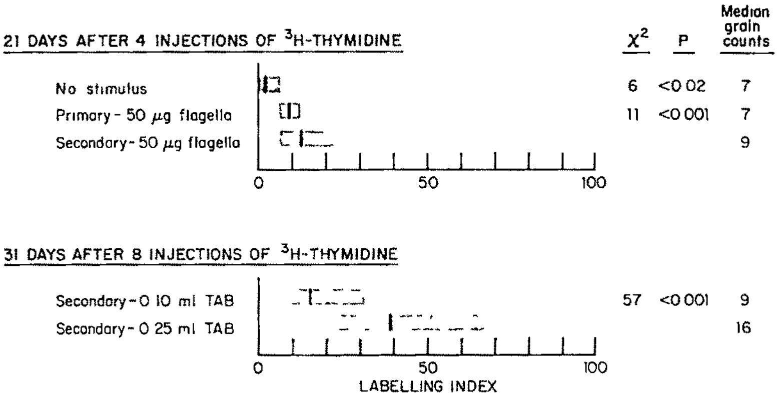

Fig 2 Histogram showing the ranges and mean values of the labelling index of mast cells in the popliteal lymph nodes after different types and degrees of immunological stimulation and after the two different courses of tritiated thymidine injections The values for χ^2 and P in the first group of experiments were calculated from the ratio of labelled unlabelled mast cells in the primarily stimulated lymph nodes compared with the non-stimulated lymph nodes, and from the secondarily stimulated lymph nodes compared with the non-stimulated nodes The median grain counts of the labelled mast cells which were present in the first experiment are considered to indicate similar degrees of labelling in each of the three groups

Fig 2 compares the range and mean values for the labelling index of mast cells 21 days after tritiated thymidine had been administered during a 2 day course when all values were highest in this experiment There were significantly greater proportions of labelled mast cells in nodes which were stimulated before labelling than in unstimulated nodes The difference between the labelling indices following a primary and those following a secondary stimulus was not significant Only one time point, 31 days after labelling, was available for comparing the labelling indices of mast cells after different secondary doses of TAB vaccine had been given A significant increase in the proportion of mast cells which were labelled after a stimulus of 0 25 ml of TAB vaccine was found compared with the proportion which were labelled after a stimulus of 0 10 ml (Fig 2) There was also an increase in the median grain count with the larger dose of TAB vaccine, despite the fact that an identical dose of tritiated thymidine had been given

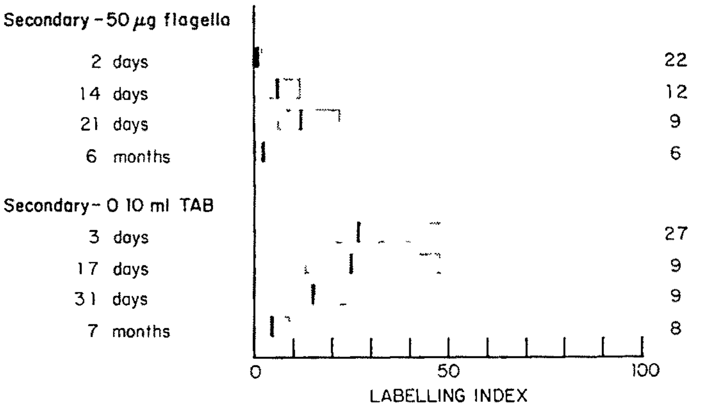

Fig 1 Histogram showing the ranges (shaded areas) and means (dark lines) of the values of the labelling indices of mast cells in popliteal lymph nodes at various times after the last dose of tritiated thymidine was administered in two experiments In the first experiment the tritiated thymidine was given in four injections in each foot pad on the third and fourth days after a secondary stimulus of 50 μg of *Salmonella* flagella In the second experiment the tritiated thymidine was given in eight injections in each foot pad on days 1–4 following a secondary stimulus of 0 10 ml of TAB vaccine The median grain counts of the labelled mast cells which were present at each time are also shown and are discussed in the text

The labelling index of mast cells in popliteal nodes of rats 33 days after labelling and 3 days after exposure to 850 rads of X-irradiation was significantly decreased in comparison with values from non-irradiated, control rats (Fig 3) Because the labelling index does not represent absolute numbers, it is not known whether the decrease in labelling index indicates radio-sensitivity of the labelled, long-lived mast cells or proliferation of new, unlabelled cells Fig 3 also shows that treatment of rats for up to 4 weeks with prednisone, cyclophosphamide, 6-mercaptopurine or actinomycin *D* did not change the labelling indices of mast cells At this time all the rats had signs of drug toxicity[3]

This work shows that mast cells actively proliferate after antigenic stimulation and that this proliferation is at least partly dependent on the degree of the stimulus The data also confirm, however, the earlier reports that mast cells, once mature, are long-lived and stable[5,6]. The labelling index and median grain counts of the mast cells decreased only over long periods of time, and none of the cytotoxic drugs, only X-irradiation, caused any change in the labelling index

Combs *et al* [7] have shown that mast cells in the foetal rat proliferate both from unidentified precursor cells and by mitosis of identifiable, but immature, mast cells Our data suggest that two mechanisms for mast cell proliferation could also exist in adult rats The delay of 21 days before the appearance of maximum labelling index following an injection of *Salmonella* flagella is best explained by assuming that cells which are not identifiable as mast cells were stimulated to divide and slowly differentiate The rapid appearance of large numbers of labelled mast cells after injection of TAB vaccine containing endotoxin, however, is more consistent with stimulation and division of mast cells themselves

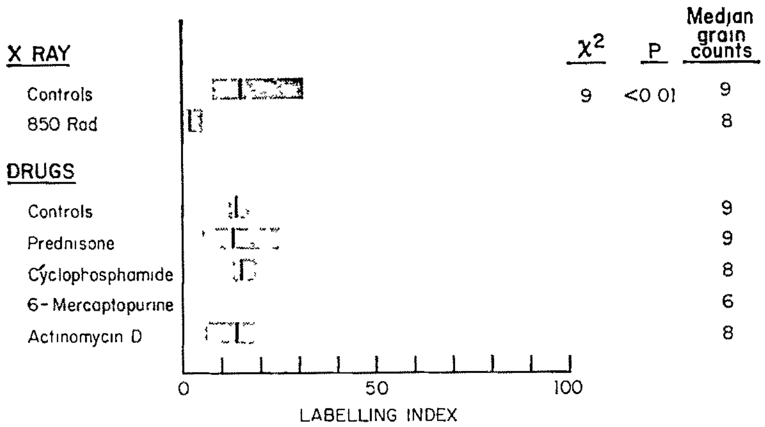

Fig 3 Histogram showing the ranges and mean values of labelling index of mast cells in popliteal lymph nodes after varied cytotoxic treatment The X-irradiation was given 30 days after irradiation The values for χ^2 and *P* were calculated for the ratios of labelled unlabelled cells in the control and irradiated groups of rats The drugs were given in progressively increasing doses intraperitoneally starting 30 days after labelling[3] The data shown are from rats which were killed after having received drugs for 4 weeks All the rats showed evidence of drug toxicity at this time The differences in labelling indices among the various drug-treated groups are not significant

In summary, the percentage of mast cells which were labelled after tritiated thymidine had been administered increased if the labelling followed antigenic stimulation This increase was in part dependent on the degree of the stimulus. The percentage of long-lived mast cells in lymph nodes decreased after X-irradiation, but not during treatment with several cytotoxic drugs

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[1] Miller, J J, III, *J Immunol*, 92, 673 (1964)
[2] Miller, J J, III, and Cole, L J, *J Immunol*, 98, 982 (1967)
[3] Miller, J J, III, and Cole, L J, *J Exp Med*, 126, 109 (1967)
[4] Allen, A M, *Anat Rec*, 139, 13 (1961)
[5] Walker, B E, *Nature*, 192, 980 (1961)
[6] Blenkinsopp, W K, *Nature*, 214, 930 (1967)
[7] Combs, J W, Lagunoff, D, and Benditt, E P, *J Cell Biol*, 25, 577 (1965).

Analysis of Antibodies against Synthetic Angiotensin II by the Passive Haemagglutination Technique

FOR passive haemagglutination studies, antigens are usually adsorbed to tanned[1], formalinized[2-5], glutaraldehyde-fixed[6] or chromium chloride-treated red cells[7] Alternatively, antigenic material can be covalently bound to native or fixed erythrocytes by various coupling reagents, including bis-diazotized benzidine[8-11], 1,3-difluoro-4,6-dinitrobenzene[12], tolylene-2,4-diisocyanate[13] and carbodiimide (CDI)[14].

Several of the techniques are rather elaborate and have many limitations and difficulties, and so we chose to assay haemagglutinating antibodies against synthetic angiotensin II The octapeptide was coupled to native sheep red cells by means of CDI Furthermore, immune agglutination of peptide-treated red cells was used to investigate antigenic determinants of angiotensin II

Sheep blood was collected in modified Alsever's solution (compare ref 16) Red cells (SRBC) were washed three times with phosphate buffered saline (PBS) and standardized photometrically To each of four tubes containing 24 8 ml of distilled water 0 2 ml of an approximately 25 per cent (v/v) SRBC suspension was added and the average absorbency ($A_{\bar{x}}$) was determined in an Eppendorf photometer (filter 546) Corrections for volume were made in accordance with the relationship $V_f = V_i\, A_x/0\ 350$ (V_f, final, V_i, initial volume)

Suspensions contained $4\ 4 \times 10^9$ cells/ml as determined by Coulter counting For haemagglutination tests, photometrically standardized suspensions were prepared to contain $2\ 4 \times 10^8$ cells/ml

Synthetic Asp $(\mathrm{NH_2})^1$-Val^5-angiotensin II ('Hypertensin', Ciba) (AII) was conjugated to native erythrocytes by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (CDI) (Ott Chemical Co) In multifactorial-type experiments optimal concentrations of SRBC, AII and CDI were determined, always using the same rabbit anti-AII serum pool CDI concentrations greater than 10 mg/ml caused haemolysis, whereas an excess of AII was associated with agglutination during coupling For a variety of concentrations of AII and a constant amount of CDI (10 mg/ml) a 25 per cent SRBC suspension yielded optimal conjugation conditions, judged by haemagglutinin titres The following procedure was finally adopted equal volumes of standardized 25 per cent cell suspension, AII (10 mg/ml PBS) and freshly dissolved CDI (10 mg/ml PBS) were added in this order, the mixture being kept at room temperature for 60 min with frequent stirring After incubation the cells were washed twice with EDTA. Cells coated with angiotensin and otherwise identically treated control cells without peptide, referred to as AII-CDI-SRBC and CDI-SRBC, respectively, could be stored for at least 15 days at 4° C without any detectable change in performance.

Haemagglutination was determined using the Takatsy outfit (Cooke Engineering Co.). To 0.025 ml. of serum, diluted in EDTA, 0.025 ml. of standardized (0.8 per cent) AII-CDI-SRBC or CDI-SRBC (in EDTA) was added. The plates were incubated overnight at 4° C. The extent of agglutination was scored from 4+ to negative[16]. Titres were expressed as logarithms to the base 2 of the reciprocal of the final dilution showing agglutination. All reactions were carried out in triplicate or quadruplicate and the following controls were always included: test sera were assayed against CDI-SRBC (containing no peptide), and the erythrocytes coated with peptides were tested with normal serum and with diluent only.

Antisera against AII were obtained after multiple injections of AII-CDI-protein conjugates incorporated in complete Freund's adjuvant[15,17] or after administration of AII-CDI-SRBC prepared as already described. Rabbits and chickens were injected on days 0, 1 and 10 with 1 ml. of a 10 per cent (v/v) cell suspension, given intravenously. Five days after the last injection the sera of both species exhibited specific anti-AII haemagglutinin titres of 5 and 6.

All the antisera used for haemagglutination were heat-inactivated and absorbed with SRBC and CDI-SRBC. Normal control sera were heat-inactivated and absorbed with SRBC only. Absorption of antisera with CDI-SRBC was essential, for haemagglutination regularly revealed antibodies to CDI or to CDI-erythrocyte determinants, whereas the presence of these antibodies could not be demonstrated immunochemically[15]. As compared with anti-AII, antibodies directed against CDI caused quantitatively and qualitatively different haemagglutination reactions. Instead of an evenly dispersed film of red cell agglutinates covering the bottom of the wells, 4+ patterns of anti-CDI were characterized by coarse cellular aggregates of different size and shape, localized in the central parts of the wells.

For haemagglutination inhibition tests, AII or its analogues and fragments were diluted in EDTA containing 1 per cent normal inactivated rabbit serum (NRS) which had been absorbed with SRBC. Two-fold serial dilutions of the peptide inhibitor were made in two groups of four rows each, each group beginning with a different concentration to give staggered endpoints. To 0.025 ml. of various dilutions of inhibitor, 0.025 ml. of rabbit anti-AII-CDI-protein diluted 1 : 64 in EDTA-NRS was added, the mixture was incubated for 30 min at 37° C. Finally, 0.025 ml. of a 0.8 per cent standardized AII-CDI-SRBC suspension was added and the plates were incubated overnight at 4° C. The smallest amount of AII, analogue or fragment, respectively, which caused complete inhibition of haemagglutination was considered to be the endpoint (extinction concentration, EC).

Results of inhibition tests are shown in Table 1. On a molecular basis, analogues or a fragment of AII differing from AII only in the N-terminal position 1 inhibited haemagglutination almost to the same extent as AII. The hexapeptide 3–8, lacking positions 1 and 2, and two analogues differing from AII in positions 4 and 5 showed a somewhat reduced inhibitory effect. On the other hand, the heptapeptide-amide 1–7, lacking the C-terminal phenylalanyl residue, inhibited haemagglutination to a much reduced extent.

Table 1. ANALYSIS OF ANTIBODIES AGAINST ANGIOTENSIN II BY HAEMAGGLUTINATION INHIBITION TESTS

Inhibitor	EC (p-mole)†	Relative EC‡
Asp $(NH_2)^1$ Val^5-angiotensin II*	2.9	1.0
β-L-Asp^1 Val^5-angiotensin II	3.9	1.3
β-D-Asp^1 Val^5-angiotensin II	5.2	1.8
$Succinyl^1$ Val^5-angiotensin II	5.5	1.9
Heptapeptide 2–8	5.8	2.0
Hexapeptide 3–8	15.4	5.3
Asp $(NH_2)^1$ Ile^5-angiotensin II	18.6	6.4
Asp $(NH_2)^1$ Phe^4-angiotensin II	18.5	6.4
Heptapeptide-amide 1–7	966.6	333.3

* Asp $(NH_2)^1$-Arg^2-Val^3-Tyr^4-Val^5-His^6-Pro^7-Phe^8 (Hypertensin, Ciba).
† Extinction concentration (10^{-12} mole).
‡ EC/EC_{AII}.

These results confirm previous findings[15,18] obtained by different techniques and stress again the great importance of the C-terminal phenylalanine and the relative unimportance of the N-terminal for immunological specificity.

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[1] Boyden, S. V., *J. Exp. Med.*, **93**, 107 (1951).
[2] McKenna, J. M., *Proc. Soc. Exp. Biol. and Med.*, **95**, 591 (1957).
[3] Ingraham, J. S., *Proc. Soc. Exp. Biol. and Med.*, **99**, 452 (1958).
[4] Csizmas, L., *Proc. Soc. Exp. Biol. and Med.*, **103**, 157 (1960).
[5] Daniel, T. M., Weyand, J. G. M., and Stavitsky, A. B., *J. Immunol.*, **90**, 741 (1963).
[6] Bing, D. H., Weyand, J. G. M., and Stavitsky, A. B., *Proc. Soc. Exp. Biol. and Med.*, **124**, 1166 (1967).
[7] Jandl, J. H., and Simmons, R. L., *Brit. J. Haematol.*, **3**, 19 (1957).
[8] Pressman, D., Campbell, D. H., and Pauling, L., *J. Immunol.*, **44**, 101 (1942).
[9] Coombs, R. R. A., Howard, A. N., and Wild, F., *Brit. J. Exp. Pathol.*, **33**, 390 (1952).
[10] Stavitsky, A. B., and Arquilla, E. R., *J. Immunol.*, **74**, 306 (1955).
[11] Gordon, J., Rose, B., and Sehon, A. E., *J. Exp. Med.*, **108**, 37 (1958).
[12] Ling, N. R., *Immunology*, **4**, 49 (1961).
[13] Gyenes, L., and Sehon, A. H., *Immunochemistry*, **1**, 43 (1964).
[14] Johnson, H. M., Brenner, K., and Hall, H. E., *J. Immunol.*, **97**, 791 (1966).
[15] Dietrich, F. M., *Immunochemistry*, **4**, 65 (1967).
[16] Dietrich, F. M., *Immunology*, **10**, 365 (1966).
[17] Hedwall, P. R., and Dietrich, F. M., *Experientia* **23**, 952 (1967).
[18] Goodfriend, T., Fasman, G., Kemp, D., and Levine, L., *Immunochemistry*, **3**, 223 (1966).

Mechanisms in Early and Late Phases of Tolerance induced by Parabiosis in Adult Mice

PARABIOSIS between *CBAT6T6* inbred mice with the $(CBA \times C3H)F_1$ hybrid $(=T6\sim F_1)$ ($\sim$ is the symbol for parabiosis) gives considerable latitude in the study of the early phase of non-reactivity (specific immunological tolerance) induced by antigen because the cross circulation develops slowly between the partners[1] and the *CBA* and *C3H* inbred mouse strains from which the partners are derived are identical at the *H-2* histocompatibility locus. This constitutes a weak antigenic combination and, in conjunction with the gradual appearance of the cross circulation, the resulting (one-way[2]) immunological reaction between the partners is mild, so that many survive for subsequent study of the mechanism by which tolerance of the foreign antigens present in the hybrid partner is induced in the parental partner.

Clearance of $(CBA \times C3H)F_1$ hybrid erythrocytes labelled with chromium-51 suggested that the parental partner was sensitized to the *C3H* antigens of the F_1 hybrid partner early in parabiosis and before tolerance (judged by skin graft survival, to be discussed later) appeared. $(CBA \times C3H)F_1$ hybrid blood was washed once in phosphate buffered saline and 0.5 ml. of sodium chromate-51 isotope, with 0.05 mc. of activity/ml., was added to 0·5 ml. of packed cells and incubated at 37° C for 20 min. The cells were washed three times in phosphate buffered saline to remove free label and 0.04 ml. of labelled cells was injected intravenously into the parental partner. Equilibrium between the two partners appeared within a few hours. Samples of tail blood (0.02 ml. from each) were taken on alternate days, lysed in distilled water and the activity of both samples was measured in a well-type scintillation counter. The sum of each was expressed as percentage decay compared with the sum of a sample

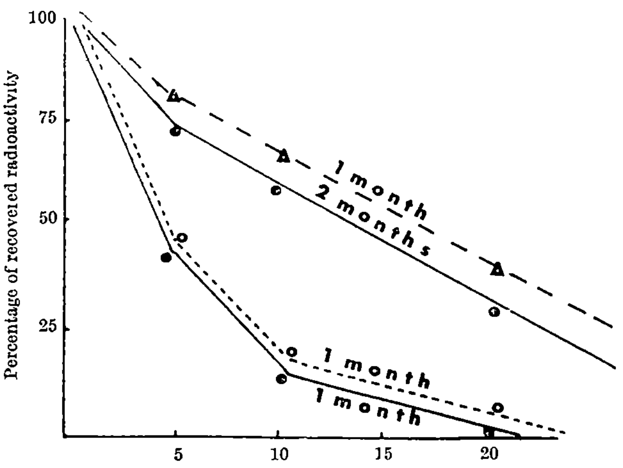

Fig 1 Elimination of chromium-51 labelled F_1 hybrid erythrocytes in parabiosis ●—●, $T6 \sim F_1$, ○ - - - ○, $T6 \sim T6$, △ - - - △, $F_1 \sim F_1$

taken 10 min after the first injection Convincing arguments against re-utilization of isotope complicating the issue have been put forward by Bainbridge *et al* [3]

In syngeneic $F_1 \sim F_1$ parabionts, elimination was always as expected from the known time of survival of mouse erythrocytes (28–35 days) Syngeneic $T6 \sim T6$ partners eliminated most of the labelled cells by 10 days In $T6 \sim F_1$ partners in parabiosis for 1 month, elimination (which presumably occurred in the parental partner) was the same as in $T6 \sim T6$ syngeneic parabionts After 2 months in parabiosis, elimination was the same as for $F1 \sim F1$ parabionts (Fig 1)

Sensitization in mice to foreign transplantation antigens to which they have previously been exposed can be detected by the accelerated rejection of skin grafts of the same genotype as the sensitizing antigen Mice were therefore parabiosed and separated at short intervals and the parental partner grafted with F_1 hybrid skin After a month in parabiosis, *C3H* parental partners rejected F_1 hybrid skin grafts in 9 days, which is the same as the second set rejection in single sensitized *C3H* mice (Table 1) It was not possible to demonstrate sensitization by accelerated skin graft rejection in *CBAT 6T6* parental partners Thus the sequence of events in both these experimental systems supports the suggestion that the parental partner goes through a period of sensitization to the foreign transplantation antigens of the hybrid partner (to which they subsequently become tolerant) during the early phases of parabiosis by recirculation of parental lymphoid cells which have been sensitized in the F_1 hybrid partner[4,5]

Table 1 REJECTION TIMES IN DAYS (EP) OF *CBAT6T6* AND *C3H* PARENTAL PARABIONTS GRAFTED WITH $(CBA \times C3H)F_1$ HYBRID SKIN

Each group represents 5 to 10 mice

Days in parabiosis with F_1 hybrid	*CBA* EP	*C3H* EP
3	11	12
6	13	10
14	17	10
22	17	9
28	> 190	—
40	> 129	10
53	—	> 133

		First set	Second set
Rejection times in parental strain mice	*CBA*→*C3H*	12	9
	C3H→*CBA*	11	8

Sensitization of *CBAT6T6* parental partners was studied further by estimating the "immunological potential" of spleen cells from parabionts after various times in parabiosis in lethally irradiated $(CBA \times C3H)F_1$ hybrids It was argued that sensitized *CBAT6T6* parabiont cells would cause rapid death by the severity of the graft versus host reaction No antibiotics were given to the irradiated mice and only intravenously injected spleen cells which are well known to contain all the necessary stem cells were used to restore them Several experiments tended to show that reactive parental cells were sensitized in the hybrid partner in the early phase of parabiosis and rendered tolerant in the later phase

Table 2 shows the effect of treatment with spleen cells from highly tolerant parabionts on lethally irradiated hybrids compared with similar hybrids treated with normal parental and hybrid spleen cells in doses of 1×10^6 and 5×10^6 cells By 14 days a graft versus host reaction had killed nearly all the hybrids treated with normal *CBAT6T6* spleen cells at both doses Those given 1×10^6 cells just managed to survive for 14 days and because it was clear they would not live for longer than a day or so they were killed at this time Mortality was eliminated with spleen cells from tolerant and F_1 hybrid mice and none died during the following 10 weeks Table 3 gives results of another experiment showing a similar pattern of long term survival of the restored irradiated hybrids, but in this case several irradiated F_1 hybrids given normal *T*6 spleen cells survived, probably because of the weak antigenic combination In this situation, secondary irradiation disease need not always be uniformly lethal[6]

Table 2 POTENTIAL OF GRADED DOSES OF PARABIONT SPLEEN CELLS IN RESTORING LETHALLY (1,000 R) IRRADIATED F_1 HYBRIDS

Survival/number at 14 days

No of cells	Source of spleen cells		
	Tolerant	Normal	Normal F_1
1×10^6	7/8	6/6*	5/5
5×10^6	9/9	1/11	5/5

* All moribund

Table 3 WEEKLY SURVIVAL OF IRRADIATED F_1 HYBRIDS RESTORED WITH SPLEEN CELLS FROM TOLERANT, NORMAL *CBAT6T6* AND F_1 HYBRID MICE

Weeks	0	1	2	3	4	5	6	6+
Tol	20	20	19	14	14	14	14	14
*T*6	15	14	8	8	6	6	6	5
F_1	15	15	15	15	15	15	15	15

5×10^6 spleen cells were injected intravenously.

The effect of *CBAT6T6* parabiont spleen cells after 9, 17 and 28 days in parabiosis in irradiated F_1 hybrids is shown in Table 4 No deaths occurred in the hybrid hosts treated with 9 day parental spleen cells Apart from the considerations about the mortality in irradiation chimaeras mentioned here, it is tempting to suggest that this represents a low zone of dosage causing paralysis as in other systems[7-9] The induction of tolerance in the later stages of parabiosis would then represent the high zone of dosage following a period of sensitization Spleen cells from 17 day old parental parabionts caused a 100 per cent mortality by 2 weeks, undoubtedly because of parental lymphoid cells sensitized against the *C3H* element in the hybrid which have recirculated to the parental partner Elimination of mortality after 28 days in parabiosis suggests that tolerance is appearing

Table 4 WEEKLY SURVIVAL OF IRRADIATED F_1 HYBRIDS RESTORED WITH SPLEEN CELLS FROM *CBAT6T6* PARABIONTS, SEPARATED AT INTERVALS OF 9, 17 AND 28 DAYS

Days in parabiosis	Weeks 0	1	2	3	4	5	6	6+
9	5	5	5	5	5	5	5	4
17	10	10	5	0				
28	5	5	5	5	5	5	5	5

It is concluded that in the strain combinations used here the parental partner becomes sensitized to hybrid antigens before the state of tolerance appears Other workers have shown that single post-natal mice injected with foreign cells go through a phase of sensitization before becoming tolerant[10,11] Further experiments are being conducted in parabiosis systems (transference of the tolerant state, for example) to try to find out whether the tolerant state is caused by elimination or paralysis of reactive lymphoid cells

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[1] McBride, R A , Nisbet, N W , and Skowron-Cendrzak, A , *Transplantation*, 5, 569 (1967)

[2] Simonsen, M , in *Progress in Allergy* (edit by Kallos, P , and Waksman, B H), 349 (Karger, Basel and New York, 1962)

[3] Bainbridge, D R , Brent, L , and Gowland, G , *Transplantation*, 4, 138 (1966)

[4] McBride, R A , and Simonsen, M , *Transplantation*, 3, 140 (1965)

[5] Nisbet, N W , *Nature*, 214, 94 (1967)

[6] Micklem, H S , and Loutit, J F , in *Tissue Grafting and Radiation* (Academic Press, New York and London, 1966)

[7] Dresser, D W , *Immunology*, 5, 378 (1962)

[8] Mitchison, N A , *Proc Roy Soc* , B, 161, 275 (1964)

[9] Bainbridge, D R , and Gowland, G , *Ann NY Acad Sci* , 129, 257 (1966)

[10] Brent, L , and Gowland, G , *Nature*, 196, 1298 (1962)

[11] Gowland, G , *Brit Med Bull* , 21, 123 (1965)

MICROBIOLOGY

In vitro Agglutination of Bovine Erythrocytes infected with *Babesia argentina*

THE presence of antibodies capable of agglutinating intracellular schizonts of *Plasmodium knowlesi* has been reported[1] and a parasite agglutination test described[1-2] This communication reports a similar test for *Babesia argentina* using heavily parasitized bovine erythrocytes

Splenectomized calves, 3–6 months old, were infected with *B argentina* by intravenous inoculation of blood from carrier cattle When 2 per cent of the erythrocytes in jugular blood were infected, blood was collected in bottles containing EDTA (disodium salt of ethylenediamine tetraacetic acid) as anticoagulant If the peak parasitaemia was below this level, blood was transferred to another splenectomized calf and the procedure repeated until the 2 per cent parasitaemia was obtained

One per cent erythrocyte suspensions in physiological saline were prepared which contained approximately 80 per cent of their cells infected with *B argentina* The infected cells were first concentrated from parasitized blood by a method described by Mahoney[3] Appropriate control suspensions were also prepared from normal blood taken from each calf before infection These suspensions of infected and uninfected cells were used as antigens for the agglutination test

The agglutination test was conducted in 'Perspex' haemagglutination trays Sera were diluted 1/5 in physiological saline containing 1 per cent normal calf serum and heated at 56° C for 30 min Doubling dilutions of serum were prepared and to 0 2 ml of each dilution an equal volume of the infected cell suspension was added A control test using the uninfected cell suspension was included with each serum The plates were shaken and allowed to stand at room temperature for 3 h

Reactions were read macroscopically and microscopically (× 100), the latter after resuspending by gentle shaking. Macroscopic reactions were graded as follows + + + +, a mat of cells completely covering the bottom of the cavity without any ring of cells around its periphery The surface had a granular appearance and the edges of the mat were sometimes folded, + + +, similar to the + + + + reaction but with a thin ring of cells around the periphery, + +, a distinct ring of cells with only a slight granular appearance, +, a thick ring of cells with a diameter approximately half that of the mat in the + + + + reaction, ± or –, a discrete button or thick ring of cells was present

The titre of the serum was taken as the highest dilution showing a + + reaction macroscopically

Microscopically the amount of agglutination was estimated at the 1/5 dilution as follows + + + +, many clumps of over 200 cells, + + +, many clumps of over 50 cells, + +, less numerous clumps of over 20 cells, +, odd clumps of less than 20 cells

Parasites from five isolations of *B argentina* from geographically separated natural outbreaks in New South Wales were used to prepare suspensions Isolates were maintained in individual carriers at the laboratory and designated with the name of the locality from which they came (Table 1) A sixth isolate (Gayndah) was obtained from Dr D F Mahoney, CSIRO, Yeerongpilly Occasionally before inoculation, parasites were preserved by freezing infected whole blood to –79° C and storing at this temperature

Antisera were collected from the same six carrier animals used to provide isolates for suspensions but were taken 2 to 6 weeks later They were stored at –20° C without preservative

The results of testing these suspensions with homologous and heterologous antisera are shown in Table 1. With each suspension the highest titre and greatest degree of clumping was obtained with the homologous antiserum Reactors with the heterologous antisera were slight.

Preliminary studies on the specificity of the reaction have been undertaken Of 450 non-infected animals tested with the Gayndah isolate suspension only four (0 9 per cent) showed any agglutination at the 1/10 dilution and only one (0 2 per cent) at the 1/20 dilution The greatest amount of clumping recorded was + + at the 1/5 dilution with one serum Similar results were obtained with 100 of these sera when tested with the other five isolate suspensions

The reaction appeared to be highly specific for each isolate The isolates used were possibly antigenic variants of one or more strains of *B argentina* arising by a process similar to that demonstrated by the agglutination test in chronic *P knowlesi* infections[2] The test described here is at present being used to investigate the possibility of similar antigenic variation in *B argentina* infections If basic strain antigens can be identified and strains of the parasite classified, the technique would be valuable in epidemiological studies

The effect of these agglutinating antibodies on immunity also needs to be investigated Agglutinins in *P knowlesi* were considered to be important in terminating relapses, but another immune response was also thought to be present and partially able to inhibit relapses[2] A similar state of affairs may exist with *B argentina* infections

Table 1 AGGLUTINATION TEST RESULTS OF SERA FROM SIX CATTLE INFECTED WITH SEPARATE ISOLATES OF *Babesia argentina* AGAINST ANTIGENS FROM THE SAME ANIMALS

		Antigen					
	Isolate	Gayndah	Cougal	Piggabeen	Dalguigan	Wiangaree	Roseberry
Serum	Gayndah	*1,250 (+ + + +)†	0 (–)	0 (–)	40 (+ +)	0 (–)	0 (–)
	Cougal	160 (+ +)	5,000 (+ + + +)	40 (+)	20 (+)	10 (+)	40 (+)
	Piggabeen	0 (–)	0 (–)	640 (+ + + +)	10 (+)	5 (+ +)	5 (+)
	Dalguigan	20 (+ +)	—	—	1,250 (+ + +)	0 (–)	40 (+ +)
	Wiangaree	0 (–)	0 (–)	0 (–)	0 (–)	320 (+ + + +)	0 (–)
	Roseberry	10 (+)	20 (+ +)	0 (–)	20 (+)	0 (–)	5,000 (+ + + +)

* Reciprocals of agglutination test titres

† Size and number of agglutinated clumps at the 1/5 serum dilution

It would seem that the development of an effective vaccine able to produce a sterile immunity for *B argentina* would depend on the importance of these antigens and their variability.

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[1] Eaton, M D , *J Exp Med* , 67, 857 (1938)
[2] Brown, K N , and Brown, I N , *Nature*, 208, 1286 (1965)
[3] Mahoney, D F , *Exp Parasitol* , 20, 232 (1967)

CYTOLOGY

Growth-promoting Effect of Lysolecithin on Chinese Hamster Cells *in vitro*

WHILE the importance of amino-acids[1,2], salts[3] and vitamins[4] for growth of mammalian cells in culture is well established, the role of lipids remains uncertain With the exception of free fatty acids[5], no naturally occurring lipids such as glycerides, phospholipids or cholesterol have been shown to promote growth of mammalian cells *in vitro*

Lysolecithin was until recently considered to be a toxic haemolysing substance generated in the blood by the action of snake venom In 1960, however, Lands[6] showed that lysolecithin was an intermediate in the synthesis of lecithin in rat liver homogenates Further, lysolecithin was found in both rat[7] and man[8] to be a normal constituent of plasma and to be associated exclusively with the albumin fraction[9] Stein and Stein[10,11] then showed that albumin-bound lysolecithin was incorporated by intact cells and utilized in the synthesis of lecithin

I have found that albumin-bound lysolecithin has a definite growth-promoting effect on Chinese hamster cells in culture The effect seems to be caused by the uptake of intact lysolecithin and not by breakdown products

The lysolecithin (Koch-Light Laboratories, Ltd), a 1-acyl phosphatidylcholine prepared from egg lecithin by degradation with snake venom phospholipase, was shown to be pure by thin layer chromatography Analysis by gas-liquid chromatography showed that 94 per cent of the fatty acid moiety was saturated, the principal components being palmitic acid (71 per cent) and stearic acid (21 per cent) The unsaturated acids (6 per cent) were chiefly oleic (2 per cent) and linoleic acid (2 per cent) Bovine plasma albumin (Armour, Fraction V) was extracted by the method of Goodman[12] and a lysolecithin-albumin complex was prepared and purified by the method of Stein and Stein[13]. The lysolecithin content of the albumin was determined by thin layer chromatography of the albumin phospholipids followed by phosphorus analysis[14]

Chinese hamster cells, line *CHEF*, were routinely grown in the Dulbecco-Vogt modification of Eagle's medium[15] supplemented with 20 per cent calf serum Experimental cultures were inoculated with 8×10^4 cells in each Petri dish (diameter 32 mm) in 1 5 ml of medium and allowed to attach After 4 h the medium was removed and the experimental medium added The plates were then incubated for 3 days Cell numbers were determined at the beginning and at the end of this period by counting the cells within five squares etched into the bottom of the plates

The experimental medium was identical with the usual medium except that it was free of serum and contained 1 per cent extracted albumin with and without added lysolecithin The final concentration of lysolecithin was 75 µmoles/l of medium, and the molar ratio of lysolecithin albumin was 1 2 (assumed molecular weight of albumin 67,000) Without addition the concentration of lysolecithin in the medium was less than 1 µmole/l

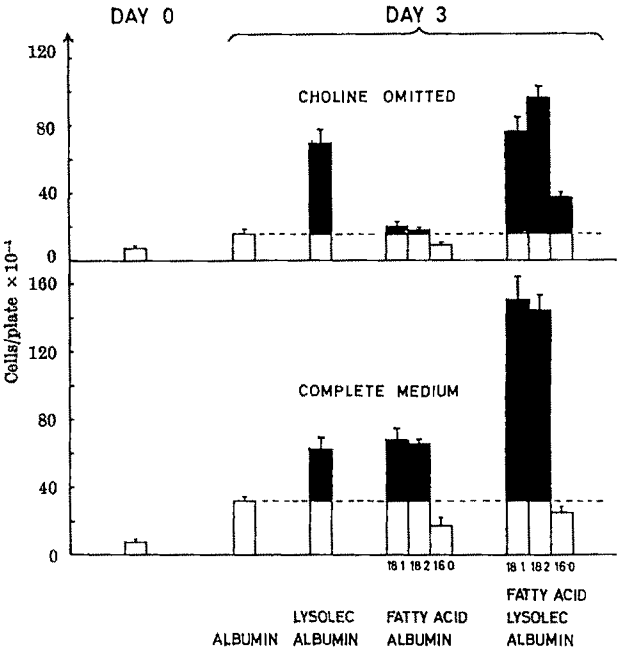

Fig 1 Effect of albumin-bound lysolecithin and fatty acids on growth of Chinese hamster cells *in vitro* The columns represent the average of four experiments each including eighteen plates, the vertical bars being the standard error of the mean The black portion of the columns indicates the increase in cell number due to the presence of albumin-bound lipid Oleic (18 1), linoleic (18 2) and palmitic (16 0) acids are designated by the number of carbon atoms and double bonds in the molecule[18]

The addition of lysolecithin to the medium (Fig 1) increased growth significantly ($P < 0\ 01$) This effect was evident both in the presence and absence of choline, but the effect was greater when choline was omitted from the medium

Fatty acids have been shown to promote growth of cells in culture[5] and some cells have lysolecithinases (acyl hydrolases) associated with their surfaces[11,16], so the question arose as to whether the growth-promoting effect was caused by free fatty acids liberated by the action of lipase or to the uptake of lysolecithin as such To examine this question individual high purity fatty acids (Applied Science Laboratories, Inc) were tried as substitutes for lysolecithin The fatty acids were combined with extracted albumin and their concentration determined by titration[17] The final medium contained 75 µmoles of free fatty acids/l , while without additions the concentration was less than 3 µmoles/l

In the presence of choline, the unsaturated acids, oleic and linoleic, stimulated growth markedly, whereas the saturated palmitic acid inhibited growth (Fig 1) About 94 per cent of the fatty acids of lysolecithin were saturated, and more than 70 per cent was palmitic acid, so that it seems unlikely that the observed effect of lysolecithin could be caused by its fatty acid moiety in the form of free fatty acid Furthermore, the maximal concentration of unsaturated free fatty acids attainable, if all the lysolecithin were hydrolysed, was found to be too low to promote growth (my unpublished results)

Lysolecithin has been found to be an intermediary in the synthesis of lecithin in other cells, and so these results suggest that lysolecithin stimulates growth by promoting synthesis of lecithin The following observation seems to support this hypothesis. Equimolar amounts of lysolecithin together with oleic or linoleic acid, in the complete medium, stimulated growth to a degree that exceeded the sum of their individual effects ($P < 0{\cdot}01$ by paired comparison) (Fig 1) This synergism suggests that lysolecithin and unsaturated fatty acids have a common mechanism of action. By direct combination, that is by acylation of lysolecithin as described by Lands, a diacylphosphorylcholine would be formed with a saturated 1-acyl and an unsaturated 2-acyl group, which is the naturally occurring form of lecithin. The inhibitory effect of palmitic acid suggests that the cells have a very limited capacity for desaturating fatty acids.

The notion that lecithin synthesis was important for cell proliferation was further supported by the following finding. The two unsaturated fatty acids caused increased growth only when choline was present—that is, when lecithin synthesis was possible, but not when lecithin synthesis was limited by the omission of choline from the medium (Fig. 1). Whether lecithin itself, when added to the medium, can promote growth is under investigation.

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[1] Eagle, H., *J. Biol. Chem.*, **214**, 839 (1955).
[2] Levintow, L., Eagle, H., and Piez, K. A., *J. Biol. Chem.*, **227**, 929 (1957).
[3] Eagle, H., *Arch. Biochem. Biophys.*, **61**, 356 (1956).
[4] Eagle, H., *J. Exp. Med.*, **102**, 595 (1955).
[5] Ham, R. G., *Science*, **140**, 802 (1963).
[6] Lands, W. E. M., *J. Biol. Chem.*, **235**, 2233 (1960).
[7] Newman, H. A. I., Lin, C. T., and Zilversmit, D. B., *J. Lipid Res.*, **2**, 403 (1961).
[8] Phillips, G. B., *Proc. US Nat. Acad. Sci.*, **43**, 566 (1957).
[9] Switzer, S., and Eder, H. A., *J. Lipid Res.*, **6**, 506 (1965).
[10] Stein, Y., and Stein, O., *Biochim. Biophys. Acta*, **116**, 95 (1966).
[11] Stein, O., and Stein, Y., *Biochim. Biophys. Acta*, **137**, 232 (1967).
[12] Goodman, D. S., *Science*, **125**, 1296 (1957).
[13] Stein, Y., and Stein, O., *Biochim. Biophys. Acta*, **106**, 527 (1965).
[14] Parker, F., and Peterson, N. F., *J. Lipid Res.*, **6**, 455 (1965).
[15] Eagle, H., Oyama, V. I., Levy, M., and Freeman, A. E., *J. Biol. Chem.*, **227**, 929 (1957).
[16] Munder, P. G., Ferber, E., and Fischer, H., *Z. Naturforsch.*, **20** *b*, 1048 (1965).
[17] Dole, V. P., and Meinertz, H., *J. Biol. Chem.*, **235**, 2595 (1960).
[18] Dole, V. P., James, A. T., Webb, J. P. W., Rizack, M. A., and Sturman, M. F., *J. Clin. Invest.*, **38**, 1544 (1959).

Odontogenesis: Cell–Cell Interactions *in vitro*

EMBRYONIC interactions of epithelial and mesenchymal tissue before organogenesis have been observed in several organ systems[1-4]. These phenomena have been tested by isolating embryonic tissues from their *in vivo* environment and transplanting them in an artificial environment from which no morphogenetic signals can be received. The chorio-allantoic membrane (CAM) of chick provides a versatile site for the maintenance and further differentiation of embryonic organs[5,6]. One such organ, the tooth primordium, arises as a result of interactions between epithelial and mesenchymal tissues and offers a model system for developmental studies.

Recently, studies of the trans-filter interaction of embryonic rodent incisor tissues have shown that overt differentiation occurs in both separated epithelial and mesenchymal tissues[7]. These results and those of numerous other investigators[8-12] suggest that the interaction of epithelial and mesenchymal tissues is essential for tooth formation.

To examine the morphogenetic potential of cells from these tissues, an experiment was designed to evaluate cell–cell interactions before histogenesis. Maxillary incisors were removed from the embryos of New Zealand white rabbits at the twentieth day of gestation. At this stage of tooth development both enamel and dentine formation have begun in the coronal portion of the organ, and most tissues which have been formed show various degrees of differentiation (Fig. 1).

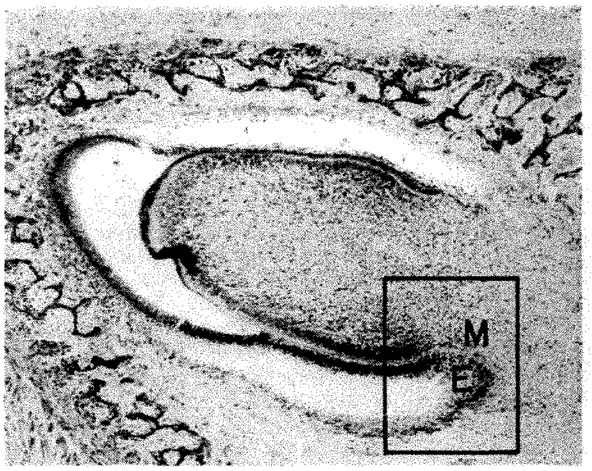

Fig. 1. Maxillary incisor in the embryo of New Zealand white rabbit at day 20 of gestation before excision. The whole tooth rudiment is encapsulated in alveolar bone. The rectangle contains the cervical loop region from which dissociated inner enamel epithelium and mesenchymal papillae cells were obtained. *E*, Epithelial cells; *M*, mesenchymal cells (× 50).

Apical or cervical regions of these incisor tooth germs were excised. The isolated cervical regions were trypsinized to dissociate the inner enamel epithelium from the mesodermal papilla by the methods of Moscona[13]. The isolated epithelial and mesenchymal tissues were further treated with 0·4 per cent trypsin to obtain single cell suspensions. The isolated cells, in a calcium-magnesium free phosphate buffered saline at *p*H 7·2, were pipetted onto the CAM of 8-day embryonated hens' eggs and incubated at 37·5° C for periods of up to 10 days. Three sets of experiments (twenty grafted eggs in a group) were used to test epithelial cells only, mesenchymal cells only and the interaction when both cell types were mixed together in culture. Tissue and cell dissociations were monitored by phase microscopy in order to ensure that there were relatively clean cell suspensions of both inner enamel epithelium and mesodermal papillae. At various periods during the experiment, xenogeneic cell preparations were dissected with the adjacent CAM and were immediately placed in Hollande-Bouin's solution. The tissues were sectioned at 5μ and stained with periodic acid–Schiff's (PAS) reagent. There is a variety of cell types within the original separated tissue preparations; it might therefore be anticipated that these originally dissimilar cells would display different patterns of mobility, differentiation and survival. The test for viability of the xenogeneic cells in these culture conditions was based upon the frequency of mitotic figures. Additional quantitation of the surviving epithelial and mesenchymal cell suspensions before and after the experiment has not yet been performed. The viability of the host chick embryo was determined by daily candling.

A positive interaction is considered to be represented by one or both cell types undergoing morphological differentiation.

When either one of the cell suspensions was cultivated independently on the CAM, no indication of tooth formation was observed (Fig. 2). Both homogeneous xenograft groups exhibited a continued proliferation of cells, reflected by numerous mitotic figures. The isolates appeared as randomly dispersed cells with neither apparent orientation nor continued differentiations; no re-aggregation of the isolates was observed.

When both cell preparations were mixed and suspended on the CAM, an induction was observed which resulted in the initiation of tooth formation (Fig. 3). A well defined PAS-positive interface, analogous to that reported to occur *in vivo*[14-15], separated the interacting cells. In several xenografts within this group, the cell–cell interaction was preceded by the bud stage of tooth development. The similarity between *in vivo* and *in vitro* development is shown by condensation of the mesenchymal cells, a specific PAS-positive future dentino-enamel junction, and regularity of the tall columnar inner enamel epithelial cells. The temporal differentiation of these epithelial cells is shown *in vivo* and *in vitro* by differentiation of squamous cells into cuboidal then columnar inner enamel epithelial cells with a subsequent alteration in nuclear polarity. The differentiation of the mesodermally derived odontoblasts is undoubtedly related to interaction with the inner enamel epithelium. Earlier workers had postulated this fundamental relationship, primarily because of the observed differentiation of inner enamel epithelium into ameloblasts before differentiation of odontoblasts[16-18]. The maximum duration of these experiments (10 days) might not allow sufficient time for the mesenchymal cells to differentiate into odontoblasts *in vitro*.

The analysis of these events during tooth development *in vitro* shows that the morphogenetic potential is closely related to the properties of both interacting cell types, not exclusive to either one. Thereafter, tooth formation must be regarded as a complex temporal differentiation involving both types of interacting cells. After re-aggregation, the events *in vitro* show a striking developmental similarity to those occurring *in vivo*.

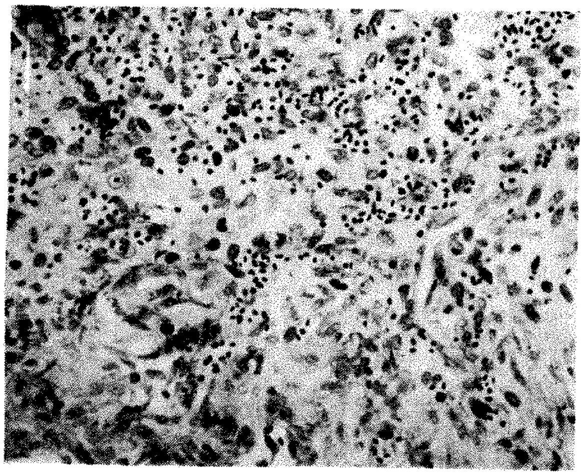

Fig. 2. Mesenchymal cell suspension explanted onto the CAM for 10 days of cultivation. The isolated cell suspensions of either mesenchymal or epithelial tissue fail to initiate tooth formation and do not re-aggregate or orientate when cultivated on the CAM (× *c*. 370).

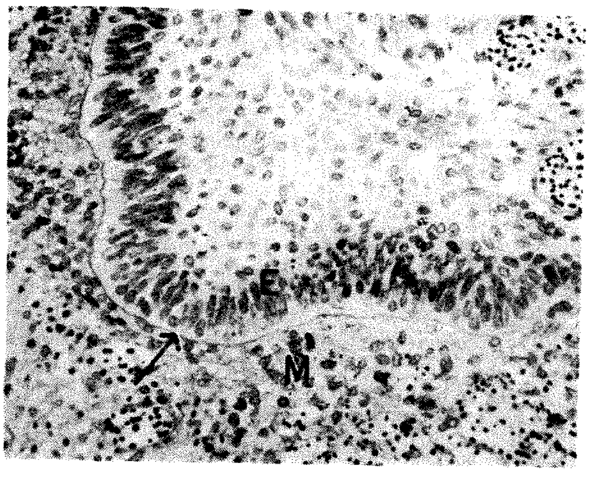

Fig. 3. When both cell preparations were mixed and cultivated for 10 days on the CAM, tooth formation was initiated. *E*, Inner enamel epithelium; *M*, mesenchymal papilla. Arrow points to PAS-positive future dentino-enamel junction (× *c*. 370).

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[1] Saunders, J. W., Cairns, J. M., and Gasseling, M. T., *J. Morphol.*, **101**, 57 (1957).
[2] Rawles, M. E., *J. Embryol. Exp. Morphol.*, **11**, 765 (1963).
[3] Auerbach, R., *Develop. Biol.*, **2**, 271 (1960).
[4] Grobstein, C., *J. Exp. Zool.*, **124**, 383 (1953).
[5] Glasstone, S., *J. Anat.*, **88**, 392 (1954).
[6] Slavkin, H. C., and Bavetta, L. A., *Experientia* (in the press).
[7] Koch, W., *J. Exp. Zool.*, **165**, 155 (1967).
[8] Hay, M. F., *Arch. Oral Biol.*, **3**, 86 (1961).
[9] Lefkowitz, W., Bodecker, C. F., and Mardfin, D. F., *J. Dent. Res.*, **32**, 749 (1953).
[10] Paynter, K. L., and Hunt, A. M., *Arch. Oral Biol.*, **9**, 611 (1964).
[11] Pourtois, M., *J. Embryol. Exp. Morphol.*, **12**, 391 (1964).
[12] Slavkin, H. C., and Bavetta, L. A., *Arch. Oral Biol.* (in the press).
[13] Moscona, A. A., *Exp. Cell Res.*, **22**, 455 (1961).
[14] Hunt, A. M., and Paynter, K. J., *Arch. Oral Biol.*, **8**, 65 (1963).
[15] Zussman, W. V., *Oral Surg., Oral Med. and Oral Path.*, **21**, 217 (1966).
[16] Glasstone, S., *Proc. Roy. Soc.*, B, **126**, 315 (1938).
[17] Huggins, C. B., McCarroll, H. R., and Dahlberg, A. A., *J. Exp. Med.*, **60**, 199 (1934).
[18] Fleming, H. S., *J. Dent. Res.*, **31**, 166 (1952).

PHYSIOLOGY

Sialotonin: Vasopressor Substance in Cat Submaxillary Saliva

THE potent vasodepressor effect of cat's submaxillary saliva after intravenous injection into the cat was first noted by Secker[1,2]. After some controversy about the alleged similarity of this substance to acetylcholine[3-5], it is now clear that it is a glycoprotein, that is salivary kallikrein[6,7].

The same workers[1-5] also occasionally observed an evanescent and variable vasopressor effect of cat's submaxillary saliva which preceded the depressor effect. The study of this apparent pressor agent was never pursued, probably because of difficulty in observing it with convincing regularity and in separating it chemically from the depressor substance, kallikrein.

We have now established the reasons for the erratic pressor (or hypertensive) effect of cat saliva when injected intravenously in the cat, and have separated it from kallikrein. The chief difficulty of studying this substance by its systemic pressor effect is that the vasoconstriction is confined to certain vascular areas, in particular the intestinal blood vessels.

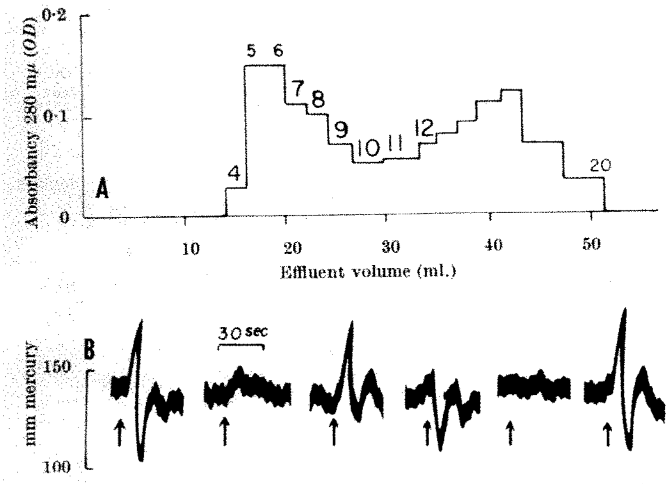
We have called this vasoconstrictor compound sialotonin. A substance which resembles it has been described by Werle and von Roden[8], who found a large-molecular hypertensive agent in extracts of the submaxillary gland of the dog but not of the cat. The arterial blood pressure of the dog rather than that of the cat was used to assay the substance.

Whereas kallikrein in submaxillary saliva is secreted in much higher concentrations with sympathetic than with chorda-lingual nerve stimulation[9], sialotonin is secreted only with chorda-lingual stimulation. Sialotonin was undetectable in saliva obtained by sympathetic nerve stimulation. Feldberg and Guimarais[3] also noted this relationship between nerve stimulation and the pressor and depressor effects of the respective salivas. We therefore obtained saliva by chorda-lingual nerve stimulation in order to study the pressor agent.

The intravenous injection of 0·02 ml. of chorda saliva (usual dose 0·2 ml., diluted 1 : 10) usually produced a sharp rise in arterial blood pressure lasting 5–15 sec, followed by a more prolonged fall caused by kallikrein. The increase in blood pressure varied from 25–50 mm of mercury and usually persisted with repeated injections. It was not affected significantly by the intravenous injection of atropine (500 µg/kg), phenoxybenzamine (500 µg/kg), hexamethonium (1 mg/kg) or carboxypeptidase *B* (3–4 mg/kg). It was abolished completely, however, if the diluted saliva was previously incubated with trypsin (100 µg/ml.) or chymotrypsin (100 µg/ml.) for 30 min. The increase in blood pressure was also usually reduced or abolished by laparotomy and gentle handling of the abdominal viscera of the cat used for the assay.

Sialotonin is a large-molecular compound, probably a protein or glycoprotein. It is heat-labile, non-dialysable and rapidly inactivated by incubation with trypsin or chymotrypsin (100 µg/ml., 30 min). Sialotonin and kallikrein were eluted in the same fractions of saliva applied to columns of 'Sephadex *G*-25' and '*G*-50.' A distinct separation was obtained, however, from columns of 'Sephadex *G*-100' after the application of cat's chorda saliva (usually diluted 1 : 2) in 0·15 molar NaCl as solvent. The optical densities of successive fractions from the column and their respective assays on the cat's arterial blood pressure are shown in Fig. 1. In this figure the original chorda saliva shows the vascular effects of both sialotonin (pressor) and kallikrein (depressor) whereas a distinct separation of these effects is seen in the pharmacological actions of fractions 7–8 and 9–11, respectively.

Whereas salivary kallikrein is readily obtained as a stable freeze-dried material, we have as yet not succeeded in preparing a stable, freeze-dried preparation of sialotonin. It is stable for months, however, if kept frozen in solution, either as the crude saliva or in eluates (in 0·15 molar NaCl) from columns of 'Sephadex'.



Sample:	Chorda saliva (1 : 10)	Fraction 4	Fractions 7–8	Fractions 9–11	Fraction 12	Chorda saliva (1 : 10)
OD of sample:	0·132	0·016	0·106	0·061	0·070	0·132
Injection vol. (ml., i.v.):	0·3	0·5	0·2	0·4	0·4	0·3

Fig. 1. *A*, Optical densities of successive fractions after application of cat's chorda saliva to a 1·5 × 20 cm column of 'Sephadex *G*-100'. The solvent was 0·15 molar NaCl, *p*H 8·2. *B*, Response of arterial blood pressure (cat) to intravenous injection of above fractions. Original chorda saliva shows activity of both sialotonin (pressor) and kallikrein (depressor). Sialotonin only, however, was present in fractions 5–8, whereas kallikrein appeared in fractions 9–11.

Using an electromagnetic flow-meter with the probe placed around different arteries, we made the following observations, which indicate that the effect of sialotonin on systemic arterial blood pressure after its intravenous injection is caused by a highly specific constrictor action which has so far been observed only on the vessels of the intestine. Injections of partially purified sialotonin, or of 0·002–0·02 ml. of diluted cat's chorda saliva, close-arterially, through the superior mesenteric artery, produced an immediate brief reduction (15–30 sec) in blood flow through this artery, indicating that the blood flow through the intestine was reduced by as much as 80 per cent. Similar experiments on blood flow through the lower limbs revealed no vasoconstrictor action of sialotonin. Our preliminary observations therefore suggest that the action of sialotonin is predominantly on the intestinal blood vessels. We are continuing the study of its vasoconstrictor action in more detail. So far, our studies failed to demonstrate a marked constrictor action of sialotonin on the blood vessels of the submaxillary gland itself.

Sialotonin can easily be distinguished from known pressor agents such as catecholamines, indolealkylamines, angiotensin and other relatively small-molecular compounds by its chemical and pharmacological properties. It is distinguishable from renin by the fact that it fails to release angiotensin on incubation with plasma and by its brief and regional vasoconstrictor effects. It does, however, resemble the hypertensive agent described by Werle and von Roden in extracts from the submaxillary gland of the dog[8].

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[1] Secker, J., *J. Physiol.*, **81**, 81 (1934).
[2] Secker, J., *J. Physiol.*, **82**, 293 (1934).
[3] Feldberg, W., and Guimarais, J. A., *J. Physiol.*, **85**, 15 (1935).
[4] Gibbs, O. S., *J. Physiol.*, **84**, 33 (1935).
[5] Larson, P. S., *J. Pharmacol. and Exp. Therap.*, **54**, 341 (1935).
[6] Frey, E. K., Kraut, H., and Werle, E., *Kallikrein* (Padutin), (Ferninand Enke, Stuttgart, 1950, 1968).
[7] Schachter, M., *Ann. Rev. Pharmacol.*, **4**, 281 (1964).
[8] Werle, E., and von Roden, P., *Biochem. Z.*, **301**, 328 (1939).
[9] Beilenson, S., Schachter, M., and Smaje, L. H., *J. Physiol.*, **177**, 61P (1965).

Action of Iontophoretically Applied Tetrodotoxin on Cortical Nerve Cells

Tetrodotoxin is the potent poison found in puffer fish of the sub-order Gymnodontes and newts of the genus *Taricha*[1]. In low concentrations, tetrodotoxin suppresses action potentials in a variety of excitable tissues including frog myelinated nerve fibres, frog muscle fibres[2], lobster and squid giant axons[3,4] and cardiac muscle[5]. The action potentials of some other tissues, such as crustacean muscle fibres, the taenia coli and vas deferens of guinea-pigs are not affected by tetrodotoxin[6–8]. Although evoked potentials in the cerebral cortex are depressed by topically applied tetrodotoxin[9], its effect on action potentials of nerve cells in the central nervous system has not been demonstrated.

In previous investigations, tetrodotoxin has been applied in perfusion solutions or administered by intravascular injection. Iontophoretic application of drugs allows their site of action to be determined with greater certainty in complex structures such as the central nervous system. Tetrodotoxin has been used to study synaptic transmission at the neuromuscular junction[10]. Its interaction with various forms of excitation of cortical neurones has now been assessed.

Tetrodotoxin is an amino perhydroquinazoline compound with a molecular formula of $C_{11}H_{17}N_3O_8$. It is sparingly soluble in water as a monoacidic base[1]. In early experiments, one barrel of the multiple barrel micropipettes used in the investigation was filled with an aqueous solution of 10^{-3} g/ml. of tetrodotoxin (Sankyo Co., Ltd.). The activity of tetrodotoxin was so marked that it was impossible to prevent diffusion of excessive quantities from the electrode. Satisfactory application and diffusional control have been obtained by preparing solutions of 10^{-6} to 5×10^{-6} g/ml. tetrodotoxin in 200 mmolar sodium chloride. The passage of cation applying currents through such a solution seems to deliver small amounts of tetrodotoxin.

Tetrodotoxin depressed the excitability of cortical neurones. An example of tetrodotoxin depression of the invasion of an antidromic spike into a pyramidal tract neurone in the pre-cruciate cerebral cortex is shown in Fig. 1. The spike was considered to be antidromically propagated because it had a constant latency of 2·9 msec, followed medullary pyramid stimulation at frequencies of 300 stimuli/sec and had a refractory period for the cell soma of less than 2 msec (Fig. 1*B*). With shorter intervals between stimuli, invasion of the soma-dendritic spike failed, leaving the initial segment spike (Fig. 1*C*). Spike generation failed completely when the interval between stimuli was 1·2 msec (Fig. 1*D*). The application of minute amounts of tetrodotoxin caused a progressive graded reduction in the size of the antidromic spike and ultimately a complete failure of invasion (Fig. 1*E*). Recovery occurred within approximately 24 sec of the termination of tetrodotoxin application.

Tetrodotoxin also prevented the generation of synaptically evoked responses and reduced or abolished the excitant effects of L-glutamate and acetylcholine on cortical nerve cells. It did not affect the inhibitory actions of acetylcholine or 5-hydroxytryptamine. An example of depression of L-glutamate firing is shown in Fig. 2. Application of tetrodotoxin by a current of 20 n.amp for 40 sec more than halved the rate of firing evoked by standard pulses of L-glutamate. Recovery after application of tetrodotoxin took approximately 3 min. Larger currents passed through the barrel containing tetrodotoxin caused greater reductions or even abolition of L-glutamate induced firing. L-Glutamate firing of pyramidal tract cells was usually abolished by amounts of tetrodotoxin insufficient to prevent invasion of an antidromic spike.

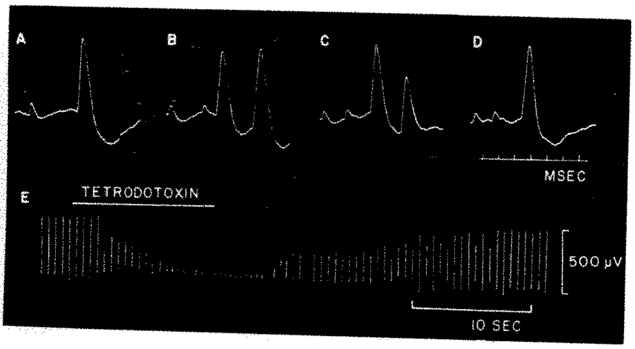

Fig. 1. Antidromic spikes, recorded from a cortical neurone, evoked by single (*A*) and paired stimuli (*B–D*) delivered to the ipsilateral medullary pyramidal tract. *E*, Antidromic spikes of same neurone recorded from a stationary cathode ray oscilloscope, using spike intensification, on slowly moving film. During application of tetrodotoxin (4 n.amp passed for period indicated by horizontal bar) antidromic invasion gradually failed. The residual response in *E* is the intensified stimulus artefact.

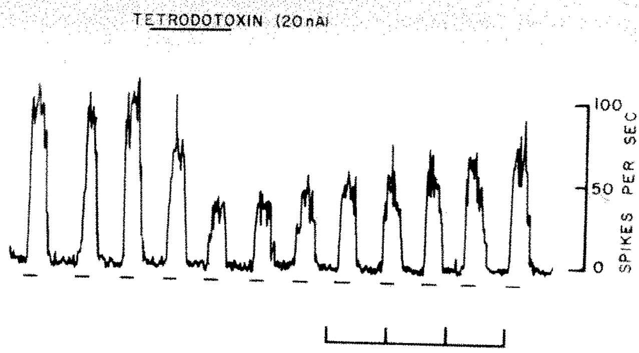

Fig. 2. Tetrodotoxin depression of the firing of a cortical neurone evoked by L-glutamate. Discharge frequency of the neurone was recorded by means of an electronic counter and ink recorder. Periods of L-glutamate and tetrodotoxin application are indicated by horizontal solid lines below and above traces, respectively.

These observations indicate that neurones of the mammalian central nervous system can be included in the group of tissues which have excitable processes sensitive to tetrodotoxin. The graded reduction in antidromic spike size shown in Fig. 1 suggests that the area of electrically excitable membrane available for spike generation was progressively reduced. Reductions in the magnitude of responses to L-glutamate and acetylcholine also indicate that excitability was reduced by tetrodotoxin, with the development of a higher threshold for spike initiation.

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[1] Kao, C. Y., *Pharmac. Rev.*, **18**, 997 (1966).
[2] Furukawa, T., Sasaoka, T., and Hosoya, Y., *Jap. J. Physiol.*, **9**, 143 (1959).
[3] Narahashi, T., Moore, J. W., and Scott, W. R., *J. Gen. Physiol.*, **47**, 965 (1964).
[4] Nakamura, Y., Nakajima, S., and Grundfest, H., *J. Gen. Physiol.*, **48**, 985 (1965).
[5] Dudel, J., Peper, K., Rudel, R., and Trautwein, W., *Nature*, **213**, 296 (1967).
[6] Hagiwara, S., and Nakajima, S., *Science*, **149**, 1254 (1965).
[7] Nonomura, Y., Hotta, Y., and Ohashi, H., *Science*, **152**, 97 (1966).
[8] Hashimoto, Y., Holman, M. E., and McLean, A. J., *Nature*, **215**, 430 (1967).
[9] Frank, G. B., and Pinsky, C., *Brit. J. Pharmac. Chemother.*, **26**, 435 (1966).
[10] Katz, B., and Miledi, R., *Proc. Roy. Soc.*, B, **167**, 8 (1967).

Small Molecular Weight Vitamin B_{12} Binders in Normal Human Serum

IN an investigation of vitamin B_{12} binders in human serum, three peptide-like binders were isolated by electrodialysis and by chromatography.

Radioactive B_{12} was obtained from Philips-Duphar, Holland, and had a specific activity of 30 mc./mg. To samples of fresh, pooled normal human serum, 300, 1,000 or 3,000 $\mu\mu$g of cobalt-57 labelled vitamin B_{12}/ml. of serum was added. The samples were dialysed at 40° C against twenty times their volume of distilled water for 5 days; the water was changed twice a day and toluene was added as preservative. Serum without additional labelled B_{12} was similarly treated. Euglobulins were precipitated during dialysis and the volume of dialysed serum was increased approximately three times as a result of osmotic pressure. The *p*H of the dialysed serum was 7·0.

Seventy ml. of dialysed samples, both precipitate and supernatant, was placed in 27/32 in. unmodified dialysis tubing (Thomas, Philadelphia) and the tubes were im-

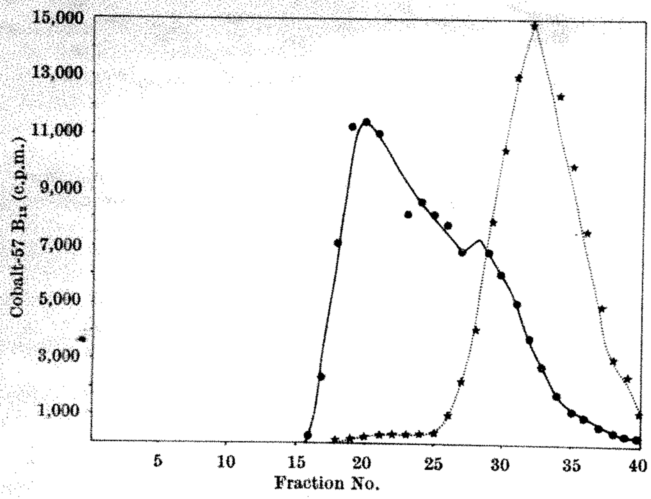

Fig. 1. Radioactive profile of concentrated electrodialysate on a 'Sephadex *G*-50' column, equilibrated and eluted with water. - - ★ - - represents the peak which was obtained when free radioactive B_{12} was chromatographed on the same column. Fractions of 2 ml. were collected and counted in a well-type scintillation counter.

mersed in 190 ml. of distilled water in a 'Pyrex' centrifuge bottle. A piece of platinum wire which was immersed along the entire length of the tubing served as anode, and another piece of wire which was placed in the surrounding water parallel to and 3 cm away from the anode served as the cathode. Electrodialysis of samples proceeded for 24 h at 150 V and 55–65 m.amp. Because experiments were performed in a cold room, temperature in the dialysis tubing did not exceed 8° C.

After completion of electrodialysis, the *p*H of the serum at 25° C was 4·5 while that of the electrodialysate was 7·0. The tubing content was homogenized by stirring and the vitamin B_{12} content of this suspension as well as that of the surrounding water (electrodialysate) was determined microbiologically using the *Euglena gracilis* method when radioactive B_{12} had not been added, or radiologically in a Baird atomic scintillation spectrometer when radioactive B_{12} had been added. The electrodialysate was concentrated to approximately 15 ml. and chromatographed on a 'Sephadex *G*-50' column (30 × 2·2 cm) using water as the developing solvent. The peak of radioactivity which was obtained from this column, and which coincided with the positive effluent of Folin's reagent[1], was further chromatographed on a 'Sephadex *G*-200' column (30 × 2·2 cm) by elution with water.

Samples of the concentrated electrodialysate were chromatographed on Whatman No. 4 paper in a butanol : acetic acid : water (65 : 10 : 25) solvent or in a butanol : acetic acid : water (60 : 20 : 20) solvent[2]. Paper chromatograms were dipped in 0·5 per cent ninhydrin in ethanol[2] to detect peptide-like substances or else were treated with periodic acid[3] to detect carbohydrates. Ninhydrin positive spots from paper chromatograms were eluted with 10 per cent acetic acid and were subsequently dried for further analysis.

A sample of the concentrated electrodialysate was hydrolysed in 6 normal hydrochloric acid for 22 h at 110° C (ref. 4) and dried products of hydrolysis were chromatographed on Whatman No. 1 paper in liquid phenol : water, 100 : 20 v/v, in the presence of 1 : 1 acetic acid[2]. Samples of naturally occurring amino-acids were developed parallel to the sample and dried chromatograms were dipped in 0·25 per cent ninhydrin in acetone.

In the process of electrodialysis a portion of vitamin B_{12} passed through the dialysis tubing and appeared in the electrodialysate, as shown in Table 1. Because samples were dialysed for 5 days before electrodialysis, free vitamin B_{12} cannot be expected to be present in the samples when dialysis is complete[5]. Vitamin B_{12} in the electrodialysate was therefore either dissociated before passing through dialysis tubing or else passed through it in the form of a complex with another substance.

Table 1. PRESENCE OF VITAMIN B_{12} OR RADIOACTIVITY OF COBALT-57 B_{12} IN DIALYSING TUBING AND ELECTRODIALYSATE

Radioactive B_{12} added to serum	Activity remaining in dialysis tubing Counts/ 5 min	Activity remaining in dialysis tubing $\mu\mu$g of vit B_{12}*	Activity in electrodialysate Counts/ 5 min	Activity in electrodialysate $\mu\mu$g of vit B_{12}*	Percentage of total activity appearing in electro-dialysate
None		6,095		1,740	22
300 $\mu\mu$g/ml.	953,800		171,000		15
1,000 $\mu\mu$g/ml.	3,704,000		621,500		15
3,000 $\mu\mu$g/ml.	10,625,000		3,131,000		23

* Microbiological assay by the *Euglena gracilis* method. These data are for 25 ml. of initial serum. The volume of dialysed serum used in each experiment was 70 ml. and the volume of the electrodialysate was 190 ml.

Because of the elution pattern which was obtained when the concentrated electrodialysate was chromatographed on the 'Sephadex *G*-50' column (Fig. 1), the vitamin B_{12} which was present in electrodialysate appeared in a bound form. The dotted line in Fig. 1 indicates the radioactive peak which was obtained when a sample of free vitamin B_{12} was chromatographed on the same column in the same experimental conditions. The combined total radioactivity (all samples under the solid line in Fig. 1) from the 'Sephadex *G*-50' column was rechromatographed on a 'Sephadex *G*-200' column, and two peaks of radioactivity, coinciding with the positive effluent, Folin's reagent, were obtained (Fig. 2). The first peak which corresponded to a complex of molecular weight between 10,000 and 20,000 as estimated by a comparison with elution patterns of ribonuclease, lysozyme and pepsin, represented a small part of the total radioactivity; the second peak corresponding to a complex of molecular weight 4,000 and 8,000 appeared ahead of the peak which was obtained from free vitamin B_{12} of approximately the same total radioactivity chromatographed on the same column in the same experimental conditions. Because of the presence of carbohydrates and aromatic amino-acids in the peptide-like substances, as will be discussed in the following paragraphs, these estimates of molecular weight are approximate[6].

The ninhydrin reagent revealed five positive zones on paper chromatograms the R_F values of which, in the two solvent systems employed, are given in Table 2. Zones 1, 2 and 4 contained vitamin B_{12} although partial dissociation of the vitamin B_{12} from the complexes occurred to the extent of 50–60 per cent, obviously caused by organic solvents. Eluted substance from zone 1 appeared in the position of the first radioactive peak when chromatographed on a 'Sephadex *G*-200' column; eluted substances from zones 2 and 4 appeared in the

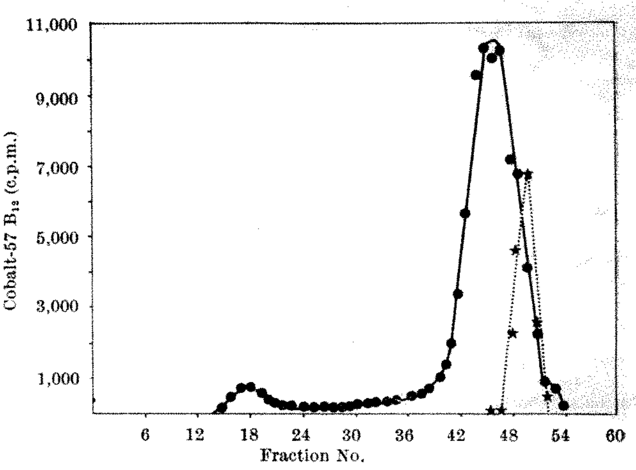

Fig. 2. Radioactive profile of peak from the 'Sephadex *G*-50' column re-chromatographed on a 'Sephadex *G*-200' column which was equilibrated and eluted with water. - - ★ - - represents free radioactive B_{12} chromatographed on the same column. Fractions of 2 ml. were collected and counted in a well-type scintillation counter.

Table 2. *RF* VALUES FOR FIVE NINHYDRIN POSITIVE ZONES

Zone	*RF* Butanol : acetic acid : water (65 : 10 : 25)	Butanol : acetic acid : water (60 : 20 : 20)
1	0·09	0·10
2	0·23	0·21
3	0·33	0·30
4	0·59	0·36
5	0·65	0·74

The solvent ran 45 cm on Whatman No. 4 paper.

position of the large radioactive peak from the 'Sephadex *G*-200' column (Fig. 2).

The indication that the vitamin B_{12} appeared to be in a bound form, in addition to the observation that complexes were ninhydrin positive, suggests the possible existence in normal serum of vitamin B_{12} binding peptides. Furthermore, ninhydrin positive zones gave positive reactions when treated with periodic acid, indicating the presence of carbohydrates. The nature of these carbohydrates is not yet known.

In the acid hydrolysate of the serum electrodialysate, cysteine, arginine, threonine, tyrosine, methionine, phenylalanine and leucine or isoleucine were tentatively identified. It is not yet known which amino-acids were present in each of the five ninhydrin positive zones appearing on paper chromatograms.

These results, although preliminary, suggest that vitamin B_{12} binding peptides are present in normal human serum. Because these peptides were not dialysable but could be electrodialysed, they were either products of protein decomposition occurring during the electrodialysis, or else were adsorbed onto the proteins by electrostatic forces. If the latter hypothesis is correct, complexes between proteins and peptides–vitamin B_{12} groups were electrostatic in nature and dissociated at the voltage used in the electrodialysis, while bonds between the peptides and vitamin B_{12} were not electrostatic.

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[1] Layne, E., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **3**, 447 (Academic Press, New York, 1964).

[2] Block, R. J., Durrum, E. L., and Zweig, G., *A Manual of Paper Chromatography and Paper Electrophoresis*, 110 (Academic Press, New York, 1958).

[3] Koiw, E., and Gronwall, A., *Scand. J. Clin. and Lab. Invest.*, **4**, 244 (1952).

[4] Light, A., and Smith, E. L., in *The Proteins* (edit. by Neurath, H.), **1**, 1 (Academic Press, New York, 1963).

[5] Bertcher, R. W., Meyer, L. M., Yarmus, H., and Mulzac, C., *Acta Haematol.*, **23**, 287 (1960).

[6] Whitaker, J. R., *Anal. Chem.*, **35**, 1950 (1963).

Histaminase Activities in the Plasma of Subhuman Primates and Man

MEASUREMENT of the concentrations of histamine in the plasma of rhesus (*Macaca mulatta*) and squirrel (*Saimiri sciureus*) monkeys after administration of the histamine-releasing agent, compound 48/80, or histamine has suggested that the rhesus monkey is more active in degrading histamine than the squirrel monkey[1]. Direct determination of histaminase activity in plasma showed that the rhesus monkey exhibits more activity of this enzyme than any other species tested, including man[2]. Squirrel monkeys and man were found to exhibit similar levels of this enzyme activity in the plasma[2]. In addition, squirrel monkeys, but not rhesus monkeys, responded to compound 48/80 by exhibiting an elevation of circulatory histamine[1], as does man[3].

The importance of subhuman primates as models of man in biological research led us to survey other simian species to find whether high activities of plasma histaminase are unique to rhesus monkeys. We obtained from this institute heparinized plasma from additional squirrel and rhesus monkeys and from stump-tailed (*M. speciosa*) and pig-tailed (*M. nemestrina*) macaques. Plasma obtained from outside laboratories was from greater galago (*Galago crassicaudatus panganiensis*), lesser galago (*G. senegalensis braccatus*), cotton-topped marmoset (*Saguinus oedipus*), night monkey (*Aotus trivirgatus*), talapoin (*Miopithecus talapoin*), anubis baboon (*Papio anubis*), yellow baboon (*P. cynocephalus*), western baboon (*P. papio*), gibbon (*Hylobates lar* and *H. pileatus*), chimpanzee (*Pan troglodytes*) and gorilla (*Gorilla gorilla*). Plasma was also obtained from volunteers from our laboratory. Frozen heparinized plasma was shipped from outside sources and assayed within 2 weeks of the time of bleeding. Our previous studies with plasma from squirrel or rhesus monkeys or from man have indicated that storage in the frozen state for up to 4 weeks caused no change in histaminase activity[2]. Furthermore, redetermination of histaminase activities in individual squirrel or rhesus monkeys or in human subjects up to 6 months after the initial determination showed that these activities were relatively stable individual characteristics. Protein concentrations in the plasma were determined[4] to allow expression of histaminase activities in each gram of plasma protein. Histaminase activities were measured as described before[2]. Only physical restraint was employed when most of the animals were bled. Exceptions were the first group of chimpanzees listed in Table 1, which received phencyclidine hydrochloride (1·0 mg/kg) intramuscularly, and the gorillas, which received the same anaesthetic agent intramuscularly in a dose of 1·5 mg/kg. In addition, all but two of the gorillas of Table 1 received atropine sulphate (0·01 mg/kg). Phencyclidine hydrochloride in a concentration of 1·6 μg/ml. did not inhibit the colour detection system for measuring histaminase activity in the absence of plasma with added hydrogen peroxide. The same concentration of added phencyclidine hydrochloride did not inhibit the activity of histaminase in the plasma from a rhesus monkey. The activity of histaminase in the plasma of an anaesthetized rhesus monkey 15 min after intramuscular injection of phencyclidine hydrochloride (1·1 mg/kg) was 92 per cent of the activity measured before the drug was given.

Table 1 summarizes the results obtained in the animals surveyed. The plasma protein concentrations were within

Table 1. MEAN BODY WEIGHT, PLASMA PROTEIN CONCENTRATION AND PLASMA HISTAMINASE ACTIVITY OF THE SPECIES STUDIED (± *SD*)

Species	Body weight (kg)	Plasma protein (g %)	Histaminase activity*
Prosimian			
Greater galago, 5♂, 5♀	1·2 ± 0·1	6·2 ± 0·5	232 ± 93
Lesser galago, 5♂, 4♀	0·29 ± 0·05	6·2 ± 0·3	141 ± 38
New World			
Cotton-topped marmoset, 5♂, 5♀	0·43 ± 0·04	5·6 ± 0·4	22 ± 10
Squirrel monkey, 8♂, 3♀	0·69 ± 0·15	7·3 ± 1·1	24 ± 5
Night monkey, 3♂, 3♀	0·92 ± 0·09	7·0 ± 0·5	39 ± 27
Old World			
Talapoin, 1♂, 2♀	1·2 ± 0·4	7·8 ± 0·7	62 ± 6
Rhesus monkey, 8♂, 4♀	3·9 ± 0·6	7·6 ± 0·6	195 ± 50
Stump-tailed macaque, 2♂, 5♀	3·2 ± 0·6	7·3 ± 0·6	217 ± 48
Pig-tailed macaque, 14♂, 1♀	6·8 ± 2·6	8·4 ± 1·1	133 ± 33
Anubis baboon, 4♂, 6♀	7·4 ± 3·7	8·2 ± 0·6	253 ± 44
Yellow baboon, 10♀	14·5 ± 0·7	8·4 ± 0·5	228 ± 70
Western baboon, 2♂, 8♀	10·4 ± 1·3	8·1 ± 0·4	198 ± 41
Gibbon, 5♂, 5♀	4·2 ± 1·7	6·4 ± 0·9	119 ± 47
Chimpanzee, 3♂, 4♀	24 ± 14	6·8 ± 0·6	77 ± 14
Chimpanzee, 6♂, 6♀	18 ± 6	8·0 ± 0·6	113 ± 24
Total chimpanzee, 9♂, 10♀	20 ± 10	7·5 ± 0·8	100 ± 27
Gorilla, 2♂, 7♀	36 ± 11	6·1 ± 0·4	61 ± 24
Man, 7♂, 3♀	69 ± 7	6·9 ± 0·6	24 ± 8

* mμmole/l. of histamine oxidized/min/g of plasma protein.

normal limits where such limits are known. No consistent sex differences were found in plasma histaminase activities, and so all subjects of one species were treated as a single group. The group of gibbons comprised two of the species *H. pileatus* and eight of *H. lar*; they were combined because no marked differences between the two species were noted. In addition, in all species studied except the gorilla (discussed later), no association between body weight and plasma level of histaminase activity was found. Although some groups contained immature animals, no separation of animals by body weight was therefore made in reporting results. Two groups of chimpanzees from different sources were studied. The first group, which had received the anaesthetic phencyclidine hydrochloride, showed slightly lower activities than the second group, which received no drugs. In addition, there was a small difference in the concentration of plasma protein between the two groups of chimpanzees. The biological significance of these differences in such a small number of animals is, however, questionable; the mean levels for all the chimpanzees studied are therefore presented.

It is immediately apparent from Table 1 that the prosimian and the Old World species exhibited relatively high levels of histaminase activities compared with the three New World species and man. Thus the rhesus monkey is not unique in possessing high plasma histaminase activity. On the contrary, the current observations suggest that man and New World species are unusual among the primates in this regard. In an earlier survey of common laboratory species[2], mice and rats were found to have essentially no histaminase activity in the plasma; in guinea-pigs and dogs, the activity was similar to that in man and squirrel monkeys; in cats and rabbits, it was two to three times higher than in man, but only about one-third of that found in rhesus monkeys.

Although fourteen gorillas were surveyed, results for only nine are included in Table 1. The remaining five (three male and two female) showed no histaminase activity in the plasma; all had received phencyclidine hydrochloride, but only one had atropine sulphate. Their mean plasma protein concentration was 5·7 ± 0·4 g per cent, similar to that of the nine reported in Table 1, but their mean body weight was 19 ± 2 kg, approximately half that of the group showing plasma histaminase activity. It is not clear why this group of five gorillas failed to exhibit plasma histaminase activity. In no other primate species were subjects found with no such activity.

Why man exhibits such a dichotomy between plasma histaminase activities and his phylogenetic relationship with the subhuman primates is puzzling. More detailed studies on blood specificities[5], quantitative immunochemical correspondence of albumins[6,7], electrophoretic patterns or erythrocytic esterases[8], and the molecular structure of myoglobin[9] of subhuman primates and man have provided clear support for the phylogenetic classification, which is based on anatomical and organismal comparisons. Nevertheless, studies of the primary structure of primate haemoglobins[10] suggest that not all biological molecules or activities should be expected to follow absolutely the phylogenetic relationships. Of particular interest in this regard is the recent report[11] that two prosimian species, the tree shrew (*Tupaia glis*) and the slow loris (*Nycticebus coucang*), can synthesize ascorbic acid, whereas no Old or New World subhuman primate studied so far has shown this ability.

The current results with plasma histaminase activities suggest that for investigations of drugs or toxic agents expected to influence the physiological or pharmacological role of histamine in man, New World subhuman primates may be better test animals than Old World ones.

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[1] Peters, J. H., Gordon, G. R., and Ferguson, S. A., *Life Sci.*, **6**, 1163 (1967).
[2] Gordon, G. R., and Peters, J. H., *Proc. Soc. Exp. Biol. and Med.*, **124**, 42 (1967).
[3] Sicuteri, F., Michelacci, S., and Franchi, G., *Intern. Arch. Allergy*, **22**, 408 (1963).
[4] Gornall, A. G., Bradawill, C. J., and David, M. M., *J. Biol. Chem.*, **177**, 751 (1949).
[5] Moor-Jankowski, J., and Weiner, A. S., *Science*, **148**, 255 (1965).
[6] Hafleigh, A. S., and Williams, jun., C. A., *Science*, **151**, 1530 (1966).
[7] Sarish, V. M., and Wilson, A. C., *Science*, **154**, 1563 (1966).
[8] Tashian, R. E., *Amer. J. Human Genet.*, **17**, 257 (1965).
[9] Hudgins, P. C., Whorton, C. M., Tomoyoshi, T., and Riopelle, A. J., *Nature*, **212**, 693 (1966).
[10] Buettner-Janusch, J., and Hill, R. L., *Science*, **147**, 836 (1965).
[11] Elliot, O., Yess, N. J., and Hegsted, D. M., *Nature*, **212**, 739 (1966).

Fine Structure of the Sensory Endings in the Vomero-nasal Organ of the Slow-worm *Anguis fragilis*

THE structure of the sensory endings of the vomero-nasal organ of vertebrates (Jacobson's organ) has been the subject of some controversy in past years[1-3]. Although the receptor cells seem to resemble closely those of the olfactory epithelium, the presence of cilia on their free ends is arguable. The problem has not yet been investigated by electron microscopy, and it is of interest to report the fine structure of these endings in a reptile, *Anguis fragilis*, where the vomero-nasal organ is particularly well developed.

Sections show that the receptor endings in this animal are completely lacking in cilia, but are provided with numerous microvilli (Fig. 1). The microvilli are long, and those of neighbouring receptors intermingle to form a complex meshwork above the epithelial surface. When thick sections of the same material are viewed by light

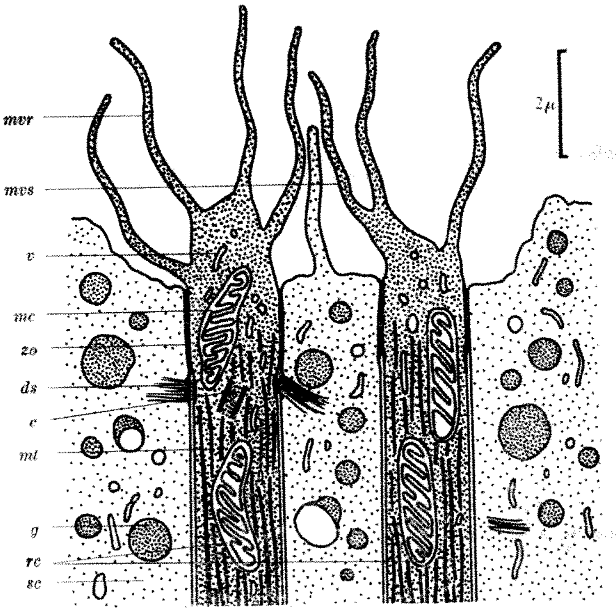

Fig. 1. Diagram of surface of vomero-nasal organ in vertical section, showing two receptor endings with microvilli. *c*, Centriole; *ds*, desmosome; *g*, secretory granule; *mc*, mitochondrion; *mt*, microtubule; *mvr*, microvillus of receptor cell; *mvs*, microvillus of supporting cell; *rc*, receptor cell dendrite; *sc*, supporting cell; *v*, vesicle; *zo*, zonula occludens.

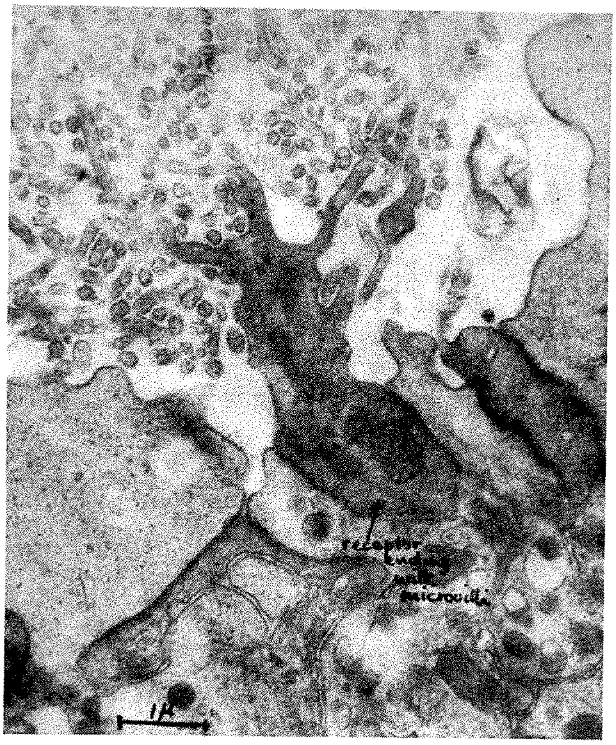

Fig. 2. Micrograph of oblique section through the receptor tip of the vomero-nasal (Jacobson's) organ.

microscopy this meshwork presents an indeterminate appearance suggestive of poorly fixed cilia, an appearance which may have been the cause of the disagreements in the literature.

In other respects the endings resemble those of vertebrate olfactory receptors, the mitochondria, microtubules, and small vesicles being constant features of the cytoplasm. The supporting cells which surround the receptors bear occasional microvilli and contain numerous densely staining secretory granules.

Other chemoreceptors bearing microvilli have been described, examples being the olfactory receptors in birds[4,5] where microvilli are found in association with cilia, and one type of olfactory receptor in a species of teleost fish[6]. These findings emphasize that cilia are not indispensable to the chemosensory process. It remains to be seen whether or not other vertebrates have endings on their vomero-nasal receptors similar to those of *Anguis*.

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[1] Allison, A. C., *Biol. Revs.*, **28**, 195 (1953).

[2] Cordier, R., in *The Cell*, **6** (edit. by Brachet, J., and Mirsky, A. E.), 313 (Academic Press, New York, 1964).

[3] Moulton, D. G., and Beidler, L. M., *Physiol. Revs.*, **47**, 1 (1967).

[4] Brown, H. E., and Beidler, L. M., *Fed. Proc.*, **25**, No. 2 (1966).

[5] Graziadei, P., and Bannister, L. H., *Z. Zellforsch.*, **80**, 220 (1967).

[6] Bannister, L. H., *Quart. J. Microsc. Sci.*, **106**, 333 (1965).

Striated Muscle in the Cortex of Foetal Guinea-pig Thymus

Striated muscle cells, referred to as "myoid" cells, have been described by light microscopists in the medulla of the thymus of a variety of vertebrates[1]. "Myoid" cells are rare, however, and most reports of the ultrastructure of the mammalian thymus make no mention of them. Van de Velde and Friedman identified "myoid" cells in a variety of mammals[2,3] but did not show them in the normal mammalian thymus. They and others, however, showed electron micrographs of striated muscle in non-mammalian thymus and in a lymphoepithelial thymoma in a patient with myasthenia gravis[3-5].

During a light and electron microscopic study of osmium-fixed thymus from numerous foetal and post-natal guinea-pigs, only one "myoid" cell was seen. Ultrastructurally, this cell (Fig. 1) showed the typical cross striated appearance of skeletal muscle and seemed to be contracted[6]. There were accumulations of glycogen just inside the cell membrane and also scattered in between the myofilaments. No nuclei were visible in the profile studied, nor was there any evidence of nerves in the vicinity of this cell. A further unusual feature was the location of this cell in the cortex. A study with the light microscope of an adjacent "thick" section (1μ) stained with toluidine blue confirmed the cortical position of the cell (Fig. 2).

The ultrastructural appearance of the cell described and of "myoid" cells illustrated by other authors[3-5] is typical of striated muscle and the term "myoid" cell may there-

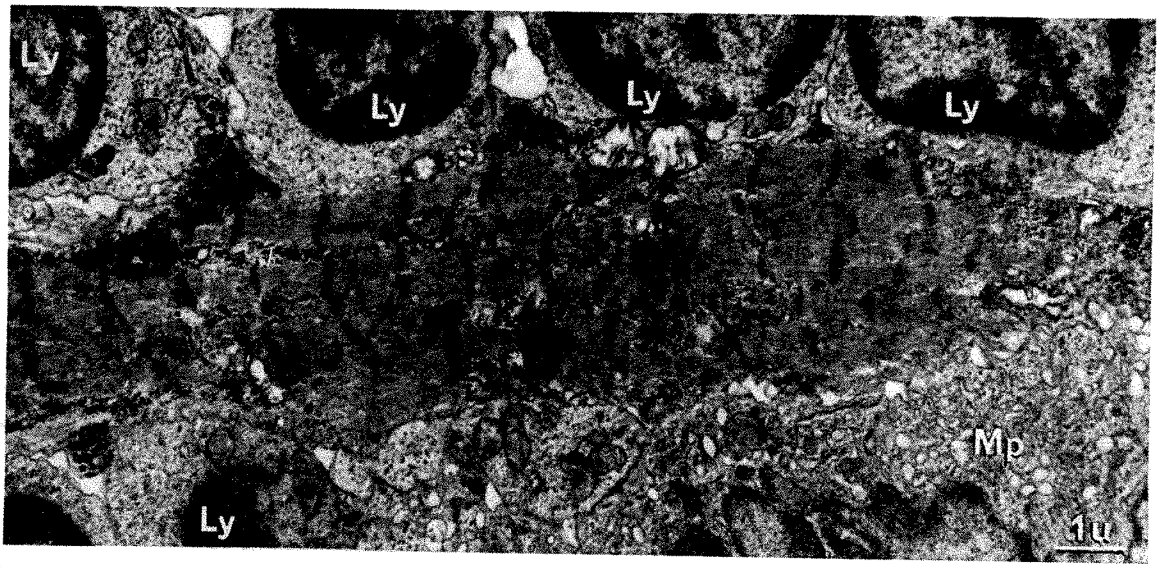

Fig. 1. Electron micrograph of a thymic striated muscle cell. The cell is surrounded by small lymphoid cells (*Ly*) and by a macrophage (*Mp*). Osmium, uranyl acetate, lead citrate. (× *c*. 10,500.)

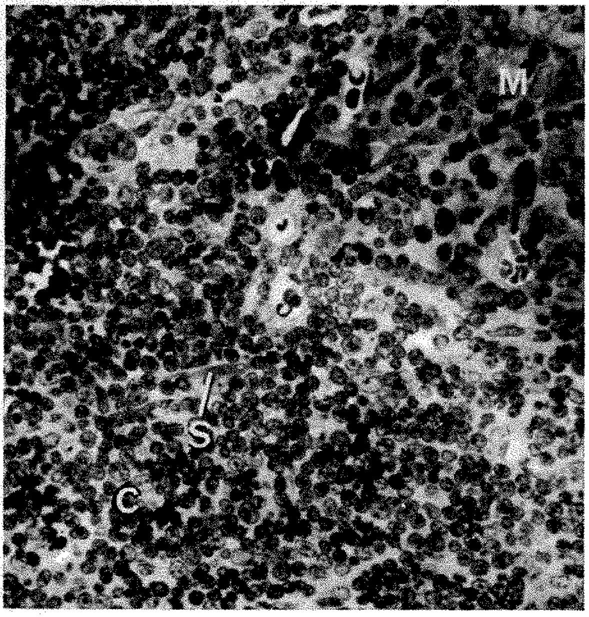

Fig. 2. Light micrograph of a "thick" section showing part of the thymus containing the striated muscle cell (*S*) illustrated in Fig. 1. Note the distinction between cortex (*C*) and medulla (*M*) and the striated muscle cell in the cortex close to the cortico-medullary junction. Osmium, embedded in araldite, toluidine blue. (× *c*. 300.)

fore be redundant. It has been demonstrated by immunofluorescence that a common antigen is shared by striated muscle and by some "thymic epithelial cells"[7], and it has been suggested that the fluorescent thymic cells may be "atrophic, rounded up, striated muscle cells which had lost their morphological identifying features"[4]. In this study, however, no cells which could be classified as being intermediate between striated muscle and thymic epithelium were seen with the electron microscope, and all epithelial cells could be recognized as such because they contained irregularly arranged bands of tonofibrils and were joined to one another by desmosomes. No evidence of persisting cross striation was seen in any of the epithelial cells.

It has also been suggested that thymic striated muscle may act as a local source of antigen for the "self-recognition" of skeletal muscle[8]. In view of the uncommon occurrence of thymic striated muscle, such a function may be difficult to sustain. It seems more likely, as Van de Velde *et al.*[3] suggested, that the skeletal muscle cells are incorporated into the thymus as myoblasts, possibly as an accidental event during embryonic development of the thymus.

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[1] Bargmann, W., in *Handbuch der Mikroskopischen Anatomie des Menschen* (edit. by von Möllendorff, W.), **6**, sect. 1, 63 (Springer-Verlag, Berlin, 1943).

[2] Van de Velde, R. L., and Friedman, N. B., *J. Amer. Med. Assoc.*, **198**, 287 (1966).

[3] Van de Velde, R., and Friedman, N., *Anat. Rec.*, **157**, 392 (1967).

[4] Strauss, A. J. L., Kemp, jun., P. G., and Douglas, S. D., *Lancet*, i, 772 (1966).

[5] Bockman, D. E., and Winborn, W. B., *J. Morph.*, **121**, 277 (1967).

[6] Huxley, H. E., in *The Cell* (edit. by Brachet, J., and Mirsky, A. E.), **4**, 365 (Academic Press, New York, 1960).

[7] Van der Geld, H. W. R., and Strauss, A. J. L., *Lancet*, i, 57 (1966).

[8] Mackay, I. R., and Goldstein, G., *Clin. Exper. Immunol.*, **2**, 139 (1967).

Radioimmunoassay of Angiotensin

HABER *et al.*[1] showed that antibodies elicited in rabbits by copolymers of angiotensin with poly-L-lysine can be used for radioimmunoassay of angiotensin. The following are details and some results of the method we have developed. Comparing the binding affinity of three different rabbit antisera to Val^5-angiotensin II amide ('Hypertensin', Ciba) and three analogues, we found that these antisera do not quantitatively behave identically. This fact has important consequences for the standardization of immunoassays of angiotensin in human plasma as long as human angiotensin is not available as a chemically pure substance. (Val^5-angiotensin has the amino-acid sequence of bovine angiotensin, while Ileu^5-angiotensin represents the structure of horse, hog and possibly human angiotensin.)

Vallotton *et al.*[2] found no differences in binding affinity to Val^5-angiotensin II amide and Ileu^5-angiotensin II amide with a similar method; in contrast, our antisera distinguished between Val^5-angiotensin II amide and Ileu^5-angiotensin II amide. Dietrich[3] also found this difference with another technique. (The difference between Ileu^5-angiotensin II and Ileu^5-angiotensin II amide seems to be of no importance in this respect, because the amide group has scarcely any immunological influence (Table 1).)

If human angiotensin is Ileu^5-angiotensin, the use of different antisera in immunoassays of human angiotensin may lead to different results, when Val^5-angiotensin II amide is used as a standard.

Antibodies were produced by immunizing rabbits with copolymers of Asp(NH$_2$)^1-Val^5-angiotensin II ('Hypertensin', Ciba) and poly-L-lysine coupled with 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (ethyl CDI) according to the method of Goodfriend *et al.*[4]. About 1 mg of the product was suspended in 0·8 ml. of a 0·9 per cent sodium chloride solution and emulsified with 0·2 ml. of complete Freund's adjuvant. Each rabbit was injected intramuscularly with 1·0 ml. After three injections at monthly intervals, three out of ten animals yielded sufficient antibody titres for our experiments.

^{125}I-Asp(NH$_2$)^1–Val^5-angiotensin II was prepared according to the chloramine *T* method of Hunter and Greenwood[5], using 0·5 mc. Na^{125}I for 5 μg angiotensin. The free iodide was separated from ^{125}I-angiotensin by means of an 'Amberlite *IRC* 50' column in H$^+$ cycle (0·5 × 0·5 cm) and removed with 0·2 molar ammonium acetate, *p*H 6·5. The labelled angiotensin was eluted with 0·05 normal ammonium hydroxide; it was then adjusted to *p*H 8·5 with *tris*-hydrochloric acid buffer and stored at −20° C. To diminish radiation damage and adsorption to the glassware, human serum albumin (HSA) was added to give a final concentration of 250 mg/100 ml. The labelled material had a specific activity of 60–70 mμc./ng. Antiserum added in excess bound 90 per cent of the labelled angiotensin. Various samples of labelled angiotensin were stored at a different *p*H (4·0–9·0) for 1 month at −20° C. The *p*H had no detectable influence on the quality of the labelled material.

Standard solutions of angiotensin containing 2·5, 5, 10, 20 or 40 ng of Asp(NH$_2$)^1–Val^5-angiotensin II in 1·5 ml. were prepared by serial dilutions with a mixture of two volumes of 0·3 molar ammonium hydroxide and one volume of 1·4 molar *tris* buffer brought to *p*H 8 with hydrochloric acid (HAS added). This mixture was chosen

Table 1. RATIO (± *S.D.*) OF EQUIMOLAR AMOUNTS NECESSARY TO REDUCE THE INITIAL BINDING TO 50 PER CENT

Compound	Amino-acid sequence	Ratio Antiserum No. 6019	 7046	 7051
A	Asp(NH$_2$)–Arg–Val–Tyr–Val–His–Pro–Phe	1	1	1
B	Asp–Arg–Val–Tyr–Val–His–Pro–Phe	1·08 ± 0·04	1·1	1·1
C	Asp(NH$_2$)–Arg–Val–Tyr–Ileu–His–Pro–Phe	1·43 ± 0·13	1·9	2·5
D	Arg–Val–Tyr–Ileu–His–Pro–Phe	1·80 ± 0·15	2·3	4·2

for two reasons: (1) the binding of the antigen and the antibody is at an optimum between *p*H 7·5–9·5; (2) ammoniacal eluates containing angiotensin from cation resin columns (often used to isolate angiotensin from human plasma) can be tested directly after mixing with a *tris* buffer. In this way evaporation to dryness and subsequent redissolving of the eluate[6] are not necessary. The antiserum was diluted with normal rabbit serum (as a source of carrier gamma globulins) to an antibody concentration which provided a 50–60 per cent binding of 0·025 μc. ^{125}I-angiotensin and di-Na-EDTA were added to a final concentration of 0·06 molar to inhibit aminopeptidase activity.

A mixture of ammonium hydroxide and *tris* buffer (1·5 ml.), containing the angiotensin, was mixed in a glass tube with 0·05 ml. of the ^{125}I-angiotensin solution (about 0·025 μc.) and incubated with 0·5 ml. of diluted antiserum. After incubation for 1 h at room temperature, 2·05 ml. of saturated ammonium sulphate (4° C, *p*H 8) was added. After standing for 4 h at 4° C, the mixture was centrifuged (at 4° C) until the supernatant was clear. The supernatant was discarded. The precipitate was washed once with a half-saturated ammonium sulphate solution (4° C, *p*H 8) and dissolved at room temperature in 2 ml. of phosphate buffered saline (*p*H 7·4). The radioactivity of the sample was then measured.

The results were expressed as a percentage of radioactivity bound to antigen, "100 per cent" representing the initial binding (no unlabelled angiotensin added). Equimolar amounts of Asp^1-Val^5-angiotensin II (compound *B*), Asp(NH$_2$)^1-Ileu^5-angiotensin II (compound *C*) and des-Asp^1-Ileu^5-angiotensin II (heptapeptide 2-8, compound *D*) were compared with Asp(NH$_2$)^1-Val^5-angiotensin II (compound *A*), in respect to the binding affinity.

In Fig. 1 the mean values of nine different series of determinations (each series in triplicate), obtained with an antiserum produced by one rabbit (No. 6019), are shown. The standard deviation of the individual determination was ±7 per cent.

For each series the ratio of equimolar amounts of compound *B*, *C* and *D* relative to *A*, necessary to reduce the initial binding to 50 per cent, was calculated. The mean values are given in Table 1.

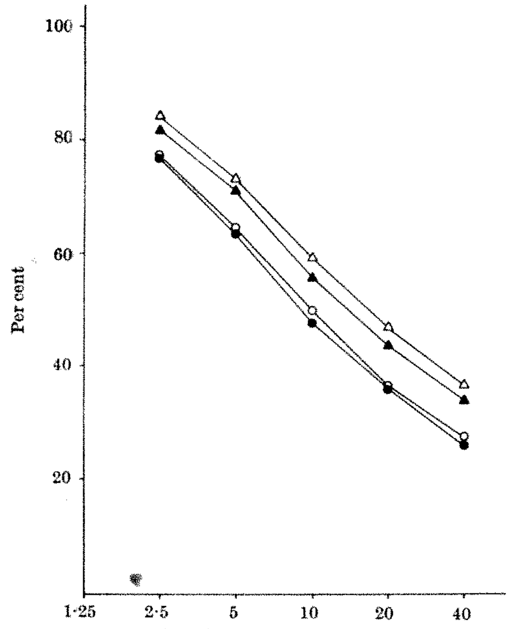

Fig. 1. Displacement of radioactivity from the antibodies by increasing amounts of Asp(NH$_2$)^1-Val^5-angiotensin II (compound *A*, plotted in ng per tube) compared with equimolar amounts of compounds *B*, *C* and *D*. 100 per cent represents the initial binding. ●, Asp(NH$_2$)^1-Val^5-angiotensin II; ○, Asp^1-Val^5-angiotensin II; ▲, Asp(NH$_2$)^1-Ileu^5-angiotensin II; △, des-Asp^1-Ileu^5-angiotensin II.

The differences between the various compounds cannot be explained by impurity of the substances because the Val^5-angiotensin II amide antibodies produced by three different rabbits (Nos. 6019, 7046 and 7051) possess different immunological affinities to compounds *B*, *C* and *D*.

We thank Dr F. Gross, Ciba, Basle, for his gifts of compounds *A* and *B* and Miss W. Kloosterziel and R. van Aalst for technical assistance. Professor E. Havinga, University of Leiden, provided compounds *C* and *D*.

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[1] Haber, E., Page, L. B., and Jacoby, G. A., *Biochemistry*, **4**, 693 (1965).
[2] Vallotton, M. B., Page, L. B., and Haber, E., *Nature*, **215**, 714 (1967).
[3] Dietrich, F. M., *Immunochemistry*, **4**, 65 (1967).
[4] Goodfriend, T. L., Levine, L., and Fasman, G. D., *Science*, **144**, 1344 (1964).
[5] Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495 (1962).
[6] Boucher, R., Veyrat, R., de Champlain, J., and Genest, J., *Canad. Med. Assoc. J.*, **90**, 194 (1964).

Axoplasmic Transport of ^{14}C-Noradrenaline and Protein in Splenic Nerves

MOST of the noradrenaline in postganglionic sympathetic neurones is bound within amine granules concentrated in the axon terminals[1]. These structures, which contain protein, are involved in the synthesis, storage and release of noradrenaline[2]. When a constriction is placed on the nerve trunk, noradrenaline accumulates proximal to it. This has been ascribed to the arrested distal transport of the granules from their site of manufacture in the cell body[3], a view which is supported by electron microscope evidence of a corresponding accumulation of granular vesicles[4]. Their flow rate in cat sympathetic nerves of 10 mm/h, however, derived from the rate of noradrenaline accumulation[3], is about 200 times that obtained for protein movement in somatic nerves (1 to 5 mm/day)[5]. Because the calculation assumes that no noradrenaline is synthesized, lost to or taken up from the circulation in the movement of the storage granules down the axon and at the site of constriction, an alternative method, labelling both noradrenaline and protein with isotopes, was used on the cat splenic nerves.

At laparotomy under ether anaesthesia, either ^{14}C-noradrenaline (5–10 μc.; 17·2 mc./mmole) or ^{14}C-leucine (5 μc.; 305 mc./mmole) was injected into the coeliac ganglion by multiple puncture of the capsule through a silicone grease seal, using a microsyringe with an electrolytically polished tip of 200μ. The cats were pretreated with iproniazid (50 mg/kg, intraperitoneally), and the preganglionic splanchnic nerves were cut, in order to minimize losses caused by metabolism and release, respectively. Two fine silk constrictions were placed 1 cm apart on a branch of the splenic nerves about 5 cm from the ganglion. After 6–24 h, the splenic nerves were stripped off the artery, cut into consecutive 5 to 10 mm pieces and homogenized in perchloric acid containing ethylenediamine tetra-acetic acid. Extracts containing catecholamines were counted for total radioactivity in aqueous scintillator[6]. Paper chromatography[7] showed that most of the radioactivity was present as noradrenaline and, to a lesser extent, normetanephrine. Proteins were washed with lipid solvents and dissolved in formamide. A sample was estimated for total protein, and the rest counted for incorporation of ^{14}C-leucine.

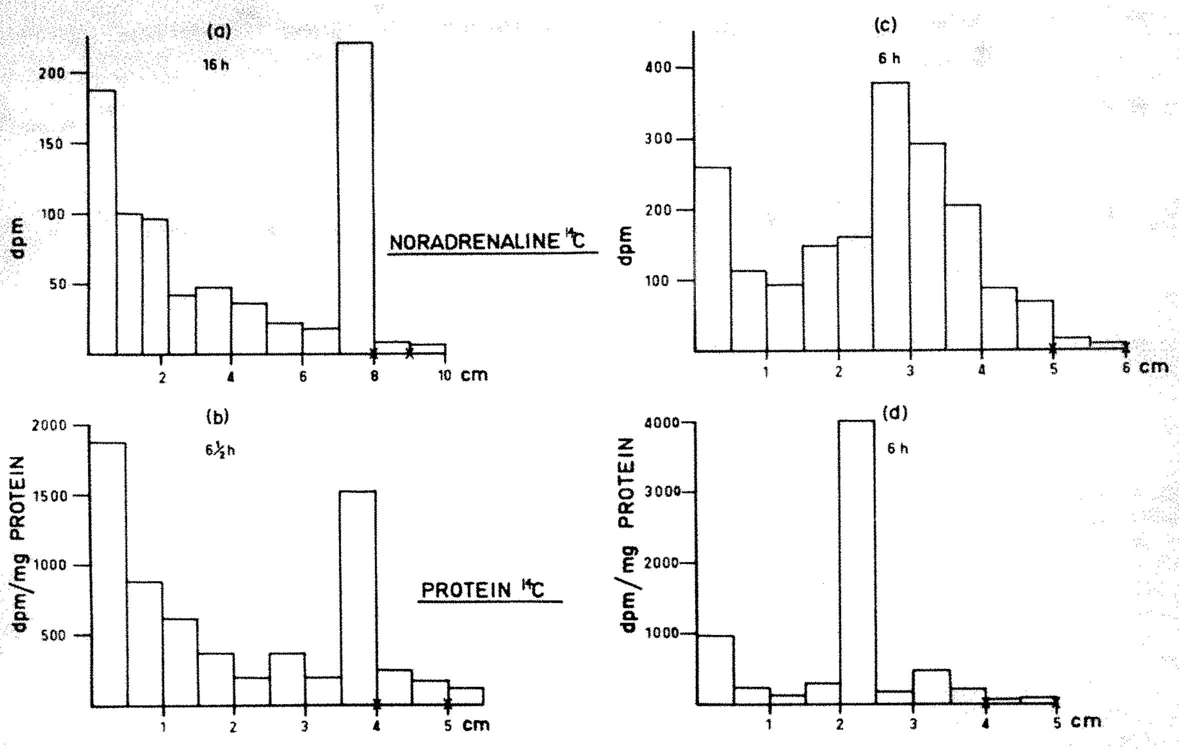

Fig. 1. Transport of ^{14}C-noradrenaline (*a* and *c*) and ^{14}C-protein (*b* and *d*) in cat splenic nerves. Abscissa: distance from coeliac ganglion. ×, Site of constriction. The distances shown in these four experiments are the minimum as measured *in situ*, and are subject to an error of up to 5 mm.

There was an accumulation of both labelled noradrenaline and protein just proximal to the upper nerve constriction but not the lower (Fig. 1 (*a*) and (*b*)). This indicates that a neural transport mechanism rather than uptake from the circulation was responsible for the accumulation.

Left for longer periods, the portion of spleen supplied by the untied branch of the nerve contained three to four times as much ^{14}C-noradrenaline as that supplied by the constricted branch, showing that labelled noradrenaline had reached the nerve terminals in the spleen by axonal transport as well as through the circulation. With shorter periods of 6–8 h, it was possible to locate peaks of both ^{14}C-noradrenaline and protein before they reached the constriction (Fig. 1 (*c*) and (*d*)).

The flow rate of approximately 5 mm/h obtained from these peaks is half that estimated previously for cat sympathetic nerves[3], but is considerably greater than any yet demonstrated for protein. If the noradrenaline is transported in granular vesicles, there may be two protein transport rates operating in the axon as observed in goldfish optic nerves[8]; perhaps a slow rate for structural proteins such as neuro-filaments, which the time scale of our experiments would not resolve, and a much faster one for soluble proteins and cytoplasmic particles like synaptic vesicles.

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[1] Euler, U. S. von, and Hillarp, N. Å., *Nature*, **177**, 44 (1956).
[2] Stjärne, L., *Acta Physiol. Scand.*, **67**, 441 (1966).
[3] Dahlström, A., and Häggendal, J., *Acta Physiol. Scand.*, **67**, 278 (1966).
[4] Kapeller, K., and Mayor, D., *Proc. Roy. Soc.*, B, **167**, 282 (1967).
[5] Lubinska, L., *Prog. Brain. Res.*, **13**, 1 (1964).
[6] Bruno, G. A., and Christian, J. E., *Anal. Chem.*, **33**, 1216 (1961).
[7] Roberts, M., *J. Pharmac. Pharmacol.*, **14**, 746 (1962).
[8] Grafstein, B., *Science*, **157**, 196 (1967).

Fine Structure of Nerve Fibres in Smooth Muscle of the Vas Deferens in Normal and Reserpinized Rats

IT is evident from Thaemert's[1] review that the intra-axonal granular vesicles are generally considered to be the site of storage of catecholamines in autonomic nerves. It has also been shown[2–7] that reserpine has a depleting effect on these amine stores in both peripheral and central nervous systems. No quantitative data on the effect of reserpine on various types of granular vesicles have been published, so a study of the effect of this drug on the nerve fibres of the vas deferens of the rat was undertaken.

Twelve male albino rats (200–250 g) were used in this work. Four were untreated controls, four received intraperitoneal reserpine (10 mg/kg) and were killed 6 h later, and four received three daily intraperitoneal injections of the same amount of reserpine and were killed 6 h after the last dose. The animals were killed by a blow on the head. Small pieces of vas deferens were quickly removed, fixed in buffered osmium tetroxide, dehydrated in acetone and embedded in 'Araldite'. Ultrathin sections were stained with lead citrate and examined in a Siemens Elmiskop 1*B* electron microscope.

The general organization of the smooth muscle cells and of the spatial relationships between nerve fibres and muscle cells in the rat vas deferens was found to be similar to that reported by Richardson[8]. The muscle is arranged in an outer longitudinal and an inner circular layer. Passing between the muscle layers are small groups of axons enclosed by Schwann cell cytoplasm and surrounded by a thin investment of connective tissue. These nerve bundles in turn branch into smaller groups, or even single axons, which run in the narrow spaces between smooth muscle cells.

There was a striking variation in the density of innervation of the rat vas deferens between the prostatic and epididymal ends (Table 1), the prostatic end having the most nerve fibres. The inner circular layer was relatively more densely innervated towards the epididymal end.

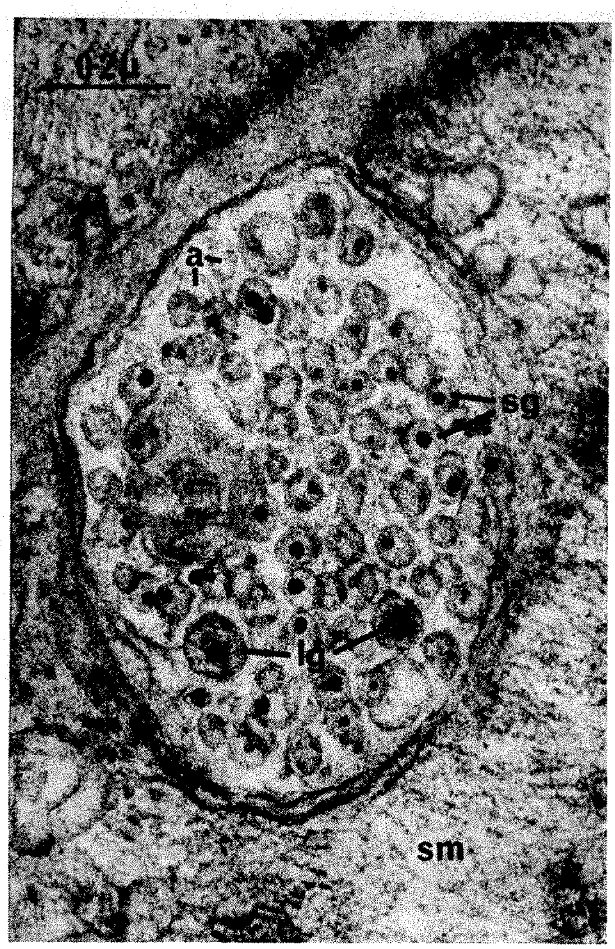

Fig. 1. An axon from a normal rat vas deferens, adjacent to smooth muscle cell (*sm*): agranular vesicles (*a*), small granular vesicles (*sg*) and an occasional large granular vesicle (*lg*). (× *c*. 85,000.)

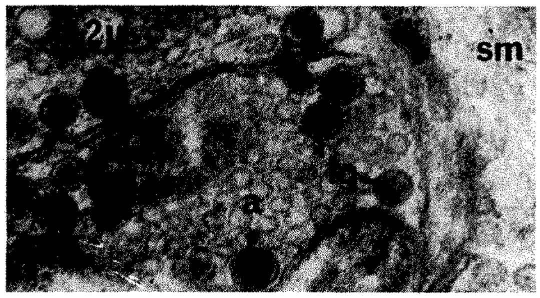

Fig. 2. Two axons from a reserpinized rat containing several large granular vesicles and agranular vesicles. (× *c*. 60,000.)

The axons contained the usual organelles: small membrane bounded vesicles, neurofilaments, neurotubules and mitochondria.

The neurofilaments have a diameter of about 60 Å and course through the axoplasm in all directions. They sometimes seem to interconnect vesicles and mitochondria by an extensive branching network (Fig. 1). In some axons neurotubules are especially numerous and often branch. They measure 200–250 Å in external diameter and contain a less electron dense core. They are usually orientated in the direction of the long axis of the nerve fibre. At intervals along their length there are small central aggregations of material of greater electron density. Small vesicular elements were frequently seen, which could be neurotubules cut in cross-section. The vesicles are of two types (Fig. 2). First, there are agranular vesicles enclosing a homogeneous material of low electron density. These are of fairly uniform size, 450–600 Å in diameter, with an occasional large vesicle 1000–2000 Å in diameter. Second, there are granular vesicles which fall into two classes. The first is a small vesicle (400–600 Å in diameter) containing a small dense inclusion (150–200 Å) which may be oval, rod-shaped or in the form of a doublet. The second is a large vesicle (800–1200 Å in diameter) which contains a spherical granule of moderate electron density (550–850 Å). All types of vesicles may be present in the same axon, but few axons exclusively contain agranular vesicles.

Table 1. AVERAGE NUMBER OF AXONS IN EACH OF SIX 75μ GRID SQUARES

	Prostatic end	Middle third	Epididymal end
Inner circular layer	79	63	33
Outer longitudinal layer	73	58	20

Fig. 3 shows vesicle population counts of 250 axons in normal animals and 250 axons in animals reserpinized for 6 h. In both groups the axons were selected at random from electron micrographs of the outer longitudinal layer of the middle third of the vas deferens. In normal rats there were approximately equal numbers of granular and agranular vesicles; 96 per cent of the granular vesicles were of the small, dense cored variety and 4 per cent were large with less dense centres.

Single or repeated doses of reserpine caused a marked reduction in the numbers of granular vesicles in nerve endings. This depleting effect was confined to the small granular vesicles (Fig. 4). The small granular vesicles were reduced from 48 per cent of the total in normal rats to 15 per cent of the total in the treated animals. The population of the large granular vesicles increased. The number of agranular vesicles was greatly increased ($P < 0·001$), but the total number of vesicles was not altered significantly.

It seems that the chief storage site of noradrenaline is in the small granular vesicles, but some of these storage sites seemed to be insensitive to depletion by reserpine in the doses given. The increase in agranular vesicles repre-

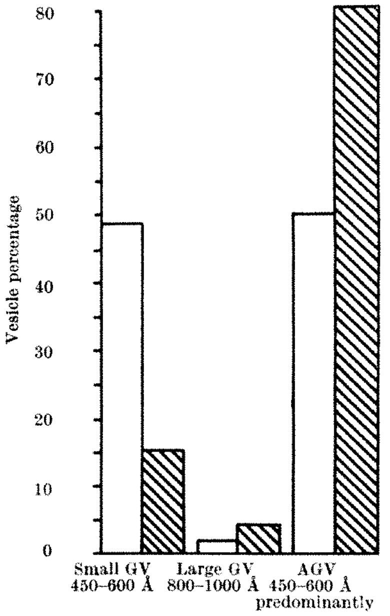

Fig. 3. The percentage of small granular vesicles, large granular vesicles and agranular vesicles in normal animals (white columns), and in rats reserpinized for 6 h (hatched columns). The absolute numbers of vesicles counted in each 250 axon series were 10,963 in normal animals and 11,305 in treated animals.

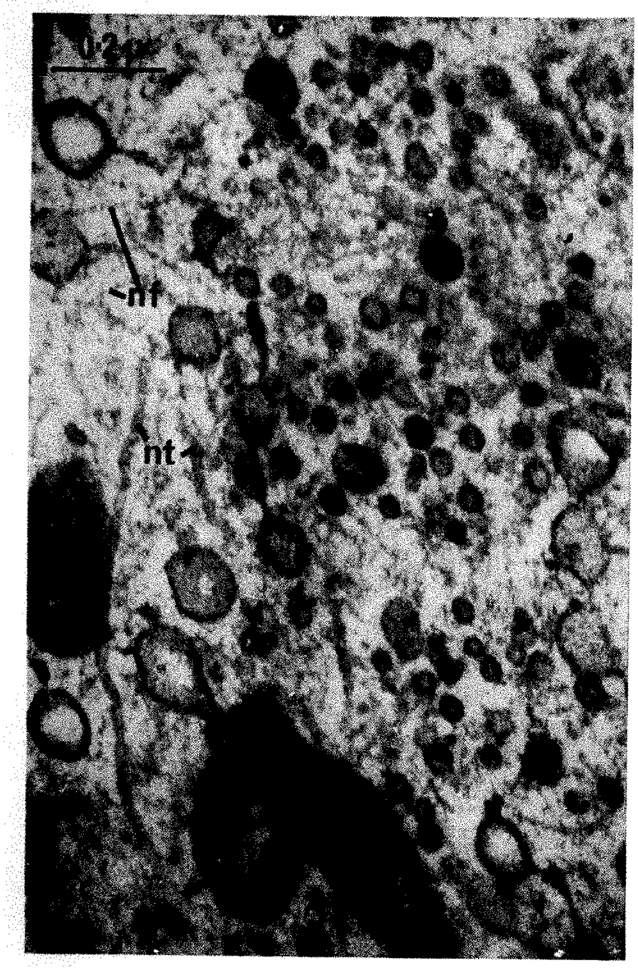

Fig. 4. An axon from a reserpinized animal. Neurotubules (*nt*) and neurofilaments (*nf*), which interconnect other organelles, are contained in the axoplasm. (× *c.* 75,000.)

sents vesicles of the same size which were granular before reserpinization. The larger granular vesicles, rather than being depleted by reserpine, increased in number.

In view of these findings, and those of Richardson[9] and Grillo[10], who showed, respectively, that the cholinergic axons of the dilator pupillae muscle and the superior cervical ganglion contained large granular vesicles, it seems possible that these larger granular vesicles in the vas deferens are a storage site for acetylcholine. The presence of both acetylcholine and noradrenaline within the same axons would lend support to the hypothesis of Burn and Rand[11] that both noradrenaline and acetylcholine are present in the same nerve fibre.

But the large granular vesicles may contain neither acetylcholine nor noradrenaline. It is possible that the inclusion is an unidentified neurotransmitter or its precursor. Alternatively, a different form of binding of a known neurotransmitter may be involved.

It is likely that the neurotubules, present in large numbers and many of which branch, are concerned with the function of intra-axonal transport. The theory of the distal flow of axoplasm is well substantiated and it is possible that neurotransmitter substances are manufactured at least partly in the cell body and transported to the periphery by means of the neurotubules. Silva[12] reported the presence of irregular dilatations along the course of intra-axonal neurotubules and suggested that these dilatations may represent a stage in the formation of the vesicles.

The observed variation in the density of innervation along the length of the vas deferens of the rat shows that extra care is necessary in interpreting quantitative results obtained by electron microscopy.

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[1] Thaemert, J. C., *J. Cell. Biol.*, **28**, 37 (1966).
[2] Richardson, K. C., *Biochem. Pharmacol.*, **12** (Suppl.), 10 (1963).
[3] Burnstock, G., and Merrillees, N. C. R., *Proc. Second Intern. Pharmacol. Meeting, Prague* (edit. by Bulbring, D.), 1 (1964).
[4] Devine, C. E., and Laverty, R., *Proc. Univ. Otago Med. Sch.*, **43**, 17 (1965).
[5] Hokfelt, T., *Experientia*, **22**, 56 (1966).
[6] Bloom, F. E., and Barnett, R. J., *Nature*, **210**, 599 (1966).
[7] Van Ordern, L. C., Bloom, F. E., Barnett, R. J., and Giarman, N. J., *J. Pharmacol. Exp. Therap.*, **154**, 185 (1966).
[8] Richardson, K. C., *J. Anat.* **96**, 427 (1962).
[9] Richardson, K. C., *Amer. J. Anat.*, **114**, 173 (1964).
[10] Grillo, M. A., *J. Cell. Biol.*, **27**, 136a (1965).
[11] Burn, J. H., and Rand, M. J., *Nature*, **184**, 163 (1959).
[12] Silva, D. G., *Anat. Rec.*, **158**, 21 (1967).

Types of Motor Units in the Skeletal Muscle of *Xenopus laevis*

INVESTIGATION of the histochemical and contractile characteristics of muscle fibres in hind limb muscles of *Xenopus laevis* and *Bufo viridis* has shown that the fast group of muscle fibres can be subdivided[1]. Some fast fibres, usually those of largest diameter, are low in oxidative enzymes and have few fat droplets. On direct electrical stimulation the contractile mechanism in these fibres rapidly fatigues. The second group of fast fibres consists of cells of smaller diameter showing a high activity of oxidative enzymes and containing many fat droplets. These fibres maintain a contracted state for prolonged periods if repeatedly stimulated. Electron microscope observations (unpublished results of R. S. Smith and S. Page) confirm the histochemical findings in that the two types of fibres contain differing numbers of mitochondria and fat droplets, and also demonstrate a higher concentration of glycogen in the latter group of fast fibres.

Slow muscle fibres isolated from *Xenopus laevis* have been found to be histochemically[1], functionally[1,2] and structurally (unpublished results of R. S. Smith and S. Page) distinct from the two types of fast fibres. In our previous study[1] of the group of slow fibres, we were unable to find evidence that the group could be subdivided, although Shamarina[3] has presented evidence which indicates that a subdivision does exist in the frog.

The present investigation was begun with the following intentions: (1) to determine whether fast motor units are homogenous; (2) to attempt to detect sub-groups within the slow fibres; and (3) to explore the relationship between the type of motor unit and the diameter of the motor nerve.

The iliofibularis muscle of *Xenopus laevis* was used throughout. As in other members of the Anura[4], the iliofibularis muscle of *Xenopus* is zoned[1]. The large and fatigable fast fibres occupy the outer parts of the muscle, while the less fatigable fast fibres and slow fibres are in the centre of the muscle. This organization assists in assessing the position and thus the likelihood of homogeneity of fast motor units.

The nerve–muscle preparation was kept in Ringer's solution at 20°–23° C in a 'Perspex' recording chamber of the type described before[1]. A single nerve fibre was dissected from the nerve trunk and stimulated electrically with rectangular pulses of 1 msec duration and amplitude about 2 dB above threshold. The contraction of the motor unit was recorded isometrically by a strain gauge attached to one tendon of the muscle, the other tendon being fixed. The strain gauge output was displayed on a strip chart

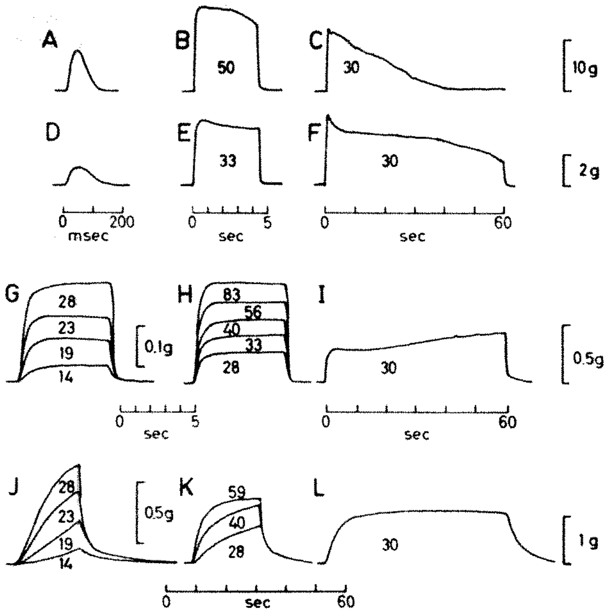

Fig. 1. Mechanical activity of single motor units in the iliofibularis muscle after their motor nerves had been stimulated. The number on each trace indicates the rate of stimulation in stimuli/sec. Traces *A* and *D* are twitch responses to single stimuli. *A*, *B* and *C*: responses of a fast motor unit in the peripheral part of the muscle. *D*, *E* and *F*: responses of a fast motor unit in the centre of the muscle. *B* and *E* are fused contractions. Stimulus train for both *C* and *F* 60 sec duration. Third row, *G*, *H* and *I*, and fourth row, *J*, *K* and *L*, are responses of slow motor units of the faster and slower types, respectively. Ordinates, grams force, on the right of each group of records to which they refer. Abscissae, seconds, immediately below each group of seconds to which they refer.

recorder ('Sanborn 320'). During recording, the muscle could be observed through a binocular stereomicroscope, which enabled an estimate to be made of the position of the motor unit within the muscle. Immediately after the experiment a photomicrograph was taken of the isolated nerve fibre, and the diameter of the fibre was then measured to the nearest micron from the photographic negative. Twenty satisfactory preparations were obtained. Individual motor units within the subgroups were typically alike, but the number of observations in each group was small; thus, in this preliminary report, the ranges of values only will be given.

A preliminary study of cross-sections of the nerve to the iliofibularis muscle showed that the diameters of myelinated nerves ranged from 2 to 20μ. Single motor nerve fibres, which covered the range 4 to 20μ, were obtained in these experiments. The results of stimulating the single nerve fibres were grouped into four classes (Fig. 1). The top row of Fig. 1 shows the response of a motor unit composed of fibres in the peripheral part of the muscle to: *A*, a single stimulus; *B*, stimulation at fusion frequency; and *C*, maintained stimulation at 30 stimuli/sec. Units of this type characteristically showed complete fatigue within about 30 sec of stimulation at a rate of 30 stimuli/sec. These units were large and gave maximum tetanic tensions of 16·8–29 g with fusion frequencies typically at about 50 stimuli/sec. The nerve fibres supplying the units were of diameter 17 to 20μ.

The second row of records (Fig. 1, *D*, *E* and *F*) presents corresponding results obtained from a fast motor unit in the centre of the muscle. These units were not as readily fatigued as those in the periphery of the muscle; they were also smaller, maximum tetanic tensions ranging from 1·0 to 13·7 g at fusion frequencies of 25 to 35 stimuli/sec. Nerve fibres supplying this group of motor units ranged from 9 to 16μ in diameter.

While the contraction times measured from single twitches in these two types of fast motor units did not differ markedly in this series of experiments, the half decay times of twitches obtained from units in the central part of the muscle were longer (40–65 msec) than those (30–40 msec) obtained with fast motor units in the periphery of the muscle.

It seems that the group of fast fibres forming a motor unit exhibit together properties sufficiently similar to the individual fibres in the same muscle, as was studied in previous work[1], to permit the conclusion that the units are homogeneous.

A slow motor unit will be defined for the present purposes as a unit which would not give a measurable contractile response after a single suprathreshold stimulus to the nerve supplying the unit, but responded to repetitive stimulation with graded contractions. This definition assumes that the motor unit has undergone a long period of rest before stimulation. The slow motor units, observed in these experiments, were separated into two types, both of which were situated in the central part of the muscle.

Responses typical of slow motor units supplied by nerves of diameters 6–10μ are illustrated in Fig. 1*G*, *H* and *I*. The nested curves in records *G* and *H* show the responses of this type of unit to repetitive stimulation at frequencies between 14 and 83 stimuli/sec. It should be noted that the rate of rise of tension in the records of Fig. 1*H* is comparable with that obtained with the fast motor unit of Fig. 1*E*. Maintained stimulation at 30 stimuli/sec gave rise to responses as shown in Fig. 1*I* and Fig. 2*A*; after reaching an initial plateau the tension showed a secondary climb.

Slow motor units supplied by nerves 4 to 5μ in diameter responded to repetitive stimulation in the manner illustrated in Fig. 1*J*, *K* and *L*. The rate of rise of tension was slow and no late increase in tension was seen on maintained repetitive stimulation. Both types of slow units were small; the maximum tetanic tensions ranged from 230 mg to 1·5 g at stimulation rates up to 100 stimuli/sec.

The group of slow motor units with the faster mechanical activity (Fig.1*G*, *H* and *I*) had unusual properties. If the train of stimuli was stopped during the late rise in tension, then small discrete contractions could be recorded in response to single test stimuli (Fig. 2*B*). This effect was never shown by the slower group of slow motor units (Fig. 1*J*, *K* and *L*). The processes behind the late rise in tension, seen during maintained stimulation in the faster group of slow motor units, are presumably connected with those responsible for the discrete responses to single stimuli. If it is assumed that this type of motor unit is homogeneous in its component muscle fibres, an assumption for which there is as yet no evidence, then a possible explanation for these effects would be found in the properties of the neuromuscular junction. Thus the discrete contractions, which could be elicited in the immediate post-tetanic period, could represent large local contractions caused by potentiation of junctional transmission. Other explanations, invoking propagated activity in the muscle fibres, are conceivable but, owing to the low

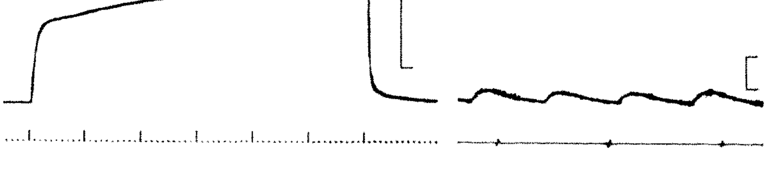

Fig. 2. Responses of a slow motor unit of the type shown in Fig. 1, *G*, *H* and *I* to repetitive stimulation of its motor nerve at 30 stimuli/sec, *A*, and to a group of four test stimuli in the immediate post-tetanic period, *B*. Abscissae: seconds and 10-sec markers in *A*, seconds in *B*. Ordinates: 250 mg force in *A* and 25 mg force in *B*.

amplitude of the discrete contractions (Fig. 2*B*), would require unusual assumptions as to the properties of the muscle fibres involved.

The evidence presented here shows that within both the fast motor unit group and the slow motor unit group motor units may show considerable differences in properties. In both the fast and slow groups of motor units, there seems to be a relationship between the properties of the motor unit and the diameter of its motor axon. The observations would satisfy several hypothetical systems from the one extreme, at which it is assumed that all properties of motor units are continuously graded from slow on through fast, to what is perhaps the other extreme at which the assumption is made that changes in properties take place step-wise and that both fast and slow motor units are divisible into two distinct types. This latter view, which is implicit in the arrangement of Fig. 1, has the merit that it lends itself to experimental testing.

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[1] Lännergren, J., and Smith, R. S., *Acta Physiol. Scand.*, **68**, 263 (1966).
[2] Lännergren, J., *Acta Physiol. Scand.* (in the press).
[3] Shamarina, N. M., in *The Effect of Use and Disuse on Neuromuscular Functions* (edit. by Gutmann, E., and Hnik, P.) (Czech. Acad. Sci., Prague 1963).
[4] Sommerkamp, H., *Arch. Exp. Pathol. Pharmak.*, **128**, 99 (1928).

BIOLOGY

Interaction of Trematodes by Predation within Natural Double Infections in the Host Snail *Indoplanorbis exustus*

THOUSANDS of snails infected with trematodes have been surveyed in the Kuala Lumpur area[1-5]. *Lymnaea rubiginosa* (Michelin), the commonest and most carefully studied snail, is infected with at least thirteen species of trematode larvae: four echinostomes, three plagiorchids, two strigeids, one *Spirorchis*, one *Trichobilharzia*, one *Fasciola* and one psilostome. Multiple infections were, however, very rare and certain trematode combinations were not found[7] and none of the few double infections found involved species with redial development[2]. The only double infections found were those in which both parasites underwent sporocyst development (producing strigeid and schistosome furcocercous or plagiorchid xiphidiocercous cercariae, personal communication from K. J. Lie).

Specimens of *Lymnaea rubiginosa* collected in the field and shedding strigeid, schistosome or plagiorchid cercariae (larvae with sporocyst patterns of development) were exposed to *Echinostoma* miracidia. These experiments confirmed that there was an "antagonism" between the trematode with only sporocyst generations and an echinostome with rediae[4]. There was no demonstrable host restriction on penetration of miracidia of the second trematode or early development of the second infection[7,8]; antagonism or interference between the two parasites occurred at later developmental stages. Production of cercaria by flukes of the sporocyst developmental type ceased soon after this stage was reached. The authors suggested that the motile rediae grazed on the schistosome sporocysts, for sporocysts were not concentrated in regions where active rediae were found. Alternatively, the rediae seemed able to kill the sporocysts by a toxic substance and then to feed on the dead larvae[8]. Similar reactions were observed in echinostome–echinostome (redia + redia) double infections[10]. Later studies with Brazilian echinostomes and *Schistosoma mansoni* in albino *Biomphalaria glabrata* confirmed and extended these findings[11].

We studied double infections of *Echinostoma malayanum* Leiper 1911 and *Schistosoma spindale* in *Indoplanorbis exustus* in a further effort to explore the interaction of trematode with trematode, using a different parasite host system, but our efforts to observe a reaction in laboratory infections were frustrated because of the 35–40 days required by *E. malayanum* to develop in the snail.

Conditions required for a large-scale experiment with doubly infected snails were provided naturally by a population of exceptionally large *I. exustus* (up to 20 mm in diameter) discovered at the outlet of an abandoned tin-mining pond at Kampong Pandan, near Kuala Lumpur. Five collections totalling 650 snails were made in February 1966. Among these, twelve were actively shedding *E. malayanum* and *S. spindale* cercariae simultaneously. Dissection showed true double infections in only ten out of the twelve; the other two had echinostome infections, but schistosome cercariae had entered the mantle cavity from the outside or may have adhered to the surface of the shell. Later these fork-tailed cercariae reappeared in the water to simulate a normal shedding of cercaria. Dissection of all snails collected throughout the month demonstrated a true double infection in forty-seven of them (Table 1). In addition to the ten snails shedding both types of cercariae, we found double infections in thirty snails that were shedding only schistosome cercariae, and in three snails shedding only echinostome cercariae. Four other snails also proved to be doubly positive, although they were shedding neither cercaria at the time. The schistosome infections in these hosts were largely displaced by a vigorous development of echinostomes.

One snail was shedding fork-tailed cercariae of *S. spindale* as well as amphistone cercariae, and four were shedding schistosome and xiphidiocercariae, indicating a twin sporocyst-type of infection. In the latter group only three snails were infected on dissection, a further indication that "shed" larvae found in the water with snails collected in the field may be unreliable.

These unexpected double infections in the wild were the first such discovery among many thousands of snails from dozens of sites. They also appeared at a level well

Table 1. NATURAL DOUBLE INFECTIONS IN *Indoplanorbis exustus*

Snails shedding the following cercariae			Snails infected with the following trematode combinations (based on individual snail dissections)									
			Trematode types in snails									
Cercariae shed	No. of snails	% of total	S + E	S + A	S + X	S	E	A	X	Pos.	Neg.	Total
S + E	12	1·8	10				2			12		12
S + A	1	0·2		1						1		1
S + X	4	0·6			3				1	4		4
S	176*	27·1	20			128				158	18	176
E	82*	12·6	3				62			65	17	82
A	10	1·5						10		10		10
X	1	0·2							1	1		1
Pos.	286	44·0	43	1	3	128	64	10	2	251	35	286
Neg.	364	56·0	4			10	24	8		46	318	364
Total	650	100·0	47	1	3	138	88	18	2	297	353	650
		% of total:	7·2	0·2	0·5	21·2	13·5	2·8	0·3	45·7	54·3	100

Data from 650 snails, Kampong Pandan, Malaysia, February 1966. S, *Schistosoma spindale*; E, *Echinostoma malayanum*; A, amphistome cercariae; X, xiphidio cercaria.

* Recorded "shed" includes snails into which cercariae swam and emerged up to 24 h later. This method of analysis proved unreliable and so was considered a first approximation only. True infection levels and incidence can only be determined by dissection and examination of each test snail.

above that expected assuming a probability of double infections to be random, that is the product of the infection rates for each of the separate species.

In the dissected snails, *S. spindale* was present in 21·2 per cent of the 650 snails, *E. malayanum* in 13·5 per cent to give a predicted level, based on random distribution, of 2·9 per cent double infections. Actually 7·2 per cent, or two and a half times the expected proportion, of the *Indoplanorbis* collected from this site were simultaneously infected with the two flukes. Double sporocyst infections (schistosome plus plagiorchid) occurred in 0·46 per cent of the total (3 of 650 snails), whereas prediction by random distribution would give only 0·06 per cent of these double infections. Only one snail was shedding xiphidiocercariae, whereas three were shedding xiphidio and furcocercous cercariae simultaneously. Double infections of the schistosome with an echinostome in a greater proportion than that predicted by random distribution may have occurred entirely by chance, or it may have been caused by reduced host resistance to a second infection in snails already carrying a different infection. In either case, it was remarkable to find so many double infections, considering the rapid loss of doubly infected snails in our laboratory conditions. It also attests to our lack of understanding of the optimum conditions required by infected snails.

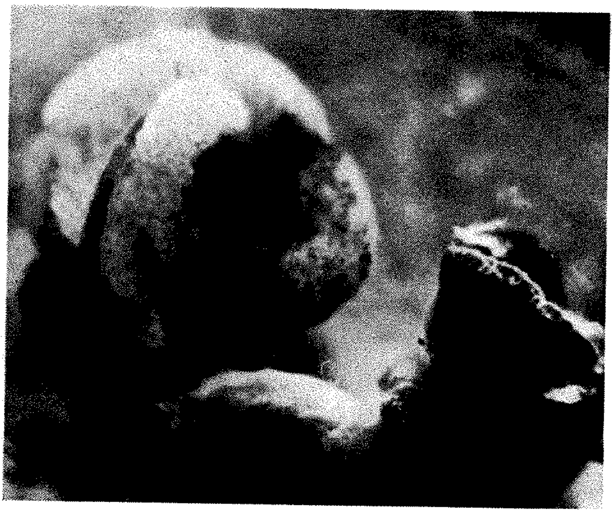

Fig. 1. Grape-like cluster of sporocysts of *Schistosoma spindale* alongside gut of *Indoplanorbis exustus*.

During dissection of the doubly infected snails, we made extended observations of the living and active rediae on and in the intact body of the de-shelled snail. Daughter rediae could be seen crawling over the surface of the digestive organ, particularly within the arch of the whorl, and over the ovotestis near the tip of the viscera. In areas of redial concentration, few if any schistosome sporocysts were observed. Typically, a heavy mass of sporocysts, threaded into the tissues, was concentrated in the midzone of the digestive gland or was hanging from this region in grape-like clusters (Fig. 1). Rediae were moving about the periphery of this mass, but a few could also be seen among the sporocysts. Some rediae were lying next to sporocysts and apparently consuming them.

A series of observations of infected snail tissue confirmed that sporocysts were being devoured by rediae. Infected tissues were removed intact and preparations were studied microscopically both with and without a coverslip. Despite this disturbance, larval activity in and around the snail tissues continued for about 30 min.

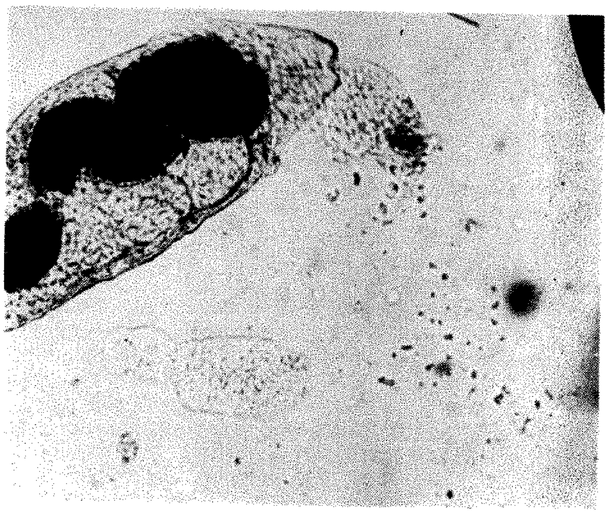

Fig. 2. Daughter redia of *Echinostoma malayanum* ingesting a sporocyst of *S. spindale* it has captured.

One redia had a third of a sporocyst lying within its pharynx, another a sporocyst in the oral opening; a third had almost the entire larva consumed, with only a small portion still protruding (Fig. 2). Cannibalistic activities are known among echinostome rediae. Wesenberg-Lund[12] describes daughter rediae feeding on other daughter rediae within the mother rediae; Nasir[13] found remains of rediae in the gut of *E. nudicaudatum* rediae, both in mother rediae and in daughter rediae free in the snail tissues.

Most surprising, however, was our observation that rediae can capture and ingest swimming cercariae, although the cercariae are far more active and motile than the rediae. Three such cases were observed: one redia made a movement towards a thrashing schistosome cercaria, snatched at it rapidly and captured it by the midbody, swallowing the entire cercaria within a minute or two. Another redia held the body of a cercaria within its pharynx, forked tail still protruding (Fig. 3). Still another caught a schistosome cercaria by the tail, with the anterior body portion protruding and attempting to escape. Undoubtedly the highly muscular pharyngeal apparatus of the redia provides a sucking structure that serves the larva admirably for both capture and swallowing

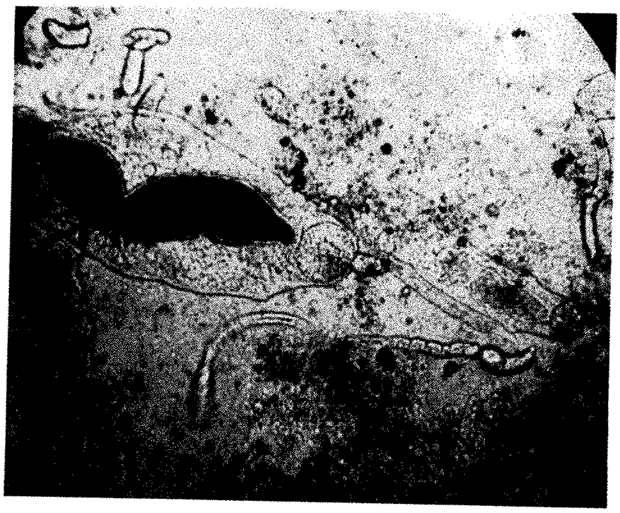

Fig. 3. Redia of *E. malayanum* consuming a cercaria of *S. spindale* it has captured. Tail of cercaria with fork collapsed visibly protruding from mouth of redia.

of its parasitic prey as well as for ingestion of host tissues. There was, however, no indication of cannibalism.

Clearly, the "antagonism" between *S. spindale* and *E. malayanum* is direct predation in this instance. How the schistosome larvae managed to survive in this series of hosts long enough to show both kinds of cercariae in the same snail is not known. A critical factor was the precise stage of development of each parasite species at the time the snails were collected. The outlet of the tin-mining pond where the *Indoplanorbis* were found is part of a hyperendemic region of *S. spindale* infection in goats and cattle maintained by the local Indian community. Conceivably, snails in this pond were free of *Echinostoma malayanum* (carried chiefly by pigs and rodents), and a sudden influx of infected faeces about 6 weeks before our first February collection may have exposed them to large numbers of *E. malayanum*. A group of heavily infected swine usually maintained elsewhere could have provided the echinostome miracidia had they been moved into this pond area, a not infrequent occurrence.

The doubly infected snails which we examined showed that the echinostome infection was ascending and the schistosome one descending. Where only schistosome cercariae were being shed, the echinostomes were seen chiefly as young daughter rediae. Where both cercariae were being shed, the echinostome infection was far heavier. Where only echinostomes were producing cercariae, the remaining schistosome sporocysts were few and scattered, obviously nearly eliminated. All doubly infected snails were the largest hosts (15–20 mm), whereas single infections were found chiefly among the 10–15 mm snails. This growth phenomenon, though not uniform or constant, has been reported in a number of snail species parasitized with larval trematodes[14,15].

We surveyed the Kampong Pandan area both before and after the discovery of the doubly infected snails. *E. malayanum* is present at a relatively low level in this region (up to about 10 per cent of the snails in an average collection). *S. spindale* is usually present in a larger percentage of the *Indoplanorbis*, reaching about 50 per cent in the vicinity of dairies where grazing cattle are numerous (and there are few echinostomes present). Snails with double infections were found later in the same site, but in a smaller proportion of snails examined than in the collection analysed here.

Later another double infection at a high level was found in a colony of very large *Indoplanorbis* snails in a roadside stream near Kampong Bohol, 6·5 miles from Kuala Lumpur. This second find in *Indoplanorbis* and our failure to locate a double infection in *Lymnaea* suggest that trematode repression of one species by another is at least in part a matter of balance between parasite and host, varying with the degree of adaptation of each species to its particular host, as emphasized by Lie *et al.*[6]. An example of conditions which appear to favour the predator is found in the combination of *Echinostoma audyi* (or *Echinoparyphium dunni*) and *Trichobilharzia* in *Lymnaea rubiginosa*. An opposite example, in which a well adapted schistome is difficult to eradicate from its snail host by an apparently less well adapted species of echinostome, is the *S. mansoni–Echinostoma barbosai* system in *Biomphalaria glabrata*[7]. The natural infections reported here from *Indoplanorbis*, *E. malayanum* plus *S. spindale*, represent an intermediate stage of adaptation in which the predator can wipe out its neighbouring parasite but does not necessarily do so before schistosome cercarial production begins. The success of this part of the reproductive phase is of special significance if double infections are to be considered as potential biological weapons against human schistosomiasis.

We think that the following points from these observations are important. (1) The trematode redial stage is not only a parasite within its snail host but can also act as a predator that seeks and devours developmental stages of other flukes in the same snail.

(2) There is need for exploration of this phenomenon as a possible supplementary biological weapon against human schistosomes, one that might function within the snail host without the entire snail population having to be destroyed. To develop the tools for such a potential biological control, however, each area of human or animal disease must be evaluated ecologically. It is necessary to determine the appropriate predator–parasite and prey–parasite balance and their mutual adaptation to a particular host. It is possible to introduce a new parasite into the environment to attack a resident schistosome focus. This should be approached only after the predator's anti-schistosome and host–parasite characteristics have been fully tested in laboratory and field-plot studies. We undertook a small field study in Malaya to test the feasibility of this approach, using the combination of *S. spindale* and *E. malayanum*[16]. Initial success of this effort shows that further search for such a biological weapon is justified. It will, however, require specific tailoring and application of each combination of trematode and host to the area in which it is applied.

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[1] Lie, K. J., *Trop. Geog. Med.*, **15**, 17 (1963).
[2] Lie, K. J., *Trop. Geog. Med.*, **16**, 61 (1964).
[3] Lie, K. J., and Umathevy, T., *J. Parasitol.*, **51**, 781 (1965).
[4] Lie, K. J., and Umathevy, T., *J. Parasitol.*, **51**, 793 (1965).
[5] Lie, K. J., and Umathevy, T., *J. Parasitol.*, **52**, 449 (1966).
[6] Basch, P. F., and Lie, K. J., *Med. J. Malaya*, **20**, 59 (1965).
[7] Basch, P. F., and Lie, K. J., *Z. f. Parasitenkunde*, **27**, 260 (1966).
[8] Lie, K. J., Basch, P. F., and Umathevy, T., *Nature*, **206**, 422 (1965).
[9] Basch, P. F., and Lie, K. J., *Z. f. Parasitenkunde*, **27**, 252 (1966).
[10] Lie, K. J., Basch, P. F., and Umathevy, T., *J. Parasitol.*, **52**, 454 (1966).
[11] Lie, K. J., *Nature*, **211**, 1213 (1966).
[12] Wesenberg-Lund, C., *Mem. Acad. Royale Sciences Lettres Danemark*, series 9, V, 1 (1934).
[13] Nasir, P., *Proc. Helminth. Soc.*, Washington, **29**, 115 (1962).
[14] Rothschild, M., *J. Mar. Biol. Assoc.*, UK, **20**, 537 (1936).
[15] Pan, C., *Amer. J. Trop. Med. Hyg.*, **14**, 931 (1965).
[16] Heyneman, D., and Umathevy, T., *Med. J. Malaya*, **21**, 373 (1967).

Effect of β-Indolylacetic Acid on the Growth and the Auxin Catabolism of *Lactobacillus bulgaricus*

ENDOGENOUS auxin may act in the regulation of bacteria growth[1], although its precise role is as yet poorly understood[2]. For example, *Lactobacillus* can grow without auxin[3]; however, in the presence of β-indolylacetic acid (IAA), its multiplication can be strongly modified[4]. In the following experiments, *Lactobacillus bulgaricus* (strain 1416, Liebefeld Institute of Dairy Research) was used and the medium was *Lactobacillus* broth *AOAC* (Difco). For each series of growth measurements, tubes containing 10 ml. of medium (with IAA) were inoculated with 1 ml. of a young culture of *Lactobacillus* (standard: 0·416 ml. of protein). The cultures were incubated at 37° C and the growth was measured after 6 and 12 h. Two methods were found most satisfactory for the growth determinations. The first is related to a direct measurement of the increase in turbidity[5], using the 'Lumetron 402 *E*' with a double photoelectric cell. The second is based on the analysis of the total protein content[6]. The protein was determined by precipitating the washed

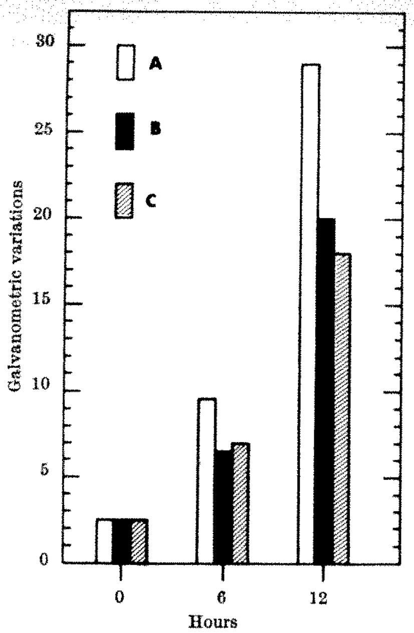

Fig. 1. Growth of *Lactobacillus bulgaricus* (in terms of turbidity: galvanometric variations). Time of incubation, 0, 6 and 12 h. *A*, Control; *B*, 1×10^{-5} molar β-indolylacetic acid; *C*, 1×10^{-3} molar β-indolylacetic acid.

bacteria cells with trichloroacetic acid. Comparing the turbidity with a series of standard solutions of serum–albumin, precipitated with trichloroacetic acid, a good correlation was found. Further examination of this technique will be discussed elsewhere.

It can be seen in Fig. 1 that the growth (in terms of turbidity) increases with time. After 6 and 12 h, IAA causes an inhibition for the two concentrations tested. In Fig. 2, the increase with time in protein content is shown. IAA produces an initial repression of the growth. But, after 8 to 10 h, the logarithmic growth phase[4] of the *Lactobacillus* treated with $1 \cdot 10^{-5}$ molar IAA continues to increase while with 1×10^{-3} molar IAA or without IAA the growth rate is lower and quickly approaches the stationary phase.

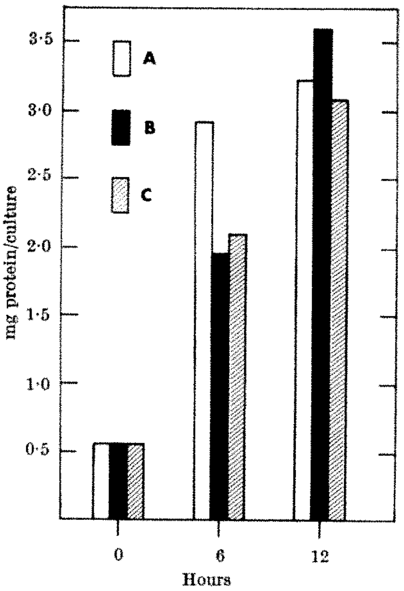

Fig. 2. Protein content of *Lactobacillus bulgaricus* (in mg of protein/culture). Time of incubation, 0, 6 and 12 h. *A*, Control; *B*, 1×10^{-5} molar β-indolylacetic acid; *C*, 1×10^{-3} molar β-indolylacetic acid.

To understand the action of exogenous auxin on the growth of *Lactobacillus*, the IAA-oxidase of this bacteria was analysed. It is well known that IAA-oxidase catalyses the oxidation of IAA[7]; conversely, IAA alters the *in vivo* activity of IAA-oxidase[8,9]. These facts suggest that the mutual interaction between hormone and enzyme may be important in the control of growth[10,11].

The preparation of the IAA-oxidase has been described before[12]. *In vitro*, IAA destruction assays were carried out with lyophilized *Lactobacillus* and cold buffer (0·1 molar; *p*H 6·1). The mixture was first centrifuged (3,500*g*, 15 min), the extract was added to the buffer for a second centrifugation (8,000*g*, 10 min) and the supernatants were made up to 10 ml. with buffer. Two ml. of the active solution was mixed with 2 ml. of deionized water and 4 ml. of cold buffer. At zero time, 2 ml. of IAA (50 μg/ml.) was added. The mixture was incubated in a metabolic shaking incubator (28° C) in complete darkness. Initial and residual IAA was determined by colorimetric analysis (modified Salkowski reaction)[13].

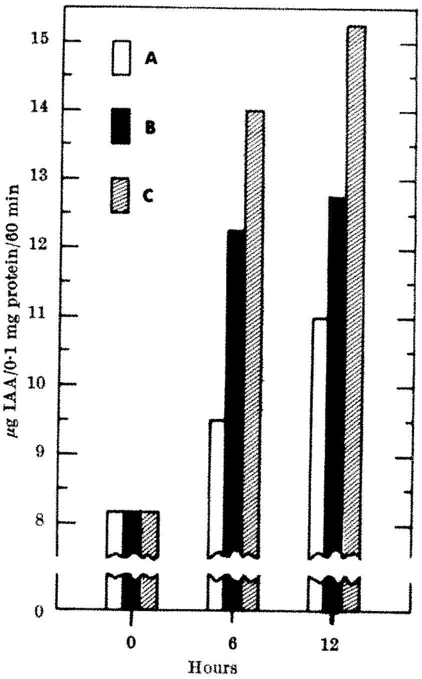

Fig. 3. Activity of β-indolylacetic acid-oxidase (expressed in μg of β-indolylacetic acid destroyed/0·1 mg of protein and for 60 min) from *Lactobacillus bulgaricus*. Time of incubation, 0, 6 and 12 h. *A*, Control; *B*, 1×10^{-5} molar β-indolylacetic acid; *C*, 1×10^{-3} molar β-indolylacetic acid.

Analyses of the IAA-oxidase activity clearly show (Fig. 3) that (1) the enzyme activity increases with the duration of culture, and (2) the exogenous IAA causes a strong stimulation of the IAA-oxidase which is greater for the high IAA level.

As it has been observed, for plant tissues[14,15], it is possible to enhance the activity of the IAA-oxidase, prepared from *Lactobacillus*, by incubating cells in IAA. If, on the other hand, the activity of these enzymes is increasing, the level of the endogenous auxins is decreasing, as was also shown in plant cells[10,11]. Finally, if the auxin content is decreasing, the growth will also be reduced. Such "adaptive increase" of the IAA-oxidase activity could explain the reduction—which was observed in the present experiment—of the growth of *Lactobacillus* caused by IAA.

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[1] Pilet, P. E., in *Les Phytohormones de Croissance* (Masson, Paris, 1961).

[2] Okabe, N., and Goto, M., *Ann. Rev. Phytopath.*, **1**, 397 (1963).

[3] Snell, E. E., *Bact. Rev.*, **16**, 285 (1952).

[4] Blanc, B., and Pilet, P. E., *CR Acad. Sci.* (*Paris*), **261**, 2509 (1965).

[5] Kavanagh, F., in *Analytical Microbiology* (Academic Press, New York, 1963).

[6] Kabat, F., and Mayer, M. M., *Experimental Immunochemistry* (Thomas, Springfield, 1958).

[7] Ray, P. M., *Ann. Rev. Plant Physiol.*, **9**, 81 (1958).

[8] Siegel, S. M., Porto, F., and Frost, P., *Arch. Bioch. Biophys.*, **82**, 33 (1959).

[9] Pilet, P. E., *Rev. Gén. Bot.*, **66**, 450 (1959).

[10] Siegel, B. Z., and Galston, A. W., *Plant Physiol.*, **42**, 221 (1967).

[11] Pilet, P. E., *Fourth Inter. Conf. Plant Growth Substances*, 167 (Iowa Press, Ames, 1961).

[12] Pilet, P. E., and Collet, G., *Méthode d'Analyse du Catabolisme Auxinique* (Zwahlen, Lausanne, 1962).

[13] Pilet, P. E., *Rev. Gén. Bot.*, **64**, 106 (1957).

[14] Galston, A. W., and Dalberg, L. Y., *Amer. J. Bot.*, **41**, 373 (1954).

[15] Pilet, P. E., *CR Acad. Sci.* (*Paris*), **259**, 1183 (1964).

Non-local Nature of Habituation in a Rotifer and Protozoan

HABITUATION, defined as a "waning of a response as a result of repeated stimulation"[1], can be considered to be a form of learning if fatigue, injury and local sensory adaptation can be ruled out. This is the case when an organism shows the same behaviour response to a different or more intense stimulus after habituation. The usual way to ascertain whether there is local sensory adaptation is by recording electrically the relevant sensory pathways; this is often quite difficult, either because the nerves are too small or are too numerous to record from. It is possible to design behavioural experiments to test for non-local habituation; among the invertebrates[1], however, this is only possessed by planarians[2].

Several freshwater micrometazoa were shown to habituate to a mechanical shock[3], but only the rotifer *Philodina* and the protozoan *Spirostomum* were found to be useful in a test for non-locality of the habituation response. The others either moved too fast to habituate at two different areas of their body or else were sensitive to the stimulus at only one area.

One rotifer at a time was placed in a depression slide and left there for 5 min before being presented with the stimulus. Thirty sessile rotifers were first lightly touched with a fine wire on the corona every 5 sec until they no longer contracted to three stimuli in a row. Five sec after habituation, the same stimulus was applied to the lower body area near the pedal gland until each rotifer met the previous habituation criterion. To guard against the possibility that the posterior area was initially less sensitive than the corona area, another thirty rotifers were given the stimulus in reverse order. Because the intensity of the stimulus determines the rate of habituation[3], it is important that all the stimuli should be of essentially the same magnitude to avoid any bias in the results. Unfortunately, if two people habituate the animal in a blind experiment, this only introduces more variation in stimulus intensity.

Each protozoan was also kept in a depression slide for 5 min before the experiment began. Then each one of the thirty was presented with a mechanical shock in the form of a solenoid which dropped a constant height from its de-energized coil every 5 sec until the animal no longer contracted to three stimuli in a row. Taking advantage of the fact that when a *Spirostomum* is cut in two both halves can still contract to a stimulus[4], each protozoan was cut transversely into approximately two equal halves with a razor blade. Fifteen seconds later, the anterior and posterior parts were habituated in the same way. To avoid the possibility that the cutting reduced the sensitivity of the parts to the stimulus, thirty unhabituated (naive) protozoa were cut in half; 15 sec later both parts were habituated. It is important that the cutting should be done cleanly and quickly or else the animals become insensitive to subsequent stimuli for the next few minutes. Furthermore, the protozoan must be cut into two nearly equal pieces, for a larger piece is more sensitive to the stimulus than a smaller one[4].

The results are presented in Tables 1 and 2. The heading "No. of stimuli to habituate" shows how many stimuli it takes before the organisms do not respond to three more stimuli in a row. In the rotifer, after the corona had been habituated with an average of 8·8 stimuli, the lower body area needed only three stimuli before the animal ceased to contract. When the lower body area was stimulated first and habituated with 8·2 stimuli, the corona area needed only 2·4 stimuli to habituate the animal. These differences are significant at $P = 0{\cdot}001$, as judged by the *t* test for the difference between correlated means, as are those differences (by the standard *t* test) between the number of stimuli it takes to habituate one specific area first and the number it takes to habituate the same area after habituating the other area. The experiment with *Spirostomum* demonstrates that while it takes nine stimuli to habituate the whole organism, only about three are needed to habituate its severed anterior and posterior pieces. By the *t* test for correlated means, this is significant at $P = 0{\cdot}001$. It takes more stimuli than this ($P = 0{\cdot}005$) to habituate the anteriors and posteriors from a previously naive organism. While there is relatively large variation in the number of stimuli needed to reach habituation in each of the areas stimulated, it should be noted that fifty-four of the sixty rotifers showed some retention of habituation when re-tested in another area, and, for the protozoa, twenty-seven out of thirty anterior pieces and twenty-five out of thirty posterior ones showed retention.

Table 1. HABITUATION IN *Philodina*

	No. of stimuli to habituate			
	1. Corona	2. Body	1. Body	2. Corona
Mean	8·8	3·0	8·2	2·4
Standard deviation	5·5	3·1	5·6	2·2

Table 2. HABITUATION IN *Spirostomum*

	No. of stimuli to habituate				
	Whole organism	Anterior	Posterior	Naive anterior	Naive posterior
Mean	9·0	3·1	3·6	7·0	9·6
Standard deviation	6·9	2·9	3·1	4·1	6·1

When *Philodina* is habituated in the area of the corona so that it no longer contracts to touch, the lower body area near the pedal gland becomes partly habituated. This lower body area is not any less sensitive to the stimulus than the corona because, when the body is habituated first, the corona also becomes partly habituated. When a *Spirostomum* is habituated so that it ceases to contract to a mechanical stimulus and is then cut in half transversely, the anterior and posterior pieces are also partly habituated. Habituating the cut pieces of a naive protozoan requires more stimuli than the pieces from a previously habituated one. The anterior and posterior parts from a previously habituated protozoan are equally sensitive (3·1 as compared with 3·6) to the stimulus, but a naive anterior is less sensitive ($P = 0{\cdot}01$) to the stimulus than the naive posterior (7·0 as compared with 9·6). Neither, however, are significantly different (at $P = 0{\cdot}05$) from a whole organism in terms of the stimuli needed to reach habituation.

After habituation of *Spirostomum*, or its anterior and posterior halves, to the mechanical stimulus, it still contracts either to a more intense stimulus which is given in the same way, or to direct touch on its body. The rotifers, too, after habituation to the touch stimulus, will contract either to a mechanical shock which is delivered by the solenoid arrangement or to a more intense touch stimulus. These organisms also return within expected statistical limits to their normal levels of sensitivity several minutes after habituation[3]. It can be concluded therefore that habituation in these two organisms cannot be explained by fatigue, injury or local sensory adaptation. This conclusion, as well as the small size and phylogenetic simplicity of these organisms, and the ease of obtaining habituation, suggests that these animals could be used in studies concerning the physiology of learning and memory.

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[1] Thorpe, W. H., *Learning and Instinct in Animals* (Methuen, London, 1963).
[2] Westerman, R. A., *Science*, **140**, 676 (1963).
[3] Applewhite, P. B., and Morowitz, H. J., *Yale J. Biol. and Med.*, **39**, 90 (1966).
[4] Clark, A. M., *J. Exp. Biol.*, **22**, 88 (1946).

ANIMAL BEHAVIOUR

Rats prefer the Home Cage to the Runway following Intermittent but not Consistent Reinforcement

SEVERAL sources of evidence suggest that, in both the Skinner box and runway, an intermittent schedule of reinforcement could be a source of aversion. Time-out studies show that when a pigeon or rat can turn off a stimulus which is associated with a particular reinforcement schedule, the frequency and duration for which it does so vary inversely with the probability of reinforcement[1,2]. Furthermore, when rats or pigeons are given a choice between equal schedules of reinforcement which are predictable in one case and unpredictable in another, they choose the former[3,4].

Although aversion has been attributed to the intermittent schedule in the runway[5], no direct test of this interpretation seems to have been made. A simple test can be made by offering a choice between the runway and, for example, the home cage. Intermittently reinforced rats run faster when placed in the runway, but if given a choice between entering the runway or going back to the home cage, they choose to go to the home cage rather than to the runway, and do so more frequently than a consistently reinforced group do.

The subjects were twelve 90-day-old male albino rats of the Sprague–Dawley strain. They were randomly divided into two equal groups: a continuously reinforced group (group *C*) and a partially reinforced group (group *P*). The apparatus consisted of a straight alley (60 × 6 × 9 in.) of unpainted redwood covered with clear 'Plexiglass'. Interruption of any of four photobeams started and/or stopped one of three standard electric clocks. The first clock measured the time taken over the first 6 in. of the alley, the second clock measured the time taken to run over a distance of 32 in., and the third clock measured the time taken over the last 6 in. (goal time). A 7 × 7 in. 'Plexiglass' platform was permanently attached on one side to the start box; the other side could be hooked to the specially designed home cage. The rat was placed on the platform and was allowed to step down into either the starting box or else its home cage.

The rats were deprived of food for 23 h and were housed individually in the special home cages. All rats were given 'Purina' lab chow in the home cage for 30 min each day, approximately 30 min after the last test trial. Water was always available. Following pretraining, each rat was given eighty-eight trials in the runway, four each day, at an average inter-trial interval of approximately 8 min. Group *C* received four Noyes pellets of 45 mg on each of its four daily trials, whereas group *P* received the same number of pellets on half of its trials. The choice procedure was initiated on the day following the last acquisition trial.

Three kinds of trials were used in the choice procedure: (*a*) standard trials of a kind which had been given (rat placed in start box and removed at the end of the run); (*b*) choice trials; (*c*) forced trials. At the start of each day the rat was placed on the platform and given a choice. If it chose the runway it was then given three standard trials; if it chose the home cage it was then given four standard trials (making a total of four runway trials in both cases). Next, a second choice was administered followed by a forced trial, and then a third choice was given, followed by a forced trial. Forced trials served to equate the number of runway trials for the two groups. By placing the rat on the platform and blocking the entry to either runway or home cage, the rat was forced to take the alternative it had not taken on the preceding choice trial. The reinforcement schedule was preserved throughout the procedure; that is, group *C* received reinforcement on all trials, while group *P* received 50 per cent random reinforcement on choice and forced trials as well as on standard trials. In brief, the rat was given three choices each day between entering the runway or returning to the unbaited home cage; one such choice was given at the start of the day, and two others were given following a block of standard runway trials.

Figs. 1 and 2 show the performance during the first eighty-eight trials before the choice procedure was introduced. The findings were as follows: group *P* surpassed group *C* in the speed of the start (as well as speed of run, which is not presented because it was comparable with speed of start), whereas group *C* was faster in the goal box. An analysis of variance on the speed of start before and after the reversal showed that the reinforcement by trials interaction was significant ($F = 5{\cdot}479$, df $= 1/10$, $P < 0{\cdot}05$). This supports the conclusions suggested by Fig. 1 as well as previously reported outcomes[6]. A similar analysis of variance which was performed on speeds over the last 6 in. for the last 3 days of acquisition trials showed that the chief reinforcement effect was significant ($F = 4{\cdot}984$, df $= 1/10$, $P < 0{\cdot}05$), while the interaction (reinforcement by trials) effect was not ($F < 1{\cdot}0$).

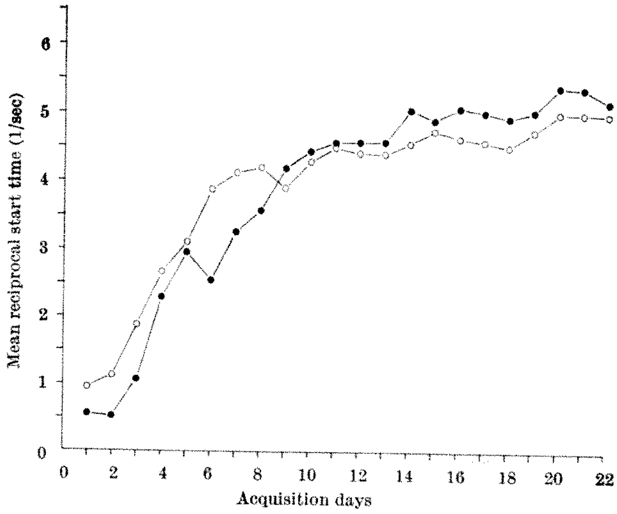
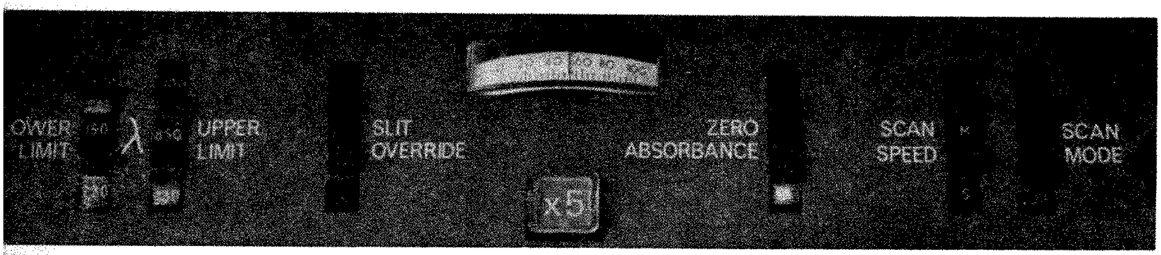


Fig. 1. Mean reciprocal starting times; each point is based on the four trials which were given on each day. ●, *P*; ○, *C*.

Data for the three choices which were given each day are presented in Fig. 3. The hypothesis is shown to be well supported by the choice data, equally by the choice at the start of the day and by those choices following the runway trials. The first two choices can be discounted on the grounds that they served as adaptation trials; on all subsequent trials, group *C* chose the runway more



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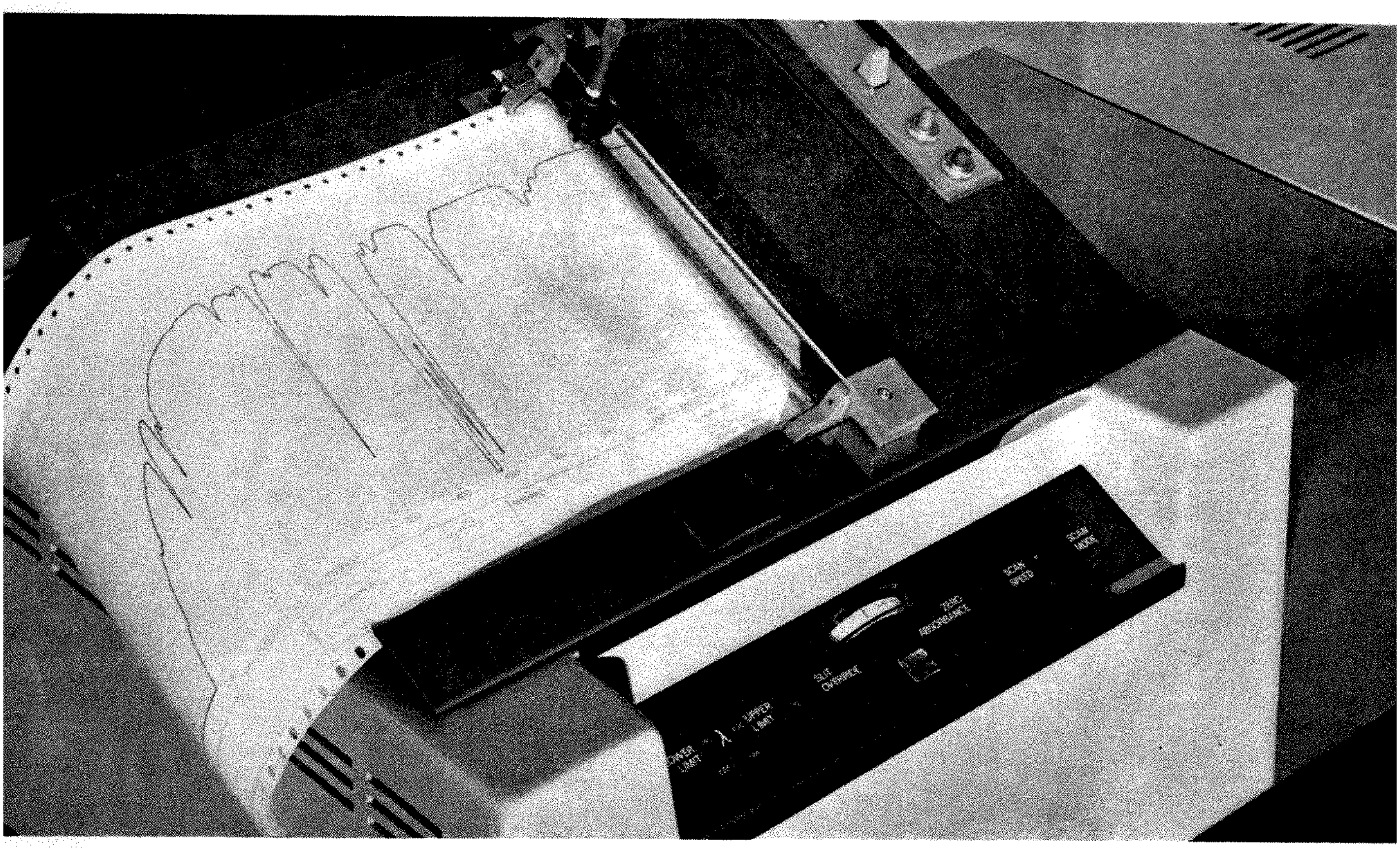

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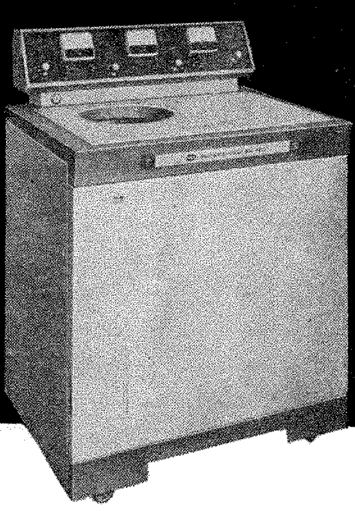

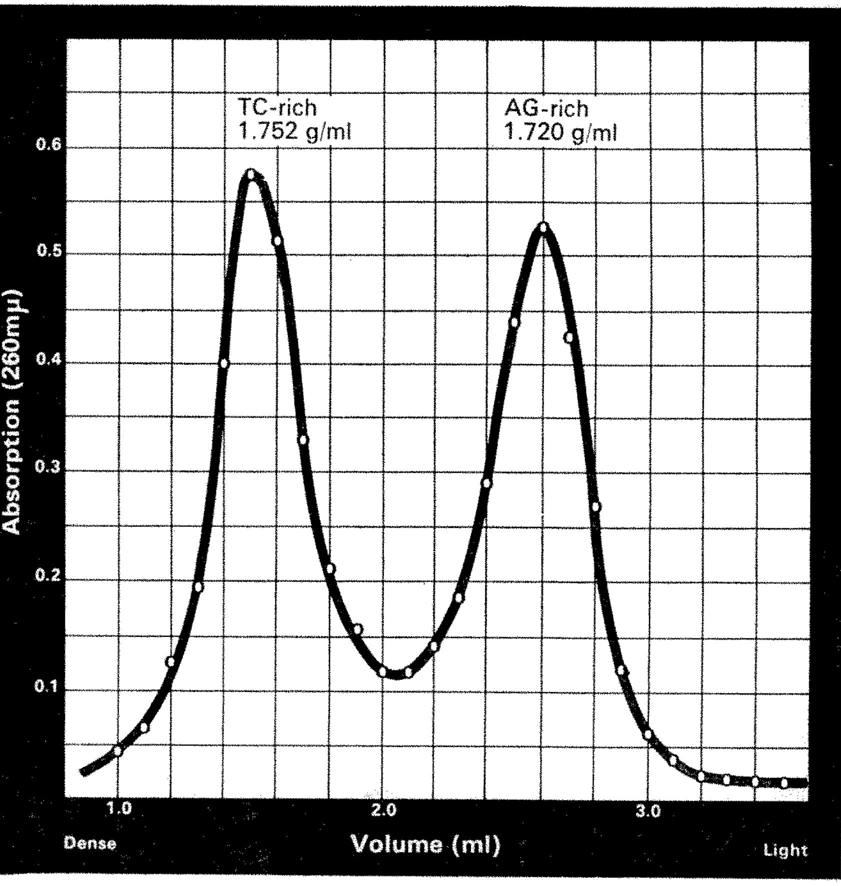

50 μg. of mouse satellite DNA in Tris-HCl buffer was treated with sodium lauryl sulphate at pH 12.7 and centrifuged in a caesium chloride gradient (initial density 1.765 g/ml at 25°C) for 20hr. at 42,000 r.p.m. at 25°C.

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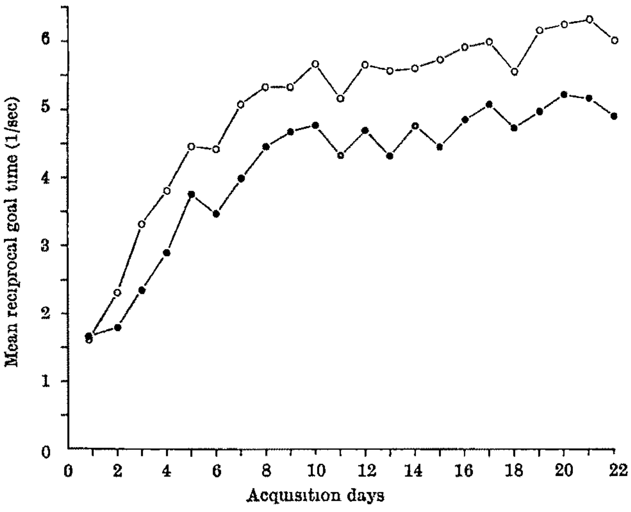

Fig 2 Mean reciprocal goal box times, each point is based on the four trials which were given on each day ●, *P*, ○, *C*

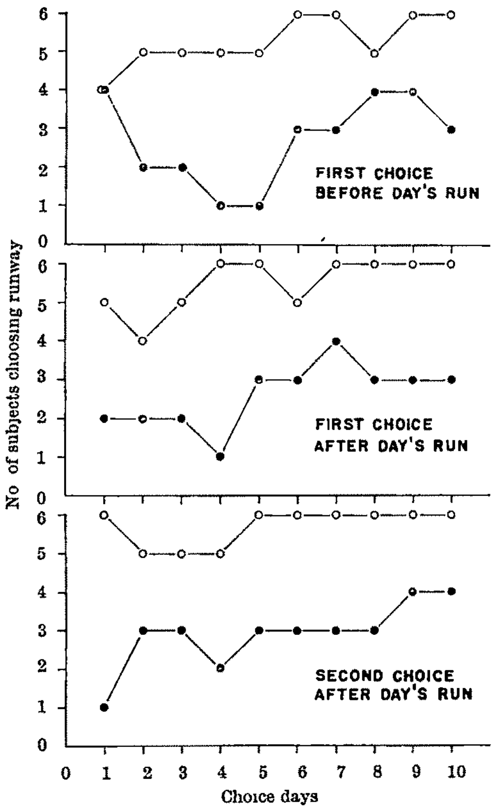

Fig 3 Number of subjects choosing to enter the runway on each of three choices which were given each day ●, *P*, ○, *C*

often than group *P*. This difference was highly significant on the first day of choice ($P<0.007$ by Fisher's exact test), and was maintained over all subsequent choices For example, with the last ninety choices (days 6–10), group *C* chose the runway eighty-eight times, compared with only fifty times for group *P* Except for one rat in group *P* which always chose the runway, the individual choice behaviour of the group *P* rats was compatible with the group average one always chose the home cage while the other four chose about equally between the two alternatives

Do the choice data provide clear evidence of the acquired aversion of the runway as a result of the associated intermittent schedule? Consider some alternative interpretations, for example, "probability matching" This interpretation is encouraged by the approximate agreement between the proportion of runway choices which were made by the two groups and the proportion of these choices which were reinforced But this reasoning cannot be depended on for several reasons First, if choice were determined by reinforcement the groups should diverge increasingly over the course of the 10 days of choice In fact, they differed as much on the first day as on succeeding days (Fig 3) Indeed, the difference which was already evident on the second choice of the first day could not be attributed to differential reinforcement of choice behaviour because on the first choice the groups were treated alike That is, on the first adaptation trial an equal number of subjects from both groups chose the runway, and the runway trial was reinforced not only for group *C* but also for group *P* The data for choice must therefore reflect the previous reinforcement which was given for runs in (and not choice of) the runway (A further reason for discounting this interpretation is that a proper test of probability matching requires that the alternatives which are offered should be equal except for their probabilities of reinforcement, it is not apparent that the runway and home cage comprise alternatives that differ only in their probabilities of reinforcement)

Another objection is that group *P* chose the home cage not because the runway was more aversive for the rats in this group than for group *C* but because the home cage was more attractive for these than for the rats in group *C* This objection, however, does not explain why group *P* rats should value the home cage more than group *C* rats when in fact both groups were similarly treated in the home cage (but, of course, differently in runway) Finally, the choice data might be attributed to the difference in total amount of food which was received by the two groups, but such reasoning would lead to the prediction that group *C* should run faster than group *P*, which is contradicted by this study and other similar ones

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[1] Azrin, N H, *Science*, 133, 382 (1961)
[2] Thompson, D M, *J Exp Animal Behav*, 7 1 (1964)
[3] Bower, G, McLean, J, and Meacham, J, *J Comp Physiol Psychol*, 62, 184 (1966)
[4] Prokasy, W F, *J Comp Physiol Psychol*, 49, 131 (1956)
[5] Amsel, A *Psychol Rev*, 69, 306 (1962)
[6] Goodrich, K P, *J Exp Psychol*, 57, 57 (1959)

APPLIED SCIENCE

Plant Competition and Crop Yield

Shinozaki and Kira[1] by combining a logistic equation of population growth (equation (1)) with the law of constant final yield (equation (2)) derived equation (3) to describe the relationship between plant density and yield

$$\frac{1}{w}\frac{\mathrm{d}w}{\mathrm{d}t} = \lambda(t)\left(1-\frac{w}{W}\right) \qquad (1)$$

or

$$w = \frac{W}{1+ke^{-T}} \text{ and } T = \int \lambda(t)\mathrm{d}t$$

$$W\rho = Y = \text{constant} \qquad (2)$$

$$\frac{1}{w} = a + b\rho$$

$$a = \frac{e^{-T}}{w_0} \text{ and } b = \frac{1 - e^{-T}}{Y} \qquad (3)$$

where at a given time t, w is the dry weight of each plant and W is the asymptote of w, λ is the coefficient of growth which is independent of plant density ρ, k is the integration constant, determined by the initial conditions and independent of plant density, Y is the final yield/unit area, and independent of density, and w_0 is w at $t=0$ The law of constant final yield, however, may not always be satisfied (data to be published elsewhere) and the yield in higher densities may decline In these conditions, equation (3) cannot describe the yield–density relationship The validity of the logistic equation (equation (1)), as tested for different crops[1,2], has proved to be justified so far, and it seems that it is the law of constant final yield which needs further modification

As stated by Shinozaki and Kira[1], the law of constant final yield is equivalent to equation (4) if the power, α, is taken as unity at the end of the growing season

$$\log W = \log K - \alpha \log \rho$$

or

$$W = K\rho^{-\alpha} \qquad (4)$$

where K and α are constants This relationship is valid when $\rho \gg C$, where C is the plant density at which competition starts, before this point W is independent of ρ If $\alpha = 1$, then $K = Y$

Equation (4) can be modified and re-written as

$$\log W = \log A - \alpha \log (\rho/C)$$

or

$$W = A(\rho/C)^{-\alpha} \qquad (5)$$

where A is the weight per plant when $\rho \leqslant C$, that is, in absence of competition When $\alpha = 1$, the law of constant final yield can be written as

$$W\rho = A\,C = Y = \text{constant} \qquad (2)$$

We propose that equation (5), which is a more general form of equation (2), can be combined with equation (1) and the following equation can be derived

$$\frac{1}{w} = a + b\rho^{a} \qquad (6)$$

where

$$a = \frac{e^{-T}}{w_0} \text{ and } b = \frac{1-e^{-T}}{AC^{a}}$$

and considering that $y = w\rho$, we got

$$y = \frac{\rho}{a + b^{a}} \qquad (7)$$

Equations (6) and (7), as any other formula given for competition, are valid only when $\rho \gg C$ (that is when there is competition at the end of the growing season) When $\alpha = 1$, equation (6) or (7) becomes the same as equation (3) which was derived by Shinozaki and Kira[1], and also independently by Holliday[3] and de Wit[4], and the law of constant final yield is satisfied When $\alpha > 1$, equation (7) suggests that the yield/unit area reaches a maximum at a certain density and declines with further increasing density.

In plant density experiments with different varieties or with different degrees of environmental growth factors, parameter b in equation (6), containing A and C, would possibly change more with the potential of the environment, while the dominant factor in parameter a might be the genetical character, however, this generalization will be modified with time Changes in α with the treatment suggest that there is an interaction between plant density and the level of treatment applied If the logistic equation of growth is valid for the growth of a plant part, equation (6) or (7) can also be used to describe the relationship between the yield of a plant part and density Furthermore, it can be suggested that when the basic assumptions given here are valid for competition in mixed communities, equation (6) or (7) can be used to describe the relationship between plant density and yield of individual species or varieties Equation (9) is a combination of the law of allometry (equation (8)) and equation (6)

$$w = Hw_p^{\theta} \qquad (8)$$

where w is weight per plant, w_p = weight of plant part, H and θ are constants

$$w_p^{-\theta} = a_1 + b_1\rho^{\alpha} \qquad (9)$$

where $a_1 = Ha$ and $b_1 = Hb$

and when $\alpha = 1$, as suggested by Shinozaki and Kira[1], equation (9) becomes

$$w_p^{-\theta} = a_1 + b_1\rho \qquad (10)$$

Equation (9) or (10) describes the way plant density affects the distribution of dry matter into plant parts, and it can be proved that by the end of the growing season $\theta \rightarrow \alpha/\alpha_p$, where α and α_p are the power α calculated for total plant weight and weight of a plant part from equation (6) or (7) If the data for a plant part are directly fed into equation (9), for each set of data, more than one set of values for θ and α can be obtained But by calculating α from equation (6) for total dry matter for each plant its value can be fixed in equation (9) and the correct value of θ can be obtained

Equation (11), which is very similar to equation (9), is suggested by Bleasdale and Nelder[5] to describe the relationship between plant density and plant weight

$$w^{-\theta} = K + \beta\rho^{\Phi} \qquad (11)$$

where θ, K, β and Φ are constants They have substituted a flexible curve given by Richards[6] for the logistic equation of growth, and, as they state "with analogous arguments to those of Shinozaki and Kira" (presumably by employing the law of constant final yield), they have derived their equation. Because of the difficulty in getting correct values of the powers (θ, Φ or α) by fitting the data directly to any equation of this type (equation (9) or (11)), Bleasdale[7] modified equation (11) by taking $\Phi = 1$ The form of this modified equation is similar to that of equation (10)

If the (λ) type logistic equation (equation (1)) is not satisfied the general logistic equation of growth can be employed as the basis for deriving equation (6) In this case the form of the equations (6) or (7) will be modified In most cases, however, addition of one extra term ($C\rho^{\beta}$) to equation (6) can give a close approximation

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[1] Shinozaki, K , and Kira, T , *J Inst Polytech , Osaka City Univ* , D 7, 35 (1956)

[2] Tadmore, N H , and Orsham, G , *Israel J Bot* ,13, 234 (1964)

[3] Holliday, R , *Nature*, 186, 22 (1960)

[4] de Wit, C T , *Soc Exp Biol Symp* ,15, 313 (1960)

[5] Bleasdale, J K A , and Nelder, J A , *Nature*, 188, 342 (1960)

[6] Richards, F J , *J Exp Bot* ,10, 290 (1959)

[7] Bleasdale, J K A , *J Hort Sci* , 42, 51 (1967)

BOOK REVIEWS

A LONG TIME AGO

The Origin of Life

By J. D. Bernal. (The Weidenfeld and Nicolson Natural History.) Pp. xvi + 345 + 29 plates. (London: Weidenfeld and Nicolson, 1967.) 55*s* net.

THIS book contains the results of a lifetime of thinking by one of the world's most distinguished crystallographers about the nature and origin of life. It presents a highly personal point of view, but it is all the better for that because, in the author's own words, "we are here trying to settle a question of a different character from those of the rest of science; it is not merely a description . . . but an attempt to carry out an intellectual reconstruction based on assumptions of inner logic themselves drawn from experimental science here and now". Biologists have been familiar with these historical exercises since the time of Darwin; not many physical scientists can handle them with the skill and necessary modesty shown by Professor Bernal.

A subject like the origin of life is difficult to treat in any logical order owing to the diversity of relevant evidence, and the book needs to be read as a whole to appreciate the attitude of mind that it displays. We are given "a reasonable, continuous, hypothetical story, a myth of the origin of life" that is consistent with what is known at the present time. Even so, two postscripts are needed in order to mention some very recent discoveries. The underlying philosophy becomes clear in the seventh chapter where, in a brief discussion of the old idea of all new life coming from seeds which are everywhere present, we read: "To my mind it seems to have the same philosophical emptiness to say that life was specially created as to say that it came from somewhere else and that it had been there all the time". It is taken as axiomatic that life arose on the surface of the Earth and that, if life once made itself, it must not be too difficult to make it again if the right chemical and physical conditions can be re-created in the laboratory. This has far-reaching implications on contemporary thought, which are discussed in the last chapter, but the sparkle of an original mind illuminates the whole writing and the thread of ideas is clear. The spontaneity of the chemical processes involved, their great but limited variety and the complexities of their interactions with one another at all levels of organization—these are the underlying concepts that set the pattern for much detailed discussion of what may have happened in the course of the Earth's history.

Bernal acknowledges clearly, by reprinting in full in an appendix, the important articles by Oparin (1924) and Haldane (1929) that started the modern study of the origin of life, but right from his own first major contribution in 1947 he departed decisively from the Oparin scheme in thinking of cells as a late stage in the process, preceded by about 2,000 million years of chemical evolution of macromolecules in "sub-vital areas" not defined by membranous boundaries. Stage I of the story takes us from the formation of the Earth by agglomeration of dust to a "primitive soup" of organic monomers. Much of this has already been reproduced in the laboratory, and the variety of conditions in which amino-acids, nucleotides and sugars can be formed suggests that it has occurred in many places in the solar system, including particularly the body which gave rise to the carbonaceous meteorites. This possibly important source of organic substances is discussed in an appendix by Dr G. Mueller, who stresses that the absence of water and of optical activity distinguishes this synthetic system from its terrestrial counterpart; the much publicized microstructures are dismissed as mineral artifices or terrestrial contaminants. Stage I ended about 5,000 million years ago and so far there is common ground with most other writers on the subject. Stage II, the period of chemical evolution, contains the crucial steps. Polymerization and the genesis of macromolecular order are attributed initially to the condensing properties of mineral particles and there are some intriguing speculations on the role of transition metals as proto-enzymes and of apatite as a primitive phosphate-donating proto-coenzyme. The injection of meteorite-borne organic complexes is mentioned as an example of the type of interaction between separate synthetic patterns that may have played an even greater part later on. Here the element of personal preference enters strongly into the story, and the "alternative pathways to biopoiesis" are listed in the eighth chapter only to be rejected. Perhaps the weakest part of the argument is the author's inability to explain, except by chance, how the key phenomenon of molecular self-replication arose in the course of chemical evolution. He seems to be getting near it in his discussion of "generalized crystallography" and when he writes of "the modification of such replication by selection of molecules, a kind of natural selection which is replaced by the natural selection of organisms at later stages". It is unfortunate that he does not mention a positive suggestion that I myself made in 1953 that branching chain reactions involving phosphate radicals might provide the starting point for such a process of natural selection at the molecular level.

The formation of organelles and the coming together of organelles to form cells is allotted a further 1,000 million years in Stage III, and at some point in Stage III occurred the photosynthetic revolution, the appearance of oxygen in the atmosphere and the first fossils. The chapter entitled "From Molecule to Cell" contains much concise information but little that is new. From then on the story is left to other volumes in this series on "Natural History". The merit of this volume is not only that it bridges the gap between cosmology and biology and so emphasizes the unity of the history of nature, but also that it contains authoritative answers to many questions which must now be asked by every thinking man and woman. Some of these are given concisely in the fourth appendix, others have to be sought in the text. I will select two which are not in the summary. "Is there life on other planets?" Stage I has occurred in many places; Stage II, because it needs water, probably occurred only on the Earth and Mars; Stage III may have occurred only on the Earth. "Is there intelligence elsewhere in the Universe?" "There is a possibility that the oldest and most advanced civilizations on distant stars have in fact reached the level of permanent inter-communication. . . . This is, of course, the wildest of speculations but it has a definite *a priori* probability. For that reason it is not by any means a waste of time to keep on searching and reaching out to see if we can find any trace of intelligences existing." *Chacun à son gout!* Sacrifices to a god in every tree used to be called superstition, not science, but this is a small criticism of what is otherwise a very fine account of an important scientific topic. J. W. S. PRINGLE

MORE LYSENKOISM

Autumnization and its Genetic Interpretation

By Sandor Rajki. Pp. 87. (Budapest: Akadémiai Kiadó, 1967.) 30*s*.

THE author of this work, Sandor Rajki, is the director of the Agricultural Research Institute of the Hungarian Academy of Science at Martonvársár. He is an agricultural botanist, plant breeder and geneticist much concerned with wheat, which is the crop species he discusses in this book He was a student of T D Lysenko, whose continuing influence is very apparent His book is a commentary on work carried out at Martonvársár which is claimed to show that the inheritance of flowering habit can be modified environmentally

Wheat genotypes differ in the environmental conditions they require for the occurrence of flowering So-called "spring" forms flower without any need for exposure to low temperatures or short days, while "winter" forms need such environments as developmental triggers for the initiation of flowering Between the extremes, genotypes can occur in which flower initiation requires environmental stimuli of varying magnitudes The inheritance of the spring–winter habit difference has been studied by a number of workers (for a recent example, see Halloran, G M , and Boydell, C W , *Canad J. Genet Cytol* , 9, 632, 1967) and the chromosomal locations of many of the responsible genes have been determined The chromosomes principally implicated are *2A*, *2D*, *5A*, *5B*, *5D*, *7B* and *7D*, although other chromosomes carry genes of less profound overall effect The inheritance of the character difference is therefore complex but conventionally chromosomal, and its description is well within the compass of current cytogenetics

The essence of Rajki's claim is that when a form of spring wheat, with no low temperature–short day requirement for flowering, was sown in the autumn for a number of successive generations, its genetic constitution was so changed that subsequently exposure to these conditions was essential before flowering would occur Thus a wheat with genetically determined spring habit was changed so that it inherited the acquired character of winter habit One sowing in spring of the form supposedly converted to winter habit is said to be sufficient to cause reversion to spring habit (page 32) It is claimed that the initial stock of spring wheat from which the experiments were started was homozygous and homogeneous, so that the possibility is denied of changes occurring by selection Indeed, Rajki concludes (page 63), "Autumnization, the adequate gradual transformation in the genetically pure spring initial stock corresponding to the quantity and quality of autumn cropping, cannot be acceptably explained through a gene concept denying the inheritance of acquired properties Thus (the category of) mutation is not applicable to autumnization . "

In our laboratory, work has commenced on a genetic comparison of the initial spring form used by Rajki and one of the winter forms supposedly derived from it by autumnization. From this work it appears that the differences are almost entirely attributable to activities of chromosomes *5A*, *5B* and *5D* Differences due to these chromosomes distinguish many spring and winter wheat genotypes, so that there is nothing unusual in the genetic constitution of this winter form Rajki's interpretation would require that the inheritance of the acquired character of winter habit involved allelic changes at a number of well recognized loci and, moreover, the capacity for the reversion to the original allelic state over one generation This notion is in conflict with a vast body of evidence that shows that although mutation occurs it is a random process—the information content of the genetic material being immune from directional change by the environment

There is thus an impasse the only way that Rajki's results can be reconciled with our current knowledge of genetic organization is on the basis of increased mutation rates accompanied by stringent selection The puzzling aspect of the situation is that examples of apparently similar phenomena have been described in flax by Durrant and by Hill in *Nicotiana* In both these cases particular environments are said to have induced specific changes in nuclear inheritance The major and perhaps critical distinction is that the changes Rajki claims to induce involve specific allelic substitutions, whereas in the flax and *Nicotiana* examples such detailed changes cannot be recognized

Despite the availability of examples of analogous work in other genera, most wheat geneticists will continue to have strong reservations on the validity of the autumnization work until it has been repeated outside the sphere of Lysenkoist influence In the meantime, those plant geneticists with a taste for the esoteric, or with a liking for Lysenkoist polemics, are recommended to this volume

RALPH RILEY

SEX CHROMOSOMES

Sex Chromosomes

By Ursula Mittwoch Pp ix+306 (New York Academic Press, Inc , London Academic Press, Inc. (London), Ltd , 1967) 112*s*.

DOCTOR MITTWOCH has written a clear and unambiguous book which will be of interest to many who are not cytogeneticists but seek knowledge in an important field of cytogenetics It will be especially useful to both undergraduate and postgraduate students of human and general genetics, of biology, of medicine and to others interested in genetical problems In each of the sections that deal with different aspects of sex chromosomes, their morphology, behaviour and anomalies in different organisms, the approach is historical· The author has chosen to discuss instructive examples rather than detailed minutiae, while the main objective, that of giving an overall view of the subject matter suited to a particular group of readers, has been kept to the fore

Appropriately a good third of the book is devoted to the sex chromosomes of man and their main abnormalities, and to sex chromatin and its practical and theoretical significance This reflects the fact that, of all vertebrates man is the one in whom most is known about sex chromosomes and their anomalies Also, the sex chromatin, though not actually first discovered in man, has been most successfully exploited in practice in our species and has reopened, with new facts and hypotheses, a fundamental problem of theoretical genetics, namely, that of the mechanisms of dosage compensation of X-linked genes in different groups of organisms Sex chromosomes of other animals are given due consideration, but plants are not neglected.

Melandrium (*Silene*), for example, is well discussed as one organism in which the Y chromosome is a male determiner, as it has recently been found to be in mice and men and other mammals Equal prominence is given to the contrasting type of sex determination in *Drosophila*, based on a balance between autosomes and X chromosomes, to female heterogamety (in lepidoptera and in birds), and to odd chromosomal sex-determining mechanisms

The observations on sex-chromatin formation in female mammals, together with the parallel findings on the time sequence of DNA synthesis of the sex chromosomes and of some autosomes, or their segments, have reopened the whole subject of heterochromatin and heterochromatization The penultimate chapter gives an adequate account of the older views on this difficult subject which arose from a purely cytological description (of a special staining property of some chromosomes or of some of their parts), but has become greatly complicated by a mixture of fact and theory and by the attribution of special properties to the heterochromatic genetic elements In the final chapter, the author synthesizes some of the main observations on sex differentiation in vertebrates and speculates (drawing a parallel also from the work on *Drosophila* on the possible mode of action of the sex chromosomes in sex determination in vertebrates The thesis is that, until primary chemical differences can be demonstrated to underlie early embryonic male or female determination one ought to consider, as has been suggested by others as well, that sex may be determined by a quantitative effect, with possibly a threshold mechanism, brought about "by the agency of relatively large chromosomal regions or even whole chromosomes"

I find that these two necessarily sketchy and speculative chapters, at the end of an otherwise factual book, are useful because they aim to point out important gaps of knowledge and areas where hypotheses abound and where their testing is particularly topical P E POLANI

FURTHER DEVELOPMENTS

Advances in Morphogenesis

Vol 6 Edited by M Abercrombie and Jean Brachet Pp viii+331 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1967) 120*s*

WHENEVER I pick up a book with a title like *Advances in Morphogenesis*, I hope to find that at last someone has found some sort of key to the problem of the link between biochemical and morphological structure Perhaps I am made unduly optimistic by the remarkable advances in our understanding of the nature and functioning of the genetic code in bacteria and viruses characteristic of recent years Needless to say, I am always somewhat disappointed to find that "morphogenesis" is usually taken to mean the development of biochemical diversity, or at most a superficial account of cellular differentiation

With this limitation in mind, however, Abercrombie and Brachet's sixth volume of *Advances in Morphogenesis* is a worth-while addition to the library of anyone interested, not necessarily just in embryology in the classical sense, but in the genetics, biochemistry and physiology of developing animals and plants Indeed, only two articles are concerned primarily with morphogenesis—one by Michael Locke on pattern development in the insect integument, and one by Allsop on heteroblastic development in plants Both are useful and detailed reviews, although the pitiable state of our understanding is well illustrated by a quotation from each author "There is a continued need to recognize that the terms used in this description may have no reality in the phenomena being studied We can proceed no further until some structural, physiological, or biochemical correlation has been made" (Locke, page 82), or "There is also no conclusive evidence that physiological ageing is determined by any intrinsic ageing of the mature tissues of the plant A plausible explanation of these changes invokes a regulatory effect of the intracellular environment on gene activity But it is not even certain that changes in gene activity are necessary" (Allsop)

Astaurov's paper on cytogenetic mechanisms in silkworms is more an assessment of the relative roles of nucleus and cytoplasm in development, and various modes of parthenogenesis, than a genuine attack on problems of morphogenesis Interesting as it is, I feel that it could have been somewhat compressed without loss It contains, however, detailed descriptions of methods which could be useful to the experimenter

Wilt's article on embryonic haemoglobin synthesis, and Wischnitzer's on the ultrastructure of the amphibian egg, are neither of them strictly concerned with morphogenesis Both are very adequate reviews, and the latter is beautifully illustrated, but whether this volume is really the proper place for their publication is perhaps doubtful

"The Biology of Teratomas", by Leroy Stevens, interested me particularly, largely because it directs attention to what was to me an unusual realm of embryological investigation That primordial (male) germ cells may be able to give rise to almost all kinds of tissue in a teratoma, even if in a disorganized way, must surely have significance in relation to the ordered development of the normal embryo

Finally, L. Gallien's account of sexual organogenesis, in both invertebrates and vertebrates, is an admirable summary of advances in our knowledge of sex-determination, especially in relation to endocrine control in invertebrates. Whether the clarity of this article derives from the original French or in part from the excellent translation, it leaves a lasting impression of how good a review article can be MICHAEL BEGG

GEOLOGY OF FOLDS

Structural Geology of Folded Rocks

(Rand McNally Geology Series.) By E H Timothy Whitten Pp. xiv+663 (Chicago Rand McNally and Company, 1966) $10 50

THE crust of the Earth has had a complex deformation history as a result of past orogenic disturbances. Great advances have been made in recent years in the understanding of the fracture patterns and folded forms taken up by stratified rocks during these deformations Professor Whitten's book describes the morphology of folded rocks and structures associated with folds It aims at guiding the reader through the modern literature, much of which is scattered through a number of specialist journals, and synthesizing these data.

The appearance of the book is immediately attractive, and departs radically from many traditionally oriented texts on structural geology now available The production is excellent and the publishers are to be congratulated on the way that they have reproduced the many figures, maps and photographs The text, however, is disappointing, the writing is verbose and repetitive, and several chapters are heavily weighed down with jargon Although Professor Whitten has been industrious in his incorporation of the results of others, in his compendium of data there is often an unfortunate lack of critical discussion as to their significance This book purports to be a synthesis, but a collection of data must be integrated into some logical system before it deserves this description Rarely does the author arrive at firm conclusions or offer to pass on to the reader his own experience in any personal commitment In discussions on controversial matters (for example, the significance of foliation, page 263) he gives no clear summaries of the various alternative working hypotheses that may explain the facts

The book is profusely illustrated, of the 485 figures more than 80 per cent have been published before It is most useful to have all these illustrations together in one book, but it is a pity that their integration into the text is sometimes so poor For example, although Professor Whitten has set out to base his account of folds on actually described field relations, there seems little justification for reproducing eighty-four small field sketches of folds (Figs 182, 183 and 184) when they are discussed in only two sentences in the text Professor Whitten's original contributions to the illustrative material are mostly confined to stereographic and equal-area-projections of geometrical forms, and some simple and rather unrealistic maps showing the geology of hypothetical regions Where original diagrams have been included in the sections on slip folds (Figs 110 and 112) and superposed folds (Figs 302 and 303) the presentation of the complex three dimensional geometrical properties is so ill conceived that I seriously doubt their usefulness

Griffiths has claimed that "progress in science can generally be measured by the degree in which the subject is pervaded by mathematics" Professor Whitten supports this assertion, yet he provides no summary of the analytical methods which have been used during the past two decades and which have revolutionized our understanding of the development of rock structures. In the preface he excuses himself for making no reference to the physical aspects of rock deformation, or to the mechanical properties of rock under stress. This admission precludes any subsequent discussion of the reasoned syntheses on fold formation put forward by Biot, Ramberg and others. His description of stress, strain and the deformation process is hardly adequate to do justice to recent interpretations of the formation of minor structures in deformed rocks. Criticisms of a book on the grounds that it omits certain aspects of the subject might be regarded as unfair, but this work purports to be a modern synthesis and these omissions are a serious flaw.

Many fundamental definitions of the geometrical characteristics of folds presented are at variance with those generally accepted. Taking a specific example from the second chapter, Professor Whitten's definition of cylindroidal folds (page 40) is weak and typical of the style of many sections of his work. "if enough points on the folded stratum are observed, some will be found to coincide with most parts of a cylinder". The fundamental parameters of fold wavelength and amplitude are nowhere described, and Professor Whitten gives an extremely confused exposition of the axial-surface and bisecting-surface of a fold. He correctly defines the bisecting surface (page 40) as that which bisects the fold limbs and uses a figure (unacknowledged Fig. 4–18 from Turner and Weiss, *Structural Analysis of Metamorphic Tectonites*) to illustrate that this surface need not coincide with the axial surface. If the reader returns to an earlier section of the book (pages 6–8, Fig. 4) where the term bisecting plane of the fold limbs is first introduced it will be apparent that Professor Whitten (i) has not bisected the fold limbs to derive this plane, he has drawn the axial surface of the fold, and (ii) has misidentified the trace of the bisecting surface by drawing the line representing the projection of the hinge line of the fold in stratum α on to the horizontal plane.

The terms competent and incompetent are used by the geologist to describe the relative ductility of different rock materials. Professor Whitten re-defines these terms, competent layers develop folds of large amplitude whereas incompetent ones accommodate themselves to the spaces between the competent layers. The dimensions of a "large amplitude" are never specified, and it seems apparent that if adjacent competent and incompetent layers are found together in a harmonically formed fold, they will both show the same fold amplitude. One could proceed chapter by chapter through this book finding inaccurate definitions and illogical development. Structural geology is one aspect of Earth Science which is ideally suited to the development and application of analytical techniques. In the past so much work in this field has been entirely descriptive that any new book, if it is to have real value for present students, must surely encourage clarity of thought and precision of definition. At first sight Professor Whitten's book might seem to do this, but I find it entirely in sympathy with the older attitudes.

As well as being marred by poor explanation of the fundamental geometrical features of folds, this book also presents some incorrect analyses when describing the geometrical forms of more complex structures. For example, the section describing the effects of flexural slip folding on sediments with cross lamination (pages 522–526) is based on the faulty assumption that the dihedral angle between the inclined beds remains constant during folding. Later in this section Professor Whitten does note that this angle will change during folding, but he makes no comment as to how this might invalidate his previous solution to the problem.

Superimposed folding has been neglected in many previous textbooks on structural geology. Professor Whitten has remedied this defect and devoted nearly a quarter of his text to a discussion of the subject. He has adopted Sander's classification, $B_1 \perp B_2$ (penecontemporaneous and perpendicular fold axes) and $B_1 \wedge B_2$ (unrelated and inclined fold axes) as a framework to his descriptions. He never establishes the reasons why penecontemporaneously developed fold axes need be perpendicular or describes the strain states which can lead to synchronous folding in several directions. All field workers are agreed that the timing of the development of superimposed folds can vary greatly, but Professor Whitten gives no indication of the criteria by which the field geologist determines these age relationships. Contradictory statements on the nature of $B_1 \wedge B_2$ fold geometry are given, the geometrical relationships being described as "very complex" on page 327 and "relatively simple" on page 329. Professor Whitten illustrates the geometry of superposed flexural slip folds with a detailed investigation of a hypothetical area and makes the deduction that the later fold axes lie in a plane normal to the compression. He never defines what he means by compression in terms of stress or strain and he ignores Ramberg's (*Norsk Geol. Tid.*, 1959) and Flinn's (*Quart. J. Geol. Soc.*, 1962) pertinent studies on fold axis orientation in buckled layers which do not accord with his own deductions on this orientation. The chapter on "Examples of Superposed Folding" begins with 113 pages of illustration of the second ($B_1 \wedge B_2$) of his and Sander's two types of superposed folds. He follows this with a section of only four pages examining described instances of the first type ($B_1 \perp B_2$) most of which he suggests are not convincing examples and concluding that ". . . it is not common to find inequivocal description of $B_1 \perp B_2$ folds". The difficulty that Professor Whitten experiences in finding well documented examples of $B_1 \perp B_2$ folds leads to his comment, "This unsatisfactory situation should be viewed as a challenge to field geologists". I think that this "unsatisfactory situation" should have led the author to re-examine the validity and usefulness of Sander's classificatory scheme, and not to accept it as some kind of irrefutable dogma to which the geologist has consciously to seek out supporting evidence.

Much of Professor Whitten's own research has been centred on developing statistical techniques for determining the significance of modal analysis in coarse grained igneous rocks. As reviewer of this book I looked forward to reading the chapter on "Models and New Methods for Quantitative Fold Description" to see how his techniques might be applied in structural geology. This chapter proved a complete disappointment; what little it has to say is obscured by a mass of verbose writing and technical jargon. The geologist is extorted to "structure" his initial hypotheses into "conceptual process-response models" and eventually to evolve these into "a deterministic model in which all relevant response characteristics are incorporated into a unifying mathematical equation". Professor Whitten describes how statistical analysis has been used to describe the shapes of single folded layers and then proceeds to analyse the regional variability of these shapes within a hypothetical area by the methods of trend surface analysis. If he had applied these methods to the naturally deformed rocks illustrated in earlier sections of his book, he would have discovered their limitations, the separate layers in a single fold will practically always have different shapes, and so it will be impossible to establish a single set of statistical parameters characteristic of the folds at any one locality.

To summarize, this book is an uncritical compilation of some of the significant researches into the geometrical forms of folded rocks. It is often wordy, yet makes little attempt at reasoned syntheses of the many works from which it quotes. Because it ignores so much of the analytical development that has been made in the studies of folds during the past twenty years, it is my view that the book is unbalanced and dated in its approach. It is, however, well illustrated and does offer a good visual compendium on the variations of the forms of folded strata to those geologists who do not have ready access to the original descriptions.

J G RAMSAY

HAMILTON'S PRINCIPLE

Hamilton's Principle and Physical Systems

By B R Gossick. Pp xiii+247. (New York: Academic Press, Inc., London: Academic Press, Inc (London), Ltd, 1967.) 68s.

MOST books on classical mechanics discuss the Lagrangian formulation of mechanics and then usually include a demonstration that Lagrange's equations can be derived from Hamilton's principle. Although it is often stated that problems of electrical systems can be expressed in a similar mathematical form this is not usually developed at any length in such books.

The author of this book is anxious to provide a text giving a treatment of mechanical and electromagnetic systems which emphasizes the similarity of approach used in dealing with problems arising in both. In brief he attempts to do this by giving a general form of Hamilton's principle and using this to provide the appropriate framework for a discussion of the mechanical and electrical examples selected in the book.

There are six chapters. The first consists of a three page historical introduction. The second provides a background of mathematics (mostly concerned with Fourier series and Laplace transforms) which is used from time to time in later chapters.

The third chapter discusses the calculus of variations and gives an account of Hamilton's principle for general physical systems. The theory is given a concrete interpretation in the fourth chapter where conservative mechanical and electrical systems are considered.

The fifth chapter, which constitutes a third of the book, deals with non-conservative linear systems. The only mechanical system treated here (which occupies two pages) is that of a rigid body with damping. The last chapter deals with the Lagrangian density.

The author claims in the preface that a comprehensive and elementary presentation of mechanical and electromagnetic systems is given. Students unfamiliar with the material will not find this a suitable text. The presentation is not self-contained and is incomplete. For example, when the Euler angles for a rigid body are introduced in the fourth chapter the only explanation is a diagram which would be unintelligible. On the other hand, for those with an appropriate background of physics a considerable part of the book is redundant.

In view of the length and emphasis of the fifth chapter and a comment in the preface that the application of Hamilton's principle to non-conservative linear systems has not previously appeared in other texts it seems reasonable to suppose that this chapter is the main reason for the appearance of the book.

Although the book is well printed and clearly set out, I find it difficult to suggest what class of readers would find it of greatest value.

M J WILFORD

TESTING IN SCHOOLS

Teachers and Testing

By David A Goslin. Pp xix+195. (New York: Russell Sage Foundation, 1967.) $7.50.

THE supposed advantages and alleged deficiencies of intelligence and objective achievement tests (forced multiple-choice, true/false, matching, completion, and so on) have been widely debated. Less attention has been paid to the immediate social context in which such tests are given: particularly to how schools and teachers use tests, and are themselves influenced by them. This book reports a well-conducted survey of these sociological aspects of testing. Although American, it should be of relevance and interest to teachers, testers and education policy makers in Britain. American children average three standardized tests every year of their schooldays. If we are following this trend, we should pay attention to how it will affect our schools and teaching.

A large sample of public secondary school teachers, counsellors and pupils received questionnaires covering a wide range of issues: beliefs about tests, teachers' psychometric sophistication, the intended and actual use made of tests in selection, grading and vocational guidance, the dissemination—or lack of it—of test scores, the extent to which pupils are pre-trained for testing, the weight of test scores in evaluation, and so on. Numerous summary tables accompany the text but are not always adequately summarized within it.

Despite the thoroughness of the investigation there are three methodological deficiencies. First, in this type of work the interesting questions often emerge only after quantities of data have been collected. A pilot study would have enabled subsequent concentration of resources and treatment of pertinent issues in greater depth. Second, there is little said about how subjects perceived the investigation, although the author himself acknowledges the extent of extraneous social influences on expression of opinion. It is not clear, for instance, whether or not teachers thought their personal views on testing might filter back to their employers. Third, there is reliance almost entirely on self-reported questionnaire data. The one exception was an interesting experiment—using made-up school records—to see what weight teachers attached to test scores in contrast to other recorded information, in placing pupils in *A* or *B* streams. There was a marked disparity between teachers' reports of what they generally do, and what they did in the experiment. It pinpoints the weakness of over-reliance on questionnaires and the need for more imaginative and realistic methods of investigation. It also undermines confidence in the rest of the study. The author describes other points of dissonance and disagreement that seem to reflect an underlying sense of uncertainty among teachers about the place of testing in their work. There are many examples. Of those teachers who strongly advocate letting their pupils have a general indication as to their IQ, a quarter had never given their pupils any such information. The number of tests a school gives, and its stated test policy, are unrelated to how much actual use is made of test results in the school. Teachers generally want to withhold test scores from parents, while parents urgently want to know them.

Two major effects of testing on teaching are mentioned, but too briefly and peripherally for their importance. First, despite their protestations to the contrary, it was found that teachers do often coach pupils for tests. Moreover, the more schools are oriented towards college admission, the more likely they are to give such preparation and assistance. The chances of going to college for pupils in other schools may therefore be affected. The second major effect is on the school's own examining, where the multiple-choice form of question has replaced other forms, such as the essay. It is conceivable that this predominance may lead to students being rewarded for achieving complete coverage of the syllabus, rather than for exercising their individuality in selection and expression. The way in which exams impose their own constraints and patterns on teaching, learning and subsequent intellectual development needs to be further investigated. The dog should wag the tail, and not the tail wag the dog.

MALCOLM PARLETT

OBITUARIES

Dr H. R Nettleton

Harold Redmayne Nettleton, who died on December 19, 1967, in his eighty-fourth year, was a distinguished exponent of classical physics in both teaching and research

He took his London B Sc from Leeds in 1903 and was appointed in 1906 as an assistant lecturer at Birkbeck College, where he took his M Sc in 1916 and D Sc in 1922

The raising of the status of the college to that of a School of the University of London in 1920, and the consequent establishment of chairs and readerships, owed much to the scholarly labours of a small band, among whom Nettleton was notable At this time his researches were largely on the determination of thermal conductivities of liquids and on the Thomson effect He continued to be active in research during the professorships of P. M S Blackett and of J D Bernal, turning his attention latterly to the absolute determination of electrical resistance, here he devised and carried out, with very modest facilities, an elegant commutating d c. method, similar in some respects to that subsequently used by the US National Bureau of Standards. In one of his last papers, published jointly with the late Professor S Sugden in 1939, on the magnetic susceptibility of nickel chloride, he described a simple method, still too little known, for the accurate measurement of magnetic fields.

A keen chess player and cricketer in his day, and an ardent champion of women's suffrage, he was devoted to Birkbeck College, which he served actively until his retirement as senior lecturer in 1952 During the Nazi period, he was a friend of the scholars who found a refuge at Birkbeck, and to generations of Birkbeckians a father confessor, a role in which he triumphed over formidable deafness But, above all, he will be remembered by his students for his superb lectures, minutely prepared and constantly revised, which delighted as much by their clarity and by their apparent ease as by their nice touches of humour, delivered with dry seriousness.

Both his students and his colleagues drew inspiration from his aesthetic flair for rational experimental design and from his absolute scientific integrity. Giving up active physics on his retirement, he kept to the very end his interest in current affairs and in friendship, which he cultivated with the same devotion and attention to detail that characterized his scientific work He will be widely missed

W Ehrenberg

University News : London

Dr M G M Jukes, lecturer in the University of Edinburgh, has been appointed to the chair of veterinary physiology tenable at the Royal Veterinary College The title of professor of botany has been conferred on Mr P R Bell in respect of his post at University College

Appointments

Mr James Ian Smith, under-secretary in the Department of Agriculture and Fisheries for Scotland, has been appointed a member of the Agricultural Research Council in succession to the late Mr Ralph Hamilton Law

Mr J A Nelder, of the National Vegetable Research Station, has been appointed head of the Rothamsted Experimental Station's Statistics Department in succession to Dr F Yates

Meetings

Problems of the Pebble Bed and Granular Materials, March 6–7, Nuclear Research Establishment, Julich (Dr D Bedenig, Brown Boveri/Krupp Reaktorbau GmbH, Experimentelle Entwicklungsabteilung, Postfach 589, D-517 Julich, Bundesrepublik Deutschland)

Theoretical Mechanics Colloquium, April 2–5, University of Oxford (Colloquium Secretary, Dr A. B Tayler, Mathematical Institute, St Giles, Oxford).

Recent Developments in Flame Physics, April 30–May 1, Department of Chemical Engineering and Chemical Technology, Imperial College (Professor F J Weinberg, Department of Chemical Engineering and Chemical Technology, Imperial College, Prince Consort Road, London, SW7)

Fluidics Conference, May 8–10, Torino (Mr H Stephens, British Hydromechanics Research Association, Cranfield, Bedford)

Precision Electromagnetic Measurements, June 25–28, National Bureau of Standards Laboratories in Boulder, Colorado (Mr Donald D King, Aerospace Corporation, PO Box 95085, Los Angeles, California).

Chemistry of Natural Products, July 8–13, London (The Secretary, 5th International Symposium on the Chemistry of Natural Products, c/o The Chemical Society, Burlington House, London, W1).

Competing Spectrochemical Techniques, July 10–12, University of Keele (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1).

Electrical Contact Phenomena, July 15–18, University College of Swansea (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London, SW1)

Photobiology, August 26–31, Dartmouth College, Hanover (The Secretariat, Vth International Photobiology Congress, Argonne National Laboratory—202, Argonne, Illinois)

Problems of Cooley's Anaemia, September 9–11, New York City (Dr H Fink, Cooley's Anaemia, Coney Island Hospital, Brooklyn, New York, NY 11235)

Volcanoes and their Roots, September 7–9, 1969, University of Oxford (Dr J D. Bell, Department of Geology and Mineralogy, Parks Road, Oxford)

Erratum The communication "Electrophoretic Study of Glutathione Reductase in Human Erythrocytes and Leucocytes", which appears on page 256 of this issue of *Nature*, is by Jean-Claude Kaplan and Ernest Beutler, and not only by Jean-Claude Kaplan as stated at the end of the article

Erratum In Table 2 of the article "*In vitro* 'Lipid' Metabolism in the Developing Human Foetus" by Jacques F Roux, A Grigorian and Y Takeda (*Nature*, 216, 819, 1967), the figures under the column headed "Brain" should read 100, 300, —, 340 and 720.

Erratum In the communication "Kinetics of Association of Carbon Monoxide with Haemoglobin at Low Temperature" by R Banerjee, P Douzou and A Lombard (*Nature*, 217, 23, 1968) the second and third sentences of the sixth paragraph on page 24 should read "The value of this constant, which we shall call l'_4, is found to be 7×10^3 M^{-1} sec^{-1} at $-55°$ C This is not far from what would be expected from Gibson's value of 6 9×10^6 at 19° C and activation energy (10 kcal) determined in aqueous solution"

Erratum. In the communication entitled "Detection of an Anisotropic Soft X-ray Background Flux" by C S Bowyer, G. B Field and J E Mack (*Nature*, 217, 32, 1968) the formula in line 14 of the penultimate paragraph should read $\int N_e{}^2\,dl = 0\ 7$ and not $\int N_a{}^2\,dl = 0\ 7$

FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, January 22

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 10 a m—Colloquium on "Microwave Integrated Circuits", "Microwave Solid State Sources"

Institute of Actuaries (at Staple Inn Hall, High Holborn, London, WC1), at 5 p m—Mr H A R Barnett "A Theory of Mortality"

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m—Discussion Meeting on "Microwave Electrostatic Wattmeter", opened by Professor H E M Barlow, FRS

University of London (in Lecture Theatre A, Mechanical Engineering Building, Imperial College of Science and Technology, Exhibition Road, London, SW7), at 5 30 p m—Professor Bertil Matern (Roval School of Forestry, Stockholm) "Statistical Models of Two-Dimensional Variation" (further lectures on January 24 and 26)

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 6 p m—Mr W B Gosney "Modern Refrigeration" (First of three Cantor Lectures Further lectures on January 29 and February 5)

Tuesday, January 23

University College London (in the Anatomy Theatre, Gower Street, London, WC1), at 1 20 p m—Dr W G Chaloner "Evidence of Life in Meteorites" *

Imperial College of Science and Technology (in Lecture Theatre A, Department of Mechanical Engineering, Exhibition Road, London, SW7), at 5 30 p m—Professor K F Roth, FRS "The Large Sieve" (Inaugural Lecture)

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m—Discussion Meeting on "The Importance of Cleanliness in Power Plant Erection"

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, SW1), at 6 p m—Professor A M North "The Effect of Polvmer Chain Flexibility on the Rates of Fast Macromolecular Reactions"

Society for Analytical Chemistry, Special Techniques Group (joint meeting with the Polarographic Societv at Imperial College, South Kensington, London, SW7), at 6 30 p m—Meeting on "Stripping Techniques" Speakers Mr B Fleet and Mr P Kane

Wednesday, January 24

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m—Mr F G Taylor and Mr J D S Hinchcliffe "Cables for Aircraft—Design and Development", Mr P W C Jones "Cables for Aircraft—Installation Systems and Experience", Mr R F Brazier "Cables for Aircraft—Pressure-Type Connections"

Challenger Society (at the Linnean Societv, Burlington House, Piccadilly, London, W1), at 5 45 p m—230th Scientific Meeting Mr P R Walne "Artificial Cultivation of Shell Fish" (Buckland Lecture)

Institution of Electronic and Radio Engineers, Telecommunications Group (at 8–9 Bedford Square, London, WC1), at 6 p m—Mr G Parker "Studio Colour Equipment"

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m—Dr G E R Deacon "Progress in Ocean Technologv" (Thomas Lowe Gray Lecture)

Society of Chemical Industry, Food Group—Nutrition Panel (joint meeting with the Oils and Fats Group, at 14 Belgrave Square, London, SW1), at 6 15 p m—Dr A Gorton "Some Biochemical Aspects of Oils and Fats"

Society of Environmental Engineers, Packaging Group (at the Euston Tavern, 73 Euston Road, London, NW1), at 6 30 p m—Mr J Raeburn "Containerization, a New Environment for the Packaging Engineer"

Thursday, January 25

Fertilizer Society (at the Geological Societv, Burlington House, Piccadilly, London W1), at 2 30 p m—Professor E W Russell "The Place of Fertilizers in Food Crop Economy of Tropical Africa"

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m—Discussion Meeting on "Electrical Propulsion for Space Vehicles"

Institution of Mechanical Engineers, Automatic Control Group, and the Control and Automation Division of the IEE (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m—Discussion Meeting on "Orientation Control of Large Structures"

Thursday, January 25—Friday, January 26

Challenger Society and representatives from the Marine Laboratories (Natural Environment Research Council Scheme) (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, SW7)—Scientific Papers

Friday, January 26

British Biophysical Society, Physical Biochemistry Group (at the Laboratories of the Chester Beatty Research Institute, Clifton Avenue, Belmont, Sutton, Surrey), at 11 a m—Meeting on "Zonal Centrifugation—Its Application to the Isolation of Macromolecules, Viruses and Sub-Cellular Organelles"

Colour Group (Great Britain), Photobiology Group (at the Middlesex Hospital Medical School, London, W1), at 2 p m—Meeting on "New Light Sources for Photobiology"

Saturday, January 27

Inner London Education Authority (at the Hornlman Museum, Forest Hill, London, SE23), at 3 30 p m—Mr Stanley Cramp "An Ornithologist in Jordan" *

Monday, January 29

Institution of Electronic and Radio Engineers, Joint IEE/IERE Medical and Biological Electronics Group (at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 6 p m—Colloquium on "Diathermy"

Institution of Mechanical Engineers, Medical Engineering Working Party (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m—Discussion Meeting on "The Physics and Medical Applications of Lasers"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Temporary Assistant Lecturer or Lecturer in the Department of Engineering in the field of geotechnology—The Registrar, The University, Leicester (Januarv 22)

Assistant Lecturer in the Department of Philosophy—The Registrar, The University, Leicester (January 26)

Technician (with experience in general histological techniques and at least HNC or equivalent) for the Histology Laboratory—The Secretary, Houghton Poultry Research Station, Houghton, Huntingdon (January 26)

Lecturer in the Department of Mathematics to be responsible for the teaching of statistics to social scientists—The Registrar, The University, Nottingham (January 29)

Lfcturer or Assistant Lecturer (with a special interest in protein crystallography) in Crystallography—The Secretary, Birkbeck College (University of London), Malet Street, London, WC1 (January 29)

Temporary Lecturer/Senior Lecturer (with a good honours degree or Dip Tech or equivalent in mathematics, and preferably some teaching experience) in Mathematics at Britannia Royal Naval College, Dartmouth, Devonshire—Ministry of Defence, Navy Department CE1(N), 368 Old War Office Building, London, SW1 (January 29)

Lecturer (with initial qualifications in either physics, mineralogy or chemistry, with experience of, or interest in, the application of modern techniques to soil studies) in Soil Science—The Secretary, The University, Aberdeen (February 3)

Chief Technician in the School of Biological Sciences to take charge of the Teaching Laboratories of the School, duties will include the supervision of technical staff, the requisitioning of supplies and assisting in the organization of practical classes—The Laboratory Superintendent, School of Biological Sciences, University of East Anglia, University Plain, Norwich, NOR 24B (February 5)

Senior Lecturer (I) or Lecturer (II) in the Department of Histology—The Registrar, The University, Liverpool, 3, quoting Ref RV/305/N (February 7)

Chair of Physics at Chelsea College of Science and Technology—The Academic Registrar (A), University of London, Senate House, London, WC1 (Februarv 9)

Junior Research Fellowship without restriction of subject—The Secretarv, Merton College, Oxford (February 10)

Lecturer or Assistant Lecturer in Applied Mathematics—The Registrar, The University, Liverpool, quoting Ref RV/236/N (February 10)

Research Fellowship in Science subjects (including Mathematics and Engineering)—The Secretary of the Fellowships Committee, Trinity Hall, The University, Cambridge (February 15)

University Lecturer in Physiology—The Deputy for the Waynflete Professor, University Laboratory of Physiology, Oxford (February 15)

Second Chair of Applied Mathematics—The Registrar, The University, Newcastle upon Tyne (February 17)

Chair of Geography—The Registrar, The University, Sheffield (February 19)

Senior Lecturer or Lecturer in Psychology in the Department of Philosophy, University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, WC1 (February 20)

Senior Lecturer/Lecturer Grade I in Diagnostic Pathology, a Senior Lecturer/Lecturer Grade I in Morbid Anatomy and Histopathology, a Lecturer Grade I in Chemical Pathology, and a Lecturer Grade I/Lecturer Grade II in Clinical Chemistry at the College of Medicine of the University of Lagos—The Inter-University Council, 33 Bedford Place, London, WC1 (February 28)

Wayne Kerr Chair of Measurement Science with particular reference to Electrical Metrology—The Academic Registrar (LFG), University of Surrey, Battersea Park Road, London, SW11 (March 1)

Chair of Bacteriology—The Registrar, The University, Senate House, Bristol, 2

Assistant Professor (with training and experience in the genetics of micro-organisms, particularly in fungal genetics) in the Department of Botany—Dr C J Hickman, Department of Botany, University of Western Ontario, London, Ontario, Canada

Associate Professor (science or medical graduate with a Ph D in biochemistry, and several years of teaching experience) of Biochemistry—Professor P H Jellinck, Head, Department of Biochemistry, Queen's University, Kingston, Ontario, Canada

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Department of Education and Science National Lending Library for Science and Technology Current Serials received by the NLL, March 1967 Pp viii+423 (London H M Stationery Office, 1967) 40*s* net [711

Report on Concrete Practice Part 2 Site Supervision and Testing Pp 60 (London Cement and Concrete Association, 1967) 15*s* [811

University of Manchester Calendar 1967–68 Pp xxxv+459 (Manchester Manchester University Press, 1967) [811

Road Research Laboratory RRL Report LR80 An Improvement to the Combination Method of Reducing Delays in Traffic Networks By D I Robertson Pp 6 (Crowthorne, Berks Road Research Laboratory, 1967) *Gratis* [811

Ministry of Technology and Fire Offices' Committee Joint Fire Research Organization Fire Research 1966 Report of the Fire Research Steering Committee with the Report of the Director of Fire Research Pp vii+100 +8 plates (London H M Stationery Office, 1967) 9*s* net [911

Office of Health Economics Early Diagnosis Paper, No 1 The Early Diagnosis of Raised Arterial Blood Pressure By W W Holland Pp 14 2*s* 6*d* Early Diagnosis Paper, No 2 The Early Diagnosis of Visual Defects By P. A Graham Pp 14 2*s* 6*d* (London Office of Health Economics, 1967) [911

Radio 1 and Radio 2 By Robin Scott (BBC Lunch-time Lectures Sixth Series—1) Pp 16 Local Radio Some Questions Answered Pp 11 (London British Broadcasting Corporation, 1967) [911

University of Strathclyde Calendar 1978/68 Pp 580 (Glasgow University of Strathclyde, 1967) [911

Schools Council Working Paper No 15 Counselling in Schools—a Study of the Present Situation in Great Britain Pp v+76 (London H M Stationery Office, 1967) 7*s* net [1011

Road Research Laboratory RRL Report LR 65 Traffic Survey Equipment for Measuring Journey Time and Stopped Time By R A Vincent Pp 20 (Crowthorne, Berks Road Research Laboratory, 1967) *Gratis* [1011

The Image of Technology By Sir Solly Zuckerman (The Fourth Maurice Lubbock Memorial Lecture) Pp 24 (London Oxford University Press, 1967) 3*s* 6*d* net [1311

The Great Debate Some Basic Issues in U S Foreign Policy Pp 14 (London United States Information Service, 1967) [1311

Thirteenth Annual Report of the Tweed River Purification Board for the year ended 31st December, 1966 Pp 43 (Newtown St Boswells Tweed River Purification Board, 1967) [1311

Cross-Channel Collaboration a Study of Agreements between British and Continental Firms By Charles de Hoghton Pp 64 (London Political and Economic Planning, 1967) 10*s* [1311

Trade Unions in France Pp 16 (London Ambassade de France, Service de Presse et d'Information, 1967) [1311

Political and Economic Planning Annual Report 1966–67 Pp 25 (London Political and Economic Planning, 1967) [1411

Imperial College, City and Guilds College 1964–67 Research Report Pp xi+116+15 plates (London Imperial College, City and Guilds College, 1967) [1411

Agricultural Research Institute of Northern Ireland Fortieth Annual Report, 1966–1967 Pp 32 Results of Recent Experiments Pp 26 (Hillsborough, Co Down Agricultural Research Institute of Northern Ireland 1967) [1511

The Severn Estuary The Key to the Future of a Region By T L Shaw and R G Morgan Pp 15 (Bristol T L Shaw and R G Morgan, The University, 1967) [1511

Steelmaking Furnace Refractories—Proceedings of the 68th Steelmaking Conference, held at the Royal Hotel, Scarborough, April 25–26, 1967 Pp 136 (London The British Iron and Steel Research Association, 1967) 30*s* [1611

Meteorological Office Scientific Paper No 27 The Determination of the Extraterrestrial Constant of a Dobson Spectrophotometer By R A Hamilton and J M Walker Pp 11 (London H M Stationery Office, 1967) 3*s* 9*d* net [1711

Other Countries

Reviews of National Science Policy—Japan Pp 231 (Paris Organization for Economic Co-operation and Development, London H M Stationery Office, 1967) 20 francs, 30*s* , $5 [1311

Rubber Research Institute of Malaya Annual Report, 1966 Pp 112 (Kuala Lumpur Rubber Research Institute of Malaya 1967) $3 [1311

Transactions of the American Philosophical Society, New Series—Vol 57, Part 7 The Community Library—a Chapter in English Social History By Paul Kaufman Pp 65 (Philadelphia The American Philosophical Society, 1967) $2 [1311

Studia Forestalia Suecica Nr 51 Comparative Studies on the Germination of Scots Pine and Norway Spruce Seed under Different Temperatures and Photoperiods By S K Kamra Pp 16 3 kr Nr 52 Physiology of Fungi Attacking Birch and Aspen Pulpwood By Björn Henningsson Pp 54 8 kr Nr 53 Interactions Between Micro-organisms Found in Birch and Aspen Pulpwood By Bjorn Henningsson Pp 32 Nr 54 Microbial Decomposition of Unpeeled Birch and Aspen Pulpwood during Storage By Björn Henningsson Pp 32 5 kr (Stockholm Skogshogskolan, Royal College of Forestry, 1967) [1311

Institut Royal Météorologique de Belgique Statistiques Quinquennales Observations Aerologiques, Station d'Uccle, 1961–1965 Pp 175 (Uccle-Bruxelles Institut Royal Météorologique de Belgique, 1967) [1311

Companhia de Diamantes de Angola (DIAMANG) Servicos Culturais Dundo–Lunda–Angola Museu do Dundo Subsidios para o Estudo da Biologia na Lunda Publicacoes Culturais No 76 Estudos Diversos, XXXI. Pp 149 (Lisboa Companhia de Diamantes de Angola, 1967) [1311

India Council of Scientific and Industrial Research Annual Report of the National Institute of Oceanography, 1965–66 (New Delhi National Institute of Oceanography, 1967) [1411

Canada Department of Energy, Mines and Resources Geological Survey of Canada Memoir 347 Bache Peninsula, Ellesmere Island, Arctic Archipelago By R L Christie Pp 63 (18 plates) $2 Paper 65–29 Geology of the Passamaquoddy Bay Region, Charlotte County, New Brunswick By L M Cumming Pp v+36 (6 plates) $1 Paper 66–24 Geological Notes, Itchen Lake Map-Area, District of Mackenzie By H H Bostock Pp v+13 $1 50 Paper 66–28 Contwoyto Lake Area (North Half), District of Mackenzie By L P Tremblay Pp iv+19 $1 Paper 67–16 Published References to Champlain Sea Faunas 1837–1966 and List of Fossils By Frances J E Wagner Pp v+82 $0 75 Paper 65–27 Mississippian Subsurface Geology, Rocky Mountain House Area, Alberta By H L Martin. Pp v+14 $3 50 Paper 67–41 Canadian Upper Mantle Report, 1967 Pp vi+245 $3 50 Paper 67–50 Field and Laboratory Methods used by the Geological Survey of Canada in Geochemical Surveys No 9 Tin in Soils and Stream Sediments 'By A Y Smith Pp 11 $1 50 Economic Geology, Report No 22 Geology of Iron Deposits in Canada Vol 2 Iron Deposits in the Appalachian and Grenville Regions of Canada By G A Gross Pp 111 (13 plates) $3 (Ottawa Queen's Printer, 1967) [1511

Annals of the New York Academy of Sciences, Vol 143, Article 1 Biology of the Mycoplasma. By L Hayflick *et al* Pp 1–824 (New York New York Academy of Sciences, 1967) [1511

Population Bulletin, Vol 23, No 4 (October 1967) Soviet Population Theory from Marx to Kosygin—a Demographic Turning Point ? Pp 85–116 (Washington, D C Population Reference Bureau, Inc , 1967) [1511

United States Atomic Energy Commission 1967 Financial Report Pp vi+50 (Washington, D C United States Atomic Energy Commission, 1967 Available from U S Government Printing Office) $0 50 [1511

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India Meteorological Department Symposium on Hydrometeorology of India with special reference to Flood Forecasting and Warning, held at the Meteorological Office, New Delhi, 23–24 March, 1964 (*Indian Journal of Meteorology and Geophysics* Supplement) Pp iv+378 (Delhi Manager of Publications, 1966) Rs 41 50, 96*s* [1511

Seibersdorf Project on Food Irradiation Research International Programme on Irradiation of Fruit and Fruit Juices—First Activity Report, 1st January, 1965–31st December, 1966 Pp 49 (Paris Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 1967) [1611

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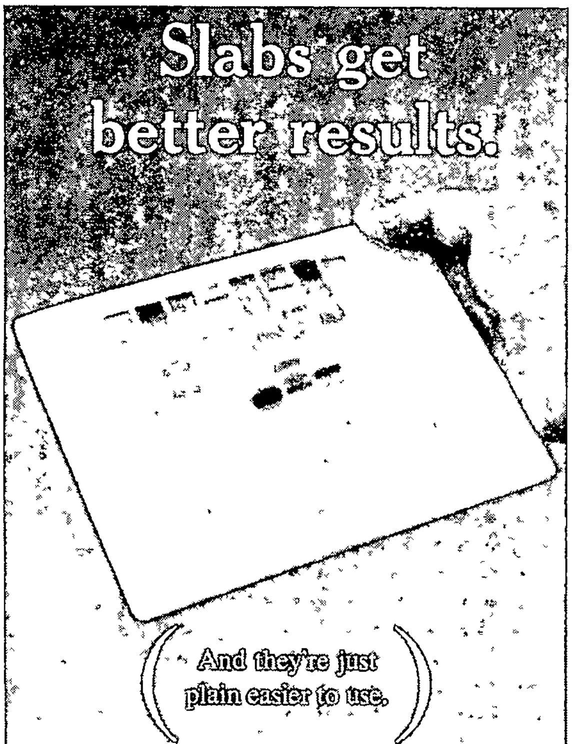
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Keep Your Powder Clean

THE Russian and American disarmament negotiators pulled a considerable rabbit out of the hat when they returned to Geneva a week ago and made public the agreement of their two governments on a draft of a non-proliferation treaty. Only a few weeks earlier, when the proceedings broke up for the holidays, it seemed very much as if the negotiators had reached yet another obstacle on the road along which they have been trudging for the best part of two years. The new and more specific form of the treaty upon which the Soviet Union and the United States are apparently agreed is designed specifically to meet the now familiar argument, put forward last year by the delegation from West Germany, that any non-proliferation treaty should allow the safeguards organization of Euratom to be responsible in Western Europe for the supervision of the safeguards procedures necessary to ensure that non-nuclear powers do not divert fissionable material to military ends. The German argument, supported by the Italian delegation, stems partly from the view that any other arrangement would be at once a slur on Euratom and an opportunity for industrial espionage. The objection to this view, held most vigorously by the Soviet Union but supported by a good deal of logic, is that it would be improper to allow membership of a private club such as Euratom to exempt a nation from the rules and regulations of an international treaty.

The new draft of the treaty which has appeared at Geneva is not so much a solution of this problem as a means of postponing it. Instead of spelling out the way in which a non-proliferation treaty would be policed, the new draft merely requires that signatories should accept some form of control and inspection which must be negotiated with the International Atomic Energy Agency beginning within six months of ratification. It is understood that nations will be able to negotiate with Vienna individually or in groups. No doubt they will also be free to settle for whatever system of control the IAEA chooses to specify. On the face of things, this is a clever move. For one thing, it will demonstrate that the IAEA remains ultimately responsible for the approval and design of all systems of control. At the same time, the new form of words will presumably enable the Euratom safeguards organization to assume responsibility for safeguards in much of Western Europe. With luck, no doubt the members of Euratom (with the exception of France) will be anxious for assurances on this point before they are prepared to sign. They would, however, be well advised not to push their luck too far. It is still something of a surprise that the Soviet Union has been prepared to modify in any important way its earlier insistence that the IAEA should be the only organization trusted with inspection.

Elsewhere, it is probably only fair to recognize that the loosening of the inspection clause in the draft treaty will eventually imply a somewhat less rigorous system of inspection than was originally foreseen, which may be a good thing and not the opposite. For if the IAEA delegates control of fissionable material in Western Europe to the safeguards organization of Euratom, it will be exceedingly difficult for it simultaneously to impose on independent signatories a system of inspection operated by its own men. There is evidence enough already that nations like India are exceedingly sensitive about the way in which the proposed treaty would make permanent the class distinction which at present exists between the nuclear powers and the others. They are unlikely to accept arrangements which would create another distinction between two classes of non-nuclear powers—those in Euratom and the rest. In these circumstances, if and when the treaty is ever ratified, the IAEA is likely to find itself encouraging the formation of regional self-inspection systems elsewhere than in Western Europe, perhaps reserving for itself chiefly the right of verification. This is probably a sound development for its own sake, if only because it has never been easy to see how the integrity of the rapidly growing nuclear power industry could be effectively guaranteed in fine detail from Vienna. By playing its cards well in the months following the ratification of a non-proliferation treaty, however, the IAEA could easily establish for itself a commanding position in the supervision of nuclear reactors throughout the world without incurring the trouble, the expense and the risks of a system of inspection which is meant to be thoroughly comprehensive. In other words, a weaker system of inspection than was at first proposed should help not only to bring the non-proliferation treaty within reach but to make international inspection workable.

That said, there can unfortunately be no assurance that an agreed text will emerge in the months immediately ahead from the disarmament committee at Geneva. The representatives of the super-powers have been putting a cheerful face on things, but the conference has not yet begun to tackle what may prove to be the most serious problem of all—the unwillingness of entirely peaceable countries to sign away their freedom to follow a course of military development which has become, as it happens, entirely respectable among the most powerful nations. More practically, there are questions of whether nations such as India can look for some degree of protection against attack in return for agreement to forgo the manufacture of nuclear weapons

If the smaller powers push these arguments strongly in the weeks ahead, the non-proliferation treaty could be held up for another year or more It is also only sensible to acknowledge that the smaller powers would be entirely within their rights in asking that the treaty should show either greater respect for their right to make decisions on defence policy on their own behalf or, preferably, that it should include some restriction of the right of nuclear powers to keep on manufacturing fissionable material for military purposes The willingness of the United States and the United Kingdom to throw their civil nuclear plants open to inspection is a gesture but not a real concession on this point In the same way, the inclusion in the new draft treaty at Geneva of a general undertaking that signatories will press forward to the objective of general and complete disarmament (or GCD as it is known among the specialists) will be most often interpreted as a formality In these circumstances, even the more realistic draft treaty which has now been designed may prove too stiff a dose of segregation for many of the smaller nations The next few weeks should tell

In for a Penny, in for a Pound

THE European Molecular Biology Organization (EMBO) has made a serious claim on public attention and even sympathy in the years since its small beginning in the early sixties. From the start—which is conventionally taken to be a conference at Ravello in September 1963—it has been plain that there is a valuable job to do in moving people from one European laboratory to another. EMBO has done well to collect enough money to start the ball rolling (and the Volkswagen Foundation, which has provided most of the support, deserves not to be forgotten). It has been something of an object lesson for other informal societies seeking some means of influencing events to see how much, in the last resort, depends on good organization Because EMBO seemed, at the beginning, to be as much a cosy self-congratulatory club as a more conventional learned society, it is not surprising that it provoked scepticism and even resentment, one of the indirect benefits of the funds which have been made available for fellowships in the past few years is that EMBO has been given a chance to demonstrate that it is a thoroughly open-minded society That done, it is only natural that most European governments should now be willing to support on a continuing basis the scheme for a mixture of travelling fellowships of various kinds In spite of the devaluation of sterling, even the British Government seems cheerful about this prospect The chances are that an international agreement on continuing support will be signed and sealed in April

The laboratory on which EMBO has set its heart (see page 314) is altogether a different proposition This, at least, is what some governments have been tempted to suppose For one thing, a laboratory employing several hundred people is a much more permanent undertaking of the kind from which governments almost instinctively fight shy It has also inevitably been asked why the molecular biologists are so keen on having a laboratory when there is no ready-made excuse such as the need to operate an expensive piece of equipment in collaboration—the prime justification of CERN In this connexion, the sceptics are well within their rights to point out how easy it is for laboratories which are established in a vacuum to remain in a vacuum more or less indefinitely The world is littered with institutions like that But a more powerful reason why governments have frequently hung back from the creation of an EMBO laboratory has been the fear that the establishment of a new centre of excellence in molecular biology could only serve still further to deplete the scarce supply of molecular biologists in national universities This, of course, is an argument which carries weight only with countries which already have a significant stock of molecular biologists and are anxious to do everything they can to husband it, but even then the reactions of similar governments are not entirely predictable In Britain, for example, the feeling in the Medical Research Council that an EMBO laboratory would thin out still further the laboratories already crying out for experienced men and women has been one of the reasons why the British Government has been conspicuously cautious in its discussions of the laboratory In France, by contrast, there have been no such inhibitions, to tell from some of the things which Mr M Schumann, the Minister of Scientific Research, says, the EMBO laboratory comes next in importance only to the *force de frappe* and the *plan calcul*

What is the truth ? And how should European governments react to the proposals now being made to them ? Perhaps the most obvious thing to say is that the documents which have been prepared in support of the case for the laboratory are an eloquent disproof of a good many of the doubts about the laboratory which have from time to time been raised The need for a concentration of resources is particularly well put For one thing, sheer propinquity is a blessing in a field in which the most skilful operators are often specialists in quite unrelated disciplines But it is also clear that molecular biologists are itching to undertake a number of large scale projects which share something of the monumental character of undertakings on high-energy physics Dr F Crick's "Project K" for making an exceedingly detailed study of the *K*12 strain of *Escherichia coli* is only one of many examples of how concentration may make it possible to tackle problems which have previously been inaccessible. Given this, and the fact that EMBO has at present such a high reputation that it would probably be able to attract the kinds of people necessary to ensure the success of an international laboratory, there is probably no need to fear that the project for an international laboratory would founder on intellectual grounds

The fear that an international laboratory would drain scarce talent from existing institutions is necessarily harder to deal with, if only because so much is bound to depend on estimates of how studies in molecular biology are likely to be stimulated by the creation of an international laboratory. Professor V. Weisskopf (page 317) is able to claim that the growth of CERN has not been accompanied by a decline of high-energy physics elsewhere in Europe, and what he has to say is powerful reinforcement for the case in favour of the European laboratory. At the same time, however, it is only possible to guess at what would have happened to high-energy physics in Europe if CERN had not been built. Most probably, the thirteen member nations would have been egged on by competition to spend more, in aggregate, on this branch of physics, but would not have had such good value for money. And in this spirit, of course, the possibility that a European laboratory for molecular biology may drain away people from national laboratories—unrealistic though it may be—is almost irrelevant. What matters is that the people concerned should remain in circulation as innovators and as teachers. If that could be assured, the EMBO laboratory would be at worst a more economical way of doing business than the present system in which funds for the support of research in molecular biology are dispersed throughout the universities of Europe. In reality, there is probably a good deal in the claim of the EMBO council that a European laboratory would be a means by which contributing governments could gain markedly from quite small extra investments. In the long run, it would even be reasonable for them to expect that a successful EMBO laboratory would restrain the strictly national demand for an expanding budget.

All this, however, is hair-splitting of a kind. The truth is that the sums of money necessary for the funding of the EMBO laboratory, large though they may seem in comparison with the scale on which biological laboratories are habitually financed, are an exceedingly small part of what Europe as a whole chooses to spend on research of all kinds. Even if the EMBO venture should turn out to be a sheer waste of money—and all the evidence runs the other way—no great damage would have been done. In the circumstances it will be a great misfortune if the governments whose representatives are now trudging back from Geneva make too much of the issues of principle involved, and neglect the wider opportunities which the EMBO proposals offer. And these, in the long run, are plain enough. Sooner or later it will be necessary to organize a great deal of European science on a co-operative basis. The sooner it is possible to set about the building of common institutions, the sooner Europeans will begin to profit from their common geography.

In that sense, EMBO and its laboratory are splendid opportunities for experiment.

No Academic Spending Spree

THE University Grants Committee has now published details of the allocations of recurrent grants to universities in Great Britain over the next five years. The figures, which were sent to universities at the end of last year, are published in the UGC Annual Survey for the academic year 1966–67 (HMSO, 3*s*. 3*d*.), together with the memorandum which the UGC sent the universities to explain "the strategic attitude" which the UGC has had to adopt in order to make a division of the resources. Sir John Wolfenden is cheerful about the reception the memorandum received from the universities—at least, he said in presenting the report, there had been "no major explosion from the universities" so far. The grants themselves confirm that the growth of universities is likely to move more slowly in the next five years than it has done in the last five. "Plain living and high thinking" was Sir John's recipe for the next quinquennium.

RECURRENT GRANTS FOR BRITISH UNIVERSITIES

	£ (million)
1967–68	150·79
1968–69	152·85
1969–70	158·57
1970–71	164·82
1971–72	171·10

(From 1968–69 onwards, the figures make no provision for equipment for teaching and research, which will be dealt with by separate grants.)

The memorandum lays emphasis on the need to expand undergraduate numbers, in particular in the arts and social sciences. Sir John defends this decision by pointing out that, in 1964, the actual numbers of science students in the first-year sixth forms of British schools declined. Despite this, the UGC has provided for a slight increase in the numbers of science students at universities. But social science and the arts, where the pressure on university places is greatest, must be allowed to expand the fastest. The UGC has not fixed limits on the number of postgraduates which universities can provide for, but the grants awarded do make certain assumptions about the proportion of undergraduates to postgraduates. As the report puts it, "It is, of course, open to any university to admit more than the number of students indicated in its separate allocation letter, if by internal economies, increased 'productivity' or any other means it thinks it can rightly do so".

The UGC has set in train a survey of the reasons why students entering universities fail to finish the course —the "drop-out" or wastage rate. At present in Britain, the wastage rate is running at about 14 per cent, but there is considerable variation both between departments and between universities. Suspicion has sometimes been voiced that some universities admit more students than they can possibly handle, and use the first year to weed them out. The survey, Sir John said, would try to sort out whether there was any truth in suggestions of this sort, and would try to make some estimate of the reasons for wastage. Vice-chancellors, he added, tended to be difficult about publishing this kind of information, so that discussion would be needed before it was finally decided what form the published survey should take.

In making the allocations to universities, the principal criterion is the number of students in any university. This year, for the first time, the UGC has also taken into account unit costs, and staff : student ratios. Veterinary students come out as the most expensive, with a unit cost of £1,136, and social scientists are the cheapest at £255. The staff : student ratio is most favourable in veterinary science, at 1 : 4·6, and least favourable in education, at 1 : 12·5. Physical science, the report says, has a unit cost of £376 and staff : student ratio of 1 : 11·3, but biological sciences are more expensive, with unit costs of £518 and staff : student ratios of 1 : 8·7.

Superannuated Dons

An alternative arrangement to the Federated Superannuation System for Universities receives consideration in a recent working party report, published by the Department of Education and Science. Headed by Sir George Maddex, the party represented a number of associations and committees and the government departments concerned. The alternative scheme which the committee considered is the terminal salary scheme.

The existing FSSU system is based on policies of assurance so that benefits reflect the actual amounts received as salary during an individual's career. This has led, the report maintains, to inadequate pensions. According to the alternative arrangement, a scheme would be adopted under which pension benefits would be directly related to the teacher's salary at retirement, thus giving weight to economic conditions at that particular time. The rates of benefit would be guaranteed, but the contributions would be fixed on a provisional basis. Because of a variety of reservations from members, however, the working party has been unable to make a clear recommendation in favour of either system.

The report includes an examination of the basic differences between terminal salary and insurance policy schemes, and it also considers the implications of tax and the National Insurance graduated pension scheme; the report compares them by working out the benefits in a number of typical cases. On a salary of £2,000 a year, for example, assuming a contribution of 5 per cent, the net charge borne by a teacher according to the terminal salary scheme would be £67 18*s*. 4*d*., whereas according to the existing scheme it would be £83 10*s*. 0*d*. The saving in net outlay in this case is 0·75 per cent of salary.

Figures of future FSSU benefits which are expressed as a percentage of benefits under a terminal salary scheme show that most teachers entering university service for the first time would do better with FSSU; the figures are, however, based on assumptions, certain of which, the report maintains, are of questionable validity. The working party agreed, however, that, if an alternative to the present system is required, the terminal salary scheme is the best that is possible in existing conditions.

Marriage of Convenience

The announcement that the British Motor Corporation and Leyland Motors are to merge provides Britain with a motor company of international size. It is also a company with several built-in handicaps, the most serious of which may turn out to be the way in which its factories are scattered about the countryside. Although this alone is likely to be a limitation on the amount of rationalization that is possible, Sir Donald Stokes, by common agreement the dominant force in the new company, will have plenty of room left for manoeuvre. His principal limitation in the short term is likely to be a shortage of funds, and the £25 million dowry with which the Industrial Reorganization Corporation blessed the union will come in very useful indeed.

Sir George Harriman (left) and Sir Donald Stokes.

For this reason, it is fortunate that the early decisions Sir Donald, and his Chairman Sir George Harriman, will have to make will involve saving rather than spending money. It is almost certain that BMC's extensive range of cars will be reduced quite rapidly, by the disappearance of the Daimler name and the phasing out of several other models, perhaps including the 1800. It has been said that the ranges of cars marketed by the two companies are complementary, but this is little more than the traditional incantation which is always murmured when two companies merge. In this case it is simply untrue—there is direct competition both in the sports car and saloon car ranges (the Austin 1300 and the Triumph 1300, for example) and since BMC took over Daimler there is also some overlap in commercial vehicles and coaches. So there is little doubt that some models will have to go; others will have to be introduced to challenge Ford's dominance of the middle of the range. Considerable economies are possible in dealerships and in export organization, and the intervention of Leyland makes it seem more likely that BMC will at last take advantage of the economies of scale which have been within its grasp since the formation of the Nuffield organization in 1952.

In research, neither company has anything to match the £1 million research laboratory which Ford has built. In Leyland, research continues to be done by the individual companies, with Leyland itself perhaps doing the lion's share. It has, for example, built a gas turbine truck, and has pooled its knowledge with Rover, which has also done a good deal of work on gas turbine propulsion. The twin problems of pollution

How BLMH Compares (1966 Production)

	Cars	Commercial Vehicles
General Motors	5,557,000	934,000
Ford	3,380,000	765,000
Chrysler	2,118,000	211,000
Volkswagen	1,219,000	84,000
Fiat	1,148,000	66,000
BMC+Leyland	788,000	173,000

and safety have also added to the amount of research necessary, but so far the idea of a central research laboratory for the group is no more than talk. The situation in BMH is very similar, although its body-making subsidiary, Pressed Steel Fisher, did open a central laboratory some months ago. Like most engineering companies, the motor companies have been slow to catch on to the benefits of large scale research. This is not to say that no research work is done, but rather that it is done in a diffuse way, and probably on too small a scale for such an important industry. In the short term there is little chance that this can be avoided—the immediate need for the new company is for some substantial profits.

No Naked Socialists

The Industrial Expansion Bill, heralded by cries of horror from the Confederation of British Industry, was made public last week (HMSO, 2*s*.). And despite the advance publicity—Mr John Davies, Chairman of the CBI, called the bill "mischievous and absolutely childish"—it seems unlikely to allow the Government a very strong hand in private industry. The bill provides in the first place for £100 million to be made available to help private industry in schemes which would benefit the national economy but would not be undertaken without support—the sort of support the Government has already made available to the computer companies, the aircraft companies, and more recently the aluminium smelters. The total amount available could be increased to £150 million if Parliament approved. The effect of the measure will be to allow support of this sort to be given without lengthy legislation, but in each case an industrial investment scheme will be drawn up, approved by the Treasury and finally approved by the House of Commons. The measure is hardly naked socialism, nor is it a licence for what is sometimes called "back-door nationalization".

There will, for one thing, be no power of compulsion vested in the bill, for only plans agreed between the Government and the company involved will be supported. The various ministries involved in the investment schemes will be advised by a committee to be set up by the Minister of Technology, and the bill also empowers the National Research Development Corporation and the Industrial Reorganization Corporation to assist the committee and provide advice of their own. The ministers involved will have to report annually to Parliament on the workings of the scheme.

There is no doubt that the bill enables the Government a measure of discrimination—some companies will undoubtedly be supported while others will have to do without. But this is hardly more than the Government already does through its purchasing function. In the absence of a large space or defence programme, it is argued, purchasing power alone is not enough to support the industries which need support. Hence the bill, which seeks to do in a more direct way what is achieved by space and defence programmes in the United States. Whether the attempt will be successful is uncertain—the intervention of government in private industry has not always been well judged—but even the CBI would hardly claim, for example, that Britain would now have a viable computer industry without Government support at crucial moments in the past.

The bill also provides for a number of measures which had already been announced. Among them is the loan to Cunard of £24 million to complete the Queen Elizabeth II, and the increase of the borrowing power of the NRDC to £50 million. The Shipbuilding Industry Board is to be provided with an additional £15 million, and £1 million is set aside for the purchase of the Beagle Aircraft Company.

Last Word on Torrey Canyon

Ten months after the incident, yet another report on the Torrey Canyon disaster has been prepared—this time by the committee of scientists convened by the Chief Scientific Adviser to the British Government, Sir Solly Zuckerman. Readers will be forgiven for asking whether they have heard it all before. Admittedly the information is clearly presented and very detailed, but there will be many who wonder why it has taken the committee so long to report.

The object is to collect the lessons learnt during the aftermath of the Torrey Canyon incident and to examine the measures to be taken in future, but this also has a familiar ring about it (see *Nature*, **216**, 533, 1967). As outlined in the report, the incident was tackled in six parts: how to deal with the oil remaining in the ship, how to dispose of oil on the sea, how to prevent oil reaching the coastline, how to treat beaches which might be contaminated, how to reduce dangers to marine life and how to co-ordinate the efforts of other bodies dealing with the threat to wild life.

The sequence of events and counter-measures is familiar enough, but it is interesting to note that methods for reducing pollution which were considered impractical in Great Britain were in fact used in France. For example, the French successfully sank oil with powdered chalk in the deep waters of the Bay of Biscay, where there seems to have been no damage to fisheries. Despite the heavy and continuous contamination of the coast of Brittany, detergent was not used and consequently there was far less damage to the flora and fauna.

What are the lessons learnt from the disaster? At the top of the committee's list is the fact that most of the decisions taken during the crisis had a scientific or technical basis. It suggests therefore that if there is ever another disaster on a similar scale, a team of scientists should again be appointed to co-ordinate scientific advice. Another recommendation is that every effort should be made to salvage a grounded vessel, and that the cargo should be removed by pumping wherever possible. Failing that, and if environmental conditions permit, the ship should be set on fire after opening the tanks, if necessary by bombing. The committee hopes that much of the research work which needs to be undertaken could be done on an international collaborative basis, perhaps co-ordinated through the Intergovernmental Maritime Consultative Organization.

Research is considered to be necessary into means of speedy transfer of the cargo from a stranded vessel and methods of firing oil in a stranded tanker and on the sea surface. The committee also recognizes the need to develop more effective but less toxic detergents and to investigate the effects of natural factors in the movement, dispersal and destruction of oil at sea, as well as of the factors determining the stability and formation of different types of oil–water emulsion. Other enquiries, it maintains, should be directed to the disposal of oil by sinking with high density material. The possibility of developing cheap, effective and floating oil-absorbent material and methods of collecting the oil-soaked material in open seas also needs to be examined. Gelling agents are at present expensive and cheaper ones need therefore to be developed. Furthermore, suitable coastal tankers equipped to spray detergent and to ensure that it is mixed with the oil are also desirable. Cheap and effective booms, primarily for protecting harbours and inlets, are not yet available but could prove useful if improved, while mechanical methods for removing oil from beaches and rocks could be reinforced by burning and steam cleaning—the latter having been used successfully in France. Finally, the committee maintains that further research is needed into the effect of oil and detergent on fish and other marine life, sea birds and coastal vegetation.

Too Much Steel

STEEL production capacity is considerably greater than the demand for it in OECD countries. This observation is made in the recently published OECD review of the iron and steel industries in member countries (*The Iron and Steel Industry in 1966 and Trends in 1967*, OECD, 21*s*).

Overcapacity in the steel industry has been a source of increasing concern since 1958 and in Europe in 1966 production of steel was 20 million tons below capacity. This imbalance between supply and demand has had destructive consequences. To maintain large production runs manufacturers have been forced to set export prices at low levels, which has in turn affected domestic prices in many countries. The downward pressure on prices has diminished profits and the ability to maintain a level of essential investment has been seriously impaired.

WORLD PRODUCTION OF STEEL, MILLION TONS

	1965	1966	Per cent change
ECSC	86·0	85·1	−1·0
UK	27·4	24·7	−10·0
OECD—Europe	127·2	124·3	−2·3
Canada	9·1	9·1	0·0
United States	119·3	121·7	+2·0
Japan	41·2	47·8	+16·1
OECD total	296·8	302·9	+2·0
USSR	91·0	96·9	+6·5
Other countries (excluding China)	56·1	60·1	+7·1
World (excluding China)	443·9	459·9	+3·6

World production of steel continued to increase in 1966 but in Europe showed a small decline. Japan is the only large producer whose iron and steel industry experienced a significant increase in production. In the first half of 1967 output in the OECD area continued at about the same rate as that recorded for 1966, the upward trend continued in Japan, but the decrease in the United Kingdom and Germany showed signs of levelling out.

Two basic trends discernible in the international trade in steel are, first, a levelling off or even a decrease in exports to developing countries, and, second, an increase in trade between industrialized countries. Developing countries not only lack the foreign exchange to import steel but in many cases are supporting indigenous industries. The increasing volume of trade between industrialized countries is caused partly by the need to make good shortfalls in production in the countries concerned, and partly by geographical factors, price differences and such competitive conditions as quality, delivery and after-sales service.

Investment expenditure in 1966 again reached high levels in Canada, the United States and Japan, but in Europe was lower than previous years. (In the United Kingdom, at least, the impending nationalization of the industry was probably one of the factors inhibiting investment.) Investment in Europe was usually devoted to modernization and short-term improvements in productivity. The report concludes on the melancholy note that the chronic overcapacity in the steel industry seems likely to continue for many years to come. International efforts to avoid or even alleviate the effects of overcapacity have been unsuccessful. Instead, individual countries have increasingly preferred to take steps to safeguard their own iron and steel industries.

Aluminium from Coal

THE British Government's plan to sell electricity cheaply to aluminium smelters in the hope of starting a new industry in Britain seems not to be going exactly as Mr Wilson intended. Alcan, an aluminium company based in Canada, has decided that it would prefer to buy its coal cheaply and build its own power station. The Government's intention was that the smelters should share the output of a large nuclear power station with the electricity boards, after paying a capital sum equivalent to the amount of electricity the smelter would need. Alcan has lent credence to the National Coal Board's claim that it is still possible to generate electricity more cheaply with coal than with nuclear power, and has at the same time given the electricity boards the uneasy feeling that Alcan is getting better terms than they are. Neither the coal board nor Alcan is revealing the price agreed for the coal, but it will be supplied in substantial amounts—1 million tons a year when the smelter is operating fully.

The Coal Board has also had discussions with another aluminium company, Alusuisse, which announced some weeks ago that it was interested in tendering for a smelter. So far these seem only to have been tentative, and no decision has been reached. But, from the point of view of the aluminium companies, there is much to be said for building a small power station sufficient only for their own needs. This spares them the need to satisfy the stringent demands of the generating boards, and makes the process of running the station very much easier. Moreover, for a small station, coal is likely to be competitive, while in the larger sizes the arguments in favour of expensive nuclear power stations are compelling.

The argument has been further complicated, perhaps deliberately, by the suggestion that Alcan might be willing to pay back to the British Government money it borrowed during the war to build aluminium plants in Canada. The sum involved is £50 million, and half was to be repayed in 1971, the other half in 1974. Repayment of this debt in dollars would be welcome for the beleaguered British economy. The Alcan plan would also be welcomed by the mining industry, which is eager to find new outlets for its products. The Coal Board says that most of the coal for the Invergordon smelter would come from the north-east coal field, and possibly some might come from Scotland.

So far the other aluminium companies which put in tenders for smelters—Rio Tinto Zinc and British Aluminium—are standing by their original intention to use nuclear stations. Meanwhile Alcan has other fish to fry—it is also to build a smelter in Japan, on the island of Hokkaido. The smelter, which will have a capacity of 130,000 tons (more than twice the size of the smelter proposed for Invergordon), is expected to come into full production in 1972. Production will start in 1970, and Alcan says that the Japanese market is expanding more quickly than any other. Although the 20 per cent annual expansion experienced between 1958 and 1967 is unlikely to be maintained, a more modest rate of expansion—perhaps 10 per cent a year—should still make Japan the second largest consumer of aluminium by 1972, after the United States.

Causes of Death

THE Area Mortality volume of the Registrar General's Decennial Supplement gives an analysis of deaths in England and Wales for the five years 1959–63. With the total number of deaths in the period being just over 2·7 million, the annual death rate per thousand of the population was just over 12·5 for males and just under 11·3 for females. Figures known as the Standardized Mortality Ratios (SMR), which are given to aid comparisons, express the actual number of deaths as a percentage of the "expected" number. Thus for the country as a whole the SMR is 100.

Deaths from all causes and from the sixteen most widespread diseases are first analysed into ten regions and conurbations, and then into the nature of the area, whether rural or urban of varying sizes. Then follow analyses for hospital regions, London and county boroughs and finally administrative counties, which are split into urban and rural areas. Seventeen diseases which cause fewer deaths are then listed and the figures analysed into three categories only for region, nature of area and hospital region. Separate tables are given for males and females and, as well as totals for each cause of death, the figures are split into six age groups—under 1 year, 1–4, 5–14, 15–44, 45–64 and 65 and over. A separate table gives infant death rates from seven different causes, as well as the number of stillbirths and the live birth rates for the various areas. For the five year period the total number of live births was just over 4 million, indicating an excess of births over deaths of about 1·3 million for the five years.

This supplement continues the series that has been produced by the Registrar General since 1851. It also continues to show that mortality was highest in the north-western region, as was found in the 1951 supplement. In general, rural districts had the lowest death rates, but density of population did not always produce high mortality. The SMRs for Greater London were below the national average for all causes, although those for lung cancer in the area were very high.

The large number of geological, economic and social factors involved in mortality figures complicate their interpretation. Official comment on the Area Mortality supplement is therefore to be published separately.

Patients in Hospital

THERE was an increase in the number of patients discharged from British hospitals in 1964, and a decrease in the average length of stay in hospital compared with 1962 and 1963. Men and boys stayed in hospital an average length of 18·3 days in 1964; women and girls (including maternity patients) averaged just under two days less (16·7 days). Between 1962 and 1964 there was also a drop in the number of beds occupied per million population. These are some of the findings in Part 1 of the *Report on Hospital In-Patient Enquiry* for the year 1964, which has just been published on behalf of the Ministry of Health and the General Register Office (HMSO, 31*s*.).

The report contains detailed tables prepared from the 1964 ten per cent sample of discharges and deaths recorded by National Health Service hospitals in England and Wales, except those for psychiatric, pre-convalescent and convalescent patients. The hospital in-patient enquiry has two purposes which are closely related—to provide administrative data for the hospital services in terms of diseases treated and of the characteristics of the patients who had these diseases, and to provide information which can be of help in assessing the amount, distribution and effect of illness in the community and in various sections of it. Hospitals collect all the information by completing forms for one patient in every ten on discharge or death.

The tables are a mine of information. They include analyses by sex, age, region of residence and diagnosis, and by source of admission (that is, whether from a waiting list, immediate or other type of admission) and diagnosis. Many of the tables are connected with length of stay in hospital, and from this the average number of beds used daily and in relation to the total population has been calculated. These figures are shown for diagnostic group, age, sex, marital status, region of residence and hospital department (speciality) and certain combinations of these factors. Significant conclusions could no doubt be drawn from these figures and those for 1962 and 1963. A discussion of the tables is in fact to be published later in a separate commentary volume covering the years 1962 to 1964.

An innovation in the tables is the singling out of the place where accidents happened to people admitted to hospital. Injuries, poisonings and the like are all analysed in great detail according to whether they were caused by road traffic accidents, accidents in the home, or "other" mishaps. Not surprisingly, head injuries (excluding skull fractures) account for the highest percentage (39·9 per cent) of the 102,830 victims of road traffic accidents who needed hospital treatment. Fractures of different parts of the body made up most of the rest of the total; burns, however, accounted for only 0·1 per cent of road traffic injuries. Poisons caused a high proportion of accidents in the home—33·1 per cent out of the estimated total of 133,490 home accidents noted in the enquiry. Figures are given for all kinds of poisons—even strychnine and belladonna are included in the results.

Agreement Signed

A TECHNOLOGICAL agreement between the United Kingdom and the USSR which has been talked of for some time was signed last week by the technology chiefs of the two countries (see *Nature*, **216**, 1060; 1967). The Russian delegation, which spent four days in London completing negotiations with the Ministry of Technology, was led by Academician V. A. Kirillin, Deputy Chairman of the Council of Ministers and Chairman of the State Committee of the Council of Ministers of the USSR for Science and Technology. Mr Anthony Wedgwood Benn, the minister, and Mr Fred Mulley, Minister of State at the Foreign Office, signed on behalf of the British Government. The agreement provides for investigation of topics of mutual interest to the two countries at all levels, and is seen as the beginning of a "special industrial relationship". Mr Wedgwood Benn hopes that each side will "discover what is interesting, and pursue it".

The ministers, or their deputies, will meet once a year to review the working of the agreement, with reference to the progress of scientific, technological and economic co-operation. Joint working parties will determine the fields for co-operation and will recommend specific subjects for investigation in the fields of research, development, technology and organization of industry. Contacts at all levels will be encouraged as a means of turning the agreement into action. Exchange visits for studies, training and consultation will be arranged. Facilities will be provided for research and practical experience in each country and scientific and technical information will be exchanged. It is expected that licensing arrangements will be made to suit circumstances as they arise. The agreement places no limit on the joint work that can be done, as it provides for "any form of co-operation in applied science and technology as may be mutually agreed".

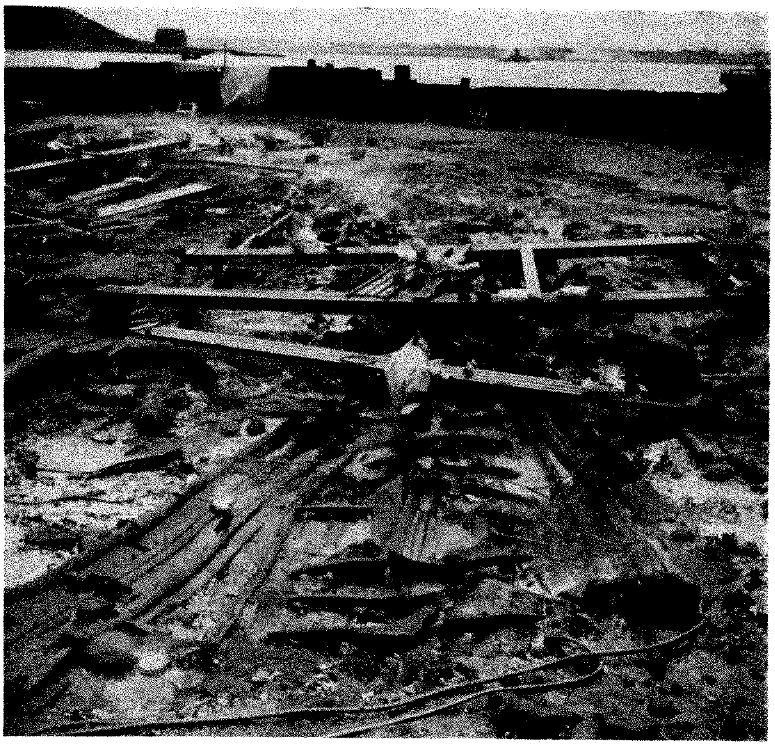
Five subjects which have been agreed, and are to be investigated by working groups, are educational technology, transport technology, materials research, information storage, processing and retrieval and non-conventional methods of food production. The ten items under the heading of industrial technology include the topics already being investigated by the Confederation of British Industry following the visit of a delegation to Russia last autumn.

Viking Ships on the Move

from Angela Croome

SIX years ago the Danish National Museum recovered the timbers of five 1,000-year-old Viking ships from Roskilde Fjord in an intensive and precisely calculated 3-month exercise which followed several years of underwater investigation. The ships, dated by carbon-14 measurements of caulking material, were known to be expendable hulks stripped down to essentials and sunk with stones across the main navigation channel to block access to the rich settlement at the head of the fjord which later became the medieval Danish capital of Roskilde. The fjord was shallow, so that a low coffer-dam was enough to enable pumping out and salvage of the timbers (see picture).

By now the first ship has completed the long and tricky "pickling" process needed to preserve its fragile timbers in air, and the work of turning these back into a ship can begin. This will take place in a specially built ship museum on the banks of the Roskilde Fjord where the first ship to complete processing is to move this week. Museum staff expect reassembly of the first ship to be completed this year when the new museum will be opening to the public. After that, visitors can watch the other ships assembled one by one. The ship being moved to the museum this week is made up of 5,000 individual pieces of wood. These have taken from 6 months to 2 years to be "rehabilitated"—the water-filled gaps must be filled with ethyl-glycol so that the wood, saturated for a thousand years in water, will not shrink when exposed to air. A cycling process in solution tanks heated to 60° C has been employed and has taken longer than originally expected. It may yet be a year or so before the remaining ships are ready for



The skeleton of one of the five tenth-century Viking ships emerges from the mud of Roskilde Fjord. When this picture was taken, in August 1962, the work of clearing the ships of mud and stones was two-thirds complete. Garden sprinklers are in use to keep the exposed wood damp.

reassembly. Each timber must conform to the original tracing in horizontal shape and size before reconstruction can begin. The third dimension can be produced by heating but not the stretching of basic length and breadth.

Each of the five ships is different. The one being moved for reassembly this week is a 40-ft. ocean-going cargo vessel such as was used by Erik the Red and his successors for the early Norse voyages to Greenland and North America—"Vinland" as the sagas called it. This is a stout, decked sailing ship of pine on an oak frame with a heightened freeboard. Another is a classical "longship" such as chieftains used on coastal forays, 90 ft. long and built of oak. Another is the coastal assault craft which is well illustrated in the Bayeux tapestry of the Norman Conquest of England (the Normans were descended from the Norsemen). The best preserved of the Roskilde Viking ships is an oak ship thought to have been used for coastal freighting in the Baltic. And there is a smaller boat which remains something of a mystery—nothing similar has been found in the records. It may have been used for net-fishing. It has neither deck nor oar-holes and is built of pine which at that period was unknown in Denmark. This suggests that it may have originated in southern Norway.

Mr Ole Crumlin-Pedersen, the naval architect who has been responsible for the finds, plans to build a full-size replica of the Atlantic craft with identical materials and a set of Viking shipwright's tools, to see how she handles at sea. He has already done this for the assault craft shown on the Bayeux tapestry and found it quite easy to load and land horses from the foreshore as shown in the tapestry.

Pattern of Research

from Our Special Correspondent

The MRC Metabolic Reactions Research Unit in the Biochemistry Department at Imperial College, London, under Professor E. B. Chain, shows a common pattern of research, as a public visit on Tuesday of this week illustrated. The pattern has four components: a belief that biochemistry done on complete organs or tissues is likely to yield results nearer to the reality of living animals than can be got from enzyme preparations; exceptional resources for making and processing analyses and counts of radioactive tracers in a multiplicity of materials; the facilities for pilot-scale fermentation on which Professor Chain insisted from the start; and the personal dominance which he still exercises. The unit has a scientifically qualified staff of 15 including attached workers and the department some 20. Interesting and potentially promising use of congenital obesity in mice is being made by Dr C. Chlouverakis. His most recent experiment shows a difference in response to insulin between diaphragm muscle and adipose tissue in these mice, and also an age effect. Muscle does not respond to insulin; adipose tissue in five month old animals does. By the age of a year the second response has disappeared and at the same time the weight of the animals levels out. Recent medical evidence on maturity-onset diabetes and obesity suggests that Dr Chlouverakis's research may be relevant to man. He plans next to look for biochemical differences associated with the tissue and age effects. Mr K. R. L. Mansford and others are working on the metabolism of complete hearts. They are interested in the partial revival of activity which follows the initial severe drop induced by oxygen deficiency. Dr H. F. Bradford, Mr K. Corbett and Dr S. P. R. Rose have gone some way towards establishing that the brain of the snail, which has cells at least ten times larger than the human brain, can be used as a simplified model from which a biochemical picture of the working of brains, including the interrelation of neurones and glia, may be in time built up. Other research, at an early stage, follows the demonstration by Dr P. Mantle in the department that agroclavin (closely related to the ergot alkaloids and in particular to LSD) has a central and specific inhibitory effect on lactation. Because agroclavin is much less toxic than LSD, study of the mechanisms by which it produces central effects, some of which appear to be shared by LSD, may give some helpful leads on the latter. This research should be worth doing in any case because of the side effects of agroclavin that may be brought to light. There is also supporting development work on automated instrumentation and data processing applied to biochemical methods of analysis and separation. Because of the opportunities that the unit and department together provide, there will be a case (in better times) for more senior posts in the department.

Applying to See Stars

The Science Research Council has set up a panel, chaired by the Astronomer Royal, to allocate time on the three large telescopes which it will eventually be supporting. These are the 74-inch reflector at Pretoria, leased from the Radcliffe Trustees for seven years from 1967, the 98-inch Isaac Newton telescope at Herstmonceux, now undergoing commissioning trials, and the projected Anglo-Australian 150-inch reflector at Siding Spring in New South Wales, half of the observing time on which will be available to United Kingdom astronomers. Observing time at the Radcliffe Observatory will be shared between the present staff, the Royal Greenwich and Cape Observatories and United Kingdom astronomers in general.

Astronomers based in the United Kingdom who wish to use the telescopes are asked to apply to the Secretary of the Panel, Dr A. Hunter, whose address is the Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, Sussex. The panel's duty is to consider applications on their scientific merits and to allocate time to individual users in accordance with SRC policy. Applications to use the Radcliffe reflector should be made one year in advance, and to use the Isaac Newton telescope six months in advance.

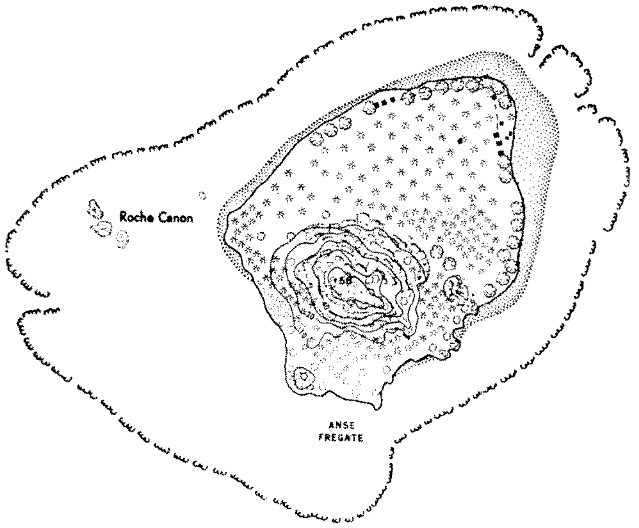
The panel itself disposes only of time, not of money; applications for grant support should therefore be made in parallel to the Science Research Council.

Cousin, not Cousine

Cousin Island is a small island of 67 acres (not to be confused with a neighbouring island, Cousine) in the Seychelles, about 800 miles north-east of Aldabra Island, which is shortly to be bought and turned into a nature reserve. That, at least, is the hope. For seven years the International Council for Bird Preservation has been bargaining to buy the island and now a firm offer for the island has been accepted. With the help of the British national appeal of the World Wildlife Fund, it is hoped that at least £50,000 will be raised to cover the purchase, to maintain the island as a nature reserve and set up a permanent research study centre.

The Seychelles are important because of the large numbers of endemic land birds they support; the fact that the Seychelles are an archipelago has probably had something to do with this. In the early eighteenth century, when the islands were first colonized, there were about a dozen endemic species on most of them. As the human settlements became larger and the islands more cultivated, the birds became rarer. Last century, at least three and possibly four of the species became extinct, and today the remaining endemic species are to be found on only one or two of the islands. The felling of trees and the introduction of rodents and cats by man are thought to have led to the disappearance and restriction in range of these birds.

Cousin Island is still fairly well stocked with endemics and from all accounts it would be ideal as a sanctuary and as a centre for studying the whole archipelago. Unfortunately, pigs are being allowed to run wild in the scrub of the north-eastern plateau with disastrous results for the bird population. It is becoming more urgent than ever, therefore, to buy the island for conservation, so that control can be kept over the vegetation and the introduced animals.



Cousin Island (Crown copyright).

It seems to be agreed that Cousin is particularly important because it is the only remaining home of the brush warbler (*Nesillas* (*Bebrornis*) *sechellanis*), of which only 50 are left. The bird breeds in dense cover, especially over the marshy area in the south-west of the island. The turtle dove, *Streptopelia picturata rostrata*, is also unique to Cousin. This endemic subspecies has been swamped in the other islands by interbreeding with an introduced form, *S.p. picturata*, from Madagascar. On Cousin, however, the endemic characters are retained to a great extent. The island is also one of the three remaining places where the Seychelles fody or toq toq (*Foudia sechellarum*) is found, and it also the only breeding place in the Indian Ocean of the rare lesser noddy (*Anous t. tenuirostris*), which otherwise breeds only on a small group off Western Australia. Apart from these rare birds, there are large colonies of the fairy tern (*Anous alba monte*), shearwaters and nesting tropicbirds (*Phaethon l. lepturus*).

There are also some giant tortoises introduced to the island by a previous owner. These are a different shape from those on Aldabra. Unfortunately the young were removed a long time ago as pets so that today only six adults remain. Apparently each tortoise has to be bought individually along with the island: it is anyone's guess how much each will fetch.

New Units for *Nature*

In accordance with the advice of the Royal Society Conference of Editors that scientific journals should adopt the SI system of units (*Nature*, **216**, 1272; 1967), *Nature* will be using the recommended symbols. This means that some of our abbreviations will be changed, as shown in the table. This will apply to all communications now accepted for publication.

Symbols previously used in *Nature*	Symbols to be adopted
sec	s
amp	A
candela	cd
steradian	sr
coulomb	C
farad	F
lumen	lm
lux	lx
cycles/sec	Hz
parsec	pc
c. (curie)	Ci

In the article referred to above, giga was shown as 10^6 and not 10^9. One atmosphere should be $1{\cdot}01325$ N m^{-2}.

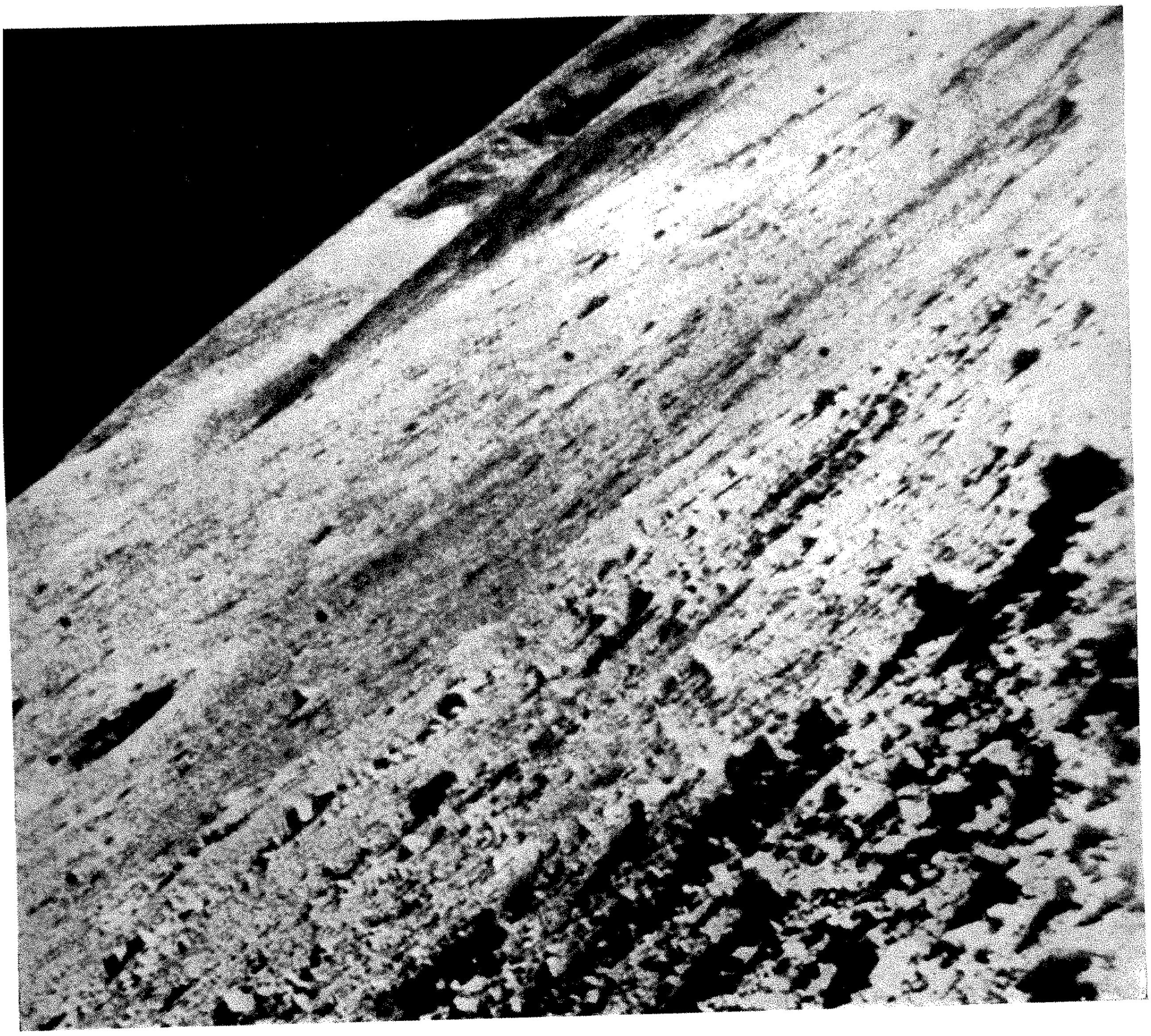
Parliament in Britain

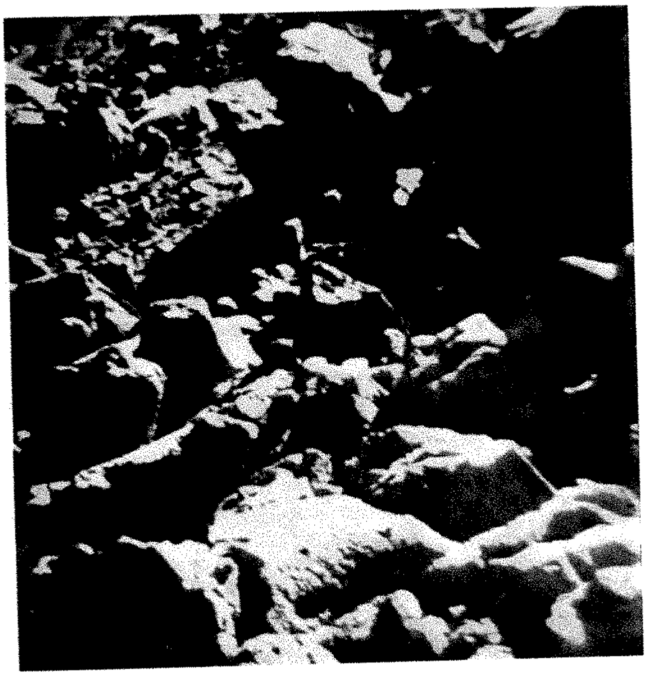
Forestry

Mr Hay, joint Parliamentary Secretary at the Ministry of Agriculture, Fisheries and Food, gave the acres planted by the Forestry Commission in England, Scotland and Wales in each of the ten years 1958–1967. In 1967, of the 52,500 acres planted, 11,200 were in England, 34,500 in Scotland and 6,800 in Wales. Now planting in Scotland was planned to increase to 50,000 acres a year by 1976. The planting programmes for England and Wales were under review. (Written answer, January 17.)

School Leavers

The Secretary of State for Education and Science, Mr P. Gordon Walker, stated that of school leavers in 1965–66, 5·5 per cent went into universities, 3·1 per cent to colleges of education, 1·1 per cent to full-time (and sandwich) degree and HND/HNC courses in further education establishments and 8·1 per cent to other full-time and sandwich courses at further education establishments, most of the latter courses being non-advanced. Between 50 and 55 per cent of boys and girls were expected to remain at school voluntarily in 1969–70 for a full year beyond the minimum statutory school-leaving age. (Written answer, January 17.)





More Moon Pictures

Pictures taken by the last of the United States Surveyor series of space flights have now been published. The spacecraft landed successfully 30 kilometres north of the crater Tycho, and the picture above shows a wide angle view of a cluster of rocks near the craft's landing site. The horizon to the north-east of the vehicle is formed by a ridge characteristic of the topography of the region, which is in the Lunar Highlands. The picture on the left shows rocky fragments in the highlands, and the centre of the picture is about 5 metres from the craft's television cameras.

NEWS AND VIEWS

No Smoking Licence

THERE may after all be some justification for the claim sometimes made by smokers that indulgence in this habit can provide a feeling of wellbeing and increase efficiency. On page 331 Drs Armitage, Hall and Morrison discuss the pharmacological basis for the tobacco smoking habit, and show that, in rats and cats at least, the nicotine in tobacco smoke can have these effects. Small injections of nicotine, in roughly the same dose as the amount taken in by a smoker smoking two cigarettes and inhaling deeply, made thirsty rats, trained to press a lever to obtain water, press the lever more frequently. The effects on the electroencephalogram showed that the activity of the cortex of the cat's brain was increased by a similar dose of nicotine. At the same time there was also an increase in the amount of acetylcholine produced by the cortex. These are both effects which characterize the normal physiological response of arousal; when animals, including people, rouse themselves from a state of decreasing consciousness, cortical activity increases and so does the output of acetylcholine from the brain. As Dr Armitage and his colleagues point out, it looks as though nicotine acts on the central nervous system, at least in small doses, by supplementing this normal arousal response and not by some effect peculiar to itself.

Larger doses of nicotine do not necessarily have the same effects and it would be tempting to connect the seemingly pleasant effects of small doses of nicotine with the finding reported by Professor A. H. Beckett (*Nature*, **216**, 587; 1967) that habitual smokers excrete less nicotine in their urine than non-smokers given the same dose of the drug; the smokers were metabolizing the nicotine to a greater extent than the non-smokers. It is of course impossible to say whether this has any connexion with the fact that it is small doses of nicotine which seem to give smokers their pleasure. There is no evidence that, like humans, cats or rats increase their capacity to metabolize nicotine after prolonged intake of the drug.

It would also be careless to say that these latest data indicate that smoking is at all a good thing. Nicotine is toxic in large doses; chronic poisoning, which usually arises from continued excessive smoking, can cause irritation of the respiratory tract, digestive and nutritional disturbances, narrowing of the blood vessels and increase in blood pressure. The carcinogenic effects of cigarette smoking are, of course, well documented by now. So, too, are the other ill effects of the habit of smoking cigarettes and even the other devices in common use for the production of tobacco smoke. The incidence of various heart ailments, for example, is known to be increased by smoking. Whatever the beneficial associations of nicotine, there is no question that it is best left alone. If that, however, is agreed, there may be great interest in what seems to be the rudiments of an explanation of why nicotine has such a hold on people.

Atomic Oxygen High Up

from our Upper Atmosphere Correspondent

MOST information on the neutral atmosphere of the Earth at heights above 150 km has been obtained from the analysis of the rate of change of the orbital period of satellites. This technique can only provide values of air density, however, and other atmospheric properties have to be inferred by fitting a theoretical model to the density data. Current atmospheric models are constructed by assuming a fixed composition at a height of 120 km, which is taken as the lower boundary, and then adjusting the temperature profile to make the model densities agree with observational values. The densities derived from satellite drag analysis can be reproduced by a wide range of boundary conditions and temperature profiles; it is for this reason that reliable composition measurements are required at heights near 120 km and that a recent review of mass spectrometer measurements of atomic oxygen in the upper atmosphere by von Zahn (*J. Geophys. Res.*, **72**, 5933; 1967) is of interest.

In current models of the upper atmosphere, the ratio of atomic to molecular oxygen at the lower boundary is assumed to be close to unity, which is near the mean of all mass spectrometer measurements. Von Zahn argues that measured argon concentrations imply a turbopause height of about 105 km and an eddy diffusion coefficient of 1×10^6 cm^2/sec; these values suggest that the atomic to molecular oxygen ratio is about 4 at 120 km. The measurement of a non-reactive species such as argon is much simpler than the measurement of atomic oxygen and more likely to be correct. It has been known for some time (*J. Geophys. Res.*, **71**, 4121; 1967) that there is severe loss and not merely recombination of atomic oxygen in "closed" ion source gauges. The use of "open" ion sources reduces the effect, but does not eliminate it. An indication that atomic oxygen measurements are too low can be inferred from the appearance during flight of a mass 44 peak, which follows closely the mass 16 peak. On the basis of laboratory calibration, only a negligible mass 44 peak would be expected and von Zahn concludes that it is produced in flight by atomic oxygen and can possibly be identified as N_2O or CO_2.

The existence of loss processes seems well established and, until they are better understood, measurements of atomic oxygen by rocket-borne mass spectrometers should be regarded as a lower limit. Densities measured by instruments aboard the aeronomy satellite Explorer 17 have been found to be systematically lower than those derived from drag studies. Since the same loss processes can also affect pressure gauges, this latest information reinforces the arguments already put forward (*Smithsonian Astrophys Obs Spec Rpt.*, 217, 1966, *Phil Trans Roy Soc*, A**262**, 172, 1967) to explain this discrepancy in terms of errors in gauge calibration

Ends in Common

from our Cell Biology Correspondent

THE base sequence of the last eleven nucleotides at the 3′-OH termini of the RNA from the closely related bacteriophages *f*2 and *MS*2 is GpUpUpApCpCpApCp-CpCpA Weith and Gilham first reported this sequence for *f*2 RNA last October in the *Journal of the American Chemical Society* (**89** **21**, 5473, 1967), and deWachter and Fiers have now reported the same sequence for *MS*2 RNA (*J Mol Biol*, **30**, 507, 1967) The two groups worked independently, and used quite different methods DeWachter and Fiers used a fairly orthodox approach, whereas Weith and Gilham adapted the periodate oxidation and base catalysed β elimination technique of Neu and Heppel (1964), and used columns of amino-ethylcellulose to absorb the 3′ terminal fragment (Lee and Gilham, 1966)

The outstanding feature of these 3′ terminal sequences is the terminal triplet CpCpA It is well known that all transfer RNAs end with this triplet, and so does tobacco mosaic virus RNA (Steinschneider and Frankel-Conrat, 1966) The 3′ terminal triplet of ribosomal 5*S* RNA from *E coli*, however, is CpApU (Brownlee, Sanger and Barrell, *Nature*, **215**, 735, 1967) and according to Lee and Gilham (1966) the 3′ termini of the majority of the ribosomal RNA from wheat germ ribosomes are GpU, GpG, ApA or ApC It is hard to believe that it is merely a coincidence that all the *t*RNAs and three viral RNAs that act as messengers terminate in CpCpA, and if it is not a coincidence, then the CpCpA triplet must serve some function DeWachter and Fiers speculate about this

They suggest the CpCpA triplet may be a device to protect the phage RNA chains from degradation by exonucleases in the cell, perhaps in the cell the CpCpA triplet acts in the same way as it does in transfer RNA and receives an amino acyl group on its 3′ OH end But it is clear from the work of deWachter and Fiers and that of Sugiyama (1965) that the phage RNA extracted from intact particles does not have an amino acyl group attached If such a group exists, it must be cleaved from the RNA before it is encapsulated It would be of great interest to know whether the CpCpA triplet of *f*2 and *MS*2 RNA is subject to metabolic turnover as is the CpCpA of transfer RNA and whether or not *m*RNAs other than phage genomes terminate in CpCpA DeWachter and Fiers further suggest that, if the CpCpA is not involved in protection from enzyme degradation, it may be part of a signal to DNA-dependent RNA polymerase to stop transcription, in which case viral RNA could be an escaped *m*RNA which has evolved into a bacteriophage but retained this signal

The other outstanding feature of the terminal sequence is the absence of an ochre, amber or UGA chain terminating codon If, as genetic evidence suggests, UAA (ochre) is the normal chain terminating codon, it is perhaps surprising that this codon does not occur at all close to the 3′ terminus In *f*2 and *MS*2 the ochre codon must be at least eleven bases in from the 3′ terminus, and quite clearly the terminal sequence cannot be involved in polypeptide synthesis

Inherited Neurological Disease

from a Biochemistry Correspondent

BIOCHEMICAL pathways in bacteria have been established by the study of mutant organisms in which a particular enzyme is missing In the same way, it is often possible to learn much about normal human metabolism from investigations of human beings who lack some of the active enzymes present in the normal human One of these "inborn errors of metabolism" causes a neurological disease known as heredopathia atactica polyneuritiformis or Refsum's disease

In 1963, more than twenty years after Refsum first described this inherited condition, Klenk and Kahlke showed that the chief biochemical abnormality in these patients was the presence of comparatively large quantities of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) in the blood The phytanic acid accumulates as a result of the partial breakdown of phytols from vegetable substances in the diet A diet with no vegetables, fruit, butter or meat-fat reduced the serum phytanic acid in two patients who had both been ill for 18 years, but only after they had been on the restricted diet for six months (L Eldjarn, K Try, O Stokke, A W Munthe-Kaas, S Refsum, D Steinberg, J Avigan and C Mize, *Lancet*, i, 691, 1966) The neurological symptoms of one patient seemed to improve as the phytanic acid level fell, but the other patient was too psychotic for neurological changes to be assessed

Animal metabolism normally breaks down phytanic acid by first oxidizing the carboxyl group to form pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) which is then broken down completely by the normal β-oxidative pathway for the degradation of fatty acids The initial oxidation is known as an α-oxidation, and the most recent observations on Refsum's disease suggest that the enzyme for this reaction is present in normal humans but is missing in patients with the disease Steinberg and his colleagues (*Science*, **156**, 1740, 1967) showed that there was practically no production of labelled carbon dioxide when uniformly labelled phytanic acid was given to two patients, while normal human volunteers readily produced labelled carbon dioxide from phytanic acid Tissue cultures of the patients' skin produced labelled carbon dioxide from pristanic acid as readily as tissue culture preparations from normal people, but did not oxidize phytanic acid to form labelled carbon dioxide The authors suggest that since α-oxidation of fatty acids has only been identified in nervous tissues in animals, deficiency of this enzyme may adversely affect nerve metabolism and the phytanic acid accumulation is a secondary effect which does not cause the symptoms

Because Karlsson, Norrby and Samuelsson (*Biochim Biophys Acta*, **144**, 162, 1967) have now described a rapid and reliable method of identification of phytanic acid in the serum triglycerides by thin-layer chromatography, this test should be given to the blood of all patients with neurological symptoms whose origin is in doubt If restrictions on the vegetable intake of these patients do not reduce their symptoms, this would suggest that α-oxidation must be of great importance in nervous tissue, and deserves further investigation

Staphylococcal Responses to Hormones

from our Microbiology Correspondent

An aspect of animal pathology which appears to deserve more intensive investigation is the effect of endocrine secretions on microbial pathogens It has been suggested that hormonal action may be important in the regulation of toxin and antigen production by staphylococci, and evidence exists that reactivation of latent infections can be affected by hormones How do microbial infections interfere with the normal functioning of the endocrine system? What effects, both primary and secondary, do such hormones have on the course of a microbial infection? Answers to some of these questions have been provided recently by Dr William Yotis, who has examined the *in vivo* and *in vitro* response of certain bacteria to synthetic progestins and oestrogens (*J Bact*, **94**, 1353, 1967) The progestational contraceptive norethindrone was studied most fully in this context, but with the ever increasing use of this form of contraception the results are of wider relevance

The growth of *Staphylococcus aureus* was inhibited significantly *in vitro* when cultured anaerobically in the presence of 10–50 μg norethindrone per millilitre The hormone caused a protraction of the lag phase and reduced the maximum population obtained, these effects were severely lessened in the presence of oxygen In a developing furuncle, however, anaerobic conditions will arise rapidly and the maximal bacteriostatic effect of norethindrone, therefore, might be anticipated *in vivo* The synthetic oestrogen mestranol exerted an insignificant effect on the staphylococci, but its combined action with norethindrone was synergistic Although this potentiated activity was not realized with other androgens and oestrogens such as testosterone 17α-oestradiol and 17β-oestradiol, progesterone showed an additive bacteriostatic effect in the presence of norethindrone In contrast to its effect on Gram positive cocci, norethindrone did not inhibit the growth of the Gram negative enterobacteria screened This selective action may be due to differential permeability phenomena, or to transformation of the hormone Bacteria are well known to transform steroids, and the inability of norethindrone to inhibit staphylococci after about 8 h of incubation may be related to such a detoxication mechanism

The *in vivo* activity of norethindrone was tested by intradermal challenge of adult female rabbits with *S aureus* Prior treatment of the rabbits with the progestin produced a marked retardation of lesion development, but once a staphylococcal infection had become established norethindrone was without therapeutic action The prophylactic action, on the other hand, was effective for as long as 6 weeks after infection Precise definition of the bacteriostatic effect of norethindrone and other hormones *in vivo* cannot yet be made, but certainly a complex interaction of factors is involved Considerable evidence now exists linking the suppression of sebaceous gland activity by hormone administration with an improvement in acne vulgaris, while stimulation of connective tissue development is another likely factor in limiting or suppressing furunculosis Further analyses of the norethindrone effect could be important to our understanding of the course of bacterial infections in animals while also providing a useful system for assessing the microbiological properties of oral contraceptives

Classifying Algae

from a Correspondent

An International Symposium, "Algae, Man and the Environment", sponsored by Syracuse University and the New York State Science and Technology Foundation, was held at the Sagamore Conference Center and main campus of Syracuse University, New York, in June last year An international group of biologists who study freshwater algae and engineers who study waste disposal and pollution were able to discuss each other's research and to consider the problems of controlling the growth of algae in a variety of situations

One of the informal discussions was devoted to the classification of blue-green algae and the designation and preservation of algal cultures A meeting under Dr R A Opperman decided that though, like bacteria, blue-green algae are prokaryotic, their classification should continue to conform to the International Rules of Botanical Nomenclature This means that names should be accompanied by formal descriptions, with good illustrations of normal form and range, and that reference material should be preserved in herbaria As additional information becomes available about biochemistry, physiology, fine structure, virus susceptibility or the composition of the DNA, this should be incorporated into the description, and used to improve the classification

The problem of borderline organisms, more or less intermediate between blue-green algae and ordinary bacteria, is unresolved, and further detailed study is needed For practical reasons it seems simplest and best (at least for the present) to consider those organisms which most closely resemble blue-green algae in form—if not in pigmentation—as apochlorotic algae, and to consider flexibacteria, along with myxobacteria and the like, as organisms whose classification should conform to the International Code of Nomenclature of Bacteria

Cultures of algae provide valuable information about the potential range of form which clones may exhibit, and physiological, biochemical and cytological characteristics are best obtained from unialgal or axenic cultures It was therefore decided that critical experiments should be carried out on designated clones The date and source of isolation of strains or clones, accompanied by illustrations, should be included in the first full length publications which report their use

Numerical designation of strains and clones has hitherto been unsystematic and confusing The meeting suggested a unified system which would necessitate a single world register of algal cultures, and is to prepare formal proposals for publication and, it is hoped, adoption at a forthcoming congress

Many clones of algae are maintained by serial transfer in public and private culture collections, where spontaneous mutations may arise and accumulate as sources of variation Cells which have been frozen and maintained at the temperature of liquid nitrogen are now believed to be stable, more or less indefinitely, so that viable cultures preserved in this way are preferable in the interests of reproducibility The meeting recommended that reliable methods for the cryogenic preservation of algae be developed and adopted as soon as they are available

New Ways in the Infrared

from our Molecular Biology Correspondent

THE study of protein conformations in solution has in recent years become harder Except perhaps for proteins containing a high proportion of α-helix, interpretation of optical rotatory dispersion and circular dichroism has become increasingly complicated, chiefly because of the discovery that various side chains can contribute sizable Cotton effects of their own This uncertainty has made the development of other methods of observing polypeptide chain conformation and its changes increasingly desirable The recent resurgence of infrared spectroscopy of aqueous solutions is therefore to be welcomed and, as will be seen, it has already produced some promising results

The new work is by Susi, Timasheff and Stevens (*J Biol Chem* , **242**, 5460, 1967), who have looked at the infrared spectra, primarily in D_2O solution, of several synthetic polypeptides and proteins It is already well known that the so-called amide I frequency of the polypeptide chain is conformation-sensitive, and that the formation of a pleated-sheet (β) structure causes a considerable shift to lower frequency A less familiar observation, which was recently reported by the same group of workers, is the distinct, though smaller, shift which occurs when the chain enters the α-helical conformation Thus, it is shown that poly-L-lysine in D_2O solution can exhibit an amide I band at 1,635, 1,645 or 1,611 cm^{-1}, depending on whether it is in the random, the α-helical, or the antiparallel pleated-sheet state In the latter form there is also a characteristic weak component, due to another vibrational mode, at 1,680 cm^{-1} (In H_2O solution there are corresponding, though displaced, frequencies, but experimental difficulties generated by the high absorption of the solvent in this range are prohibitive) These band positions may vary somewhat from molecule to molecule, and will depend, for example, on the strength of hydrogen bonding In proteins then a certain spread of frequencies is found for each conformation, but this does not appear to be sufficient to cause any serious ambiguity

In a second article Timasheff *et al* (*J Biol Chem* , **242**, 5467, 1967) survey a large number of proteins In the majority of cases the peak of the amide I band lies either at about 1,650 or near 1,637 cm^{-1} The first of these groups comprises those proteins known to have a substantial proportion of α-helical structure—as myoglobin and lysozyme, for example The second consists of proteins known or thought to contain little α-helical, or other regular, structure In almost all cases, however, prominent shoulders are observed which reveal the amide I band to be complex Thus in lysozyme, the inflexion at 1,632 cm^{-1} must reflect the β-structure known to be present, and in many cases shoulders at 1,685 cm^{-1} also indicate the presence of antiparallel β-forms Indeed, in all cases for which crystallographic data are available, the conformation is qualitatively matched by the distribution of amide I frequencies In a few instances partial resolution of components of the band maximum is observed

Some striking effects engendered by denaturation are shown Insulin, for example, has been converted into three different denatured states in methanol solution, the position of the band-peak is unchanged, thus showing that the α-helical structure persists, in alkaline solution it shifts to a position characteristic of a random coil, and on heating at low *p*H antiparallel β-sheets appear These effects are paralleled by changes in the ultraviolet Cotton effects Again, β-lactoglobulin in the native state evidently contains a large proportion of β-structure, addition of methanol leads to denaturation with progressive formation of α-helix, though a different β-structure, associated with aggregation, also appears. Again the transition follows optical rotatory dispersion changes The utility of this approach is obvious it will assuredly find wider use

Algae on Film

by our own Correspondent

"MICRO-ALGAE—a Study of Movement and Multiplication" is the title of a film shown to the Linnean Society at a meeting on January 18 The film, which is intended primarily as a teaching aid, was made by Dr P E Brandham of the Royal Botanic Gardens, Kew

The film is silent, with explanatory captions, and in the twenty-five minutes for which it runs shows some very active fresh water algae Dr Brandham minimized vibration, usually a big problem in cine-microphotography, by standing the microscope on a rubber pad and a sprung base plate For the slower movers and for showing cell division, time lapse photography was used, with apparent accelerations of from 30 to 3,840 times

First, flagellar action is shown in a dinoflagellate, *Ceratium*, and in two euglenoid and three volvocacean genera Dark field microscopy shows the flagella particularly clearly, and in the water around *Volvox* small particles can be seen moving as a result of flagellar action *Phacus* is seen moving by its characteristic rolling motion, and looking like a sting ray doing contortions *Euglena* gives a perfect display of euglenoid movement

Time lapse photography makes it possible to see blue-green algae, including *Anabaena*, gliding around, and, among the Chlorophyta, the very slow movement which brings filaments of *Spirogyra* together for conjugation can be seen Diatoms are shown shunting to and fro—the ease with which they can change direction is clearly seen The desmid *Closterium acerosum* moves by a form of jet propulsion, by secreting mucilage from one end of its thin body, like toothpaste from a tube The mucilage hardens and *Closterium* is pushed forward There are also some fine time lapse pictures of cell division in several genera, including colonial algae and desmids

Crystalline Ribosomes

from our Cell Biology Correspondent

THE spatial organization of RNA and proteins in ribosomes will remain unknown until somebody manages to produce crystalline ribosomes which can be analysed by X-ray crystallography. Techniques other than crystallography, such as electron microscopy, dye binding and spectroscopy, can of course be made to yield limited information about the structure of ribosomes. Cotter, McPhie and Gratzer (*Nature*, **216**, 864, 1967), for example, recently proposed a plausible model of ribosome structure based on spectroscopic studies. Their data indicate that the outer surface of yeast ribosomes is predominantly RNA and from this they suggested what might be called a hedgehog model of ribosome structure, with the RNA looping out on to the surface of the particle and with the protein in the centre. But, because the experimental data are so limited, such models can never be more than sensible speculation.

What are the possible sources of crystalline ribosomes? The discovery in 1966 by Byers, working at Harvard University, that hypothermia *in vivo* induces the formation of crystalline sheets of ribosomes in chick embryo cells, caused great excitement because it raised the possibility that enough crystalline ribosomes could be isolated from these cells to allow crystallographic analysis. Alternatively these ribosomes might be crystallized *in vitro*. Unfortunately both these approaches have so far failed. The ribosome crystals which Byers has isolated from cells are too small for crystallographic work, and his attempts to produce large ribosome crystals *in vitro* have not yet been successful.

Crystalline ribosomes—in this case helices of ribosomes—also occur in the cysts of the amoebae *Entamoeba invadens*. Originally these helical structures were called chromatoid bodies, but since Barker's work in 1963 it has been suspected that they are crystalline ribosomes. In the current *Journal of Cell Biology* (**36**, 45, 1968), Morgan, Slayter and Weller report experiments which firmly establish this, but it is disappointing that they have failed to isolate intact ribosome crystals. Morgan *et al.* find that the ribosomes extracted from cysts of *Entamoeba* under various ionic conditions are dimers, monomers, or 50 *S* and 30 *S* sub-units, but never crystals. They calculate from the yield of monomer ribosomes that the chromatoid bodies must be dissociated into monomer ribosomes during the extraction. Ribosome crystals can only be extracted after fixation of the cysts, and after fixation of this sort the crystals cannot be dissociated into monomer ribosomes. The fixation must irreversibly cross link and denature the ribosomes. Thus although *Entamoeba* and chick embryonic cell ribosomes crystallize *in vivo*, neither material looks like providing a source of crystalline ribosomes for X-ray crystallography.

EMBO's Plan for a Laboratory

This week at Geneva there has been a meeting of the council of the European Molecular Biology Organization (EMBO) and representatives of several European governments to discuss the long-term support of EMBO's activities. The most controversial part of these proposals is that there should be a European laboratory for molecular biology; some countries—France, for example—have come out openly in favour of the laboratory, but others are known to be only lukewarm towards it. The following is an extract from the documents prepared by the EMBO council in support of the case for a laboratory.

MOLECULAR biology is a science rich with promise for the future, with applications to medicine, public health, agriculture and industry. Europe, in its own interests, should play its full part in the development of this field. The idea that we Europeans might rely on advances at a fundamental level made elsewhere and limit our own efforts to application of these is unrealistic and would be a false economy. Effective and competitive applications presuppose a wide base of fundamental knowledge and understanding. In this interdisciplinary field the development of local laboratories and centres in the individual countries is not enough. The establishment of a central laboratory in Europe, of a size and scope beyond the present human resources of any single country, would have an immense effect, not only because of its direct contributions to basic knowledge, but also because of its influence on the local laboratories. It would place European molecular biology in a unique position relative to any other country.

A central laboratory would provide a number of services to the European biological community at large, such as pioneering in the field of high powered instrumentation, the production on a large scale of rare materials such as enzymes and organelles, the maintenance of pure strains of experimental organisms and the sponsoring of special courses and conferences. In this respect, a single laboratory would in the long run be a source of economy by eliminating useless duplication (since it would avoid doing the same thing over and over in different places). Also in the balance it would be a source of far more trained biologists than it would absorb.

We propose a laboratory with an eventual staff of about 150 scientists and engineers (of whom about 20 would be of senior grade and have tenure), 60 visiting scientists, 40 postdoctoral fellows, and 310 technicians and other non-scientific staff. It is estimated that such a laboratory would cost between 8 and 9 million dollars a year to run.

The council's argument starts from the view that the promise of molecular biology is far from exhausted with the working out of the genetic code and the determination of the structures of four enzymes. On the contrary, "this approach to the study of living organisms is still in its infancy".

First of all, many of the apparently simple processes taking place in living cells are in fact of great complexity and are understood only in the barest outline, or not at all, at the molecular level. Thus we know the three-dimensional structure of a few protein molecules, for example, haemoglobin, but not how it expresses itself in the complex functioning of the molecule; we know in great detail the double-stranded structure of DNA, but still very little of the forces involved in the dynamics of its replication. The genetic code is indeed understood, as is the general scheme for its expression in the synthesis of proteins, but the actual mechanics of the assembly of the proteins being coded for is largely uncharted territory, taking place as it does through the intermediary of the ribosome, a cellular particle of very great complexity probably consisting of at least 20 different kinds of protein molecule as well as of nucleic acid molecules of unknown function; about the structure of this complex assembly, let alone its mode of functioning, we know virtually nothing. At present our knowledge of these processes is for the most part purely descriptive and phenomenological; it is easy to draw diagrams with molecules and intracellular particles represented formally by shapes like cottage loaves or twisted hairpins, and with processes shown as ingenious flowsheets full of arrows and feed-back loops, but it is quite another thing to relate these models to an actual physical process or a molecular structure.

When it comes to more complicated systems we are even more in the dark. As human beings our primary interest lies in the functioning of complex organisms like ourselves and the plants and animals on which we depend—that is to say, of organisms characterized by highly differentiated tissues and by the possession of sophisticated inter-cellular organization. Systems of this kind, although they transcend the cellular level of complexity, will demand for their understanding the same kind of thinking and the same experimental approach which has been so successful at the lower level. After all, living beings are all constructed of the same basic ingredients, and the same principles are at work in the behaviour of the most complex as those governing the functioning of the simplest.

As Europeans we believe that Europe, in its own interests, has a particular responsibility to take part in this activity on a regional basis. Many of the key discoveries which triggered these developments were made in European laboratories. Today, the initiative has to a substantial extent passed to America, where the very large resources available for scientific research have been liberally and wisely applied in this field. The intellectual capital of Europe is not being exploited to the full on home ground. EMBO has made a number of proposals to remedy this unsatisfactory situation, some of which (for fellowships, travel grants, summer courses, etc.) have already been implemented on a small scale, and a more extended programme for future activities of this kind has been accepted in principle by the European governments represented at the European Conference on Molecular Biology held in Geneva in April 1967. In our opinion the most important remaining part of our proposals, without which the rest will not come to full fruition, is the establishment of a European laboratory of molecular biology.

Why a Laboratory?

There are good reasons for establishing large laboratories in fundamental biology, both in the interests of good research and in the interests of good training in the methods of research. In this field everyone is a specialist in some particular area or technique; the real advances come from cross-fertilization among specialists, from understanding other people's ideas and techniques, and from day-to-day exchange of ideas—in short, from the interaction of differing attitudes and skills—and all of these are best achieved by propinquity. As to training in research, it may well be argued that at the predoctoral stage the scientist should often concentrate rather narrowly on acquiring a particular set of skills, but after the PhD, when he should be forming his own ideas and developing an independent research programme, the young post-doctoral fellow needs to be in a large laboratory where work is going on in many aspects of the field.

The argument for large institutes of fundamental biology is not the same as that in the case of high-energy physics, where the need (now satisfied by CERN) was for a very powerful and expensive machine which could only be provided in a central institute; in biology the essential "plant" is, rather, an interacting group of differing techniques and talents, each only moderately expensive by itself, but in sum costing so much as to be beyond most national resources, financial or—especially—human.

The EMBO laboratory which we propose on the basis of this philosophy will have the following features:

1. It will provide a centre for the vigorous prosecution of basic research of the very highest quality within the framework of an integrated programme.

2. The senior staff of the laboratory, all of the highest calibre, will consist of a small group of permanent full-time scientists, together with a number of others who agree as a matter of principle to spend a part of each year there over a period of at least several years. The bringing together, even for limited periods, of investigators from several countries will create a real European community of scientists, to the benefit of each individual country. It will make the EMBO laboratory the effective headquarters of a concerted research programme.

3. The laboratory will perform valuable functions in connexion with the recruitment, the training and the employment of scientists and technicians.

There is another role which the EMBO laboratory will play, less fundamental perhaps, but of great practical and indeed financial significance. Because of its size and resources, it will be in a unique position to offer to other laboratories a set of service facilities which at present are for the most part not available at all, or which, if they do exist, are dispersed over a large number of centres, with resulting loss of accessibility and efficiency.

The Scientific Programme

Though it is in the very nature of scientific progress that the precise directions cannot be forecast, we believe that there will be two main areas of attention; on the one hand the following up of what we know today at the simpler level of the macromolecule and the sub-cellular particle, and the extension of the analysis to the integration of the whole cell; on the other hand, a first application of molecular concepts to the problem of the relationship and co-ordination of cells in higher organisms—it goes without saying that this is a subject of far greater complexity and of extreme technical difficulty.

The concept of information lies at the very roots of any understanding of living organisms and indeed of human behaviour, and forms the core of the fundamental problems which we propose as the basis for the scientific programme of the laboratory. However, the whole complex of issues which arises greatly exceeds the capacity of any single institution and it is necessary to be selective. We accordingly propose for the present to limit the programme of the EMBO laboratory to five main assigned themes.

Structure of biological macromolecules. It is of prime importance to explain the functional behaviour of the giant molecules of biology, at present understood only at a phenomenological level, in terms of their three-dimensional structures. Why is it that in different haem proteins the haem behaves so differently depending on its environment? What is the nature of the conformational effects that explain the way in which activators and inhibitors exert their influence on an enzyme so that it may either be brought into play or prevented from going too far? As a result of recent developments in X-ray crystallography we now know for the first time the three-dimensional structure of several respiratory pigments and enzymes at a resolution of about 2 Ångstrom units, and there is every prospect that such knowledge will be extended to many other proteins, nucleic acids, and so on in the course of the next few years. Furthermore, recent dramatic improvements in methods of synthesis of proteins and nucleic acids make it almost certain that we can soon produce long sequences to order

The molecular basis of the immune response The capacity of an animal to develop an antibody in response to a foreign protein, possibly resulting from the presence of a pathogenic organism, obviously represents one of the most important evolutionary adaptations The picture of what is involved here is just beginning to emerge in its main outlines—the way in which certain serum globulins of characteristic size and shape, which have the property of combining specifically with the foreign molecule, are produced selectively Yet we have very little detailed understanding of what is involved, whether, for example, the antibodies are produced in some way *de novo* or whether all contingencies are provided for in the genetic make-up of the animal, and, if so, just what triggers the production of the antibody The antibody problem is in part a genetic one, in part a matter of the relation of structure to function and in part a question of cellular regulation The practical consequences for medicine and public health of a better understanding of this subject can hardly be overestimated

Viruses Closely related to the problem of the immune response, particularly in its implications for medicine and public health, is that of the nature and structure of the viruses Much is now known of the highly ordered structures of these particles, which stand at the dividing line between living and non-living matter, and of the organization of the nucleic acids and proteins of which they are largely composed It is now clear that the introduction of a virus particle into an organism is equivalent to the introduction of a piece of foreign genetic information which takes control and diverts the cells from their normal function The problems presented here are of the greatest interest in relation to control mechanisms both at a genetic and a metabolic level, and on the practical side lie at the heart of an understanding of many diseases Indeed, there is reason to think that a closer study of the viruses may provide a clue to the solution of the cancer problem

Some aspects of the functioning of the nervous system The problem of the genetic code is now essentially solved However, this is only the simplest and most basic of the problems involving information theory presented by the organism The corresponding problem at a more complex and even more challenging level is the storage, transfer, reassortment and retrieval of information in the nervous systems of multicellular organisms Is the basis of these phenomena, lying as they do at the root of adaptation, learning, response and indeed consciousness itself at all the same as that of the genetic code, and if not, what is its basis ? At present we cannot even hazard a reply to this outstanding question The answer will result from still unforeseen developments in a variety of subjects such as the study of membranes and of the control mechanisms which govern their structures, electrophysiology, and the chemistry of the gate mechanisms involved in the transmission of impulses across the synapse

Project K This theme takes its name from a particular strain, known as K12, of the bacterium *Escherichia coli* Today for the first time it is realistic to contemplate embarking on a programme directed towards a total understanding of the working of a single, characteristic unicellular organism—of its chemical make-up, of the many complex chemical processes taking place within it, of the mechanisms of its energy production, of the storage and expression of its genetic information, of the mechanics of its reproduction, and of its interaction with its environment Just because the genetic code is universal, because all kinds of cells use the same chemical pathways mediated by very similar catalysts (enzymes), it follows that this total understanding of one particular cell, even though it be a micro-organism, would throw a flood of light on the whole domain of cellular organization, including the economy and behaviour of a human cell The eventual rewards of such understanding would be the possibilities of practical control of cellular processes, with possible applications in medicine, in agriculture and in industry

Table 1 RELATIONSHIP BETWEEN THE FIVE THEMES AND THE FIFTEEN GROUPS

Working groups	Structure and function of proteins	Viruses	Chemical basis of the immune response	Nervous system	Project K
Protein chemistry	×	×	×		×
Nucleic acid chemistry		×			×
X-ray analysis	×	×	×		×
Electron microscopy	×	×	×	×	×
Physical chemistry	×	×			×
Physical enzymology	×				×
Genetics		×	×		×
Differentiation	×		×	×	
Neurophysiology				×	
Immunology	×		×		
Membranes	×			×	×
Computation	×	×	×	×	×
Production	×	×	×		×
Instrumentation	×				×
Animal and plant house	×	×	×	×	×

It should not be thought that these selected problems are fixed for all time, or even for more than a few years They have been chosen on the basis of the present state of biology, as well as their special suitability for attack at an international laboratory They will inevitably give place to others as the subject evolves For example, a problem not yet ripe for study at the molecular biological level is the mode of action of hormones, in a few years this might well find a place among the major objectives of the laboratory.

The council's document goes on to describe how the proposed laboratory would be organized into fifteen working groups of various sizes, and Table 1 shows how these would be linked with the five themes singled out for special treatment in the initial programme at the laboratory

On the staff and site, the council's document includes the following observations

The overriding consideration in the appointment of staff at every level should be ability "the laboratory would not succeed, and would not be worth establishing, unless its staff were of quite first class quality". Only a few senior scientists and engineers, for the most part group heads, would have tenure These would amount to something like 10 per cent of the total scientific population, say, 25 individuals in all The rest would be appointed for limited terms, but there would be possibilities of renewal so that an occasional young man might look forward to making a career in the laboratory.

In addition to regular staff, the laboratory would have a large number of visitors and trainees, who would represent an equally important part of the programme To a large extent these would probably receive support from sources outside the laboratory, including the EMBO Foundation

Among the factors governing the eventual choice of a site for the EMBO laboratory are the following

(1) Intellectual milieu The scientific work of the laboratory would greatly benefit from distinguished intellectual surroundings, both in the strictly scientific and the general cultural sense

(2) Other amenities—climate, housing, international schools, language, countryside Such factors would be important in attracting the senior visitors (and their families) whose presence in numbers would be essential

(3) Attitude of the host country A positive and continuing welcome and enthusiasm by the host country would greatly assist the establishment and subsequent development of the laboratory

(4) Accessibility A reasonably central position and good communications by air are important considerations in view of the constant coming and going of visitors from all parts of the world

(5) Local availability of services and materials The laboratory would benefit greatly from being situated in a place where skilled labour, servicing facilities and normal supplies were readily available locally

On the basis of its estimates of the scale on which the EMBO laboratory would operate, the council has been able to construct a budget for the construction and operation of the laboratory for the first decade or so of its existence (Table 2). It proposes that the head of the laboratory (as at CERN) should be a director-general, and the EMBO council emphasizes that this official should be appointed at the time work is authorized When operating at full strength, the laboratory would employ 122 *scientists as well as* 30 *"physicists and engineers", would have* 100 *or so visiting fellows on the premises and would need some* 300 *people as supporting staff*

Table 2 ESTIMATED BUILD-UP OF EXPENDITURE

Years from authorization	Non-recurring expenditure		Recurring expenditure						Totals
	Buildings	Initial equipment	Amortization Buildings	Amortization Equipment	Salaries	Computer rental	Running costs	Travel	
1	0 5	—	—	—	0 1	—	—	—	0 6
2	2 5	—	—	—	0 3	—	—	—	2 8
3	2 5	2 5	0 4	—	1 0	—	—	—	6 4
4	0 7	2 5	0 8	0 5	2 5	0 5	0 6	0 2	8 3
5	0 5	1 4	0 8	1 1	2 9	0 5	0 7	0 2	8 1
6	0 5	0 7	0 9	1 4	3 2	0 5	0 8	0 2	8 2
7	0 5	0 7	1 0	1 6	3 6	0 5	0 9	0 3	9 1
8	—	—	1 0	1 6	4 0	0 5	1 1	0 3	8 5
Totals	7 7	7 8							

Millions of dollars at 1967 prices Year 4 would be the first full operating year, and year 8 that in which the scale of operations reached a plateau

CERN and the Proposed European Biology Laboratory

In support of the case which the EMBO council has prepared, Professor V. Weisskopf, director-general at CERN between 1960 and 1965, has provided the following argument in favour of European laboratories.

IN connexion with the plans for an international biological laboratory in Europe, it might be useful to write down the experiences which we had in running CERN, the international high energy physics laboratory. I had the privilege of being the director-general during the time when the construction period of CERN was over and when the research activities were just about to start I therefore had an opportunity to see the effect of research at CERN on European university life at a most critical time

I remember that at the time of the founding of CERN, university circles in Europe were seriously worried about the effect of CERN on the staffing of their own national laboratories Naturally it was feared that such a well equipped and attractive research opportunity would attract the best and the most productive people away from the national laboratories, and as such deprive the national laboratories of their most important people, and by this means of their most important educational effect on students of the universities It was also feared that the centre of ideas and discussions would be removed from the universities to Geneva, so that the great European research centres would be relegated to a second order position

High Standing

When CERN was developed, these reasons were taken very seriously, but they were set aside by the absolute necessity of having an international laboratory of high energy physics because of the fact that no national institution was able to collect the funds necessary to build accelerators of the size which CERN was able to provide. Although the danger for the universities was realized and appraised, it was thought that there was no other way to bring Europe back into the first line of fundamental physics research—a position which it had indisputably held until the destruction of science in Middle Europe by the Hitler regime

It was one of the most exhilarating and encouraging facts of the development of CERN that this fear did not materialize In fact, it turned out that the existence of CERN has had a positive effect on university life, far beyond any measure which could have been predicted at the outset Let me try to sketch this surprising development

First of all, the construction period of CERN did not take many of the first-class research people away from European universities Clearly a construction of this size mainly involves people of engineering interests who did not play a great part in the process of education of physics itself However, a number of personalities who have been well known and important in physics education were taken away from a few universities, particularly in Switzerland, Germany, Italy and the Netherlands The number, however, was small enough not to make a decisive effect on the educational system of physics in these countries

Young Leadership

When CERN began its research activities, the situation had changed to some extent. It was, however, the conscious purpose of the CERN administration that leadership in that kind of modern research should be mainly in the hands of young people. This is why the additional research members who came to Geneva several years before the actual research—the people who were most active intellectually in the preparation of experiments—were only partly people who had previously taught in European universities (It is clear that research of this kind must be prepared several years before it begins, CERN made the mistake of neglecting this point to some extent and as a consequence productive research started later than might have been possible) To some extent they were people who had previously emigrated to America because of the much better facilities available and who had come back because of the promise of having similar facilities in Europe. The administration put special emphasis on giving these young people the greatest possible freedom of initiative I admit that this was not an easy task because it was against the general European tradition of leaving responsibility in the hands of older persons We therefore did not always succeed, but we tried in many cases to do our best

Slow Beginning

Research at CERN started quite successfully but, as indicated here, somewhat later than would have been possible This delay had the consequence that some of the fundamental discoveries which could have been made at CERN were actually made in the United States at the

Brookhaven National Laboratory However, a few important discoveries, made at CERN but characteristically with the small machine with which the European scientists had had more experience, had lifted morale and given those in the laboratory a sense of being in the forefront of research When, after two or three years of running, CERN had taken its place among the best and most productive institutions of its kind, research was carried out by a large number of groups An important part of the research was undertaken with bubble chamber pictures taken at CERN and evaluated at installations placed at different national universities

National Universities

The influence of this type of research on the national universities was quite remarkable Activity in high-energy physics increased all over Europe The European universities were no longer forced to beg for bubble chamber pictures from American institutions; they could get them directly from CERN The bubble chamber programme was always considered as a collective programme among the European countries, in which all institutions had a voice in deciding what pictures should be taken at CERN Many university people spent a certain time at CERN installing facilities and thus became acquainted with the details of the set-up, an important condition for successful evaluation work In other kinds of experiments, such as spark chamber and counter work experiments, the chief activity was at CERN, where the participation of the universities was, to begin with, relatively small

The increase of interest in high energy physics at all European universities produced a strong demand for new positions and professorships in this field in a surprisingly short time These positions were usually filled by young people who had worked for several years at CERN, either after some period of training in the United States or directly from their home countries For example, we have provided about six full professorships in Germany, two full professorships in the Netherlands and several others in different countries of Europe. More accurate statistics are available, it would be my estimate that at least fifteen positions around Europe were filled by young people who were trained at CERN Most of these people would have ended up in the United States were it not for CERN It should be mentioned that at least three senior physicists with the rank of full professor in the United States have since taken positions at CERN or in Europe

Supplying Professors

This 'invasion" of CERN professors into European universities had a very definite effect These professors did not stop their work at CERN when they joined their universities They spend their free time mainly at CERN and they have sent assistants, graduate students and other younger helpers to CERN in order to continue their lines of research or start new and promising investigations

The effect was that, by the end of my period, more than half of the experimental work at CERN was initiated or prepared by people who had been trained at CERN but who had received professorships at national universities Thus CERN has never been and is less than ever an institution independent of the university life of Europe It is an integral part of it, and it has not only supported but has clearly increased the activity and productivity of elementary particle physics in European universities It has successfully stopped and reversed the brain brain to the United States

Let me add that this effect is by no means restricted to the large and rich countries such as France, Germany or England Quite the contrary, those countries have their own opportunities to perform high energy experiments with their national institutions such as Saclay in France, in Rutherford Laboratory in England and DESY in Germany It is my conviction that the small countries have in fact taken advantage of CERN to a far larger degree—relative to the amount of money which they have put into the centre—than have the large countries Before CERN was active, it would have been almost unthinkable that countries like Norway or Austria could have maintained such a high degree of high energy physics for the amont of money which they actually pay to the CERN laboratory

The narrow collaboration between universities and CERN has had an enormously stimulating effect on the universities and has increased considerably the number of students interested in this fundamental field But it had also created certain administrative problems for the universities Professors had to be absent from the university much more frequently than usual, costs of travel have had to be met, a new way of life for laboratory personnel has had to be created Most universities were not too sympathetic to these innovations They reacted shortsightedly, not realizing that the alternative is no modern science and the loss of their best professors, students and technicians to the United States New solutions must be found, though not much has been done so far I suggested double professorships, where alternatingly one of the pair is at the university teaching and caring for the students and the other is at CERN working at the apparatus This solution has never been tried out in Europe, although it works quite well in my present department at MIT I do not pretend that there are no other solutions to this problem Archaeologists have worked for ages at places far removed from their universities

This report may give the impression of being too sanguine and optimistic I cannot help feeling that one of the most gratifying experiences as director of CERN was to see the stimulating and invigorating effect which that institute had on European physics In comparing CERN with the proposal of a biological laboratory we must beware that the element of building a very large accelerator does not exist To my knowledge, there is no instrument in biology which a single nation could not afford to provide for itself if it considered this was necessary

However, I have tried to show that the advantages of CERN go beyond the provision for European countries of a large accelerator A productive international intellectual centre has been created where young people have found a meeting place equipped with all modern facilities Here they were immersed in an atmosphere of active research, of the highest possible level Such a place is the best breeding spot for professors for national institutions, whose scientific level will by itself adjust to the level of the centre institution

United Europe

It is also important not to forget the importance of such centres for the idea of a united Europe The spirit of CERN and of a future biological institution is the spirit of a cultural European unity which exists but has had only all too few realizations

I would fervently hope that the Biological European Institute becomes a reality, but I also hope that it will be situated in the neighbourhood of CERN These institutions should become the nucleus of a European graduate school The arguments brought forward by these remarks should have amply proved that a European Graduate School of Science would never weaken national institutions It could only have an invigorating effect on each national university by raising the level of research, by providing a pool of potential candidates for jobs, and by reversing the brain drain It is Europe that has created modern science, it is high time that Europe becomes aware of the fact that it still could be the strongest supplier of scientific intellect

Glaciology and the International Hydrological Decade

by
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Snow and ice constitute an important part of the world's reserves of water. What follows is a description of some of the plans which have been made within the International Hydrological Decade for the study of these reserves.

THE great majority of the water on the surface of the Earth is in the form of salt water in the sea, but of the fresh water some 80 per cent is in the form of snow and ice, so that it is not surprising that a study of the snow and ice on the Earth should form an important part of the programme for the International Hydrological Decade. Of course, most of this snow and ice is in the great ice sheets of Greenland and Antarctica, about which much more knowledge has been gained from work started in the International Geophysical Year; in particular, measurements of the depths of ice in these ice sheets have provided some sort of an answer to the question of just how much water there is in this form. This problem is not perhaps of immediate practical importance, but in many parts of the world where streams fed from melting ice and snow are a principal source not only of water but also of hydro-electricity, the problems of just how much snow and ice exists and how much melt water can be expected in any particular season are of great importance in planning future needs.

In order to systematize the study of these problems, the International Hydrological Decade co-ordinating council, through the Scientific Committee on Water Research (COWAR) of the International Council of Scientific Unions (ICSU), has passed several resolutions concerning glaciological problems, and the Commission of Snow and Ice of the International Association of Scientific Hydrology has made plans to put these resolutions into effect. In many cases considerable progress has been made, and documents are available setting out just what should be done in order to provide the necessary information.

Permanent Recording

One important requirement is for all nations which have permanent ice and snow, whether above the surface of the Earth or within it, to record what they have, and for this purpose a working party under Professor F. Muller (McGill University, Canada) has prepared a guide to a world inventory. This suggests that the inventory be conducted at two levels of sophistication, and that the results should be prepared in a digital form suitable for storage and handling by computer.

The technique has been tested by three pilot studies using the suggestions for the glacier inventory in three areas of different nature, and results of these pilot studies were reported at the General Assembly of the International Association of Scientific Hydrology at Bern last autumn. The first area was the south-west part of Axel Heiberg Island in the Canadian High Arctic, a region with moderate mountain relief and many different types of cold glaciers. Some 250 glaciers were included in that study. The second region was in the Waputik Range in the Canadian Rocky Mountains, where a number of valley glaciers descend from three small ice fields and where there are numerous cirque glaciers and glacierets. About 100 glaciers were included in that survey. Finally, there was the region of Mount Everest in eastern Nepal, where most valley glaciers are fed by avalanches from precipitous slopes and where the ablation areas are often thickly covered with debris. Some 450 glaciers were included in this study. For each glacier a data sheet was filled in which identified and classified the glaciers and then gave its vital statistics such as elevation range, length, maximum width, area, elevation of snow-line and so on. This information was then put into a computer format for use on an IBM 7040/7044 computer which allows 131 characters per line; in the lower degree of sophistication each glacier required two lines. On the basis of the experience of these pilot studies and of the comments of other experts on the draft, a final version of this guide has now been prepared.

It is hoped that, with this guide available, there will be a great increase in the available data on glaciers and permafrost. Three volumes of the catalogue of glaciers of the USSR have already been published, and similar work is going on for the French Alps. It is necessary that this work be undertaken by all countries with glaciers or permafrost on their territory. If this can be achieved, we can improve greatly on the present position where estimates of the area outside the polar regions covered by ice vary by more than 20 per cent.

Another obvious requirement is to measure and map seasonal snow cover, and a working group under Dr M. de Quervain of the Swiss Federal Avalanche Research Institute has been preparing a guide to enable people with relatively little experience to make worthwhile measurements which will be comparable from place to place. One of the requirements is to give clear definitions of the terms involved (just what is meant by snow-line, for example), and another is to give simple methods for measuring the total storage of water in a snow cover. A similar problem arises in the case of sea, lake and river ice, and a guide is also being prepared by a working group, under Dr E. R. Pounder of McGill University, to cover these fields.

Glacier Fluctuation

A further resolution of the IHD co-ordinating committee concerns a study of the fluctuations of glaciers. This is a field with a much older history. Observations of the advance and retreat of glacier fronts have been made for many years in many parts of the world, and the gradual development of co-ordination in this field has culminated in the recent establishment of a permanent service under FAGS (Federation of Astronomical and Geophysical Services). Ing. P. Kasser (Swiss Federal Institute of Technology) has produced the first report of this service recently. The chief development since the early international reports has been the increase of interest in changes of thickness and of mass balance of glaciers as well as of their lengths

Another project, in many ways the most ambitious, calls for combined water, ice and heat balance studies at selected glacier basins A working group under Dr M F Meier, the present president of the Commission of Snow and Ice and a member of the US Geological Survey, has selected three chains of stations The first stretches north and south through the Americas and into the Antarctic Peninsula, a second runs east–west from Tien Shan in Soviet Central Asia through the Caucasus and Alps, with further points in Canada and the Pacific Northwest of the United States, while a further chain, also roughly east–west, goes through the Polar Urals, Scandinavia, Iceland, Greenland and the Canadian Arctic to Alaska At each of the selected basins it is hoped to have a comprehensive set of measurements to cover all three balances—of ice, run-off water and heat—so that their interdependence can be studied Some countries are already doing a lot of this, about 30 of the stations being in operation in 1965–66, but often there is one of the elements left out By emphasizing the need for all to be measured on the same basins, it is hoped to encourage national authorities to support a really comprehensive programme

Polar Ice

The worst gap in our knowledge concerns the ice in the polar regions It is not known at present whether the amount of ice in either of the great ice sheets in Greenland and the Antarctic is increasing or decreasing In the case of Greenland, work at present being done by the International Glaciological Greenland Expedition may provide the answer, but for the Antarctic an even larger programme of work will be needed With the aim of preparing a co-ordinated programme for the International Hydrological Decade in Antarctica, the working group on glaciology of the Scientific Committee on Antarctic Research (SCAR) has prepared a document for the hydrological evaluation of glaciological research programmes in Antarctica. This document is also now being considered by COWAR and the IHD Co-ordinating Council

Two future international conferences are planned to discuss scientific matters concerned with these programmes Both are co-sponsored by the Commission of Snow and Ice The first, of which the other co-sponsor is SCAR, will be an International Symposium on Antarctic Glaciological Exploration, it will be held in Hanover, N H, USA, in September 1968 The second, co-sponsored by the Glaciological Society, is on the Hydrology of Glaciers, and will be held in Cambridge, England, in September 1969

It is plain that much of this work is still at the planning stage The manuals are in the final stages of preparation and the stage is set for a big increase in our systematic knowledge of the ice on the Earth The extent to which these plans will result in a completed programme will depend very largely on the extent to which the authorities in different lands realize the great advantages of knowing just what resources of water there are tied up in their seasonal and permanent snow and ice, and therefore make available the relatively small amounts of money and manpower necessary to do the work Persuading people of the importance of this is what the International Hydrological Decade is about

I would like to thank Dr M F Meier and Dr F Muller for commenting on a draft of this report, and also Professor H Hoinkes, the president of the Commission of Snow and Ice from 1963–67, for letting me see the manuscript of his presidential address to the Commission last September, in which he reviewed the position of glaciology in the International Hydrological Decade at greater length[1]

[1] Hoinkes, H, *Union Géodésique et Geophysique Internationale, Assemblée Générale de Berne*, September 25 to October 7, 1967 (in the press)

Feasibility in Age Research

by

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The present state of knowledge and the potentialities of the subject seem to justify fundamental research into the process of ageing.

In several countries investment decisions are now being taken, or are about to be taken, about future support for research into problems connected with the process and manifestations of human ageing The desirability and possible scope of an institute of age studies are under informal discussion with British universities and donors, and a committee of the United States Senate is receiving evidence with a view to planning future research policy

Developed countries have an increasing proportion of old citizens whose medical and social needs require study and palliation, and call for an increasing volume of *ad hoc* research At the same time, where a fundamental process is probably comprehensive and possibly controllable, reasonable support for a radical attack on the biology of that process offers a chance (if the attack succeeds) of shortcircuiting much piecemeal investigation or (if it succeeds to the point of comprehending a process which then appears fundamentally or practically uncontrollable) of avoiding misplaced research.

The aims of ageing research are presumably (*a*) to comprehend and if possible palliate the disorders which affect old human beings, and (*b*) to interfere, fundamentally if possible, with the "clock" which determines timewise loss of vigour either by spinning out the entire process of cradle-to-grave development, so that, without reducing physiological activity, it occupies more years· or better, to produce an effect opposite in sense to the effect produced in mice by chronic low-dose irradiation or a single large dose in early life. These procedures appear to lop off a portion of the plateau of adult vigour proportional to the dose it is an open question whether this represents an intensification of the same processes which are seen in normal ageing, or only a simulation.

An ideal "remedy" for senescence would have the reverse effect, of prolonging the same plateau, so that the vigour and disabilities of a treated individual of 85 would resemble those of an untreated individual of 80, the effect being produced across the board and without reference to particular genetic constitutions or causes of death. While remedies depending on unknown biological facts cannot be synthesized to order, this appears to be the objective of choice provided that it is not manifestly impracticable on a basis of existing knowledge.

Projects based on the prolongation of life by interrupting bodily function (artificial hibernation, cold storage) complete the list of theoretically possible approaches, but have little practical appeal outside the field of long-distance space travel. Current priorities and predictions, for British research at least, should be drawn from less paranoid fields. In these it is desirable not only to plan research for feasibility, but to predict possible changes in demographic requirements which may, eventually, result from the research of others, should we fail to give our own biologists the support needed to make the relevant advances themselves.

Wider scientific and public discussion of the prospects and direction of research into ageing is needed now, because the present state of the subject is not widely known to scientists outside the field, and planning decisions can easily be influenced by the optimistic or pessimistic tone of single theorists or papers. Research planners require to know whether or not there is a reasonable prospect in the near future of identifying a single leading process in mammalian ageing which might be open to useful interference. The consequences would be similar to those observed in the dietary retardation of rodents, that is, a postponement of death from all causes or, better, a generalized retardation of all age-associated deteriorative processes leading to a longer period of adult vigour. If there is not, or if the "leading process" is, for example, one of exposure of un-eliminated late-acting genes, or otherwise an evolutionary or statistical process only, research money would better be applied to prosthetics and geriatrics, the piecemeal alleviation of senile disabilities.

If, as I believe, the first of these alternatives is probable but by no means certain, the highest immediate research priority is to concentrate resources on research designed to distinguish between them.

I suggest that a choice between these hypotheses could be made within a reasonably short period of time, and that in view of the consequences for our long-term planning of age research, this choice should be an immediate research priority.

In the early years of the present century it was a reasonable expectation of medical researchers that the "cause and cure" of cancer (meaning the malignancies in general) might, with reasonable application, be discovered within a short time—on the model of earlier discoveries of the cause of bacterial infections. A Nobel Prize was even awarded for the discovery of such a cause—prematurely, as it appeared. Such expectations, which formed the early years of "cancer research", no longer retain credibility in quite this form against the background of wider understanding.

It is necessary to ask whether there should be a similar reappraisal of the aims of research into the nature and control of human ageing. A less popular topic than cancer research, gerontology too was begotten in simplistic optimism when Brown-Séquard attempted to rejuvenate himself with testicular extract. Although the possibility of a single large-scale influence on the realizable human lifespan cannot be excluded, on the basis of what is now known, most workers would today consider the picture of mammalian senescence to be at least as complex as that of malignant change, and some, on evolutionary or genetic grounds, have suspected that it may be practically or essentially unmodifiable by reason of diversity[1,2]. If the 'loss of programme" which increasingly upsets homoeostasis with age represents the accumulation of postponed genetic effects inaccessible to selection, this pessimism would be justified. It may be that natural selection has increased lifespan where necessary, not by increasing the reliability of cellular stability but by providing fallback "patch" mechanisms to compensate for disadaptive unreliability[3]. Such an expedient would itself result in a divergent system, like the borrowing of money to repay creditors, and one very difficult to stabilize artificially.

Judging by recent congresses and publications, most research gerontologists now have a leading general hypothesis of ageing—that it is a cellular information loss, probably in fixed cells, and originally at the molecular level, dyshomoeostasis of other kinds through loss of neuronal, endocrine and immunological information being secondary. This theory is based on the natural history of ageing in organisms generally, on radiation biology, and on stochastic mathematics applied to the life-table. The information-content of fixed-cell DNA at successive ages has not been measured, and so the theory may be wrong, but it must now be demonstrated or disposed of.

If molecular information-loss is the timekeeper of ageing, there are by consensus two hypotheses to explain its occurrence: (*a*) that it is caused by the random accumulation of chemical noise in the cellular information system, and is itself unprogrammed, (*b*) that it is a built-in consequence of differentiation, caused by the switching-off of synthetic processes which cannot be switched on again without loss of differentiation, or to the evolutionary accumulation of late-acting deleterious genes.

Prediction in age studies is closely involved with these hypotheses, which are not mutually exclusive, and it is now highly important to examine and dispose of them. If human ageing as observed in the intact subject is not timed by genetic information loss, the nature of its leading "clock" and the accessibility of this to interference remain wide open. If it is so timed, and hypothesis (*a*) is correct, the likelihood of successful interference with the underlying random process is reasonably high. Suggested sources of random damage include "hits" by radiation[4,5], cross-linking and colloid changes[6-8], free-radical effects generally[9], and release of lysosomal DNases[10,11]. All these are observable and, in model systems, modifiable. Another consequence of hypothesis (*a*) is that it does not exclude at least a useful identity between the causes of ageing, for example, in insect imagos and those observed in mammalian fixed cells, with an increase in the number of possible test systems available. The identity of low-dose radiation-induced life shortening with accelerated ageing remains a matter of controversy. The resemblance is close but not complete[12], attempts to see whether radioprotectants abolish the life-shortening effect of chronic exposure to low doses run into practical problems of maintaining drug-level with the agents now in use, and no conclusive results have been published. Accumulation of somatic point mutation, which was at one time favoured as an explanation of ageing, is now largely excluded on statistical and genetic grounds[13,14] as well as by the failure of chemical mutagens to produce radiation-like life-shortening unless they are also capable of causing damage more extensive than point mutation—particularly crosslinking[13]. Where, as in numerous papers by Curtis[15,16], "mutation" is adduced as a source of programme noise in ageing cells, the term is used to include breakage, deletion and operon loss generally. Although the rate of increase in chromosomal abnormalities is greater in short than in long-lived strains[17], the only age phenomena which seem likely to be explicable in terms of one or more point mutations are autoimmune divergence[3,11,18-20] and possibly some factors in malignancy[3,11,21]. Radiation-type life-shortening can be induced in adult *Drosophila*, which are resistant to low doses of radiation, by feeding amino-acid analogues during larval life[8,22], before the postmitotic state is reached

Hypothesis (*b*) has been most fully stated by Medvedev[23] It implies that the suppression of information or synthetic capacity which upsets homoeostasis and leads to ageing is itself a necessary process in differentiation. If this is so, the prognostic consequences are considerable. It does not mean that artificial "stretching" of the human lifespan is impossible, but that the only likely way of prolonging vigour will prove to be through stretching the development programme as a whole, on the analogy of rodent dietary restriction Such stretching would be biologically drastic, and would include (or even be confined to) the prolongation of immaturity, rather than the preservation of "adult vigour" as a steady state —which, on hypothesis (*b*), it is not

Medvedev's argument includes a revival of the Weismannian idea that life-limitation might itself be an evolved adaptation, which has provided the organism with a destructor mechanism in the form of noise-generating genes Evolutionary jobbing-backward in relation to observed lifespans is particularly unrewarding, and I have already argued that the Weismannian advantages of senescence to a species depend on a circular argument[24], although a contrary view has been developed by Sacher[25] Noise-generator genes might or might not be accessible to interference, and it seems as likely that the evolved attribute limiting lifespan, if such there be, is the general stability of the informational system to noise-accumulation at this point the argument approaches Pangloss's theorem Leaving this aside, we are left with two questions which influence our expectation of probable interference with the rate of human senescence Is mammalian dyshomoeostasis with age timed by loss of molecular information ? If so, is this loss a result of random noise-accumulation, in fixed or in clonally replicated cells, or of the finitude of the switching-sequence involved in epigenetics, or of both ?

Until these questions are resolved, further research on ageing and the likelihood and extent of any control of the human lifespan can only be assessed "blind", and so the immediate programme of age research needs to be directed to them There is a fair body of evidence in favour of the concept of information loss, and of that loss being random in character, but there is a methodological bias in favour of these conclusions built-in by the involvement of age studies with radiation effects (which may not be analogous to natural ageing) and the mathematical preoccupations of radiobiologists Direct examination of the functioning of fixed-cell DNA by the detection of nonsense-materials[26] or by the evidences of compensatory synthesis to keep up with failing accuracy of copying[27-30] is already in hand, on the lines originally suggested by Maynard Smith[14,27,31] Production of nonsense materials, if demonstrated, would accord better with stochastic damage than with the switching-off of genes The only conclusive short-cut experiments are those which might point the way by demonstrating that cellular information can be protected, through the study of substances which prolong the life of fixed cells The complementary demonstration, depending on life-shortening[22], is inconclusive for obvious reasons Attempts to prolong mammalian life with antiradiation drugs and antioxidants have so far been unsatisfactory[9] in execution rather than in conception Apart from the papers of Harman[32], whose longest-lived mice on antioxidants failed to equal the best untreated performance for the strain, the single, and relatively small, prolongation in lifespan so far reported in mammals followed the administration of an immunosuppressant[33]

There is available a repertoire of treatments which could, if the practical difficulties of administration to a suitable and standardized organism can be overcome, dispose both of the general hypothesis of noise-accumulation, and of sub-hypotheses concerning the parts played, for example, by cross-linking, lysosomal DNase, and consequent autoimmune phenomena

If it could be shown that a theoretically accountable substance (antioxidant, anti-cross-linking agent, DNA ligase potentiator or lysosome stabilizer) is capable of prolonging the mature lifespan of a simple organism beyond the maximum normally observed in outbred individuals, this would in itself constitute a useful advance Demonstration of Medvedev's alternative hypothesis would involve considerably subtler techniques, recent work on serial gene expression in ciliates[34] foreshadows these, and indicates that the hypothesis could be extended from fixed cells to clones Meanwhile the whole question of clonal stability in somatic cells, an old gerontological source of optimism dating from Carrel, remains open.

Concentration of fundamental projects in these questions could at least dispose of them, and a concerted attack on these lines seems desirable—gerontology at the moment has more bricklayers than architects, and the most important new evidence might well come from outside the circle of age researchers, provided the general biologists in other fields are aware of the situation In view of the importance of work on cellular information and epigenetics to biology as a whole, there is little risk of wasting a research investment at such a fundamental level If within 10 years it has become evident that protection of cellular information at the level demanded by "adult vigour", or useful stretching of development to extend that vigour, is fundamentally or practically unlikely in man, researchers on age processes would at least be released to pursue palliative concerns, such as the re-examination of autoimmune divergence or the correction of hormone deficits, traditional topics of gerontology which in any case require re-examination, while the chances of a degree of control over the timing and sequence of age changes as a whole seem at least good enough to merit a substantial and co-ordinated effort on a world basis

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[1] Medawar, P B , *Modern Q* , 1, 30 (1945)

[2] Strehler, B L , *Time, Cells and Aging* (Academic Press, New York, 1962)

[3] Comfort, A , *Gerontologia*, Basle (in the press)

[4] Failla, G , *Sympos AIBS*, **6**, 170 (1960)

[5] Szilard, L , *Proc US Nat Acad Sci* , **45**, 30 (1959)

[6] Bjorksten, J , *J Amer Geriat Soc* , **6**, 740 (1958)

[7] Verzár, F , *Experimental Gerontology* (C C Thomas Co , Springfield, Illinois, 1963)

[8] Von Hahn, H P , and Verzár, F , *Gerontologia* (1963)

[9] Harman, D , in Shock, N , *Biological Aspects of Aging*, 267 (New York, 1962)

[10] Allison, A C , *Proc Roy Soc Med* , **59**, 867 (1966)

[11] Comfort, A , *Lancet*, ii, 1325 (1966)

[12] Lindop, P J , and Rotblat, J , *Proc Roy Soc* , B, **154**, 332 (1960)

[13] Alexander, P , *Symp Soc Exp Biol* , **21**, 29 (1967)

[14] Maynard Smith, J , *Nature*, **184**, 956 (1959)

[15] Curtis, H J , *Curr Topics Radiat Res* , **3**, 140 (1967)

[16] Curtis, H J , *Brookhaven Lecture Series*, **34**, 1 (1964)

[17] Crowley, C , and Curtis, H J , *Proc US Nat Acad Sci* , **49**, 626 (1963)

[18] Burnet, J M , *Perspect Biol Med* , **10**, 141 (1967)

[19] Burnet, J M , *Brit Med J* , i, 338 (1965)

[20] Walford, R L , and Troup, G M , in Shock, N , *Perspectives in Experimental Gerontology* (C C Thomas Co , Springfield, Illinois, 1966)

[21] Burch, P R J , and Burwell, R G , *Quart Rev Biol* , **40**, 252 (1965)

[22] Harrison, B J , and Holliday, R , *Nature*, **213**, 990 (1967)

[23] Medvedev, Zh A , *Protein Biosynthesis* (Oliver and Boyd, 1967)

[24] Comfort, A , *Ageing, The Biology of Senescence* (Routledge and Kegan Paul, London, 1964)

[25] Sacher, G A , in Shock, N , *Perspectives in Experimental Gerontology* (C C Thomas Co , Springfield, Illinois, 1966)

[26] Orgel, L E , *Proc US Nat Acad Sci* , **49**, 517 (1963)

[27] Clarke, J M , and Mavnard Smith, J , *Nature*, **209**, 627 (1966)

[28] Samis, H V , Wulff, V J , and Falzone, J A , *Biochim Biophys Acta*, **91**, 223 (1964)

[29] Wulff, V J , *J Gerontol* , **17**, 456 (1962)

[30] Wulff, V J , Quastler, H , and Sherman, F G , *Proc US Nat Acad Sci* , **48**, 1373 (1962)

[31] Maynard Smith, J , *Proc Roy Soc* , B, **157**, 115 (1962)

[32] Harman, D , *J Gerontol* , **16**, 247 (1961)

[33] Walford, R L , *Symp Soc Exp Biol* , **21**, 351 (1962)

[34] Siegel, R W , *Symp Soc Exp Biol* , **21**, 127 (1967)

Palaeomagnetism, Potassium–Argon Ages and Petrology of some Volcanic Rocks

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Geological evidence obtained from samples of rocks from Cocos Island, Costa Rica, seems to agree with the idea that this island could have been a stepping stone for organisms migrating to the Galapagos Islands

Cocos Island lies in the eastern Pacific Ocean on the crest of the Cocos Ridge, a well defined linear bathymetric high with an average depth at its crest of about 1,000 fathoms[1] Extending south-west from Costa Rica to the Galapagos Islands, where it joins the Galapagos Fracture Zone, Cocos Ridge appears to be a fracture zone similar to the Tehuantepec Ridge, which branches from the Clipperton Fracture Zone, and to the Nasca Ridge, which branches from the Easter Island Fracture Zone. The age of Cocos Island is of geophysical interest as an indication of the age of the volcanism associated with the fracture zone, and as a measure of the minimum age of the sea floor in this tectonically active region The age is also of biogeographical interest because of the possibility that Cocos Island served as a stepping stone for plants and animals migrating to the Galapagos Islands from Central America The visit of the Galapagos International Scientific Project to Cocos Island on March 8–9, 1964, gave one of us (A C) an opportunity to collect rock samples in the vicinity of Chatham Bay (Fig 1) The results of potassium–argon age determinations, palaeomagnetic measurements and chemical analyses are reported here

In spite of more than 400 treasure-seeking and scientific expeditions to Cocos, surprisingly little is known about its geology Merrill[2] published a description and partial chemical analysis of an "andesite" collected by Agassis from the vicinity of Chatham Bay Chesterman[3] described two olivine basalts, one latite, one lapilli tuff-breccia and one "oligophyre" composed mostly of oligoclase, collected on earlier expeditions The most complete report of the geology of Cocos is that of Chubb[4], who spent 24 h there in 1924 He noted that the island was composed chiefly of massive lava flows and described an agglomeratic tuff from the south side of Wafer Bay From a study of beach pebbles collected near Wafer Bay, he concluded that the rocks there are principally labradorite–andesite and hornblende labradorite–andesite Chubb presented chemical analyses of two typical specimens and described them as similar to those of the Galapagos and especially of Rapa Macdonald[5], however, recognizing the inadequacy of the geological information from Cocos Island, did not include the Cocos rocks in the mid-Pacific volcanic province

The botanist Alban Stewart found that the flora of Cocos was that of a typical oceanic island, and that there was no botanical evidence for a land bridge connecting Cocos with the Galapagos Islands[6] He concluded that Cocos Island was younger than the Galapagos Islands because the percentage of endemic plants is smaller, an argument that seems tenuous in view of the fact that Cocos is only half as far from the mainland, has a different climate and is in different oceanic and atmospheric current systems Vinton inferred a Pleistocene age for the island from the thinness of the soils, the ungraded character of the streams and the scarcity of beach deposits[7] He further concluded that, during the Oligocene and Miocene, Cocos Ridge was a peninsula extending from Central America to within less than 200 km of the Galapagos Islands but that the peninsula submerged well before the formation of Cocos Island Channels have been discovered on the sides of Cocos Ridge, but Wilde[8] concluded that these were formed by turbidity currents rather than by subaerial erosion Furthermore, the crust underlying the ridge seems to be oceanic rather than continental[9], and the absence of guyots on the ridge indicates that it has remained submerged at least since the time of the volcanic

Table 1 CHEMICAL ANALYSES AND MOLECULAR NORMS OF TRACHYTE (4*C*400) AND ALKALI BASALT (4*C*401, 4*C*402 AND 4*C*404) FROM COCOS ISLAND

	4*C*400	4*C*401	4*C*402	4*C*404	Average oceanic[12] alkali basalt
SiO_2	58 4	48 3	48 8	48 6	47 4
Al_2O_3	19 0	19 0	18 6	19 1	18 0
Fe_2O_3	4 7	6 5	5 3	8 3	4 2
FeO	1 9	2 8	3 7	1 1	5 8
MgO	0 69	3 7	3 8	2 6	4 8
CaO	1 7	7 0	7 2	6 2	8 6
Na_2O	5 5	3 4	3 2	4 2	4 0
K_2O	4 5	1 7	2 0	2 3	1 7
H_2O-	0 81	1 8	1 6	1 8	0 6
H_2O+	1 4	1 3	1 3	1 5	0 8
TiO_2	1 1	3 4	3 5	3 3	2 9
P_2O_5	0 25	0 59	0 51	0 61	0 9
MnO	0 09	0 20	0 17	0 19	0 2
CO_2	<0 05	<0 05	<0 05	<0 05	—
Total	100 0	99 7	99 7	99 8	99 9
		Molecular norms			
Q	5 2	3 2	3 3	—	
C	2 6	0 3	—	—	
Or	26 6	10 1	11 9	13 6	
Ab	46 5	28 9	27 2	35 6	
An	6 8	31 0	30 6	26 5	
Ne	—	—	—	—	
Mt	3 2	—	2 3	—	
Hm	2 5	6 5	3 7	8 3	
Il	2 1	6 4	6 7	2 7	
Ap	0 6	1 4	1 ?	1 4	
Ol	—	—	—	—	
Di	—	—	1 5	—	
Hy	1 7	9 2	8 8	6 5	

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activity that produced the numerous seamounts on the ridge[10].

The rocks in the vicinity of Chatham Bay, like those studied by Chubb at Wafer Bay, include flows, tuff and agglomerates. East and south of Chatham Creek the flows are thick, massive and nearly horizontal and seem to extend several kilometres along the coast These flows are capped by a thick sequence of volcanic ash in the basin at the head of Chatham Creek In contrast, the flows north of Chatham Creek are thin and are interbedded with crystal lithic tuff probably of subaerial origin. Most of these flows dip seaward away from present topographic highs, suggesting initial dip rather than deformation The northern tip of Cocos consists of bedded tuffs that dip northward Nuez Island is composed primarily of agglomerates, which also dip northward away from the central mass of Cocos, but there the dips are sufficiently steep to suggest the possibility of some deformation

diopsidic augite The plagioclase phenocrysts are complexly twinned and strongly zoned, most of them are labradorite (An$_{55-65}$), but a few are as calcic as An$_{80}$ The groundmass is composed of plagioclase of composition An$_{40-45}$, olivine, diopsidic augite, iron–titanium oxides and apatite. Almost all of the olivine is altered to iddingsite A modal analysis of sample 4C401 gave 56 per cent plagioclase, 18 per cent pyroxene, 13 per cent olivine, 11 per cent opaque minerals and 1 per cent apatite The chemical analyses of these three rocks also do not differ significantly (Table 1). They all contain less than 50 per cent silica, a relatively large amount of alumina and total alkalis, and are oxidized, as can be seen by their high Fe$_2$O$_3$/FeO ratios More than 1 75 per cent titania is present, which is typical for oceanic basalts in contrast to circumoceanic basalts, which usually contain less TiO$_2$ (ref 11).

Sample 4C400 from one of the thick massive flows is a slightly porphyritic, granular trachyte that consists primarily of sodic plagioclase and sanidine with small amounts of olivine (altered to iddingsite), hypersthene, iron–titanium oxides and apatite Petrographically and chemically, it resembles the specimen from Chatham Bay described by Merrill[2] as an andesite

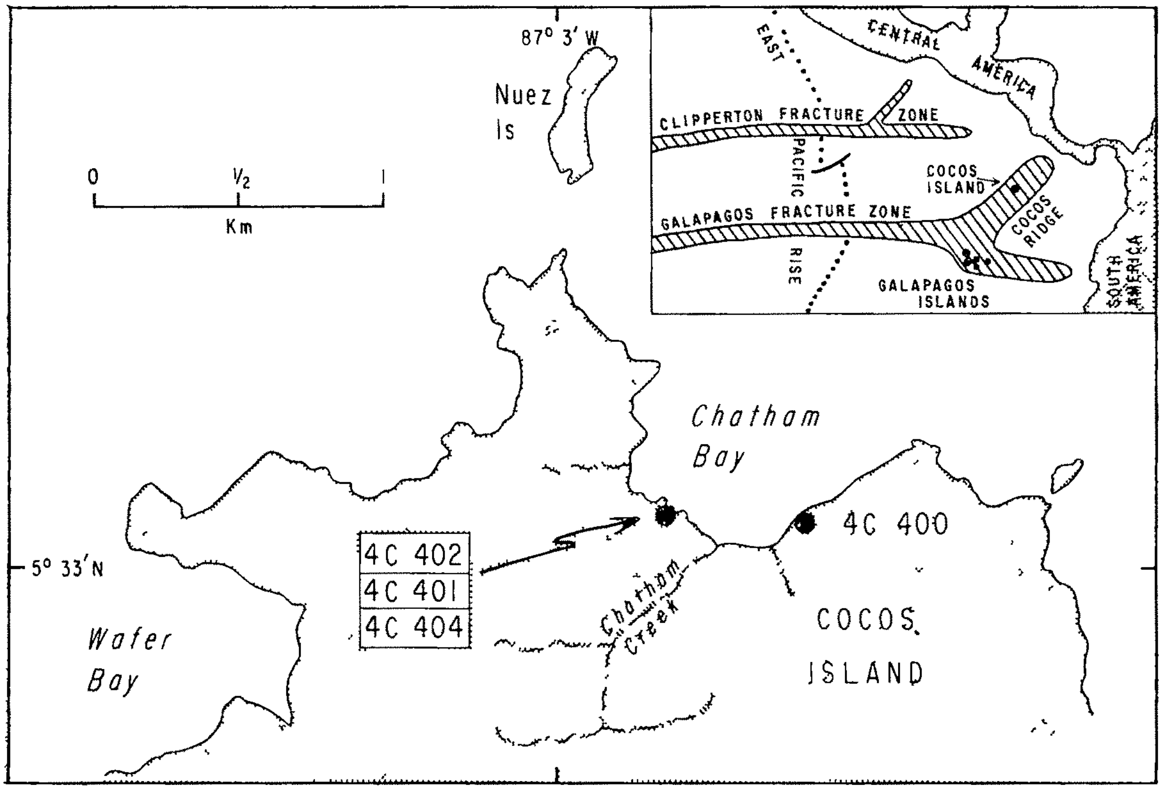

Fig 1 Index map of northern Cocos Island showing locations of analysed samples Samples north-west of Chatham Creek are shown in stratigraphic order Inset shows the relationship of Cocos Island to the bathymetric features discussed in the text

The marks of extremely active erosion characterize the present surface of Cocos Island Despite the rapid growth of vegetation, many landslide scars are present in the vicinity of Chatham Bay Mudflow and landslide deposits clog the lower reaches of the steeply incised canyon of Chatham Creek, and fans containing subangular boulders up to 2 m in diameter extend into the bay at the mouth of Chatham Creek, indicating that there has been recent mudflow activity

For this study, samples were collected from thin flows north of Chatham Creek and from one of the thick, massive flows on the south side of Chatham Bay (Fig 1) Samples 4C401, 4C402 and 4C404 from three of the thin flows are nearly identical alkali olivine basalts These samples are porphyritic with a trachytic texture and contain phenocrysts of plagioclase, olivine and, rarely,

The alkali basalts which were studied are plotted on a K$_2$O–SiO$_2$ diagram (Fig 2) together with previous analyses by Chubb[4] The analyses all fall within the field of the alkali oceanic basalts[12] No tholeite has been reported from Cocos Island The alkali basalts compare closely in composition with average "oceanic" alkali basalt[12], and A E J Engel and C G Engel[13] have pointed out that rocks of this composition predominate on the islands and the upper parts of seamounts associated with the East Pacific Rise Petrologically, Cocos Island is thus

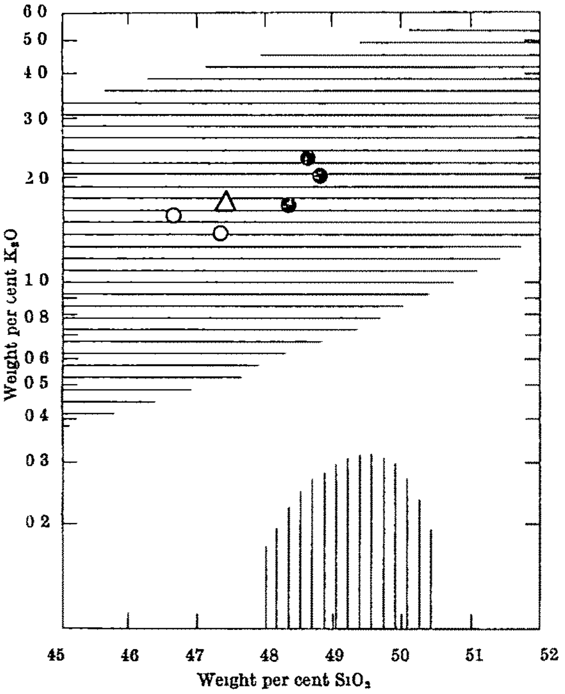

Fig 2 K_2O–SiO_2 diagram for rocks from Cocos Island Solid circles are analyses from this study, open circles are data from Chubb[4], and triangle is average oceanic alkali basalt[13] Vertically and horizontally ruled areas are approximate fields of the oceanic tholeiite and alkali basalt[13]

oceanic, strengthening the conclusion that Cocos Ridge is not a submerged part of the continent

The polarity of four or more oriented samples from each flow was measured in the field with a portable fluxgate magnetometer[14] and found to be internally consistent These field measurements were confirmed by remeasuring the remanence of one sample from each flow in the laboratory[15] and checking its magnetic stability in alternating magnetic fields[16] (Table 2) The three flows north of Chatham Creek are reversely magnetized (Table 2) Two of these, flows 4*C*401 and 4*C*402, are underlain by baked tuffaceous material that is also reversely magnetized, indicating that the reversed remanent magnetism is a result of reversals of the Earth's field and not mineralogically controlled self reversal The massive trachyte flow (4*C*400) is normally magnetized The intensity of magnetization of the alkali olivine basalt samples is typical of basalt[17], whereas that of the trachyte (4*C*400) is much smaller This is probably because the trachyte contains fewer grains of iron oxides

An examination of thin sections showed that three of the four flows were suitable[18] for whole-rock potassium–argon age measurements Argon analyses were by standard isotope dilution techniques using methods and techniques previously described[10] Potassium was determined by flame photometry using a lithium internal standard The ages (Table 3) of flows 4*C*401 and 4*C*402 are consistent with their stratigraphic relations, and the ages of all three flows are tightly grouped near 2×10^6 yr

Recent studies of the history of geomagnetic reversals indicate that the polarity structure consists of polarity epochs, which are periods of time during which the Earth's magnetic field was predominantly or entirely of one polarity, and polarity events, which are like epochs but of much shorter duration[20–22] One of the best documented events is the Olduvai normal event which occurred about 2 0 to 1 9×10^6 yr ago The normal polarity of this event is recorded (Fig 3) in lava flows from Olduvai Gorge, Tanzania[23–24], the Pribilof Islands, Alaska[25], Réunion Island in the Indian Ocean[26] and Victoria, Australia[27] From the polarity and age of sample 4*C*400, Cocos Island also appears to be a locality where volcanism occurred during the Olduvai event The duration of the Olduvai event has been estimated to be about $0{\cdot}14 \times 10^6$ yr (refs 28 and 29), whereas the spread in potassium–argon ages is nearly twice this Although undoubtedly partly caused by dating errors, the spread in ages suggests that the Olduvai event consists of multiple, widely spaced shorter events, for which some evidence has already been found in deep-sea sediment cores[29].

Table 2 PALAEOMAGNETIC DATA FOR ROCKS FROM COCOS ISLAND

Sample No	Original magnetization D	I	J	After partial demagnetization D	I	J	Polarity
4*C*400	003°	21°	$8\ 02 \times 10^{-4}$	008°	24°	$2\ 97 \times 10^{-4}$	Normal
4*C*401	182°	00°	$2\ 51 \times 10^{-3}$	182°	06°	$1\ 61 \times 10^{-3}$	Reversed
4*C*402	173°	−07°	$2\ 41 \times 10^{-3}$	173°	−08°	$1\ 20 \times 10^{-3}$	Reversed
4*C*404	170°	−01°	$4\ 27 \times 10^{-3}$	170°	−01°	$2\ 30 \times 10^{-3}$	Reversed

D, Declination, I, inclination, J, intensity in emu/cc.

Partial demagnetization was in a c field of 400 gauss

Table 3 ANALYTICAL DATA FOR POTASSIUM–ARGON AGE DETERMINATIONS ON VOLCANIC ROCKS FROM COCOS ISLAND, COSTA RICA

Sample No	K_2O (per cent)	Sample weight (g)	Argon Ar^{40}_{rad}/g (moles)	$\frac{Ar^{40}_{rad} \times 100}{Ar^{40}_{total}}$	Calculated age* (10^6 yr)
4*C*400	4 78, 4 81	9 956	$1\ 480 \times 10^{-11}$	62 8	2 09 ± 0 04
4*C*401	2 12, 2 13	10 000	$0\ 637 \times 10^{-11}$	25 9	2 03 ± 0 06
4*C*402	2 22, 2 22	8 624	$0\ 632 \times 10^{-11}$	19 0	1 93 ± 0 09

* $\lambda_\varepsilon = 0\ 585 \times 10^{-10}\ yr^{-1}$, $\lambda_\beta = 4\ 72 \times 10^{-10}\ yr^{-1}$, $K^{40}/K = 1\ 19 \times 10^{-4}$ mole/mole

The ± values are estimates of the analytical precision at the 68 per cent confidence level[26]

Analyses were made on whole-rock samples

In a similar study on the Galapagos Islands[30] we found that volcanism began at least 2 4×10^6 yr ago and has continued to the present time Thus there is at least some overlap between the volcanic activity on the Galapagos Fracture Zone and that on the Cocos Ridge On

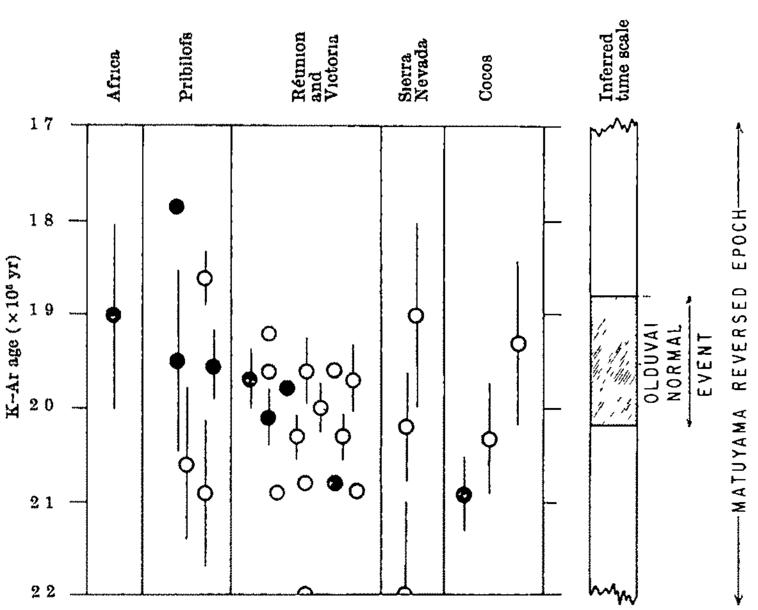

Fig 3 Comparison of data from Cocos Island rocks with worldwide palaeomagnetic–radiometric data from rocks having ages between 1 7 and 2 2×10^6 yr (refs 23–27) Solid circles indicate normal polarity, open circles indicate reversed polarity, and vertical bars are estimates of standard deviation of analytical error where available

both Cocos and the Galapagos Islands, it is possible that older rocks may exist beneath a surface cover of younger flows, so that the time when individual islands first emerged from the sea cannot be determined with certainty The geological evidence, however, does not contradict the idea that Cocos Island may have served as a stepping stone for organisms migrating to the Galapagos Islands, even though it is unlikely that the Cocos Ridge was ever a land bridge

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[1] Shumway, G , and Chase, T E , *Occasional Papers Calif Acad Sci* , **44**, 11 (1963)
[2] Merrill, G P , *Mus Comp Zool. Bull* , **16**, 235 (1893)
[3] Chesterman, C W , *Proc Calif Acad Sci* , Fourth Series, **32**, 339 (1963)
[4] Chubb, L J , *Bernice P Bishop Mus Bull* , **110**, 1 (1933)
[5] Macdonald, G A , *Bull Geol Soc Amer* , **60**, 1541 (1949)
[6] Stewart, A., *Proc Calif Acad Sci* , Fourth Series, **1**, 375 (1912).
[7] Vinton, K W , *Amer J Sci* , **249**, 356 (1951)
[8] Wilde, Pat, *Deep-Sea Res* , **13**, 635 (1966)
[9] Gutenberg, B , *Bull Geol Soc Amer* , **62**, 427 (1951)
[10] Shumway, G , *J Geol* , **62**, 573 (1954)
[11] Chayes, F , *Mineralog Mag* , **34**, 126 (1965).
[12] Engel, A E J , Engel, C G , and Havens, R G , *Bull Geol Soc Amer* , **76**, 719 (1965)
[13] Engel, A E J , and Engel, C G , *Science*, **146**, 477 (1964)
[14] Doell, R R , and Cox, A , in *Methods in Palaeomagnetism*, 159 (Elsevier, Amsterdam, 1967)
[15] Doell, R R , and Cox, A , in *Methods in Palaeomagnetism*, 136 (Elsevier, Amsterdam, 1967)
[16] Doell, R R , and Cox, A , in *Methods in Palaeomagnetism*, 241 (Elsevier, Amsterdam, 1967)
[17] Tarling, D H , *Geophys J* , **11**, 423 (1966)
[18] Cox, A , Hopkins, D M , and Dalrymple, G B , *Bull Geol Soc Amer* , **77**, 883 (1966)
[19] Lanphere, M A , and Dalrymple, G B , *J Geophys Res* , **70**, 3497 (1965)
[20] Cox, A , Doell, R R , and Dalrymple, G B , *Science*, **144**, 1537 (1964)
[21] Doell, R R , Dalrymple, G B , and Cox, A , *J Geophys Res* , **71**, 531 (1966)
[22] McDougall, I , and Chamalaun, F H , *Nature*, **212**, 1415 (1966)
[23] Grommé, C S , and Hay, R L , *Nature*, **200**, 560 (1963)
[24] Grommé, C S , and Hay, R L , *Earth and Planet Sci Lett* , **2**, 111 (1967)
[25] Cox, A , Hopkins, D M , and Dalrymple, G B , *Bull Geol Soc Amer* , **77**, 883 (1966)
[26] Chamalaun, F H , and McDougall, I , *Nature*, **210**, 1212 (1966)
[27] McDougall, I , Allsopp, H L , and Chamalaun, F H , *J Geophys Res* , **71**, 6107 (1966)
[28] Cox, A , and Dalrymple, G B , *J Geophys Res* , **72**, 2603 (1967)
[29] Ninkovich, D , Opdyke, N , Heezen, B C , and Foster, J H , *Earth and Planet Sci Lett* , **1**, 476 (1966)
[30] Cox, A , and Dalrymple, G B , *Nature*, **209**, 776 (1966)

Extragalactic Soft X-ray Astronomy

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The opacity of the galaxy to soft X-rays may be less than previously supposed This may permit a variety of extragalactic objects to be observed at ~ 50 Å Some implications of this situation are discussed here

WE wish to discuss in this article some astrophysical implications of soft X-ray observations Our discussion is motivated by new evidence which indicates that the galaxy may be more transparent to X-rays than was previously believed, and that extragalactic radiation of wavelength ~ 50 Å (or even longer) may penetrate to us without severe attenuation

The X-ray opacity of the galaxy is straightforwardly related to the column density of matter along the line of sight It has traditionally been thought that 21 cm radio measurements yield a direct measure of the column density of hydrogen atoms· the optical depth of the galaxy to X-rays can then be calculated, making allowance for contributions from molecular hydrogen, from helium, and from heavy elements in interstellar space This calculation leads to the conclusion that the optical depth at 50 Å $\gtrsim 1$ in almost all directions There is now, however, some evidence which suggests that the amount of hydrogen in interstellar space may be significantly lower than the value inferred from 21 cm data Some recently obtained ultraviolet spectra of stars in the direction of Orion reveal that the Lyman α absorption line is surprisingly narrow[1] Fischel and Stecher[2] have made a proposal which may resolve the discrepancy They show that "pumping" by blue-shifted Lyman α radiation can lead to population inversion in the hyperfine states of atomic hydrogen. Because 21 cm radiation would be amplified by the ensuing maser action, a lower column density of hydrogen is then needed to produce a given intensity in the 21 cm line

Further evidence that the interstellar hydrogen density may be lower than the conventional estimates was recently reported by Bowyer, Field and Mack[3], who have measured the X-ray flux in the 44–70 Å range over a wide area of sky They have discovered a background flux, stronger towards the Galactic Pole, which probably originates outside the galaxy If this flux is truly isotropic (as is the X-ray background at shorter wavelengths), the observed latitude dependence indicates that the opacity of the galaxy should be about three times lower than the usual estimates In consequence, the optical depth at 50 Å would be $\lesssim 1$ over a wide area of sky at high galactic latitudes Although the optical depth goes roughly as λ^3, observations at even 100 Å may be feasible in certain directions where there are "windows" in the hydrogen density. Our aim in this article is to emphasize the potential importance of very soft X-ray observations. While it will clearly be valuable to extend the spectra of non-thermal X-ray sources towards longer wavelengths, the chief interest of such observations lies in the possibility of detecting thermal emission by hot gas. Whereas such gas would need to have a temperature approaching 10^7 °K to be detectable at wavelengths shorter than 10 Å, very soft X-ray observations may reveal thermal sources at $\sim 10^6$ °K. Moreover, absorption by diffuse intergalactic matter may become detectable. We shall also make some tentative inferences from the results of Bowyer, Field and Mack, which provide the only published information on ~50 Å X-rays.

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Diffuse X-ray Background and Intergalactic Absorption

Bowyer *et al.* observed an X-ray flux near the Galactic Pole of $\sim 5\times10^{-25}$ erg cm^{-2} sec^{-1} ster^{-1} (Hz)$^{-1}$ at ~44 Å (uncorrected for galactic absorption). Even according to the conventional estimates, the optical depth of the galaxy is only ~1 in this direction. Unless this is a serious underestimate, we can set a limit to the intensity of background X-rays incident on the galaxy, and we can infer that, if the density of the intergalactic medium is $n\sim10^{-5}$ particles cm^{-3}, its temperature T must satisfy $T(z)\lesssim 5\times10^5\,(1+z)^\circ$ K at all red-shifts z.

A background flux of X-rays with a spectrum $\nu^{-\alpha}$, with $\alpha\sim1\cdot2$, is known to exist at shorter wavelengths[4]. This background is isotropic to within 10 per cent, and is probably universal. It has been commonly interpreted as inverse Compton radiation resulting from scattering of the primeval black-body radiation by relativistic electrons[5,6]. If this interpretation is correct, the spectrum would be likely to maintain the same slope right down to the very soft X-ray range. (A spectrum which flattens at longer wavelengths could be explained, but a steepening would not be expected.) A straightforward extrapolation of the observed X-ray background spectrum to ~50 Å leads to a flux of the order of three times greater than that observed by Bowyer *et al.* We can therefore conclude that either (*a*) the optical depth of the galaxy at ~50 Å does indeed have the conventionally assumed value ~1 at high galactic latitudes, and we are observing a background flux with a straight power-law spectrum*, or (*b*) the opacity of the galaxy is lower, but the background has a spectrum which flattens at long wavelengths, either intrinsically or because of extragalactic absorption. In both cases it follows that the optical depth of the intergalactic medium to soft X-rays is $\lesssim 1$, out to a red-shift of at least $z\sim1$. As Bowyer *et al.* point out, this implies that, for $n\sim10^{-5}$ particles cm^{-3}, hydrogen and helium must be at least ~99 per cent ionized. If the ionization is collisional, this requires $T\gtrsim1.5\times10^5$ °K at least out to $z\sim1$.

An interesting limit can also be set on the abundance of heavy elements in intergalactic space. If all elements are present with their "cosmical" abundance in a cool gas, Bell and Kingston[7] have calculated that the heavy elements contribute about 50 per cent as much as hydrogen and helium together to the opacity at ~50 Å (and proportionately more at shorter wavelengths). Whereas the intergalactic gas can be hot enough for hydrogen and helium to be almost completely ionized, however, it cannot (if $n\sim10^{-5}$ particles cm^{-3}) be hot enough to strip the heavy elements of their K-shell electrons which are responsible for the X-ray opacity. If the optical depth $\lesssim 1$, heavy elements in intergalactic space must have less than 2 per cent of their "cosmical" relative abundance (with correspondingly weaker limits if n is lower). We would not expect them to be present with their full local abundance relative to hydrogen[8], but this is nevertheless a highly significant result, because it sets a limit to the cumulative contamination of intergalactic space by galaxies and exploding objects during the life of the universe.

If the inverse Compton explanation for the X-ray background is the correct one, it is probable that most of the observed X-rays were emitted at very early epochs corresponding to $z\gtrsim5$ (ref. 6). This is because the black-body radiation would then have been more intense, and also because radio sources (and thus presumably relativistic electrons) may have been produced more profusely in the past[9]. We may therefore be able to set limits on the opacity of the intergalactic gas at these very large red-shifts, which would be especially interesting because all the other direct evidence for the state of ionization of intergalactic gas—the absence of Lyman α absorption in the spectra of quasi-stellar objects[10], and the 21 cm observations[11]—refers only to $z\lesssim2$. In calculating the absorption at very large red-shifts, we must allow for the facts that $n\propto(1+z)^3$, that the cross-section for absorption of a photon of observed wavelength λ by hydrogen or helium atoms is roughly proportional to λ^3 (that is, to $(1+z)^{-3}$) and that the effective path length (in the Einstein–de Sitter model) $\propto(1+z)^{3/2}$. As a result of these effects, we can only conclude that the degree of ionization of hydrogen and helium is >95 per cent for $z\sim2$–5—a rather less sensitive result than for smaller red-shifts. We still obtain an upper limit of ~2 per cent for the heavy element abundance relative to its "cosmical" value, however (if $n\sim10^{-5}$ cm^{-3}), because absorption by these elements is relatively more important at shorter wavelengths.

If an accurate spectrum of the X-ray background between, say, 1 Å and 50 Å were available, we might be able to decide whether the long wavelength flux is reduced by intergalactic absorption, and, if so, where the absorption is taking place. The possibility that the intergalactic gas was highly ionized at epochs corresponding to $z\sim5$, implying that $T\gtrsim2.5\times10^5$ °K, is an interesting further constraint on its permitted thermal history. It would also imply that radio waves from distant sources had an appreciable chance of being Thomson scattered by the gas[10].

Intergalactic Gas in Clusters of Galaxies

It is possible that the intergalactic medium is chiefly concentrated within clusters of galaxies, rather than being distributed smoothly. It has, for example, been suggested that the gas density within the Local Group (which is ~1 Mparsec in extent) may be as high as $\sim10^{-3}$ cm^{-3}. If this quantity of gas is indeed present, its temperature must be below 10^6 °K, otherwise it would emit a flux of soft X-rays in excess of what is observed. If other clusters of galaxies are similar to ours, however, they will, collectively, make a larger contribution to the X-ray background than the Local Group, because there would be several clusters with $z<1$ along any line of sight. We can therefore place an upper limit on the temperature of the gas within a typical cluster, which is slightly more stringent than in the smoothed-out case because the radiative losses per particle are proportional to the gas density. The limits on absorption would, however, be the same.

We consider separately those exceptionally large clusters, such as the ones in Virgo and Coma, which may contain large quantities of intergalactic gas, needed to satisfy the virial theorem[12]. Coma, for instance, would require $\sim3\times10^{48}$ g, and if this mass of gas were spread uniformly through a sphere of ~3.5 Mparsec diameter and had a temperature $\sim10^6$ °K, the 50 Å flux at our galaxy would be $\sim4\times10^{-26}$ erg cm^{-2} sec^{-1} (Hz)$^{-1}$

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* If we adopted alternative (*a*) we could determine how the apparent discrepancy between the hydrogen column density and the X-ray opacity depends on direction by correlating the observed X-ray flux with the 21 cm intensity. One might thus obtain some insight into the cause of the discrepancy.

This approach contrasts with that used by Bowyer *et al.* who supposed that the X-ray opacity was proportional to the 21 cm intensity—which is equivalent to assuming that the process causing the anomalous 21 cm emission is equally efficient in all directions (the inferred discrepancy being a factor ~3).

The Coma cluster lies near the Galactic Pole, where, according to Bowyer *et al.*, the optical depth of the galaxy is $\lesssim 1/3$, and such a high flux would easily have been detected by these workers, despite the fact that their beam covered ~ 5 times the solid angle subtended by the cluster. Even if the higher conventional estimate for the opacity of the galaxy is correct, this flux would still be detectable. There is no evidence for enhanced emission from the Coma direction on the map published by Bowyer *et al.*, so we can presumably conclude that, if the "missing mass" in the Coma cluster is indeed in gaseous form, its temperature must be $\lesssim 5\times10^5$ °K. The expected X-ray intensity would be even greater if, as Woolf[13] has considered, the gas were concentrated towards the centre of the cluster or were distributed inhomogeneously. If the gas were present, but cool and neutral, its optical depth would be at least 60. The Coma cluster should then cast a shadow on the X-ray background* which would be observable by a detector with two or three times better angular resolution than that used by Bowyer *et al.* In this case, however, the cluster would be observed as a strong 21 cm emission feature and it is not[15]. If the temperature were a few times 10^5 °K, the hydrogen and the helium would be almost all ionized, but there would still be a "shadow" effect caused by heavy elements if they had more than 2 or 3 per cent of their "cosmical" abundance.

Quasars and Radio Galaxies

The only two extragalactic objects which are already known or suspected to emit detectable X-rays at shorter wavelengths are Virgo A and *3C* 273 (refs. 16 and 17). Although it is difficult to identify discrete X-ray sources without better angular resolution than that used by Bowyer *et al.*, a strong source might still be detectable above the background. One of the patches of strong soft X-ray emission on the map published by Bowyer *et al.* seems to coincide with the position of *3C* 273 ($l^{\rm II} = 290°$, $b^{\rm II} = +64°$). Although this may be a coincidence or (in view of the quoted errors) a chance fluctuation, it is worth considering the possibility that the excess flux from this patch of sky does indeed come from *3C* 273. Although this source is at a high galactic latitude, it lies in a direction where the 21 cm emission is rather high: the optical depth of the galaxy in this direction is ~ 1 according to the analysis of Bowyer *et al.* and ~ 3 on the conventional analysis. Taking Friedman and Byram's estimate of the intensity of *3C* 273 at 1–10 Å, we have calculated what spectrum the source would need to have in order to produce ~ 8 counts/sec in the detector used by Bowyer *et al.*, assuming that there is no absorption along the line of sight apart from that caused by the galaxy. We find that, if the spectrum is a power law, the slope has to be ~ -3 if the opacity has the conventionally assumed value, and ~ -1.5 if the lower estimate of Bowyer *et al.* for the galactic opacity is taken.

We do not, of course, know whether the spectrum of *3C* 273 is a power law (as would be likely if the X-rays are synchrotron or inverse Compton radiation) or an exponential thermal spectrum. It does not prima facie seem unreasonable, however, to suggest that the excess counts reported by Bowyer *et al.* in the direction of *3C* 273 are in fact emitted from this source. If this is indeed so, other interesting consequences follow. Koehler and Robinson[18] have reported 21 cm absorption in the radio spectrum of *3C* 273, which they attribute to neutral hydrogen in the Virgo cluster. If these observations are correct, they imply a H I column density of $\sim 1.3\times10^{19}$ T_s atoms cm^{-2}, where T_s is the spin temperature. The optical depth at 50 Å caused by this gas alone is therefore $\sim 4\times10^{-2}\,T_s$, if there are ~ 10 per cent of helium atoms in the cloud. Assuming that the X-ray opacity resulting from the cloud is $\lesssim 1$, we deduce directly that $T_s \lesssim 25°$ K. Such a low value for T_s is not unreasonable (and was inferred by Koehler[19] from other considerations), but this is a significant constraint on the properties of the H I cloud responsible for the 21 cm absorption.

Although Virgo A was found by Friedman and Byram[16] to be ~ 5 times stronger than *3C* 273 in the 1–10 Å wavelength range, there is no evidence on the map of Bowyer *et al.* for enhanced soft X-ray emission from its position. Koehler and Robinson found that the 21 cm absorption from this source was 25 per cent weaker than in *3C* 273. Koehler[19] pointed out, however, that there may be, within the galaxy *M*87, a large amount of gas with too high a spin temperature to contribute to the 21 cm absorption. This material would absorb X-rays, and an extra optical depth as low as ~ 2 would suffice to make Virgo A appear weaker than *3C* 273 at ~ 50 Å, even if its intrinsic spectrum were similar. Soft X-ray observations of extragalactic radio sources would thus not only extend our knowledge of their intrinsic spectra, but also place restrictions on the permitted properties of the diffuse intervening medium. It has recently been reported[17] that the X-ray spectrum of Virgo A is fairly flat at ~ 8 Å. This may indicate appreciable absorption even at ~ 8 Å, or an intrinsically flat spectrum. If either of these alternatives is correct, Virgo A would be too weak to have been detected at ~ 50 Å by Bowyer *et al.*

Accretion Wakes of Galaxies

Another possible source of soft X-rays is the accretion wake of the galaxy (and possibly of other galaxies). Ruderman and Spiegel[20] have discussed the flow pattern which would arise from the galaxy's motion, with supersonic velocity, through the general intra-cluster gas. They show that there would be a weak gravitationally enhanced conical shock behind the galaxy, and that, in a direction opposite to that of the galaxy's motion (that is, along the axis of the shock), there would be a "tail" of enhanced density, where the temperature could exceed 10^6 °K. They pointed out that this "tail" could be a source of soft X-rays. Its position in the sky can be estimated from an analysis of the velocities of galaxies in the Local Group[21], and it turns out to lie in the direction $l^{\rm II} \sim 340°$, $b^{\rm II} \sim +20°$. Bowyer *et al.* do in fact find intense X-ray emission from this direction, as well as enhanced emission from the whole surrounding area of sky. This coincidence is suggestive, although it may, of course, be merely due to chance.

An apparently unrelated coincidence is the following: the centre of the radio feature known as the North Polar Spur also lies in the same direction[22]. The Spur is widely believed to be the envelope of an old supernova remnant[23] (although there are difficulties associated with this interpretation[24]), so an alternative theory to explain the X-rays from $l^{\rm II} \sim 340°$, $b^{\rm II} \sim +20°$ would be to say that they come from the debris of the same explosion which gave rise to the Spur. It would then be pure chance that the centre of the Spur lies in the expected direction of the accretion wake of the galaxy. This last remark does, however, suggest a radically different interpretation, according to which the radio Spur either delineates the conical shock wave predicted by Ruderman and Spiegel[20] or results from the impact on the galactic disk of gas streaming in from the accretion wake. The fact that the centre of the Spur, the observed region of enhanced X-ray emission, and the expected accretion wake all lie in the same direction would not then be accidental. Furthermore, such a theory would account for the strong 21 cm emission from this direction and for the fact that the zone of avoidance seems to extend further from the galactic plane in this direction

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* Gould and Sciama[14] suggested in 1964 that galaxies might cast shadows on the X-ray background. An angular resolution better than 1 degree would be needed to detect this effect. If it were possible to observe a shadow cast by a nearby galaxy, such as Andromeda, which has been mapped at 21 cm, one would be able to test whether amplification is causing a discrepancy between the 21 cm intensity and the X-ray opacity similar to that which may occur in our own galaxy. Of course such observations would also test whether the X-ray background is truly extragalactic.

High Velocity H I Clouds

Another source of diffuse X-rays near the galaxy might be associated with gas accreted by the galaxy from directions other than that of the accretion wake High velocity clouds of neutral hydrogen have been detected by 21 cm observations in the general direction $l^{II} = 70°–170°$, $b^{II} = +30°–+60°$ (ref 25) These clouds all seem to be falling into the galaxy with typical velocities of ~ -120 km sec^{-1}, and Oort[26] has suggested that they have an extragalactic origin If they have been captured by the galaxy's gravitational field, however, they should now have velocities of the order of 500 km sec^{-1} towards us, unless their speeds have been reduced in some way They may have been braked by cosmic ray pressure[27], but an alternative possibility is that the clouds have been braked by interaction with gas in the galactic halo[26] In the latter case, they would almost certainly impinge on the halo with supersonic velocities, so their motion would lead to the formation of shock waves, in which temperatures of a few million degrees would be attained[28] The gas behind these shocks would then emit soft X-rays The observed clouds lie in directions where the opacity of the galaxy is not too great, and calculations show that, if the gas density in the halo is adequate to produce the required deceleration, X-rays from the associated shocks may be detectable These X-rays may contribute to the flux observed by Bowyer *et al* away from the Galactic Pole, and future observations should enable us to set restrictions on the mass of the clouds, and perhaps to decide whether the braking is more likely to be caused by the halo gas or by cosmic rays The temperatures behind the shock waves are determined chiefly by the cloud velocities, and we would expect only very soft X-rays These would not be detectable except by the long wavelength observations which now for the first time seem feasible if the opacity of the galaxy has indeed been overestimated

Conclusions

Our discussion shows that important information on a variety of extragalactic phenomena could be obtainable from soft X-rays ($\sim$50–100 Å) if the column density of hydrogen in our galaxy were a few times less than is implied by a straightforward analysis of the 21 cm data Preliminary observational and theoretical considerations suggest that this is a very real possibility In view of the importance of the X-ray observations which would become possible, and also of the interstellar questions involved, we would urge that further observational and theoretical studies of this problem be carried out

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[1] Morton, D C , *Astrophys J* ,**147**, 1017 (1967) Jenkins, E B , and Morton, D C , *Nature*, **215**, 1257 (1967)

[2] Fischel, D , and Stecher, T P , *Astrophys J* ,**150**, L51 (1967)

[3] Bowyer, C S , Field, G B , and Mack, J E , *Nature*,**217**, 32 (1968)

[4] Gould, R J , *Amer J Phys* , **35**, 376 (1967)

[5] Hoyle, F , *Phys Rev Lett* ,**15**, 131 (1965) Felten, J E , and Morrison, P , *Astrophys J* , **146**, 686 (1966)

[6] Bergamini, R , Landrillo, P , and Setti, G , *Nuovo Cim* , **52B**, 495 (1967) Brecher, K , and Morrison, P , *Astrophys J* , **150**, L61 (1967) Rees, M J , *Mon Not Roy Astro Soc* ,**137**, 429 (1967)

[7] Bell, K L , and Kingston, A E , *Mon Not Roy Astro Soc* ,**130**, 373 (1967)

[8] Wagoner, R V , Fowler, W A , and Hoyle, F , *Astrophys J* ,**148**, 3 (1967)

[9] Davidson, W , and Davies, M , *Mon Not Roy Astro Soc* ,**127**, 241 (1964), Longair, M S , ibid ,**133**, 421 (1966) Rowan-Robinson, G M , *Nature* **216**, 1289 (1967)

[10] Gunn, J E , and Peterson, B A , *Astrophys J* ,**142**, 1633 (1965)

[11] Field, G B , *Astrophys J* , **135**, 684 (1962) Goldstein S J ibid , **138**, 978 (1963) Davies, R D , and Jennison, R C , *Mon Not Roy Astro Soc* ,**128**, 123 (1964)

[12] Limber, D N , *Problems of Extragalactic Research* (edit by McVittie, G C), 239 (Macmillan, 1962)

[13] Woolf, N J , *Astrophys J* ,**148**, 287 (1967)

[14] Gould, R J , and Sciama, D W , *Astrophys J* ,**140**, 1634 (1964)

[15] Muller, C A , *Paris Symposium on Radio Astronomy* (edit by Bracewell, R N), 465 (Stanford Univ Press, 1959)

[16] Friedman, H , and Byram, E T , *Science*, **158**, 257 (1967)

[17] Bradt, H , Mayer, W , Naranan, S , Rappaport, S , and Spada, G , *Astrophys J* (in the press)

[18] Koehler, J A , and Robinson, B J , *Astrophys J* ,**146**, 488 (1966)

[19] Koehler, J A , *Astrophys J* ,**146**, 504 (1966)

[20] Spiegel, E A , *Lectures on Interstellar Gas Dynamics* (Univ of Wisconsin, 1966)

[21] Humason, M L , and Wahlquist, H D , *Astron J* , **60**, 254 (1955) Byrnes, D V , *Pub Astro Soc Pacific*, **78**, 46 (1966)

[22] Large, M I , Quigley, M J S , and Haslam, C G T , *Mon Not Roy Astro Soc* ,**124**, 405 (1962)

[23] Hanbury Brown, R , Davies, R D , and Hazard, C , *Observatory*, **80**, 191 (1960)

[24] Bingham, R G , *Mon Not Roy Astro Soc* ,**137**, 157 (1967)

[25] Hulsbosch, A N M , and Raimond, E , *Bull Astro Inst Netherl* ,**18**, 413 (1966)

[26] Oort, J H , *Bull Astro Inst Netherl* ,**18**, 421 (1966)

[27] Puppi, G , Setti, G , and Woltjer, L , *Nuovo Cim* , **45**, 252 (1966)

[28] Savedoff, M P , Hovenier, J W , and Van Leer, B , *Bull Astro Inst Netherl* ,**19**, 107 (1967)

Directed Emission Model for Quasars

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The difficulties of accounting for the powerful emission of energy from quasars can be resolved if the emission is assumed to be concentrated in one direction

If quasar red-shifts are assumed to be cosmological, considerable difficulties arise in reconciling short term variations with the magnetic and particle energies[1] If, on the other hand, the quasars are assumed to be local[2], it is very difficult to find a satisfactory explanation of the red-shift It would therefore seem that alternative and even somewhat artificial mechanisms may be worthy of consideration The possibility of a directed emission has been informally suggested, but its implications have not been considered in detail Coherent but isotropic emission has been suggested by Ginzburg and Ozernoy[3]

If the radiation is assumed to be isotropic, an energy emission of the order 10^{46} ergs per second is required in the continuum from 10^{7}–10^{15} Hz Mechanisms may exist, however, by means of which a beam of narrow angular spread could be produced We shall assume a number of sources of this type distributed with random orientation throughout the universe and having a characteristic beam width of the order of 0 1 sec of arc, 5×10^{-7} radians We will take account only of those which are directed towards us. The value of θ is chosen somewhat arbitrarily, the beamwidth could in fact be considerably larger than the observed angular diameter of the source In the mechanism which will be proposed, our choice is limited —chiefly by the very small proper motions which are observed

Accepting the specified value, however, the energy requirement for each source is now $E = 10^{46} \times \theta^{2}/4 = 10^{33}$ ergs/sec This is comparable only with the radiation from the Sun, so that the phenomenon becomes stellar in scale, rather than galactic The number of such sources must be very much larger than the number of quasars which have been observed About one hundred have been seen in a volume which is about one-tenth of the observable universe. Assuming that this is not a gross underestimate of the true density, the total number which is now required is about $10^{3} \times 4/\theta^{2} = 10^{16}$. If the phenomenon is intragalactic, 10^{6} sources are required for each galaxy, but the probability of observing one in our own or an adjacent galaxy is only about 10^{-7} because of the narrow beam width Quasar phenomena are therefore rather common occurrences in this model, they are stellar in magnitude, and may be individually quite short-lived

Experimentally, the lifetime of each quasar need only be about 100 years, and large variations in intensity are observed on time scales which are significantly shorter than this The energy requirement per quasar is therefore about 10^{42} ergs, and the power expended in each galaxy is about 3×10^{46} ergs per year This value is independent of the assumed beam width and is modest compared with the cosmic ray energy in our galaxy, which requires an estimated injection power of 10^{48} ergs/yr[4] The similarity of these orders of magnitude is itself striking

A mechanism for such emission presents difficulties which are not insuperable The injection of a stream of high energy particles into a region of uniform magnetic field could possibly produce enough power The expansion of a relativistic gas in the presence of magnetic fields will be along the field lines[5], and it has also been shown that non-spherical expansion of shock fronts from supernovae is possible[6] In these cases, the beam width and energy requirement would be much greater than in the example which is given here, and the number of sources would be correspondingly less

An alternative possibility could be a phenomenon which is analogous to the extensive air shower, developing over distances of galactic order of magnitude and initiated by a single high energy particle or coherent bunch of particles, as suggested by Hayakawa[7] In such a cascade, the medium of development is the isotropic microwave flux[8] Electron-photon development would be determined by the inverse Compton and photon-photon pair production cross-sections[9–11], and the nuclear development by the 3/2, 3/2 resonance cross-section[12] Loss of electrons by synchrotron radiation would be relatively rapid, and the principal directed continuum synchrotron emission would come rather from mesons, nucleons and anti-nucleons Multiple scattering would arise only from irregularities of the magnetic field, so that the disk of particles would remain thin in the line of sight, and lateral spread or curvature would be small The angular beam width would be of the order mc^{2}/E, where E is the typical particle energy, but at radio wavelengths phase coherent dipole emission from the whole disk might occur, modifying both the beam width and frequency spectrum In models of this type, the power requirement is considerably less than 10^{33} ergs/sec This is because, as in the case of Čerenkov radiation from an air shower, the radiation travels with the disk and is deposited in a time short compared with the transit time The effective time contraction factor could, in principle, be as large as $1/\theta^{2}$, but in practice would probably be determined by differences in path length, with a possible contribution from the differential recessional velocity over the path traversed If the phenomenon is confined to galaxies, the contraction factor would be of the order of 1,000 for an apparent lifetime of 100 years For extragalactic emission, it could be as large as 10^{8}, a limit being set by the Hubble constant Large fluctuations in power[13] would be caused by transit through field irregularities, but these would also be contracted in time scale Power output requirements for the continuum are about 10^{23} W for a galactic effect, or for an extragalactic effect could be as low as 10^{18} W

For protons, lower limits on particle energies are set by the requirement that the deviation of the shower paths in magnetic fields must be less than 0 1 sec of arc over the total path length observed With $B = 10^{-6}$ gauss, particle energies of about 10^{25} electron-volts are required, but because synchrotron losses are large, at these energies, even for protons, rapid degradation would occur, both by nucleon-photon collisions[12] and by synchrotron losses, to effective energies in the region 10^{16} electron-volts The critical frequency for protons would be in the soft X-ray region The total energy of the phenomenon must, of course, be about 10^{42} ergs, and a very large number of particles—about 10^{26}—must be radiating at any given time Because of the coherent dipole character of the radiation at long wavelengths, some apparent structure would be expected, but this would arise from the overlapping of coherent wavefronts rather than from a real linear separation

To satisfy observational requirements, line spectra must be excited coherently as the flux which is observed in such lines as Hβ is of the order 10^{42} erg/sec, assuming isotropic emission The inherent diffraction width for coherent radiators, spread over a distance T, is $\theta = (\lambda/T)^{\frac{1}{2}}$, so that, at optical frequencies, phase coherence must be preserved over distances of about 10^{8} cm The short wave radiation of the continuum traversing a galaxy would excite interstellar gas atoms By analogy with the laser, these would then emit stimulated emission as a result of being triggered by the appropriate wavelength of the continuum The continuum consists of wave trains which, although not mutually coherent, would be of sufficient length to constrain the line emission into an angular distribution similar to that of the continuum The power requirement is about 10^{22} W, and emission would be observed from a cylindrical volume of about 10^{63} cm^{3}, which in a normal galaxy contains enough atoms to give the required emission. Because of the time contraction of the continuum, the excitation power is sufficient If the shower disk traverses the galaxy, some excitation would be caused by the particles also, but because the diameter of the disk would only be about 10^{6} cm, the number of atoms which would be affected would be relatively small Line broadening would arise from differential red-shift across the emitting galaxy, and the overall red-shift would be that corresponding to the distance of the galaxy

Not all the quasar lines which have been observed[14] have been found in interstellar spectra, but because the conditions of excitation are unusual, this difficulty may not be serious Rapid changes in line intensities, again time contracted, would be expected, as the continuum traverses interstellar clouds The presence of line emission does not necessarily imply that the continuum is produced in galaxies, for there is a significant possibility that radiation originating at cosmological distances will intersect a galaxy on its path to the observer

The polarization which would be expected in coherent phenomena, or in incoherent radiation from particles traversing the same magnetic field, would remain in this model, but would change rapidly Complete polarization would not be expected at any time as the wave trains from different regions will overlap

There are, of course, very serious difficulties in explaining the acceleration of cosmic ray particles, even to the energies of about 10^{20} electron-volts which have been observed It would probably be necessary to invoke particles such as the magnetic monopole[15], with subsequent production of monopole pairs, to obtain a sufficiently high energy

The possible contributions of monopoles to the primary cosmic radiation have been pointed out previously[16], and it would seem that there is now some tentative evidence for their existence[17] Fermi acceleration mechanisms and production cross-sections for cosmic ray monopoles have been considered by Goto[18] and Alvarez[19]

In the present case, we postulate a cascade proceeding in a region of reasonably linear magnetic field The principal production cross-section for monopole pairs for incident monopoles of 10^{18}–10^{20} electron-volts, in galactic or halo regions, is probably with the microwave flux If ν is the average production multiplicity, and l the distance traversed in interaction lengths, the number of monopoles $N = \left(\frac{\nu}{2}\right)^{l}$, because only one sign of pole will contribute to the cascade Opposite poles will be decelerated, come to rest and initiate individual cascades of their own

The rate of extraction of energy from the magnetic field is $NgBD$ ergs per yr, where N is the number of monopoles, g the pole strength (assumed $3\ 3 \times 10^{-8}$ EMU), B the field and D one light year In a field of 5×10^{-6} gauss, 10^{32} monopoles are required to extract 10^{23} Watts If we assume $\nu = 20$, and a cross-section 10^{-25} cm^2, for interaction with the microwave field, the required value of N will be reached in 3×10^4 yr These assumed values are, of course, arbitrary, but not entirely implausible (Because $g^2/\hbar c = 34$, multiple production is expected, even in electromagnetic interactions) Effects of radiation damping and magnetic space charge are complex, and could slow down significantly the development of the process Individual particle energies greater than 10^{20} electron-volts are not required in this model, for collimation would be preserved by the monopole component, and the electromagnetic radiation would be emitted by a rapidly degraded secondary electron component The diameter of the disk in this model is 10^{16} to 10^{17} cm, and the magnetic field energy swept out is sufficient to provide the observed power As in the proton cascade, time contraction occurs and structural changes may be seen at a speed apparently greater than the velocity of light

If we wish to explain the normal cosmic radiation within our galaxy as the degraded residue of this process, the total number of monopoles required to extract 10^{48} ergs/yr from the magnetic field is about 10^{43} This corresponds to an average density of 10^{-24} monopoles/cm^3, which is compatible with the experimental upper limits[17,20,21]

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[1] Hoyle, F , Burbidge, G R , and Sargent, W L W , *Nature*, **209**, 751 (1966)·
[2] Arp, H , *Science*, **151**, 1214 (1966)
[3] Ginzburg, V L , and Ozernoy, L M , *Ap J* , **144**, 599 (1966)
[4] Ginzburg, V L , and Syrovatskii, S I , *The Origin of Cosmic Rays*, 31 (Pergamon, 1964)
[5] Gold, T , *Proc Ninth Intern Conf on Cosmic Rays*, London, 132 (1965)
[6] Oda, M , and Hasegawa, H , *Proc Eighth Intern Conf on Cosmic Rays*, Jaipur, 3, 370 (1963)
[7] Hayakawa, S , *Prog Theor Phys Suppl* , **37**, 594 (1966)
[8] Penzias, A A , and Wilson, R W , *Ap J* **142**, 419 (1965)
[9] Felten J E , and Morrison, P , *Phys Rev Letters* **10**, 453 (1963)
[10] Gould, R J , and Schreder, G , *Phys Rev Letters*, **16**, 252 (1966)
[11] Jelley, J V , *Phys Rev Letters*, **16**, 479 (1966)
[12] Greisen, K *Phys Rev Letters*, **16**, 748 (1966)
[13] Low, F J , *Astrophys J* , **142**, 1287 (1965)
[14] Burbidge, E M , and Burbidge, G R , *Proc Ninth Intern Conf on Cosmic Rays*, London, 92 (1965)
[15] Dirac, P A M , *Phys Rev* **74** 817 (1948)
[16] Porter, N A *Nuovo Cim* , **16**, 958 (1960)
[17] Kolm, H , *Physics Today*, **20**, 69 (1967)
[18] Goto, E , *Prog Theor Phys* , **30**, 700 (1963)
[19] Alvarez, L W , *Physics Notes*, Berkeley, **479**, 1 (1963)
[20] Malkus, W V R *Phys Rev* , **83**, 899 (1951)
[21] Goto, E , Kolm, H H , and Ford, K W , *Phys Rev* , **132**, 387 (1963)

Pharmacological Basis for the Tobacco Smoking Habit

by

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Small amounts of nicotine injected intravenously usually increased the lever pressing activity of rats and caused a change in the EEG of cats indicative of cortical activation These results in experimental animals are consistent with the subjective impression of some smokers that inhalation of tobacco smoke causes them to be more alert and efficient

ALTHOUGH the pharmacology of nicotine has been studied in considerable detail since Langley[1] first showed that nicotine stimulated and then paralysed ganglion cells, many of the published data are of little or no relevance to the pharmacology of tobacco smoking[2] It is generally agreed that nicotine plays an important part in the tobacco smoking habit, but in spite of much discussion and research the precise action of nicotine remains unknown[3] Johnston[4], however, showed clearly that the cravings of habitual smokers could be successfully appeased by nicotine injections instead of smoking We believe that our findings in recent experiments with nicotine on cats and rats provide an objective pharmacological explanation for the tobacco smoking habit.

Nicotine Absorption during Cigarette Smoking

The nicotine content of a puff of tobacco smoke is dependent on the way in which a cigarette is smoked. As a result of a survey carried out in the United Kingdom on behalf of the Tobacco Research Council, it has been found that most smokers take between one and two puffs

Table 1 NICOTINE CONTENT OF THE MAINSTREAM SMOKE OF STANDARD CIGARETTES

Puff volume (ml)	Average time to smoke (min)	Average nicotine in the smoke per cigarette (mg)	Average nicotine in the smoke per puff (mg)
35	9	2.5	0.25
30	10.4	2.3	0.20
25	10.2	1.96	0.17
20	11	1.64	0.14

The cigarettes, made from a blend of several different tobaccos currently used in the manufacture of British cigarettes, were smoked on an apparatus described before by Armitage[5] to a butt length of 20 mm with a puff duration of 2 sec and a puff frequency of 1/min.

each minute, a puff lasting about 2 sec with an average volume of about 25 ml. We used a pump that would smoke cigarettes in this way and which was capable of introducing any selected puff volume into the lungs of an anaesthetized cat[5]. Table 1 shows how the nicotine content of a puff of cigarette smoke varied with the puff volume when the puff duration and frequency were kept constant. The larger the volume of the puff the quicker the cigarette was smoked, with more nicotine appearing in the smoke each puff. With a 35 ml puff volume there was nearly twice as much nicotine in each puff compared with a puff volume of 20 ml. In this way, a smoker takes into his mouth a total of about 1–2 mg of nicotine during a smoking time of approximately 10 min. A biological experiment is necessary to ascertain how much nicotine is absorbed and how quickly it is absorbed. We therefore compared the rise in blood pressure caused by a puff of tobacco smoke administered to the lungs of an anaesthetized cat with the rise in blood pressure caused by nicotine injected intravenously. Fig. 1 shows the effects of 40, 80 and 120 μg of nicotine injected into a femoral vein on the femoral arterial blood pressure of a cat anaesthetized with chloralose, compared with the rise in blood pressure caused by the third puff of a cigarette (25 ml puff volume, lasting 2 sec). The time course of the rise in blood pressure caused by the puff of tobacco smoke was almost identical with that resulting from an intravenous injection of nicotine. This shows that inhalation of tobacco smoke in man is an extremely efficient way of administering nicotine. The amount of nicotine in the "man sized" puff of tobacco smoke in this and other experiments caused a rise in blood pressure similar to 120 μg of nicotine base injected intravenously, that is, a dose of about 30 μg/kg. If the average man weighs 70 kg, every time he inhales a puff of tobacco smoke he can be expected to receive a dose of nicotine roughly equivalent to between 1 and 2 μg/kg administered intravenously. It is worth noting that someone smoking a cigarette has literally finger tip control of how much nicotine he takes into his mouth; by reducing the puff volume or inhaling less frequently he absorbs less nicotine.

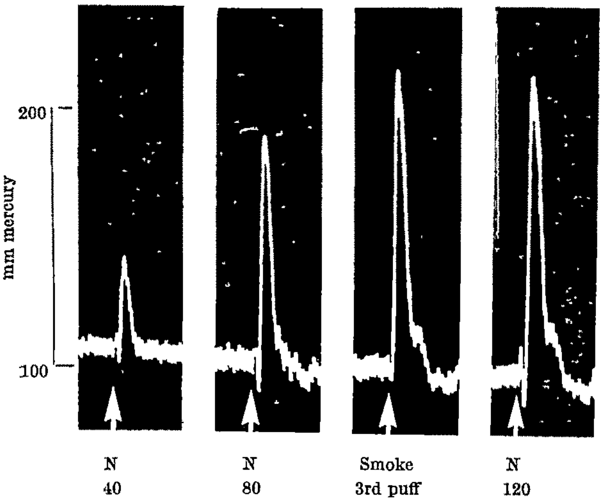

Fig. 1. Record of mean femoral blood pressure of a cat weighing 4 kg, artificially ventilated, under chloralose anaesthesia. In the first, second and fourth panels, nicotine (N) was injected into a femoral vein, the dose being 40 μg, 80 μg and 120 μg, respectively. In the third panel is shown the rise in blood pressure caused by a 25 ml puff of tobacco smoke administered to the lungs by means of a pump.

It seems likely that some people smoke in order to dose themselves with nicotine, and our particular concern has been to find out what effect these small amounts of nicotine have on a smoker. Tobacco smokers have frequently stated that they are either "tranquillized" or "stimulated" as a result of smoking a cigarette, and although this evidence is purely subjective it cannot be dismissed. To investigate this experimentally it was necessary to administer nicotine to a conscious unrestrained animal in a series of small intravenous doses and to observe its effect on some measurable aspects of behaviour. The behaviour of thirsty rats which have been trained to press a lever to obtain water is particularly sensitive to the effects of drugs acting on the brain, and we therefore decided to examine the effects of nicotine on this type of conditioned behaviour which was first described by Skinner[6]. A rat, once trained, gives a consistent day to day performance and thus serves as its own control.

Effects of Nicotine on Behaviour

Small subcutaneous doses of nicotine have been shown to increase the rate at which thirsty rats will press a lever for water rewards[7,8]. This effect is most clearly seen when rewards are made available at varying intervals, under which condition the rats press the lever at a steady rate. In a number of similar experiments, we gave nicotine intravenously as a series of small injections to a conscious unrestrained rat while it was pressing the lever in a Skinner box. For this purpose a cannula made out of polythene tubing was placed in the jugular vein during an aseptic operation and was exteriorized from the back of the neck where it remained undisturbed by the rat[9]. Fig. 2 illustrates the effect of intravenous nicotine on the lever pressing behaviour of a rat which had been trained to press a lever to get water. The lever pressing records of 3 consecutive days are shown and these were charted automatically by a pen recorder which moved upwards each time the lever was pressed. The steeper the slope of the record the faster was the rate of lever pressing. Records *a* and *c* show the lever pressing performance of the rat on 2 control days when for the first 20 min of the 90 min session the rat was given saline intravenously in a volume of 0.005 ml every 30 sec. The rat pressed the lever 558 and 714 times on these 2 days. On day 2 (record *b*) the rat was given 0.5 μg of nicotine every 30 sec for 20 min, that is, forty doses of about 2 μg/kg. Under the influence of nicotine the rat pressed the lever 1,133 times during the 90 min session, an increase of 78 per cent over its average score for the 2 control days. This dose of nicotine (2 μg/kg every 30 sec) increased the lever pressing activity of most of the rats (eleven out of eighteen, mean increase of 62 per cent), had no effect on four rats and decreased the activity of the remaining three.

Not only do the behavioural effects of nicotine vary from animal to animal but they are also dependent on the rate at which the drug is injected. Experiments have been made in which the same total amount of nicotine (80 μg/kg) was administered at different rates over a period of 20 min. In the same rat, 4 μg nicotine/kg injected every 60 sec usually caused less stimulation than 2 μg/kg every 30 sec. In a few experiments, the effects of the two regimes were the same. When the nicotine injections were given more frequently (1 μg/kg every 15 sec) the lever pressing activity of some rats was increased but the behaviour of others was depressed although they had been stimulated when the nicotine was injected more slowly.

It seems probable that the increased activity of these animals under the influence of nicotine is a manifestation of some kind of central stimulation. The effect is similar to that caused by low subcutaneous doses of nicotine, and Bovet *et al.* have shown that these doses can improve the learning ability of rats and mice in several different tests[10,11].

Effects of Nicotine on the Electroencephalogram

MacIntosh and Oborin[12] first showed that acetylcholine was spontaneously released from the surface of the brain. They collected the acetylcholine in a small cup placed on the exposed cortex and measured the amount that accumulated in a given time. This important finding initiated much research into the role of acetylcholine in the central nervous system, as a result of which there is now substantial evidence that one of its functions is that of a transmitter substance for the propagation of nerve impulses in the cerebral cortex. It has also been shown[13] that the rate of release of acetylcholine from the cerebral cortex of the cat can be related to its state of activation—in the awake animal the output of acetylcholine increases and in the sleeping animal it decreases. Simultaneous recording of the brain electrical activity confirms a positive relationship between the rate of release of acetylcholine and states of consciousness.

In an attempt to ascertain whether the increased activity of rats under the influence of nicotine was a manifestation of stimulation, we have made a series of experiments[14] on anaesthetized cats in which acetylcholine was recovered from the cortex, using the technique described by Mitchell[15], with simultaneous recordings of the electroencephalogram.

Fig. 3 shows the result of a typical experiment, on the left the acetylcholine release from the left parietal cortex in ng/20 min/cm² and on the right records of the electroencephalogram from the left and right parietal cortex (LPC and RPC) at various times during the experiment. In this experiment, the resting output of acetylcholine was in the range 2.5–4 ng/20 min/cm² and throughout this period the typical electroencephalogram is shown in *a–d*. The pattern recorded from both hemispheres resembled light sleep and consisted of slow waves punctuated by bursts of spindle activity. At the black bar, nicotine was given intravenously in a dose of 2 µg/kg every 30 sec for 20 min so that altogether forty injections of this amount were given. Shortly after the start of the 20 min injection period the electroencephalogram showed a dramatic change indicating cortical activation. The spindle bursts became less frequent in occurrence and the accompanying slow wave activity was reduced in amplitude. This electrocortical desynchronization was accompanied by an increased release of acetylcholine into the cortical cup which persisted until the end of the experiment. It should be noted, however, that the electroencephalogram had assumed its original pattern while the acetylcholine concentrations were still elevated. When twenty injections of nicotine (4 µg/kg) were given every minute for 20 min, that is, a larger dose given less frequently but the same total dose as in Fig. 3, similar effects were usually observed. In one experiment, however, there was a decrease in the acetylcholine output from about 5 ng/20 min/cm² before the nicotine was given to about 2 or 3 ng/20 min/cm² after the nicotine, and the output of acetylcholine remained at this level for more than 2 h. The electroencephalogram changed from the typical pattern of spindle bursts and slow waves before nicotine to one in which spindle burst activity was reduced and the amplitude of the accompanying slow waves was increased. These changes were indicative of a reduction in cortical activity. In ten experiments in which nicotine (2 µg/kg/30 sec) was given as a series of small intravenous injections, there was an increase in cortical acetylcholine output in seven of these experiments and there was always a positive correlation with the change in the activity of the electroencephalogram. In six experiments in which a larger dose of nicotine was given less frequently (4 µg/kg/min), cortical activation with an increased output of acetylcholine occurred in three experiments, and in two experiments there was a decrease in cortical acetylcholine output accompanied by depression of cortical activity. One of us (G. H. H.) has also shown that nicotine (2 µg/kg/30 sec) causes cortical activation and behavioural arousal in the unanaesthetized *encéphale isolé* preparation and in the conscious unrestrained cat.

It therefore seems that nicotine can have two effects; it can cause either an increase or a decrease in the amount of acetylcholine released from the parietal cortex and can cause changes in the electroencephalogram consistent either with increased or decreased cortical activity. It seems likely that the effect varies from cat to cat, and even with small changes in the dose of nicotine and the rate of injection.

There are good reasons for believing that forty intravenous injections of 2 µg/kg of nicotine given every

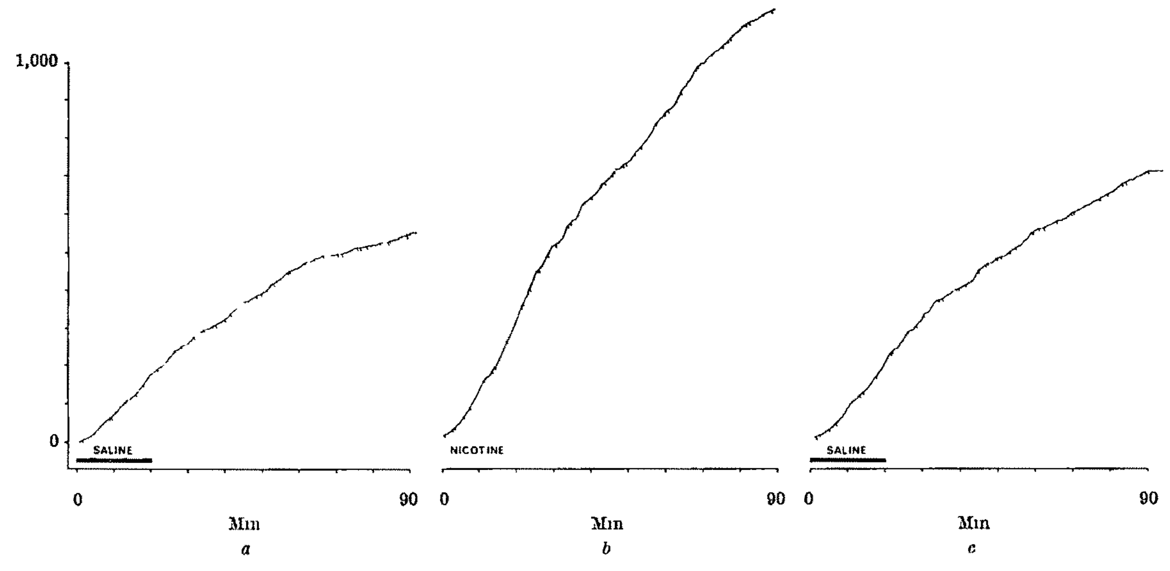

Fig. 2. The effect of intravenous nicotine (2 µg/kg every 30 sec for 20 min) on the lever pressing performance of an intravenously cannulated rat. Ordinate is number of times the lever was pressed, and abscissa is time in minutes. The small blips on the records indicate the occasions on which water was presented to the rat.

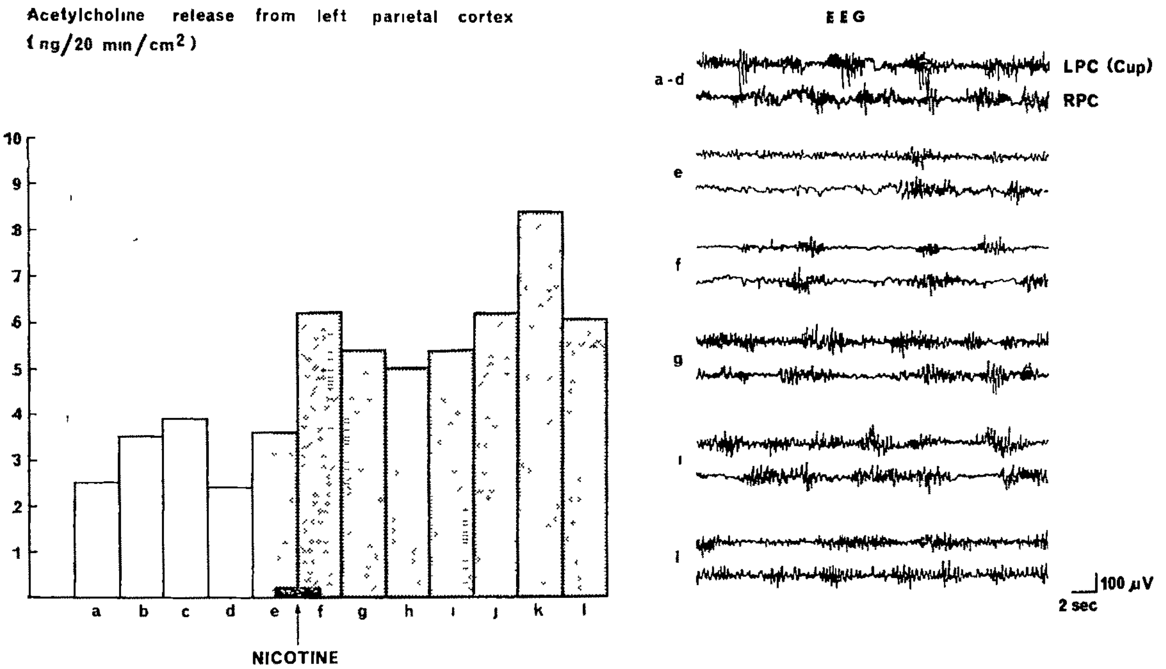

Fig. 3. Records of electrocortical activity and acetylcholine release from the left parietal cortex of a cat weighing 3.5 kg under 'Dial' anaesthesia. At the black bar, nicotine was given intravenously in a dose of 2 µg/kg every 30 sec for 20 min. Note the change in electrocortical activity (*e* and *f*) caused by the nicotine and the more prolonged changes in the amount of acetylcholine released

30 sec for 20 min is roughly the nicotine intake of a smoker, smoking two cigarettes and inhaling deeply. An increase in alertness on smoking a cigarette, that is to say an increase in cortical activity similar to that caused by intravenous nicotine in the cat, might be the explanation of the subjective impression that concentration and efficiency of the smoker are improved. We suggest that such effects of nicotine in man probably depend critically on the dose and rate of self-administration and also on whether he is excited or depressed. In this restricted sense the nicotine probably merely brings about a normal physiological response which can occur without intervention of a drug.

We are aware of the fact that the effects of nicotine that we and others (notably Domino[16] and Bovet[17]) have shown to occur in animals have not yet been conclusively demonstrated in man, and some carefully controlled experiments in human pharmacology are clearly needed.

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[1] Langley, J. N., and Dickinson, W. L., *Proc. Roy. Soc.*, **46**, 423 (1889).
[2] Comroe, jun., J. H., *Ann. NY Acad. Sci.*, **90**, 48 (1960).
[3] Van Proosdij, C., *Smoking—Its Influence on the Individual and its Role in Social Medicine* (Elsevier, Amsterdam, 1960).
[4] Johnston, L. M., *Lancet*, ii, 742 (1942).
[5] Armitage, A. K., *Brit. J. Pharmacol. Chemother.*, **25**, 515 (1965).
[6] Skinner, B. F., *The Behaviour of Organisms* (Appleton-Century-Crofts, New York, 1938).
[7] Morrison, Cathleen F., *Intern. J. Neuropharmacol.*, **6**, 229 (1967).
[8] Morrison, Cathleen F., and Armitage, A. K., *Ann. NY Acad. Sci.*, **142**, 268 (1967).
[9] Armitage, A. K., Harris, E. R., Morrison, C. F., and Sellers, C. M., *Brit. J. Pharmacol. Chemother.*, **29**, 123D (1967).
[10] Bovet, D., Bovet-Nitti, F., and Oliverio, A., *Ann. NY Acad. Sci.*, **142**, 261 (1967).
[11] Gatti, G. L., Robustelli, F., and Bucci, L., *Clinica Terap.*, **34**, 388 (1965).
[12] MacIntosh, F. C., and Oborin, P. E., *Abstr. Nineteenth Intern. Physiol. Cong.*, 580 (1953).
[13] Celesia, G. C., and Jasper, H. H., *Neurology*, **16**, 1053 (1966).
[14] Armitage, A. K., and Hall, G. H., *J. Physiol.*, **191**, 115 (1967).
[15] Mitchell, J. F., *J. Physiol.*, **165**, 98 (1963).
[16] Domino, E. F., *Ann. NY Acad. Sci.*, **142**, 216 (1965).
[17] Bovet, D., in *Tobacco Alkaloids and Related Compounds* (edit. by von Euler, U. S.), 125 (Pergamon, Oxford, 1965).

Formation and Chemical Pumping of OH Molecules

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It has been suggested that maser amplification may be the cause of intense OH lines observed in some small sources. An efficient two body pre-association of hydrogen and oxygen may lead to the formation of OH and the population inversion needed for maser amplification.

RECENTLY there have been extensive astronomical observations of four radio lines at 1612, 1665, 1667 and 1720 MHz, arising from transitions between levels of the λ doublet in the ground state of OH. The radiation in the lines is extremely strong and seems to come from small sources with brightness temperatures as high as 10^{12} °K. The line intensity ratios deviate strongly from those expected in conditions of thermodynamic equilibrium and the line width is extremely narrow. In addition, the lines exhibit varying degrees of circular and linear polarization. In order to account for the extraordinary nature of these observations, the existence of maser amplification has been suggested Recent reviews by Barrett[1] and Robinson and McGee[2] discuss the detailed observational evidence leading to this conclusion

In addition to the existence of a mechanism which leads to population inversion (and therefore a maser), there must also be a process producing the OH in appropriate astrophysical conditions In this article I suggest that the process responsible for both formation and population inversion is the relatively efficient two body pre-association of oxygen and hydrogen atoms taking place at moderately high temperatures of the order of 1,000° to 2,000° K The processes of destruction of OH must also be taken into account, for it is precisely the nature of the destruction processes which leads to the conclusion that chemical pumping as opposed to optical pumping must be responsible for the population inversion

The pre-association reaction leading to the formation is

$$\mathrm{H}(^2S) + \mathrm{O}(^3P) \rightarrow \mathrm{OH}(^2\Sigma^-) \rightarrow \mathrm{OH}(^2\Sigma^+_{v=2}) \rightarrow \mathrm{OH}(^2\Pi) + h\nu \qquad (1)$$

The potential energy diagram of the molecular states is shown in Fig 1

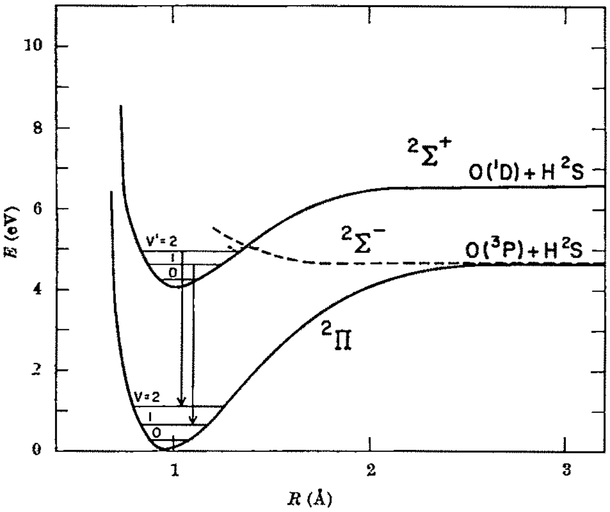

Fig 1 Potential energy curves for OH, showing the formation of ground state molecules by inverse pre-dissociation

Evidence for this reaction was first obtained by Charton and Gaydon[3], when they noticed that in low pressure flames an extremely high emission intensity originated from the $v' = 2$ level of the OH band $^2\Sigma^+ \rightarrow {}^2\Pi$ Many characteristics of an inverse pre-dissociation (pre-association), such as a strengthening of lines originating from f_1 levels and the existence of the effect only at low pressure, led to the conclusion that reaction (1) was responsible This was confirmed in an experiment by Kaskan[4], who found that the intensity of the bands is proportional to the square of the free atom density, and thus proved that a two body reaction was responsible The rate coefficient and temperature dependence measured by Kaskan is $K_1 = 3 \times 10^{-15} \exp(-3{,}700/T)$ cm³ sec⁻¹ where the rate of production of molecules is given by $N_{\mathrm{OH}} = N_{\mathrm{O}}N_{\mathrm{H}}K_1$ It should be noted that while this is slow in comparison to chemical reactions it is two to three orders of magnitude faster than other known radiative association processes[5]

The two basic types of destruction processes are chemical exchange and photodissociation Because of the large cosmic abundance of hydrogen the major chemical exchange reaction will be

$$\mathrm{H} + \mathrm{OH} \rightarrow \mathrm{H}_2 + \mathrm{O} \qquad (2)$$

with a rate coefficient[6] $K_2 = 1\ 0 \times 10^{-11} \exp(-3{,}700/T)$ Formation and destruction of OH by reactions (1) and (2) have the effect of cycling the oxygen atoms through OH and then making them available again for the production of OH Thus at any time the ratio [OH] [H] is independent of the density, but the rate of production of OH per cm³ is proportional to the square of the density There is an uncertainty in the activation energies involved of several hundred degrees, but to within experimental error the temperature dependence is the same In conditions where (2) is the dominant destruction mechanism, the abundance of OH is $[\mathrm{OH}] = 3 \times 10^{-4}\,[\mathrm{O}] = 3 \times 10^{-7}\,[\mathrm{H}]$

Recently Hughes[7] made a suggestion, based on experiments by Spindler *et al* [8], that OH may be formed through pre-association in the $v' = 0$ level The rate coefficient for this process is (see ref 8) 3×10^{-21} cm³ sec⁻¹ This is five orders of magnitude smaller than the pre-association through $v' = 2$ at a temperature of 2,000° K A consistent interpretation of these data is that the level crossing involved in these reactions occurs at an energy corresponding to $v' = 2$, and the much lower rate at $v' = 0$ is a result of the inefficiency of tunnelling through the potential barrier In the analysis of the production of OH in interstellar conditions, Hughes[7] completely neglected any destruction mechanism and thereby concluded erroneously that the OH abundance could build up indefinitely In fact, the production of OH with a rate coefficient of 3×10^{-21} cm³ sec⁻¹ is negligible because the rate of either collisional destruction or photodissociation will be ten orders of magnitude greater

The rate of photodissociation of OH, and the pumping mechanism responsible for the inversion, are closely related questions Optical pumping of OH through absorption and re-emission (at $\lambda = 3080$ Å) in the $X^2\Pi \rightarrow A^2\Sigma^+$ bands has been suggested by Perkins *et al* [9] and Litvak *et al* [10] The total f value for this band, however, is only 8×10^{-4} (ref 11) Considerations of the photodissociation rate in H II and H I regions, near hot stars, show that the molecule will be destroyed more rapidly than it will be pumped If the OH exists in an H II region, it will be exposed to radiation at $\lambda < 912$ Å which is capable of photoionizing it with a total f value of 1 The rate of destruction of the molecule by photoionization in the radiation field of a hot star will therefore exceed the pumping rate by three or four orders of magnitude The lifetime of a molecule after entering the H II region will be only 1 year, it is therefore impossible to have OH molecules in an H II region unless they are shielded from the radiation in a cooler cloud, in which case they will not actually be in an H II region but surrounded by it

The question of photodissociation in an H I region, where there is no radiation at $\lambda < 912$ Å ($E > 13\ 6$ eV), is more complicated because the potential energy curves for the unbound states can only be approximated Fig 2 shows the potential energy curves for the two bound states ($X^2\Pi_{3/2}$ and $A^2\Sigma^+$), the unbound $^2\Sigma^-$ and the $B^2\Sigma^+$ state which has a very low binding energy The curves for the X and A states and the bound portion of B ($r > 1\ 4$ Å) are empirical curves based on the RKR method from Fallon *et al* [12] The unbound $^2\Sigma^-$ curve has been estimated on the basis of a crossing of the A state at $v = 2$, and the portion of B with $r < 1\ 4$ Å has been obtained by extrapolation The shaded area represents the range of internuclear separation in which transitions from the ground state must take place according to the Franck–Condon principle All transitions to repulsive curves in this region will lead to photodissociation (All possible cases for $E > 13\ 6$ eV have been neglected because there are no photons of this energy in H I regions) It can be seen from the diagram that transitions to both $B^2\Sigma^+$ and $^2\Sigma^-$ states occur at about 11 5 eV and 7 eV, respectively In the case of the $^2\Sigma^-$ state, if the identification with the level crossing is correct, there can be no doubt that photodissociation will take place at energies less than 13 6 eV The f value for these transitions is unknown, but the transitions $^2\Pi \rightarrow {}^2\Sigma^+$ and $^2\Pi \rightarrow {}^2\Sigma^-$ are both permitted sub Rydberg transitions and should therefore have similar strength

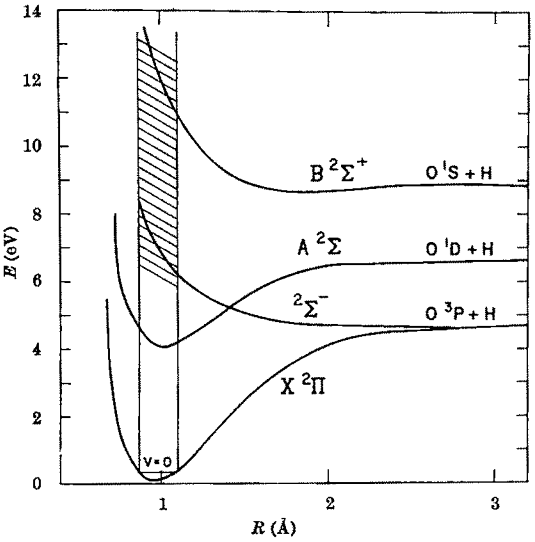

Fig 2 Potential energy curves of four OH electronic states demonstrating photodissociation transitions from the ground state to $^2\Sigma^-$ and $B^2\Sigma^+$

All the strong OH emission regions are observed near hot stars on the borders of H II regions, in these conditions the radiation field will be characterized by a temperature of approximately 30,000° K The rate of photodissociation R, for a molecule in a radiation field of this temperature, with a dilution factor $W = 10^{-16}$ has been calculated for the processes

$$\mathrm{OH}(^2\Pi) + h\nu(7\ \mathrm{eV}) \rightarrow \mathrm{OH}(^2\Sigma^-) \rightarrow \mathrm{O}(^3P) + \mathrm{H}(^2S) \qquad (3)$$

and

$$\mathrm{OH}(^2\Pi) + h\nu(11\ 5\ \mathrm{eV}) \rightarrow \mathrm{OH}(B^2\Sigma^+) \rightarrow \mathrm{O}(^1P) + \mathrm{H}(^2S) \qquad (4)$$

assuming that the f value for each transition is 8×10^{-4}. The total rate of photodissociation $R = R_3\,(1\ 2 \times 10^{-11}) + R_4\,(0\ 6 \times 10^{-11}) = 1\ 8 \times 10^{-11}$ sec^{-1} is greater than the maximum pumping rate $P = 0\ 55 \times 10^{-11}$ sec^{-1} (at $\lambda =$ 3080 Å) calculated in the above conditions for a strong inverting transition, with a strength equal to half of the total electronic f value Actually the dissociating transition is at a higher frequency than the absorption lines responsible for the pumping, and the dissociating f values and rates may even be greater than estimated

The rate for collisional destruction, per OH molecule by chemical exchange (reaction (2)) is $N_{\mathrm{H}} \times 10^{-11}$ exp $(-3{,}700/T)$ This exceeds the ultraviolet pumping and photodissociation rates when N_{H} exp $(-3{,}700/T) > 1\ 8$ For temperatures of 1,000 and 2,000° K, collisions will dominate at number densities $N_{\mathrm{H}} > 70$ and $N_{\mathrm{H}} > 11$, respectively When the rate of chemical reactions exceeds that of photo-reactions the pumping mechanism must also be chemical

An alternative to pumping by ultraviolet radiation is the selective formation of OH in the upper state of the Λ doublet. The ultraviolet pumping mechanism which has been proposed[10,11] works by selective absorption into levels of the $A^2\Sigma^+$ state Because this is the state that the molecule is forming in, a population inversion could result directly from preferential formation in those levels (of $A^2\Sigma^+$) which decay into the upper level of the Λ doublet As an example of the possibilities involved it can be shown by consideration of the selection rules involved that formation into the $J = \frac{1}{2}$, $K = 1$ negative state of $A^2\Sigma^+$ leads to a very strong population inversion with all cascades direct to $^2\Pi_{3/2}$ going into the upper level of the Λ doublet In the ultraviolet pumping scheme[10], absorption in this line leads to very strong anti-inversion because the upper level is depopulated A more complete analysis and theoretical understanding of the formation process are necessary in order to predict the population inversion as a function of physical conditions, but the existence of a mechanism leading to population inversion by selective formation makes it possible to achieve high pumping rates without requiring that all strong OH emission sources be extremely close to unobservable $O5$ stars

In the case of the extremely strong sources such as $W3$, the pumping rate required to explain the observations is approximately 0 01–1 0 cm^{-3} sec^{-1} As has been pointed out by Shklovsky[13], this could not be realized by ultraviolet pumping even if the star were in the centre of the concentration If it is assumed that each formation leads to 0 5 to 1 0 radio photons, then the formation mechanism suggested here requires an average number density of $N_{\mathrm{H}} = 10^8$ to 10^9 cm^{-3} at a temperature of approximately 1,000°–2,000° K One of the predictions that can be made from this requirement is that OH sources should be strong emitters in the infrared. Raimond[14] has pointed out that the OH emission source in Orion is coincident with a strong infrared source

The operation of the OH formation mechanism suggested here implies that the presence of OH is a necessary consequence of high temperature and density in interstellar matter resulting directly from the nature of atomic processes in these conditions The astronomical object which meets these physical conditions most readily is a protostar The highly complex OH emission sources are far more likely to be a result of the poorly understood yet universal process of stellar formation, rather than, as has been suggested by Barrett[1], the completely unknown and rare, later stages of evolution which may be called super-intelligent life

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[1] Barrett, A H , *Science*, **157**, 881 (1967)

[2] Robinson, B J , and McGee, R X , *Ann Rev Astro and Astrophys* , **5**, 183 (1967)

[3] Charton, M , and Gaydon, A G , *Proc Roy Soc* , A, **245**, 84 (1958)

[4] Kaskan, W E , *J Chem Phys* , **31**, 944 (1959)

[5] Bates, D R , *Mon Not Roy Astro Soc* , **3**, 303 (1951)

[6] Skinner, G B , and Ringrose, G H , *J Chem Phys* , **42**, 2190 (1965)

[7] Hughes, V A , *Nature*, **215**, 942 (1967)

[8] Spindler, G , Ticktin, S , and Schiff, H I , *Nature*, **214**, 1006 (1967)

[9] Perkins, F , Gold, J , and Salpeter, E E , *Astrophys J* , **145**, 361 (1966)

[10] Litvak, M M , McWhorter, A L , Meeks, M L , and Zeiger H J , *Phys Rev Lett* , **17**, 821 (1966)

[11] Golden, D M , Del Greco, F P , and Kaufman F , *J Chem Phys* , **39**, 3034 (1963)

[12] Fallon R J , Tobias, I , and Vanderslice, J T , *J Chem Phys* , **34** 167 (1961)

[13] Shklovsky, I S , *Astro Tsirk, Byuro Astro Srobshch* , No 424 (1967)

[14] Raimond, E , and Eliasson B , *Astrophys J* (in the press)

Response of Bacterial Spores and *Micrococcus radiodurans* to Ultraviolet Irradiation at Low Temperatures

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At low temperatures, bacterial spores and *Micrococcus radiodurans* show paradoxical changes in sensitivity to the lethal effects of ultraviolet irradiation.

MANY micro-organisms are supersensitive to the lethal effects of exposure to ultraviolet light when irradiated at low temperatures in the frozen state[1]. The possible nature of this supersensitivity has been discussed[2] with the conclusion that damage to the DNA not associated with the thymine dimer was probably involved. The lesion induced by ultraviolet light at low temperatures is not amenable to repair by the normal repair processes present in most bacteria, nor is it photoactivable (ref. 3 and unpublished results of J. Wilcockson). The induction of mutations in *Escherichia coli* when irradiated with ultraviolet light in the frozen state is increased at low temperatures in a manner similar to the lethal response in that there is an increasing number of induced mutations with decreasing irradiation temperature[4].

Ashwood-Smith and Bridges[1] reported that the spores of *Bacillus subtilis* were supersensitive to ultraviolet induced killing at −79° C. Donnellan (personal communication), however, had noted that the spores of *Bacillus megaterium* were much more resistant to ultraviolet radiation at −196° C than at normal room temperature. We confirmed this observation with the spores of two strains of *B. megaterium* irradiated at liquid nitrogen temperatures, but also demonstrated that these same spores when irradiated at −79° C were nearly three times more sensitive than at 25° C. These results led us to examine, in considerably more detail, the effect of temperature in the solid state on the ultraviolet sensitivity of the spores of *B. subtilis* and the vegetative cells of the extremely radio-resistant bacterium, *Micrococcus radiodurans*. The response of *E. coli* in similar circumstances[1,2], although already well known, has been included in the present results for comparative purposes.

The irradiation procedures used in this study were essentially similar to those reported earlier[4]. The ultraviolet source was a low pressure mercury lamp about 98 per cent of the output of which was at 2537 Å. The ultraviolet flux at a distance of 30 cm from the source was 65 ergs mm^{-2} sec^{-1}. All the experiments were carried out in a darkened room as a precaution against photoreactivation. The spores of *B. subtilis* were prepared on Schaeffer agar plates, collected and freed of vegetative cells by treatment with sodium lauryl sulphate and lysozyme according to the method of Yoshikawa[5]. These spores were stored at 4° C in distilled water until used. *E. coli B/r WP2* (tryptophan-requiring) was grown in nutrient broth (Oxoid) at 37° C until the latter part of the logarithmic growth phase had been reached. The cells were collected and suspended in M/15 phosphate buffer (*p*H 6·8) for irradiation. *Micrococcus radiodurans* (National Collection of Industrial Bacteria, Aberdeen, Scotland, NCIB code number 9279) was grown in nutrient broth to which had been added 0·5 per cent glucose, and these cells were also collected and suspended in phosphate buffer when in the latter phase of logarithmic growth. Suspensions of cells or spores were cooled to −196° C as previously described[1] and then warmed to the irradiation temperature. Immediately after irradiation the frozen suspensions were thawed quickly by immersing the irradiation vessel in water at 37° C and colony counts for viable cells were made on nutrient agar plates for all three micro-organisms; 0·5 per cent glucose was added to the plates used for estimating colonies of *M. radiodurans*. Corrections were made for cell death associated with the freezing and thawing procedures. About 70–90 per cent of the cells of *E. coli* survived the freeze–thaw cycle. Survival of *M. radiodurans* and the spores of *B. subtilis* was nearly 100 per cent. Each experiment was repeated three times and complete survival curves were constructed. The D_{90} values were extracted from the curves and the average values in ergs mm^{-2} were plotted on a logarithmic axis against the irradiation temperature for the three bacterial types. Presentation of the results in this manner (Fig. 1) permits easy comparison of the differing sensitivities to ultraviolet radiation and the effect of temperature on these sensitivities.

The survival of *E. coli* suspended in phosphate buffer and plotted as a function of the irradiation temperature is similar to that already published[1] in that there is a progressive increase in sensitivity with decreasing temperature down to about −80° C. At lower temperatures the sensitivity, although still considerably greater than at 25° C, becomes progressively but rather slowly less marked. Similar observations have been made by Smith and O'Leary[3]. The spores of *B. subtilis* are nearly four times more resistant at 25° C than the cells of *E. coli* at this temperature. With decreasing temperature increasing sensitivity in the solid state to ultraviolet irradiation is apparent. At −79° C the spores are 2·5 times more sensitive than at 25° C. The increased sensitivity becomes progressively less marked with temperatures lower than −80° C, and at −140° C the spores possess the same sensitivity to ultraviolet light as they do at room temperature. As the irradiation temperature continues to fall a marked and logarithmic increase in resistance is apparent. This logarithmic increase in resistance, represented by the equation $y = a + bt$ when $y = \log_{10} D_{90}$, would indicate that the resistance of *B. subtilis* spores to ultraviolet light at −269° C would be $3{\cdot}1 \times 10^5$ ergs mm^{-2} (D_{90}), and at −273° C, 4×10^5 ergs mm^{-2} (D_{90}). It is interesting that the resistance of the spores of *B subtilis* at − 190° C is the same as that of the extremely resistant bacterium *M radiodurans* at 25° C

The behaviour of *M radiodurans* at low temperatures is unlike that of any other bacterium thus far studied[1] There is little change in the sensitivity of this bacterium with phase change and low temperatures until an increase in resistance is seen at temperatures below − 60° C The very large increase in resistance between − 60° C and − 196° C follows a quadratic function represented by the equation $y = a + bt + ct^2$ At − 269° C the estimated value for the D_{90} is 1 7 × 10^7 ergs mm^{-2}, and at absolute zero 2 2 × 10^7 ergs mm^{-2} These figures assume no alteration in the shape of the dose response curve at temperatures at or near absolute zero, this assumption may well be incorrect as photochemical events at these very low temperatures could be anomalous A small number of experiments with *Serratia marcescens* indicate that it behaves towards irradiation with ultraviolet light at low temperatures in a manner similar to that of *E coli* in that the supersensitivity at − 196° C is less than at − 79° C The spores of two different strains of *B megaterium* and the spores of the fungus *Aspergillus nidulans* are considerably more resistant to lethal effects of ultraviolet light at − 196° C than at 25° C Rahn[6] has reported that the DNA of *Haemophilus influenzae* irradiated *in vitro* in a purified state is much more resistant to the inactivation of its transforming properties when irradiated with ultraviolet light at − 196° C than at 25° C

Why do the spores of *B subtilis*, the cells of *E coli* and the cells of *M radiodurans* behave so differently to irradiation with ultraviolet light at low temperatures? When *E coli* is irradiated with ultraviolet light at − 79° C fewer thymine dimers within its DNA are produced than at 25° C (ref 3), the lethal damage is much less photoreactivable[3] and there is an increase in the amount of protein cross-linked to DNA[2,3] Many bacteria, several bacteriophages and at least one species of yeast, *Saccharomyces cerevisiae*, are supersensitive to the lethal effects of ultraviolet light at − 79° C and it has been suggested that death is associated with the formation of a photoproduct not amenable to normal repair systems[2] It would seem probable that the lethal effects of ultraviolet radiation at subzero temperatures in the frozen states are dependent on the relative contribution of the three known events previously mentioned and the ability or lack of ability of an organism to repair the qualitatively different damage resulting from low temperature radiation *M radiodurans* is known to possess very efficient mechanisms for the repair of thymine dimer lesions and it may well be able also to repair low temperature lesions as well as to excise the smaller amounts of thymine dimer within DNA formed at low temperature An alternative possibility is that the low temperature lesion formed in most bacteria is not formed in *M radiodurans* We cannot explain the interesting biphasic response of spores It has been reported that a large amount of the thymine in the DNA of spores is converted into a photoproduct which is not of the usual type of cyclobutane dimer (personal communication from J Donnellan) At irradiation temperatures lower than − 80° C it is possible that the formation of the spore photoproduct or products is less efficient than at higher temperatures

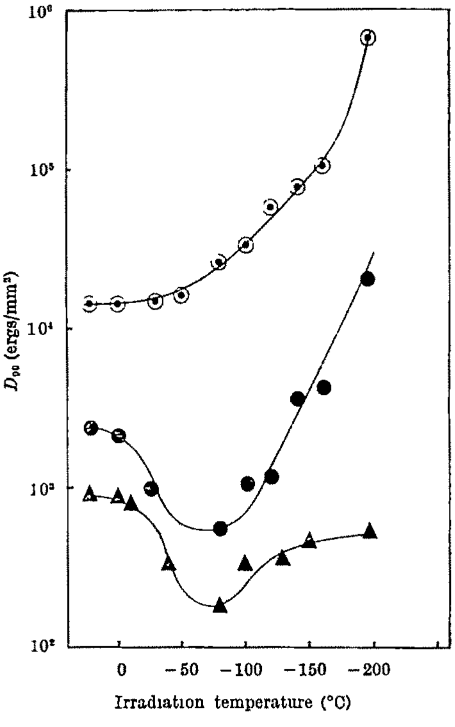

Fig 1 Comparison of sensitivities to ultraviolet radiation and the effect of temperature on these sensitivities ○, *Micrococcus radiodurans*, ●, *Bacillus subtilis* (spores), ▲ *E coli WP2*

Are the results presented in this account of any relevance to problems associated with solar irradiation of airborne micro-organisms? Sunlight is very effective in killing a number of viruses at normal temperatures[7] A recent study has demonstrated that the number of mutations induced by solar radiation at the surface of the Earth is as much as fifteen times greater when *E coli* is exposed at − 60° C than at 30° C (ref 8)

Quantitative analyses of the atmosphere at altitudes ranging from 3 to 27 km for various micro-organisms have been helped by studies with balloons[9] Yeasts, various moulds and several different species of bacteria, with the Micrococci predominating, were found at these heights At heights varying from 9–18 km the average number of micro-organisms is probably 6 per 100 m³ At 18–27 km the average number is 1 5 per 100 m³ At much lower altitudes considerable numbers of micro-organisms may be found Analysis of air over the Atlantic Ocean has revealed fungi to be widespread at concentrations as high as 200/m³ at 3 km above sea level[10] The temperature of the atmosphere decreases in the International Standard Atmosphere by 6 5° C/km up to 11 km At altitudes greater than 11 km the temperatures encountered by micro-organisms will vary in a complex manner At the outer limit of the stratosphere (48 km) the temperature will be approximately 10° C, but at the outer limit of the mesosphere (100 km) the ambient temperature will be as low as − 75° C Micro-organisms exposed to the increased ultraviolet flux, which will include a proportionately larger amount of the very damaging shorter wavelength radiations not filtered by the ozone, will almost certainly be in a desiccated, frozen or freeze-dried condition Little is known about the sensitivity of micro-organisms to ultraviolet light in these conditions The results with *M radiodurans* and bacterial spores suggest that great caution must be exercised before speculating on the ultraviolet sensitivity of airborne micro-organisms when a number of factors such as temperature and the state of the cell water are not considered

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[1] Ashwood-Smith M J, and Bridges, B A, *Proc Roy Soc*, B, 168, 194 (1967)
[2] Bridges, B A Ashwood-Smith, M J, and Munson, R J, *Proc Roy Soc* B, 168, 203 (1967)
[3] Smith, K C, and O'Leary, M E, *Science*, 155, 1024 (1967)
[4] Ashwood-Smith, M J, and Bridges, B A, *Mutation Res*, 3, 1350 (1966)
[5] Yoshikawa, H, *Proc US Nat Acad Sci*, 53, 1476 (1965)
[6] Rahn, R O, *Abs Biophys Soc*, 83 (1966)
[7] Appleyard, G, *J Gen Virol*, 1, 143 (1967)
[8] Ashwood-Smith, M J, Copeland, J, and Wilcockson, J, *Nature*, 214, 33 (1967)
[9] Bruch, C W, in *Airborne Microbes, Seventeenth Symp Soc Gen Microbiol* (edit by Gregory, P H, and Monteith, J L), 356 (Cambridge University Press, 1967)
[10] Pady, S M, and Kelly C D, *Canad J Bot* 32, 202 (1964)

LETTERS TO THE EDITOR

ASTRONOMY

Microwave Background in a Steady State Universe

It is widely believed that the microwave background radiation[1–10] discovered by Penzias and Wilson had its origin in a hot, early phase of the expanding Universe. The possibility has recently been considered[11,12], however, that it is instead a result of the thermalization of starlight by interstellar dust grains. It is claimed that this could explain the fact that the energy density of the 3° K radiation is very close to those of starlight, magnetic fields, turbulent gas motions and cosmic rays, all measured in the galactic disk, and also of the energy produced[13] by the conversion from hydrogen to helium of one-third the mean density of visible galaxies—a fraction which corresponds to the ratio of helium to hydrogen in young stars[14]. A paper by Zeldovič and Novikov[15] has already, in fact, considered and discounted this possibility, and the purpose of this communication is to draw attention to some difficulties particularly associated with the suggestions of Hoyle and Wickramasinghe[11] and of Narlikar and Wickramasinghe[12].

One obvious problem is that of accounting for the detection of the radiation at decimetre wavelengths because interstellar dust grains are likely[16] to have graphite cores surrounded by ice mantles with a diameter of about 10^{-5} cm. These grains would not usually be expected to radiate efficiently at wavelengths about 10^6 times their physical dimensions. Hoyle and Wickramasinghe[11], however, note that certain doped alkyl halide crystals have[17,18] narrow absorption bands at frequencies near 300 GHz and they suggest that interstellar dust grains would also be expected to contain impurities which might radiate at these or lower frequencies. Narlikar and Wickramasinghe[12] develop this train of thought and claim that dust grains in a galaxy could absorb optical and ultraviolet radiation from stars and re-emit the energy as strong infrared radiation in frequency bands at about 300 GHz with an intensity equivalent to about one-third of the entire luminosity of the galaxy. The observed microwave radiation is then, in their view, attributable to the integrated and red-shifted infrared emission from all the galaxies in a steady state universe. It is, in any case, apparent from the observed isotropy[19] of the radiation and the absence of any detectable galactic absorption in the microwave spectra of radio sources that the microwave background radiation cannot be merely a phenomenon confined to our galaxy. They show that such an integrated radiation component would have a spectrum $\mathcal{J}(\nu)\propto\nu^3$ and they believe that the difference between this and the observed spectrum $\mathcal{J}(\nu)\propto\nu^2$ could be a result of distinct impurities oscillating at particular frequencies such that the observed spectrum depends in a fortuitous manner on the frequencies of observation.

There are serious weaknesses in this interpretation which must be overcome before it can be regarded as a remotely plausible model for the origin of the radiation.

(1) Narlikar and Wickramasinghe carry out a calculation intended to show that the observed brightness is not inconsistent with the integrated thermalized optical radiation from galaxies in a steady state universe. Because the total energy density of the microwave radiation is about 6×10^{-13} erg cm^{-3}, it follows that the total optical radiation from galaxies in a steady state universe would have to be of at least this value if there were no thermalization. A calculation of this quantity is to be found in a recent review by Davidson and Narlikar[20] and is given as 2.4×10^{-15} erg cm^{-3}, that is, more than two orders of magnitude less than that of the microwave radiation. This discrepancy was noted in the review where it was said to be "by a factor of at least 400" (p. 587) but is not mentioned by Narlikar and Wickramasinghe. They do, however, suggest that because of the present uncertainty in the ultraviolet output of a galaxy it may be that the total ultraviolet emission of young spiral galaxies exceeds that of the visible emission by a factor of 10 to 100. Subsequent thermalization, it is suggested, could produce microwave radiation and the "microwave power could exceed the visible power by a factor of about 100". If this is so for our own galaxy, then the microwave frequencies emitted must avoid all frequency bands in which observations have so far been carried out. Penzias and Wilson[21], for example, have measured the brightness of the microwave radiation from the galactic disk at a frequency of 4.08 GHz and have shown it to be entirely consistent with lower frequency observations which set a value of $\approx10^{39}$ erg/sec on the total galactic radio emission, at least four orders of magnitude less than the total optical emission. An upper limit on the galactic radiation at 9.4 GHz can also be set from the measurements by Partridge and Wilkinson[19] which are again consistent with this value. At still higher frequencies ($\approx10^3$ GHz) Hoffmann *et al.*[22] have put low upper limits to the brightness temperature of any discrete source in the northern sky, that is, $<10°$ K for a black-body subtending 2° or more and $<0.6°$ K for an optically thin "grey-body" which gives a Rayleigh–Jeans (ν^2) spectrum in this frequency range. Any substantial increase in the energy density of microwave radiation in the galaxy would be extremely difficult to reconcile[23] with the observed energy spectrum of cosmic ray electrons which interact with radiation quanta by the Compton effect and would also, of course, destroy the agreement with the various energy densities of starlight and cosmic rays which the theory was put forward to explain in the first place.

(2) Neither paper includes a discussion of the obvious and vital differences between the optical properties of alkyl halide crystals and of interstellar dust grains of the ice coated graphite type proposed by Wickramasinghe[16]

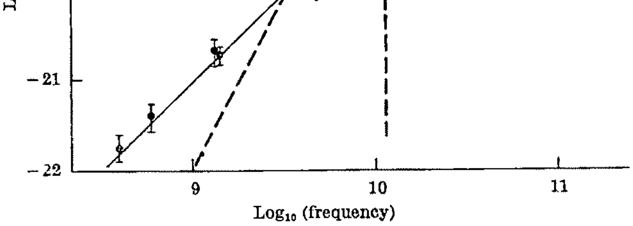

Fig. 1. The plotted points show the measured values of the brightness of the "excess" microwave background radiation[1–10]. That at 115 GHz is derived from cyanogen excitation[25,26]. The solid line is a Planck spectrum corresponding to a temperature of 2.68° K and the dashed lines[12] show the type of emission which Narlikar and Wickramasinghe believe could originate in interstellar dust grains. The units of brightness are W m^{-2} Hz^{-1} ster^{-1} and of frequency are Hz.

Because graphite is a semi-metal with a low resistivity[24] at the temperatures[16] of the grains, these will behave as Hertzian dipoles in this frequency range and radiate with power $\propto\nu^4$ rather than in discrete bands or lines. There may, of course, be impurities in the ice coating, but if these behave as do the impurities in the potassium chloride studied by Sievers[17], the absorption coefficient ($\approx 2·5$ cm^{-1}) is negligible compared with that of the graphite.

(3) At the time of submission of the communication by Narlikar and Wickramasinghe, measurements of the brightness of the microwave background had already been published[1–6] for five wavelengths, two of which were, admittedly, very close. In trying to fit the observed ν^2 spectrum with the calculated ν^3 spectra reference was made to two of these only. At the present time, measurements[1–10] at twelve wavelengths over a frequency range of 90:1 (or 280:1 if the cyanogen excitation results[25,26] are included) are available. They are all, with one possible exception[8], consistent with a Planck spectrum[7] at a temperature of 2·68° K (see Fig. 1). It would clearly require a very special distribution of impurity oscillators, each producing a spectrum $\mathcal{J}(\nu)\propto\nu^3$, to fit the observed values.

We conclude with Zeldovič and Novikov[15] that radiation from dust grains is not a satisfactory alternative to the primordial origin of the cosmic microwave radiation.

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[1] Penzias, A. A., and Wilson, R. W., *Astrophys. J.*, **142**, 419 (1965).
[2] Roll, P. G., and Wilkinson, D. T., *Phys. Rev. Lett.*, **16**, 405 (1966).
[3] Howell, T. F., and Shakeshaft, J. R., *Nature*, **210**, 1318 (1966).
[4] Penzias, A. A., and Wilson, R. W., *Astro. J.*, *NY*, **72**, 315 (1967).
[5] Welch, W. J., Keachie, S., Thornton, D. D., and Wrixon, G., *Phys. Rev. Lett.*, **18**, 1068 (1967).
[6] Wilkinson, D. T., *Phys. Rev. Lett.*, **19**, 1195 (1967).
[7] Stokes, R. A., Partridge, R. B., and Wilkinson, D. T., *Phys. Rev. Lett.*, **19**, 1199 (1967).
[8] Ewing, M. S., Burke, B. F., and Staelin, D. H., *Phys. Rev. Lett.*, **19**, 1251 (1967).
[9] Howell, T. F., and Shakeshaft, J. R., *Nature*, **216**, 753 (1967).
[10] Salomonovich, A. E., Stankevich, K. S., and Puzhano, V., reported at *I.A.U. Thirteenth Gen. Ass.*, Prague (1967) (*Astro. Zhurn.*, *USSR*, in the press).
[11] Hoyle, F., and Wickramasinghe, N. C., *Nature*, **214**, 969 (1967).
[12] Narlikar, J. V., and Wickramasinghe, N. C., *Nature*, **216**, 43 (1967).
[13] Wagoner, R. V., Fowler, W. A., and Hoyle, F., *Astrophys. J.*, **148**, 3 (1967). See pp. 23–24.
[14] Tayler, R. J., *Quart. J. Roy. Astro. Soc.*, **8**, 313 (1967).
[15] Zeldovič, Ya. B., and Novikov, I. D., *Astro. Zhurn.*, *USSR*, **44**, 663 (1967).
[16] Wickramasinghe, N. C., in *Interstellar Grains* (Chapman and Hall, Ltd., London, 1967).
[17] Sievers, A. J., *Phys. Rev. Lett.*, **13**, 310 (1964).
[18] Sievers, A. J., and Takeno, S., *Phys. Rev.*, A, **140**, 1030 (1965).
[19] Partridge, R. B., and Wilkinson, D. T., *Phys. Rev. Lett.*, **18**, 557 (1967).
[20] Davidson, W., and Narlikar, J. V., *Rep. Prog. Phys.*, **29**, part 2, 539 (1966). See p. 587.
[21] Penzias, A. A., and Wilson, R. W., *Astrophys. J.*, **146**, 666 (1966).
[22] Hoffmann, W. F., Woolf, N. J., Frederick, C. L., and Low, F. J., *Science*, **157**, 187 (1967).
[23] Verma, S. D., *Phys. Rev. Lett.*, **18**, 253 (1967).
[24] Primak, W., and Fuchs, L. H., *Phys. Rev.*, **95**, 22 (1954).
[25] Field, G. B., and Hitchcock, J. L., *Astrophys. J.*, **146**, 1 (1966).
[26] Thaddeus, P., reported at *Third Texas Symp. Relativistic Astrophys.*, New York (1967).

3C390·3, a Second Variable *N*-Galaxy

Oke[1] and Sandage[2] have shown that the *N*-galaxy 3*C*371 is variable. This communication reports the discovery that the similar system 3*C*390·3 has been varying by up to about 1·5^{m} during the past 70 years. This is interpreted as showing that at least some quasars are cosmological.

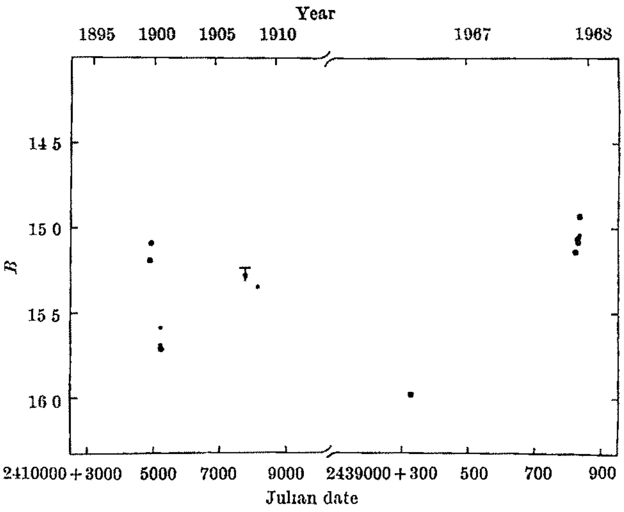

Fig. 1. Light curves of 3*C*390·3 for 1894 to 1910 and for 1966–67. The larger points have errors of less than 0·07^{m}, the smaller ones have larger errors. The arrow denotes a lower limit to the magnitude.

The radio source 3*C*390·3 is listed by Wyndham[3] as being identified with an *N*-type galaxy. Sandage[4] has obtained a spectrum of the galaxy which shows nine emission lines, some of which are 50 Å broad, giving a red-shift of 0·0569. It also shows a large ultraviolet excess similar to those found in other *N*-galaxies and quasars. We obtained one plate of the galaxy during August 1966 on the 26 in. *f*/10 refractor at Herstmonceux. Following the discovery that 3*C*371 was variable, we have obtained a further series of plates of 3*C*390·3 on the same instrument. These showed that 3*C*390·3 had varied by about 1^{m} in 16 months. This discovery prompted a search for old plates of the field of 3*C*390·3. We discovered a number of plates taken on the 13 in. astrographic refractor at Greenwich between 1894 and 1908 in connexion with the astrographic charts, eight of which were of sufficient depth and quality for photometry. These showed that 3*C*390·3 had also been varying at that time. A photographic transfer plate taken on the same 13 in. refractor, now at Herstmonceux, was used to transfer the photoelectric standards set up by Sandage[5] in the nearby field of the cluster *NGC* 188 into the field of 3*C*390·3. Past experience[6] shows that this technique yields comparison sequences good to about 0·1^{m}. All plates were measured on a Sartorius Iris diaphragm photometer. The resulting measures were reduced to give magnitudes for the galaxy by a computer programme fully described elsewhere[7].

The full results of this reduction are listed in Table 1 and displayed in Fig. 1. The modern plates were taken on Eastman Kodak IIaO emulsion without filter which gives approximate *B* magnitudes. The old plates were taken on a variety of blue sensitive emulsions, so there may be small differences of system. The observed variation, however, is so large that it is not in doubt, particularly because plates taken on different emulsions in the same month agree within their errors. Some modern plates (marked with an asterisk in Table 1) were taken deliberately out of focus in order to show that the non-stellar nature of the galaxy does not affect our results. That this is so can be seen from the agreement of plates taken on the same night. In fact, 3*C*390·3 and other *N*-galaxies are only just non-stellar on the Palomar Observatory Sky Survey (POSS) charts. Also listed in Table 1 are the photoelectric *B* magnitude published by Sandage[4] and a magnitude estimated by eye from the blue POSS chart. The most rapid variations we have recorded are 0·6^{m} in 10 months during 1899–1900 and 0·8^{m} in 16 months

Table 1

Method	Plate No	Date	Julian day	B	Error
13 in astrographic chart plate	2191	1894/ 8/31	241 3071/2	14 47	0 15
,, ,,	3548	1897/ 6/23	241 4098/9	14 93	0 12
,, ,,	4610	1899/ 8/11	241 4877/8	15 19	0 07
,, ,,	4675	1899/ 9/20	241 4917/8	15 09	0 05
,, ,,	5023	1900/ 7/12	241 5212/3	15 69	0 13
,, ,,	5054	1900/ 7/26	241 5226/7	15 71	0 05
,, ,,	5056	1900/ 7/28	241 5228/9	15 58	0 11
,, ,,	6910	1907/ 7/31	241 7787/8	>15 3	—
,, ,,	7062	1908/ 7/31	241 8153/4	15 34	0 15
POSS	+78°18h24m	1953/ 7/11	243 4569/70	~15 0	~0 2
Sandage pe	—	?1966	?	15 30	?0 02
26 in plates	E9909	1966/ 7/24	243 9331	15 97	0 03
,, ,,	E11175*	1967/11/28	243 9823	15 14	0 01
,, ,,	E11183	1967/12/ 4	243 9829	15 08	0 02
,, ,,	E11184*	1967/12/ 4	243 9829	15 06	0 02
,, ,,	E11212*	1967/12/ 7	243 9832	15 04	0 18
13 in transfer plate	D5188	1967/12/ 7	243 9832	14 94	0 05

Table 2

Name	b^{II}	z	B	B'	S_{178}	S'_{178}
3*C*390 3	+27	0 0569	14 46	14 46	43	43
Ton256	+45	0 131	16 71	15 15	<2	<12 4
PHL 1194	−51	0 298	17 43	14 39	<2	<72
3*C*47	−41	0 425	18 15	14 25	21	2,150
3*C*371	+29	0 0508	14 75	14 75	9 5	7 5

during 1966–67 It is also probable that the 0 1^m increase during November and December 1967 is real

Sandage[2] has published a two colour diagram which shows that the variable *N*-galaxies 3*C*371 and 3*C*390 3 are the two *N*-galaxies which most closely resemble quasars in their observed colours He also points out the similarity of colours and spectra of 3*C*390 3 and the quasar Ton256 (ref 4) These similarities and the so called "continuity" arguments of Heeschen[8] and Ryle and Longair[9] raise the question of the relation between quasars and *N*-galaxies In Table 2, we present the properties of 3*C*390 3 and 3*C*371 together with three sources listed as quasars by Barbieri *et al* [10] and Burbidge[11] These three quasars lie nearest to the Hubble relation in the magnitude–red-shift diagram.

We give the observed galactic latitude (b^{II}), red-shift (z), B magnitudes—at maximum for the *N*-galaxies—and flux density at 178 MHz (S_{178}) We also present B' and S'_{178}, the B magnitude and flux density at 178 MHz which would be observed if the other sources were in the same position in space as 3*C*390 3 To compute B' we have assumed that the red-shift is cosmological, that the *K*-correction for quasars given by Sandage[12] applies and that the absorption in the galaxy is given by $A_B = 0\ 12^m$ cosec b^{II} We have computed S'_{178} similarly assuming a cosmological red-shift and a *K*-correction based on a straight spectrum ($S \propto \nu^{-\alpha}$ for 3*C*47 $\alpha = 0\ 82$ from Conway *et al* [13], otherwise no values of α are available in the literature and we have assumed $\alpha = 0\ 7$) Note that for Ton256 and *PHL* 1194 the limit $S_{178} < 2$ is based on the absence of these sources from the fourth Cambridge catalogue of radio sources[14,15]

It is noticeable that the values of B' of all these sources are closely similar If the *N*-galaxies were at a distance given from the red-shifts of the quasars by the Hubble law, they would probably not seem to possess any nebulosity Indeed, one might otherwise ask why we have not observed *N*-galaxies at much greater distances Thus it is quite possible that the "quasars" listed in Table 2 are at cosmological distances and if brought closer would appear very similar to the *N*-galaxies We note that a very deep plate of another quasar, 3*C*48, by Sandage and Miller[17] does show nebulosity but beyond the range of the Palomar Observatory Sky Survey plates

The situation is not so clear in the case of S'_{178}, nonetheless it is apparent that the flux density of 3*C*390 3 is bracketed by other sources hitherto regarded as quasars It is also interesting that both 3*C*390 3 and 3*C*47 are radio doubles with very similar physical separations[9]

It seems that the arguments for the continuity of quasars and radio galaxies understate the case We propose that these three "quasars" and possibly others are, in fact, *N*-galaxies and so are at cosmological distances It is perhaps particularly provoking that 3*C*47 is an archetypal quasar, being the third[16] to have had its red-shift determined It may be, however, that quasars comprise more than one type of object so that whether the red-shifts of all quasars are cosmological is still an open question

We thank Miss Rosemary Brett for assistance in measuring the plates and Dr D. Lynden-Bell for directing our attention to the old astrographic plates We are also grateful to Mr M Rowan-Robinson for first directing the attention of one of us to the problem of *N*-galaxies

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[1] Oke, J B , *Ap J* , **150**, 5 (1967)
[2] Sandage, A R , *Ap. J* , **150**, *L*9 (1967)
[3] Wyndham, J D , *Ap J* , **144**, 459 (1966)
[4] Sandage, A R , *Ap J* , **145**, 1 (1966)
[5] Sandage, A R , *Ap J* , **135**, 333 (1962)
[6] Harding, G A , and Penston, M J , *Roy Obs Bull* , No 115, 1966
[7] Penston, M V , and Cannon, R D , *Roy Obs Bull* (in preparation)
[8] Heeschen, D S , *Ap J* , **146**, 517 (1966)
[9] Ryle, M , and Longair, M S , *Mon Not Roy Astro Soc* , **136**, 123 (1967)
[10] Barbieri, C , Battistini, P , and Nasi, E , *Pub Astro Obs Padua*, No 141 (1967)
[11] Burbidge, E M , *Ann Rev Astro and Ap* , **5**, 399 (1967)
[12] Sandage, A R , *Ap J* , **146**, 13 (1966)
[13] Conway, R G , Daintree, E J , and Long, R J , *Mon Not Roy Astro Soc* , **131**, 159 (1965)
[14] Pilkington, J D H , and Scott, P F , *Mem Roy Astro Soc* , **69**, Pt 5 (1965)
[15] Gower, J F R Scott, P F , and Wills, D , *Mem Roy Astro Soc* , 71, Pt 2 (1967)
[16] Schmidt, M , and Matthews, T A , *Ap J* , **139**, 781 (1964)
[17] Sandage, A R , and Miller, W C , *Ap J* , **144**, 1238 (1966)

Alfvén's Critical Velocity Hypothesis

IN his theory on the origin of the solar system[1], Alfvén makes an assumption which is central to the entire theory[2] This assumption is that when there is a relative velocity between a plasma and a neutral gas in the presence of a magnetic field, a strong ionization process will set in when the relative velocity reaches a critical value U_{crit} such that

$$\tfrac{1}{2} m_g U^2_{crit} = eV_i$$

where m_g and V_i are the mass and ionization potential of the gas atom Using this assumption, Alfvén has been able to predict fairly accurately the mass distribution of the planets and satellites in the solar system A study of the ionization mechanism leads to some difficulties, however, first, the threshold energy for ionization in a collision between a gas atom and an ion of the same gas is $2\,eV_i$ (ref 3) and, second, the ionization cross-section of ions is very small compared with that of electrons, except at very high energies It has been suggested[4] that the ionization must be caused by the electrons and that energy is transferred from the ions to the electrons by some kind of space charge mechanism which is not discussed in detail The purpose of this communication is to point out three simple ionization mechanisms which lend support to the critical velocity hypothesis

In Alfvén's theory the situation considered is that of a plasma held stationary by a magnetic field in a stream of neutral gas The electrons in the plasma will be heated by collisions with the gas stream, and the equipartition principle shows that with elastic collisions only the average energy of the electrons is given in terms of the gas stream velocity U by

$$\tfrac{1}{2}m_e \langle v_r^2 \rangle = \tfrac{1}{2}m_g U^2$$

Inelastic collisions will tend to reduce the electron energy, but this relationship shows that when U is near U_{crit} the electron energy is an appreciable fraction of eV_i and consequently ionization is to be expected By using an equivalent field technique[5] which is closely related to the equivalent pressure technique of Blevin and Haydon[6], the electron ionization frequency can be found as a function of U alone from the data available on the electrons' drift velocity and ionization frequency in the absence of a magnetic field The electron ionization frequency for helium is shown in Fig 1, which also gives U_{crit} for helium

The relative kinetic energies of interest measurements[7] show that the charge transfer collision cross-section for atomic helium ions in helium is much larger than the elastic cross-section, and at these energies elastic collisions show pronounced forward scattering[8] For these reasons, charge transfer collisions are the most important, and it is a characteristic of these collisions that after the collision the ion has very nearly the same velocity, that is, U, as the atom had before it The ion will then move through its Larmor orbit in the magnetic field with a constant velocity and the ion energy relative to the gas stream will then be $(m_i = m_g)$

$$m_g U^2 (1 - \cos \omega_i t)$$

The average ion energy is $m_g U^2$ and this equals the threshold energy for ionization $(2\,eV_i)$ when $U = U_{\text{crit}}$ The peak energy is $2m_g U^2$, which is 98 eV in helium when $U = U_{\text{crit}}$, and these high energies can lead to appreciable ionization by the ions The ionization frequency of the ions can be averaged around the orbits[5] of the ions and the result, based on Rostagni's[9] ionization cross-sections, is shown in Fig 1 It can be seen that the ionization frequency of the ions is greater than that of the electrons although the ionization cross-section of the ions is much smaller than that of the electrons This is because the energy of every ion is above threshold in part of its orbit, if U is large enough, while only the small fraction of electrons in the high energy tail of the electron energy distribution can ionize

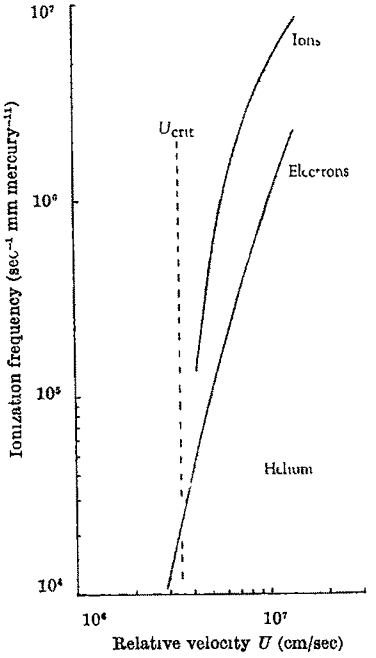

Fig 1 Plot of ionization frequency against relative velocity U between the plasma and the neutral gas for electrons and ions of helium

The atoms formed in charge exchange collisions will move in straight lines with almost the same velocity as the ion had before the collision This means that these atoms will have on average an energy, relative to the gas stream, which is roughly equal to the ion energy (In the constant mean free time approximation the "new" atom and ion energies are equal[5]) Also the ionization cross-section for atom–atom collisions[10] is similar to that for ion–atom collisions, so that the ionization frequency of the "new" atoms is similar to that of the ions The "new" atoms may contribute more to the total ionization than the ions, depending on the density of "new" atoms in comparison with that of the ions

It is suggested that the critical velocity hypothesis is a consequence of the fact that the ionization frequencies of the electrons, ions and atoms formed in charge exchange collisions all become appreciable and increase rapidly with U when U is near U_{crit} By lending support to the critical velocity hypothesis these results indirectly support Alfvén's theory of the origin of the solar system, but it is pointed out that the details of Alfvén's theory might have to be modified to take account of the possible effects of the ionization caused by the ions and atoms formed by charge exchange

Results from experiments with partially ionized crossed field homopolar discharges[11,12] can be explained if the relative velocity between the gas and plasma is limited to a value near U_{crit} (ref 12) If the theory of Drobyshevskii[13] is extended to include the effects of ionization by the ions and atoms formed by charge exchange collisions, fair agreement with experiment is obtained[5] It turns out that in these experiments the density of "new" atoms is higher than the ion density so that most of the ionization is caused by the atoms formed by charge exchange

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[1] Alfvén, H , in *On the Origin of the Solar System* (Oxford University Press 1954)
[2] Alfven, H , and Wilcox, J M , *Astrophys J* , **136** 1016 (1962)
[3] von Engel, A , in *Ionised Gases*, 68 (Oxford University Press, 1965)
[4] Alfvén, H , *Rev Mod Phys* , **32**, 710 (1960)
[5] Sherman, J C , thesis, Univ Oxford (1967)
[6] Blevin, H A , and Haydon, S C , *Austral J Phys* , **11**, 18 (1958)
[7] Cramer, W H , and Simons, J H , *J Chem Phys* , **26**, 1272 (1957)
[8] McDaniel, E W , in *Collision Phenomena in Ionised Gases*, 146 (Wiley, 1964)
[9] Rostagni, A , *Nuovo Cim* , **15/2**, 117 (1938), ibid , **11**, 34, 39 (1934), von Engel, A , in *Ionised Gases*, 70 (Oxford University Press, 1965)
[10] Hayden, H C , and Utterback, N G , *Phys Rev* , A, **135**, 1575 (1964)
[11] Fahleson, U V , *Phys Fluids*, **4**, 123 (1961)
[12] Angerth, B , Block, L , Fahleson, U V , and Soop, K , *Nucl Fusion*, suppl Pt 1, 39 (1962)
[13] Drobyshevskii, É M , *Soviet Phys Tech Phys* , **8**, 903 (1964)

PLANETARY SCIENCE

Northward Drift of India—Examination of Recent Palaeomagnetic Results

Initial surveys[1-3] of the palaeomagnetism of the Deccan traps showed that there is an apparent decrease of palaeomagnetic inclination with altitude above sea level This was interpreted[2,4] as indicating a continuous northward movement of India during the time when the traps were being extruded It was pointed out however, that the observations could also be explained in terms of partial magnetic instability causing the directions to be pulled towards the present geomagnetic field Sahasrabudhe[5] has now made a very extensive palaeomagnetic study of the Deccan traps using magnetic cleaning techniques His aim was to try to use the palaeomagnetic directions for stratigraphic purposes and he did not attempt to

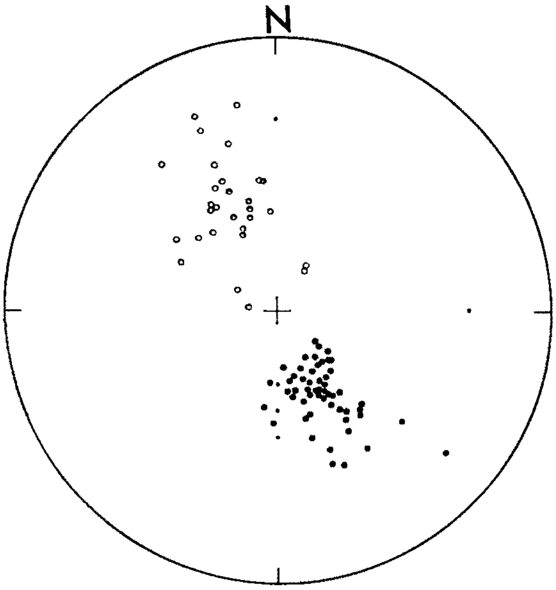

Fig 1 Stereographic projection of directions of magnetization at ninety-two sites in the Deccan traps ●, Lower hemisphere, ○, upper hemisphere

analyse his data for normal palaeomagnetic purposes I have made a complete analysis of his data and the purpose of this communication is to demonstrate that the earlier results are more likely explained in terms of viscous magnetization This conclusion leads to some consequences regarding the northward drift of India during the Tertiary

Approximately a thousand samples were analysed by Sahasrabudhe from seventy-one sites in a minimum of thirty flows and from thirteen dykes intruding them The sites were spread over the length and breadth of the area at present covered by the traps and over the entire thickness of about 4,000 ft Flows below an altitude of 2,000 ft were usually reversely magnetized (positive dips) and those above this level were usually normally magnetized (negative dips) The more detailed collection coupled with magnetic cleaning studies shows that there is no systematic change in inclination through the sequence as was suggested by the earlier results The lower (reversed) traps range in inclination in no systematic manner from +70° to +27° and the upper (normal) traps likewise range from −77° to −19° Normally magnetized dykes intrude both the lower and upper traps, but reversely magnetized dykes were not found intruding the normal ones Because the normally magnetized traps always overlie the reversed ones and are therefore stratigraphically younger, a distinction can be made between the lower and upper traps on the basis of polarity and the overall means for the two groups are given in Table 1 This analysis was not carried out by Sahasrabudhe, and for completeness I have also included results from eight sites in four areas not sampled by him[6,7] In each case, magnetic cleaning techniques were also carried out

Table 1 shows that ninety-two sites have been sampled in the flows and dykes of the Deccan traps and this is now one of the most thoroughly sampled formations in the palaeomagnetic literature The means of the two groups differ by 172 ± 8°, so that they both represent the same axis of rotation within the experimental errors The directions of magnetization are illustrated in Fig 1 The scatter of directions can readily be explained in terms of secular variation[8,9], and in neither case can it be shown[10] that the distribution of directions is non-Fisherian[11] The data therefore strongly support the contention that little or no polar movement took place during the period of extrusion of the Deccan traps and that the centre of the Indian subcontinent was situated about 30° S throughout this time

Table 1 PALAEOMAGNETIC RESULTS FROM THE DECCAN TRAPS, INDIA

	Directions						Pole position		
	No of site	Declination	Inclination	Resultant of N unit vector	Estimate of precision (ref 11)	α_{95}	Lat N	Long E	A_{95}
Upper traps (negative dips)	29	335	−45	27 185	15 4	7	37	102	8
Lower traps (positive dips)	63	152	+53	61 105	32 7	3	30	101	3
Overall mean	92	153	+51	88 114	23 4	3	33	102	3

α_{95} and A_{95} are the radii of 95 per cent circles of confidence

Recently, some potassium–argon age determinations have been made at the Department of Terrestrial Magnetism, Washington[12] Although the five determinations range from 42 to 65 × 10⁶ yr, the oldest age is from the overlying Pavagarh basalt, so there is the possibility of argon loss from some of the samples Assuming the age to be represented by the oldest determination, this places the traps at the Cretaceous–Tertiary boundary[13] Some clue as to the time covered by the extrusion of the traps is given by the fact that in spite of very detailed sampling only one reversal of the geomagnetic field is recorded in the 4,000 ft sequence Recent observations from magnetic anomalies and ocean-floor spreading since the late Cretaceous have led Heirtzler and his colleagues[14] to deduce a possible geomagnetic time scale back to 75 × 10⁶ yr. This analysis suggests that periods of normal or reversed polarity have persisted for no longer than 2 × 10⁶ yr at a time since the late Cretaceous If this is the case, then it seems unlikely that the Deccan traps cover a period of much longer than 5 × 10⁶ yr

Polar movement with respect to India since the Upper Permian is illustrated in Fig 2 It is not clear from the available data whether there is any real difference between the Upper Permian and Jurassic poles, although there is a significant difference between the Jurassic and Cretaceous poles It is quite clear from Fig 2, however, that the important polar shift with respect to India occurred between Deccan trap times and the present During this time the central part of India moved from latitude 30° S to 20° N If India had reached its present position by the time of formation of the Himalayas in, say, the Miocene, then this 50° change of latitude took place over about 50 × 10⁶ yr This corresponds to an

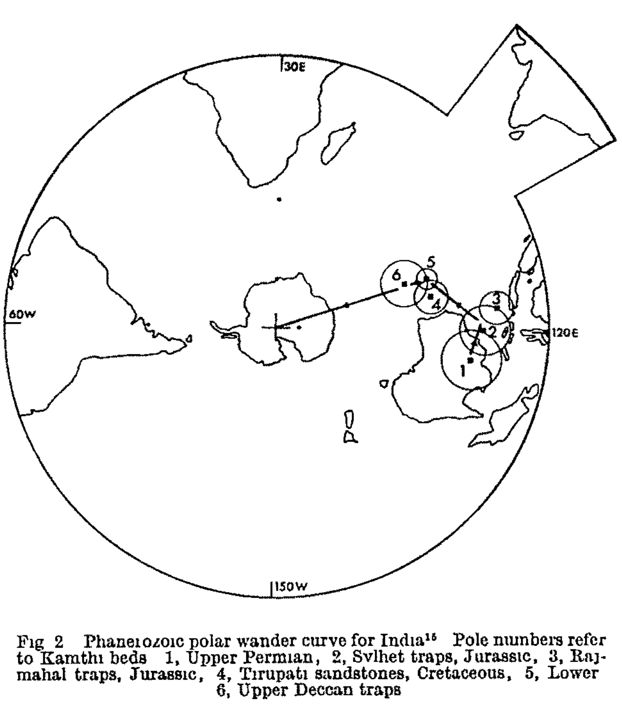

Fig 2 Phanerozoic polar wander curve for India[15] Pole numbers refer to Kamthi beds 1, Upper Permian, 2, Sylhet traps, Jurassic, 3, Rajmahal traps, Jurassic, 4, Tirupati sandstones, Cretaceous, 5, Lower 6, Upper Deccan traps

average rate of drift of India of 10 cm/yr during the Tertiary This is considerably greater than the average rate of about 5 cm/yr which I suggested[15] occurred between the Gondwanic continents during the break-up of Gondwanaland since the Upper Cretaceous It will be interesting to see in future whether these palaeomagnetic deductions of drift rates are substantiated by sea floor spreading rates south of India

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[1] Clegg, J A, Deutsch, E R, and Griffiths, D H, *Phil Mag*, 1, 419 (1956)

[2] Clegg, J A, Deutsch, E R, Everitt, C W F, and Stubbs, P H S, *Phil Mag*, suppl, *Adv Phys*, 6, 219 (1957)

[3] Deutsch, E R, Radhakrishnamurty, C, and Sahasrabudhe, P W, *Phil Mag*, 3, 170 (1958)

[4] Clegg, J A, Radhakrishnamurty, C, and Sahasrabudhe, P W, *Nature*, **181**, 830 (1958)

[5] Sahasrabudhe, P W, *Semin Geophys Invest Peninsular Shield*, 226 (Osmania University, Hyderabad, 1963)

[6] Verma, R K, and Rao, A A, *Semin Geophys Invest Peninsular Shield*, 161 (Osmania University, Hyderabad, 1963)

[7] Bhimasankaram, V L S, *Geophys J*, **9**, 113 (1965)

[8] Creer, K M, *J Geophys Res*, **67**, 3461 (1962).

[9] Gough, D I, Opdyke, N D, and McElhinny, M W, *J Geophys Res*, **69**, 2509 (1964)

[10] Watson, G S, and Irving, E, *Mon Not Roy Astro Soc*, suppl Geophys, **7**, 289 (1957)

[11] Fisher, R A, *Proc Roy Soc*, A, **217**, 295 (1953)

[12] *Annual Report for 1963–64*, 336, Department of Terrestrial Magnetism, Washington

[13] Kulp, J L, *Science*, **133**, 1105 (1961)

[14] Heirtzler, J R, Dickson, G O, Le Pichon, X, and Pittman, W C, *Trans Amer Geophys Union*, **48**, 133 (1967)

[15] McElhinny, M W, *Inter Union Geol Sci UNESCO Symp Continental Drift, Montevideo, October, 1967* (in the press)

Glasgow University Exploration Society Expedition to Inishtrahull

A GLASGOW University Exploration Society expedition to the island of Inishtrahull (lat 55° 26′ N, long 7° 14′ W), which is 5 miles from the northern coast of Donegal, was made during June 23–July 4, 1967, under the leadership of John Smart The rocks of the island have been both compared and correlated with the Lewisian gneisses of north-western Scotland[1,2], and the geological investigations carried out by the ten members of the expedition, under the direction of Dr D R Bowes and Dr A M Hopgood, are in continuation of Lewisian investigations being carried out in the Departments of Geology of the University of Glasgow and the University of St Andrews The island is approximately 1 mile long, in an E N E–W S W direction with almost uninterrupted clean coastal exposures on a broad wave-cut platform or on accessible cliffs Inland elevated parts (up to 141 ft above sea level at the western end), with outcrops of gneiss and related rocks, occur on either side of a low central area The island is less than 200 yd across in part of this central area and the gneiss complex largely covered by post-glacial bedded raised beach deposits (at approximately 40 ft O D) and soil On the high ground at the western end of the island stands the Inishtrahull Light

Quartzo-feldspathic and related gneisses make up much of the rock outcrop Some are strongly foliated and have a well developed mineral lineation other lithological units show little expression of planar or linear fabric elements One massive unit of calcium-rich epidote-bearing gneiss has a chemical composition similar to that of a calcareous pelite Its occurrence among a lithologically layered assemblage of gneisses suggests that the metamorphic complex largely represents a supracrustal series, possibly of sedimentary derivation There is evidence of metamorphic reconstitution at amphibolite facies, but over large areas the results of retrogressive deformation accompanied by considerable cataclasis constitute the dominant fabric elements of the rocks Among these crushed units are long narrow zones of cataclasite and small veins of pseudotachylite have been observed

Amphibolite occurs as small lensoid rock masses and in parts as fragments in agmatite A narrow, elongate lens of mafic rock with relict pyroxene and a ribbed outcrop, resembling that of layered ultrabasic bodies in the Lewisian of north-western Scotland[3], suggests that there was igneous activity before the development of the dominant foliation There are minor intrusions, including two sets of pegmatite veins Their respective times of emplacement in relation to the history of the gneiss complex can be determined from the various effects of metamorphic reconstitution and deformation exhibited In one case the evidence is based on the presence of an exceptionally well-displayed set of folded joints A number of unmetamorphosed dolerite dykes with an approximately easterly trend cut the gneiss complex They are presumed to be of Tertiary age

The effects of six phases of folding and associated deformation have been observed in the gneiss complex Of these, the earliest recognized was broadly co-eval with the formation of the gneissose foliation The structures which exert the dominant control on the distribution of the lithological units, and with which the development of the strong retrogression and cataclasis are related, were formed during the second deformational phase These structures deform both lithological layering and gneissose foliation The effects of the third and fourth deformational phases include some new mineral growth as well as the warping of the major structures while the final two phases are only locally expressed as very open folds In summary the sequence of geological events shown by the gneiss complex is as follows

(1) The formation of a lithologically layered sequence, possibly as the result of sedimentary processes

(2) The emplacement of mafic-rich igneous masses

(3) The development of gneisses associated with tight isoclinal folding of the lithological layers and tectonic disruption, in parts with the formation of agmatite Metamorphic reconstitution took place and the dominant foliation developed parallel to the axial planes of the isoclinal folds, hence generally parallel to the lithological layering

(4) The emplacement of basic igneous minor intrusions

(5) The formation of folds of varying style (isoclinal, asymmetrical, chevron, open) which fold the foliation and exert the most important control on the distribution of the lithological units Statistical analysis of fabric elements confirms field observations that the folds have sub-vertical axial planes and indicates that they plunge at approximately 15° towards 055° The style of the structures and the related cataclasis indicate deformation at a relatively shallow level in the crust Subsequent uprise of quartzo-feldspathic pegmatitic material from deeper levels was controlled by the axial planes of these folds

(6) Successive emplacement of acid and intermediate minor intrusions at the close of the second deformational phase Quartzo-feldspathic sheets, much sheared parallel to their margins, are cut by more mafic material which shows some planar alignment of minerals parallel to the intrusion walls

(7) The development of open folds with sub-vertical axial plane cleavage which trends N N E–S S W and cuts the minor intrusions of (6) These structures were superimposed on an already deformed foliation, and so the angle of plunge of the folds varies

(8) The formation of open folds with sub-vertical axial planes which trend N W–S E These folds are expressed on map scale as warps on the steep limbs of the 55° trending structures and have an associated fine planar mineral growth In one place a fold of this phase has been observed to deform the axial plane of a fold formed during event (7)

(9) The localized development of open concentric folds with sub-vertical E -W trending axial planes No related linear or planar fabric elements have been observed but crescent-shaped patches of quartz occur in some fold crests and troughs

(10) The localized development of very open folds plunging at low angles towards the north-east These folds are apparent only where they are superimposed on parts of the principal structure with flat-lying foliation

Quartzo-feldspathic pegmatite veins and, more commonly, quartz veins, occur and these follow the foliation on vertically disposed limbs of second generation folds They seem to be unaffected by events (7) and (8) but their relationships with events (9) and (10) are not clear

In the absence of radiometric dates, the absolute age of the gneiss complex of Inishtrahull cannot be determined There is, however, great similarity, both in rock types and also in tectonic and metamorphic histories, to the Lewisian of parts of the north-west Highlands of Scotland[4] and particularly to the Lewisian of Barra[5] and Mingulay[6,7] in the Outer Hebrides In each case (*a*) the earliest recognized phase of tectonics is associated with intense shear folding and the development of the dominant foliation and of autochthonous granitic rocks, (*b*) the principal structural pattern is related to deformation of the dominant foliation, the development of asymmetrical folds by a combination of concentric and shear movement and the later uprise of pegmatitic material along the axial planes of these folds Also (*c*) the open folds, formed largely by concentric movement, but with related development of fracture cleavage, deform both the dominant foliation and the major structures, and in the case of Inishtrahull, Mingulay and Barra, precede the further uprise of pegmatitic material Also in the case of the gneiss complexes of the three islands, the final tectonic phase is shown by very open concentric folds

While such similarities do not constitute definite proof of a correlation, and comparable features have been observed in other metamorphic terrain, it seems likely that there is general correspondence of geological histories of the Lewisian gneiss complex of the southern regions of both the north-west Highlands of Scotland and the Outer Hebrides[8] and the gneiss complex of Inishtrahull If this is proved to be the case, both the existence of Lewisian rocks as far south as Inishtrahull and the distinctive features of the gneiss complex there (particularly the evidence for a moderately shallow depth during the second deformational phase) will add considerably to our knowledge of the Lewisian.

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[1] Hull, E , Nolan, J , Cruise R J , McHenry, A , and Hyland, J S , *Mem Geol Surv Ireland* (1890)
[2] McCallien, W J , *Geol Mag* , **67**, 542 (1930)
[3] Bowes, D R , Wright, A E , and Park, R G , *Quart J Geol Soc* , **120**, 153 (1964)
[4] Bhattacharjee, C C , *Scott J Geol* , **4** (in the press, 1968)
[5] Hopgood, A M , *Krystalinikum* (in the press)
[6] Robertson, J F , Rankin J B , Bowes, D R , and Hopgood, A M , *Nature*, **204**, 25 (1964)
[7] Bowes, D R and Hopgood, A M , *Geol Soc Amer -Poldervaart Vol* (in the press)
[8] Bowes, D R , *Twenty-third Intern Geol Cong* , **4** (in the press, 1968)

Characterization of Carbonaceous Material from the Puerto Rico Trench of the Atlantic Ocean

Samples of two types of rocks containing organic material were collected from 65° 41′ 24″ W , 20° 15′ 58″ N during a cruise of the R V *Chain* of the Woods Hole Oceanographic Institution to the north wall of the Puerto Rico Trench (Fig 1) One of the samples is soft material, predominantly organic (*Ch* 34–*D*3) The other sample is hard material, a mudstone into which organic material seems to have migrated and subsequently been silicified (*Ch* 34–*D*3–182) This organic material and the associated basalts, limestones, cherts and siliceous mudstones were collected from depths of between 3,350 and 3,450 fathoms[1,2] These are the first organic samples obtained from ocean depths of this magnitude The limestones collected in the same dredge haul are Cenomanian in age[3], however, the age of the organic material is unknown

Characterization of the carbonaceous material is interesting because of its possible relationship to the principal types of terrestrial liquid and solid carbonaceous material such as coal, petroleum, and shale oil Characterization of the organic material may present clues to its origin It may have been transported to the site of deposition by living organisms, such as marine worms, which live in the deepest part of the Puerto Rico Trench, or it may have been deposited concurrently with the other sedimentary rocks, however, the carbonaceous material present in the siliceous mudstone indicates that the organic material postdates the sedimentary rocks

Another point of interest is the possible economic value of the carbonaceous materials at the bottom of the ocean, although it is very unlikely that such materials will have economic value in the foreseeable future

The original sample of semi-consolidated substance (soft material) was 15 cm × 8 cm × 3 cm in size, light in weight (real specific gravity of 1 50), porous, brown to brownish black in colour and easily ignited Thin-section analysis indicated that inorganic particles the size of clay made up 2–4 per cent of the sample X-ray diffraction analysis showed that the clay mineral was montmorillonite (expanded to 17 Å on treatment with ethylene glycol, but gave a broad peak indicating that the montmorillonite is poorly crystalline) The total content of minerals is appreciable, as indicated by the ash yield

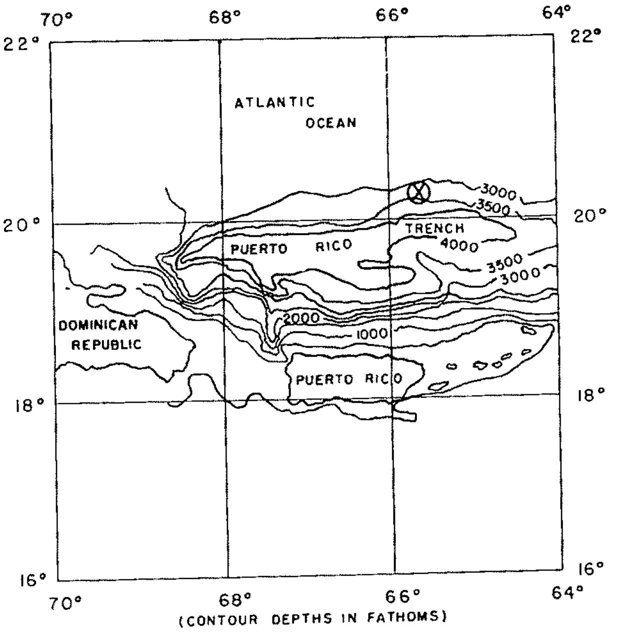

Fig 1 Location map, ⊗ shows collection site

Table 1 EMISSION SPECTROGRAPHIC ANALYSIS OF SOFT MATERIAL (SEMI-QUANTITATIVE)

Element	Weight per cent
Silicon	5
Magnesium	1–5
Aluminium	1
Iron	1
Calcium	0 5
Sodium	0 05
Titanium	0 05
Vanadium	0 05
Manganese	0 05
Chromium	0 01
Copper	0 01
Boron	Trace

determined by combustion, 27 5 per cent The metallic elements determined by emission analysis are given in Table 1

The original sample of silicified carbonaceous mudstone (hard material) was 13 5 cm × 10 cm × 2 5 cm in size was very fine grained and black to olive grey in colour, with the dense black portion (real specific gravity of 2 25) being surrounded by porous olive grey material which was poorly consolidated (real specific gravity of 2 42) The carbonaceous material within the black portion appeared to have disseminated into the siliceous mudstone and was then concentrated there as a hard, dense material as a result of subsequent silicification of the central portion of the sample The mineral portion of this sample consisted of 45 per cent quartz with minor tridymite, 1 per cent calcite that was less than 0 002 mm in size and subhedral in shape, 1 per cent smectite which was disseminated throughout, and 1 per cent chlorite which occurred as stringers and blebs Infrared spectra of the original rocks substantiated part of the X-ray diffraction analyses, but added no further information The carbonaceous material representing about 10 per cent of the sample was yellowish brown in plane light and isotropic between crossed polarizers Poorly preserved microfossils consisting of radiolaria, which were replaced by quartz, comprise 20 per cent of the sample Unfortunately, the microfossils were too poorly preserved for identification

Of several solvents investigated for extraction in organic studies, pyridine seemed to give the best results The yield of pyridine extract was 1 5 per cent for the soft material and 0 82 per cent for the hard material Dioxane extraction of the portion of the soft material soluble in pyridine gave a yield of 80 per cent of material suitable for ultraviolet spectral measurements Ultraviolet and visible and infrared spectra were obtained from the original and extracted material

Ultraviolet and visible spectra for the extracts of the soft and hard materials were identical The spectrum of the soft material is given in Fig 2 Two strong bands at 412 and 658 mμ were observed in the visible spectrum (pyridine solvent) Moderate bands were found at 695, 600 and 502 mμ, and a weak band at 557 mμ In the ultraviolet region moderate bands occurred at 250 and 301 mμ (dioxane solvent) Because of the strong bands observed in the visible spectrum an attempt was made to obtain a visible spectrum of the original rock The spectrum obtained (Fig 2, curve *C*) indicates that intense absorbers are present, it is not usual to obtain structured visible spectra directly on carbonaceous rocks

The spectra seem to be similar to spectra of porphyrins, or the related bile pigments[4] or the metal complexes of these The strongest band, 412 mμ, is the Soret band familiar in spectra of porphyrins and related materials Semi-quantitative measurements based on pure compound spectra for metal complexed bile pigments and porphyrins yield results in the range of 1 per cent for the content of the material producing this spectrum The bile pigments and the porphyrins have similar structures, both involving a group of four pyrrole rings[4] The nitrogen content of the carbonaceous materials is high enough to accommodate such structures as shown by the ultimate analyses (Table 2) A high sulphur content is also indicated in Table 2

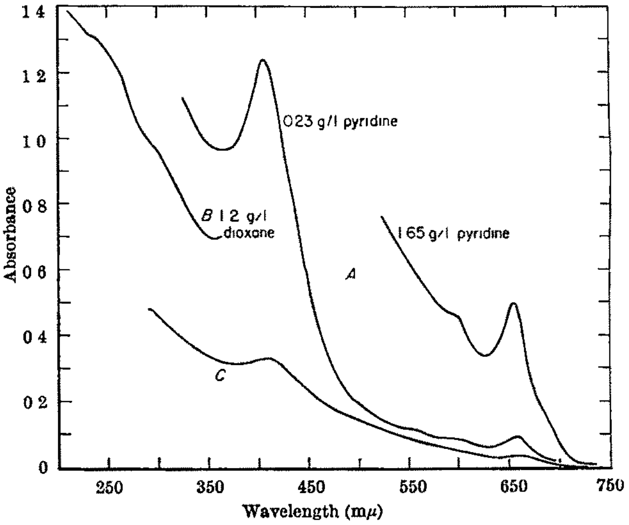

Fig 2 Ultraviolet and visible spectra of carbonaceous materials from the Puerto Rico Trench, curve *A*, pyridine extract of soft material, 1 cm path, curve *B*, dioxane solubles from pyridine extract of soft material, 2 cm path, curve *C*, potassium bromide pellet of original soft material

A visible spectrum of the kerogen concentrate from oil shale (not shown) also shows weak bands at 412 and 270 mμ and very weak bands at 637 and 312 mμ The specimens do not fluoresce under mercury 2537 Å light

Infrared spectra of extracts of the ocean rocks initially indicated some interference caused by traces of mineral matter From dilute solutions of the extracts the mineral matter was allowed to settle out, the solutions were decanted and spectra nearly free of mineral interference were obtained These spectra, shown in Fig 2 along with a spectrum of kerogen from oil shale (kerogen sample supplied by J Cummins, Bureau of Mines, Petroleum Research Center, Laramie, Wyoming), indicate intense OH absorption (about 3,400 cm^{-1}), intense aliphatic CH (about 2,900 cm^{-1}), very weak aromatic CH (3,030 cm^{-1}), strong carbonyl absorption (1,700 cm^{-1}), strong absorption caused by $-CH_3$ groups (or $-SO_2-$ groups or both) (1,375 cm^{-1}), strong absorption in the region indicating carbonyls in either acids or esters (1,200 to 1,250 cm^{-1}) and weak absorption in the aromatic band region (650 to 900 cm^{-1}) There is some indication of aromatics but the spectrum is principally that of aliphatic substances The infrared spectra of the original rocks were also recorded (not shown), the spectra of the rock samples are apparently identical to the spectra of the extracts obtained from them For the soft material it was possible, in spite of the spectral interference of the minerals, to observe all the principal spectral bands produced by the entire carbonaceous

Table 2 ULTIMATE AND PROXIMATE ANALYSIS OF CARBONACEOUS ROCKS* FROM PUERTO RICO TRENCH (SPECIFIC GRAVITIES)

Weight per cent	Moisture and ash-free basis Hydrogen	Carbon	Nitrogen	Oxygen	Sulphur	Moisture	Volatile matter	Fixed carbon	Ash	Specific gravity
Soft material	8 1	66 6	1 8	15 6	7 9	2 0	53 3	17 2	27 5	1 50
Hard material (outside part)	8	57	2	22	11	1 0	7 2	1 1	90 7	2 42
Hard material (inside part)	8	56	2	26	8	3 3	8 7	1 5	86 5	2 25

* Identification *Ch* 34–*DS*

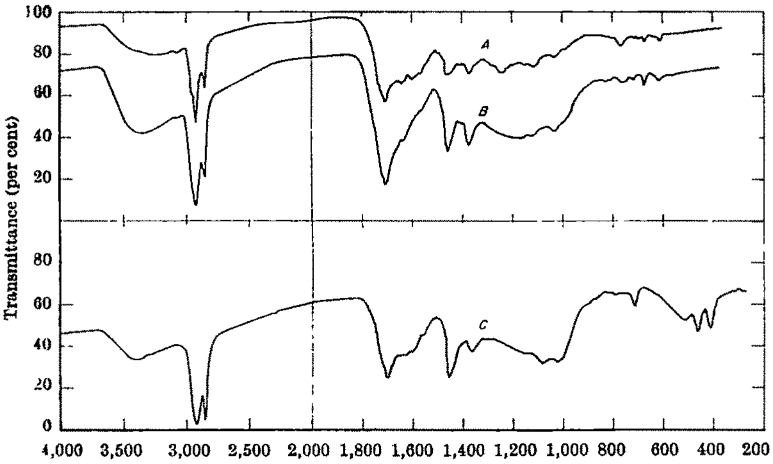

Fig 3 Infrared spectra of potassium bromide pellets curve *A*, pyridine extract of hard material, curve *B*, pyridine extract of soft material, curve *C*, kerogen concentrate from oil shale

sample For the hard material only the very intense absorption bands of the carbonaceous material were observable

Mass spectra of the rocks and of their pyridine extracts were obtained, but they were not informative Mass peaks of nearly equal intensity were observed at all masses investigated

The organic material obtained from the Puerto Rico Trench seems to be more nearly similar to kerogen from oil shale than to petroleum or coal This conclusion is based on the infrared spectra of both the extracts and the original carbonaceous rocks Neither petroleum nor coals possess intense absorption bands at the carbonyl position, 1,700 cm^{-1}, which are shown by the spectra of the ocean samples and the kerogen concentrate The highly aliphatic character of the ocean samples and the kerogen also differs from the more aromatic character of petroleum and coals The material is in a highly oxidized state, as shown by the oxygen contents and the intense OH and C=O bands in the infrared spectrum There does not seem to be any large amount of oxygen in ether type or heterocyclic structures according to the infrared spectra Two surprising findings are (1) there are many methyl groups or $-SO_2-$ groups or both, and (2) there is no indication in the infrared spectrum of the absorption characteristic of long, unbranched chains; the typical spectral band at 720 cm^{-1} does not appear The 1,375 cm^{-1} band in the spectra of carbonaceous materials is usually assigned exclusively to $-CH_3$ groups For curves *A* and *B*, however, the assignment is questionable because the 1,375 cm^{-1} band is unusually strong relative to the $-CH_2-$ band at 1,460 cm^{-1} The band shoulder at 2,950 cm^{-1} partly substantiates the assignment of 1,375 cm^{-1} to $-CH_3$ groups But, because of the presence of appreciable concentrations of sulphur and oxygen, plus the spectral indication of many oxygenated groups, it is believed that the 1,375 cm^{-1} band is partly produced by $-SO_3-$ groups

	Ocean	Kerogen
Aromatic CH	Greater	
CH_3 groups (or $-SO_2-$ groups or both)	Greater	
Long alkyl chains		Greater
OH	Greater	
Aromatics	Greater	

The infrared spectrum (Fig 3) of the soft material curve *B*, resembles somewhat the infrared spectrum of a kerogen concentrate from oil shale, curve *C* This resemblance, however, cannot be considered definitive The infrared absorption bands of both substances are too broad and ill-defined to be considered as proof of structure There are distinct differences between the spectra of the ocean samples and those of the kerogen concentrate, comparison of the spectra shows the chemical entities that predominate in the spectra of the ocean and kerogen materials

When the visible spectra of the ocean samples were compared with spectra of porphyrins, chlorins, bile pigments, and the metal complexes of these, the substance found to have a spectrum most similar to those of the ocean samples is pheophytin *a*[5], a chlorin which is a well known degradation product of chlorophyll *a* formed by the simple loss of magnesium This indicates that the organic material in the ocean samples is closely related to degradation products of plants, rather than of animals The discovery of a high magnesium content in the soft material (Table 1) tends to substantiate the close relationship of the organic portion to chlorophyll *a*

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[1] Bowin, C O , Nalwalk, A J , and Hersey, J B , *Geol Soc Amer Bull* , 77, 257 (1966)
[2] Nalwalk, A J , thesis, Univ Pittsburgh (1967)
[3] Todd, R , and Lowe, D , *Deep-Sea Res* , 11, 395 (1964)
[4] Lemberg, R , and Legge J W , in *Hematin Compounds and Bile Pigments*, 102 (Interscience Pub , Inc , New York, 1949)
[5] Schiele, F P , and Comar, C L *Bot Gaz* , 102, 463 (1941)

Trend in the Harmonic Energies of the Diurnal Variation at Two British Observatories

SEVERAL workers, the earliest of whom seems to be Schuster[1], have extensively analysed the world-wide distribution of the daily magnetic field variation, in order to obtain estimates of crustal electrical conduc-

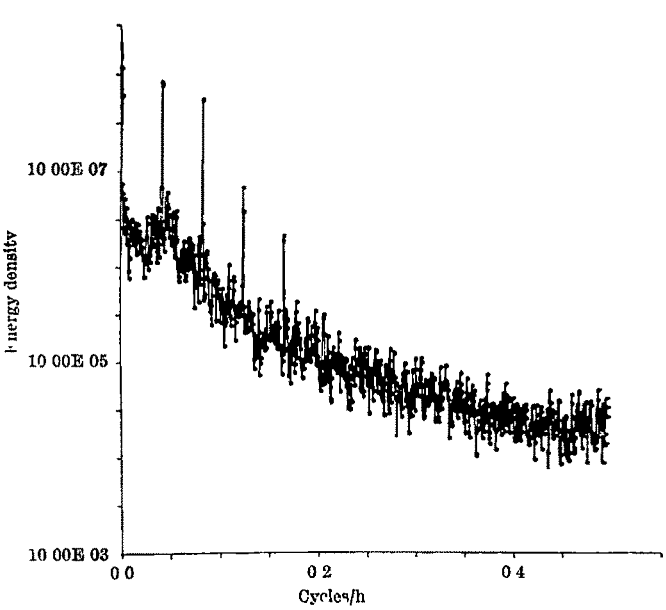

Fig 1 A spectrum of energy for variations in the vertical magnetic field for Eskdalemuir The peaks for 24 12, 8 and 6 h can be seen clearly

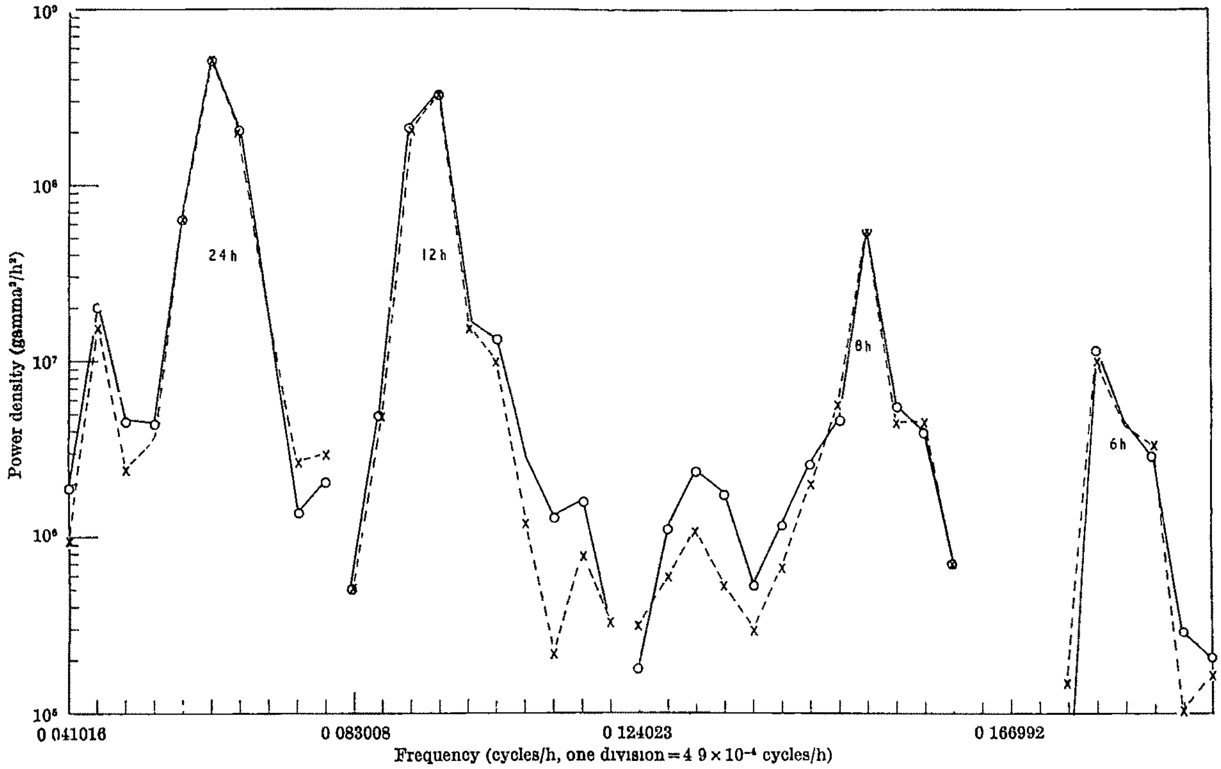

Fig. 2 The shape of the 24, 12, 8 and 6 h peaks in Fig 1 at full spectral resolution ———, Eskdalemuir, - - - - , Stonyhurst

tivity Much of this work was done before the advent of high speed computers, and may have been affected by subjective errors. The object of this communication is to present the results obtained by a full Fourier analysis of a year's data from two British observatories Comparison of these allows the errors in amplitude and phase of the spectral peaks to be estimated

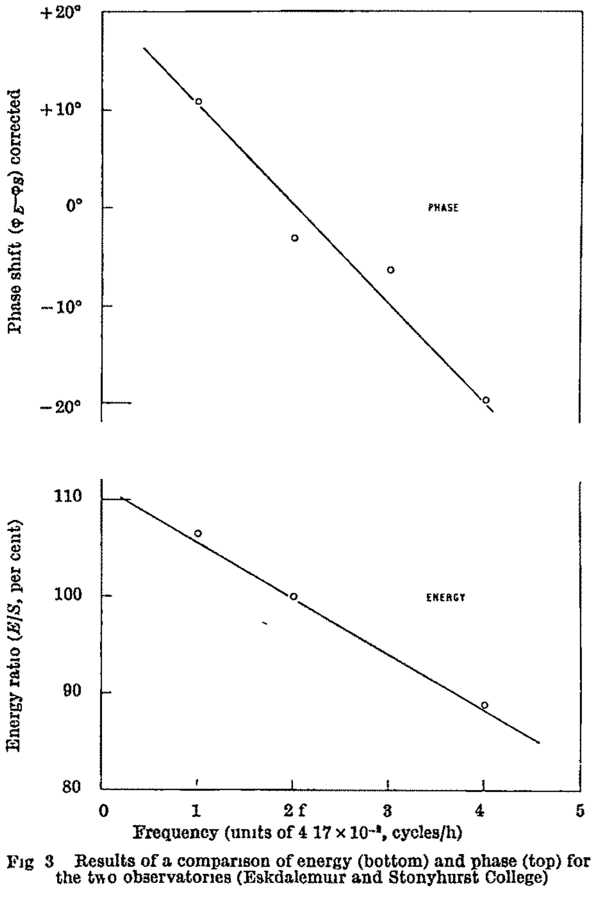

Fig 3 Results of a comparison of energy (bottom) and phase (top) for the two observatories (Eskdalemuir and Stonyhurst College)

The year 1964 was chosen as the most recent for which results are readily available The values used were (J D Jacobs and Father V E Worthy, private communications) measured at Eskdalemuir (lat 55° 19′ N , long 356° 48′ E) and at Stonyhurst College (lat 53° 51′ N , long 357° 32′ E) which are separated by 200 km along a line running N N E Both are removed from the coast, and so should be free from tidally generated fields The data used were the hourly values of the vertical field variation, and all the values taken during a year were used except for those on the disturbed days This gave a total of 7,344 hourly values A Fourier transform programme due to A Douglas (private communication) was used, and this gave estimates of energy and phase at frequency intervals of 1 22 x 10⁻⁴ cycles/h A smoothed spectrum is shown in Fig 1, the only lines which are clearly distinguishable from noise are those at periods of 24, 12, 8 and 6 hours The shape of these lines at the full spectral resolution is shown in Fig 2 It is apparent that there is good agreement between the estimated component energies, so that a detailed comparison between the two observatories should be meaningful The results of this comparison, in energy and in phase, are shown in Fig 3, where a correction has been applied to the phase estimates to allow for the 0 7° West relative longitude of Eskdalemuir

The first point about this comparison is the good consistency obtained .This is particularly striking in the case of the phase The phase zero for each frequency occurs at the time of the first reading each year and the individual phase estimates vary rapidly between ± 180° despite this the differences between the phases at the observatories are small and vary regularly It is estimated that the relative standard deviation of the estimates at the peak energies is ± 1 4 per cent in energy, ± 1 2° in phase

Riddihough[2] has shown that the variation with 24 h period in 1957 was higher at Eskdalemuir than at Stonyhurst, and the present work confirms this It is interesting that this difference falls to nothing for the 12 h variation and becomes negative at shorter periods Although the effect of oceanic edge currents would be expected to be of shorter range at shorter periods, this would not give a negative gradient in variation energy, it would only reduce the excess at Eskdalemuir The effect might be caused by the presence of a more highly conducting region beneath Eskdalemuir, which would reduce the coastal energy enhancement at all periods

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[1] Schuster, A , *Phil Trans Roy Soc* , A, 208, 163

[2] Riddihough, R P , *Nature*, 215, 720 (1967)

PHYSICS

Circulation within Monolayer Films on Aqueous Substrates at Low Surface Pressures

ALTHOUGH at high surface pressures, up to about 20 dynes/cm^2, films of long chain fatty acids on water form uniform monolayers, at zero surface pressure the film is no longer uniform but has been described by some authors as consisting of "islands" held together by the van der Waals forces of cohesion between the hydrocarbon chains[1] An examination with an electron microscope of surface films of stearic acid prepared at a surface pressure of 3 dynes/cm^2 has provided some evidence that these irregularly shaped islands exist[2]

Radio-tracer studies of oleic acid and other films on water have revealed an interesting phenomenon lending credence to the existence of islands An apparent circulation of large film elements in a regular periodic manner has been observed Although oleic acid is usually considered to be "insoluble" in water, surface films after spreading to a pressure of 5–20 dynes/cm^2 do proceed to dissolve at a rate which is a function of the surface concentration As the diffusion process continues the surface pressure drops substantially and radioactive counting of the remaining surface film gave the results shown in Fig 1

The experiments were conducted as follows A 'Pyrex' glass dish 9 cm in diameter was cleaned and placed in a polyethylene holder Distilled water (50 ml) was then added to give a water depth of 0 8 cm A Baird–Atom model 821*B* counting tube, 3 cm in diameter, was placed over the dish, and the background radioactivity counted for 1 h in 10 min intervals The entire apparatus was suspended on a heavy steel plate attached by springs to a frame in order to block out vibrations Finally, a measured amount of radioactive oleic acid in hexane was spread on the water surface with an Alga micrometer syringe The counting rate was monitored for 24 to 170 h

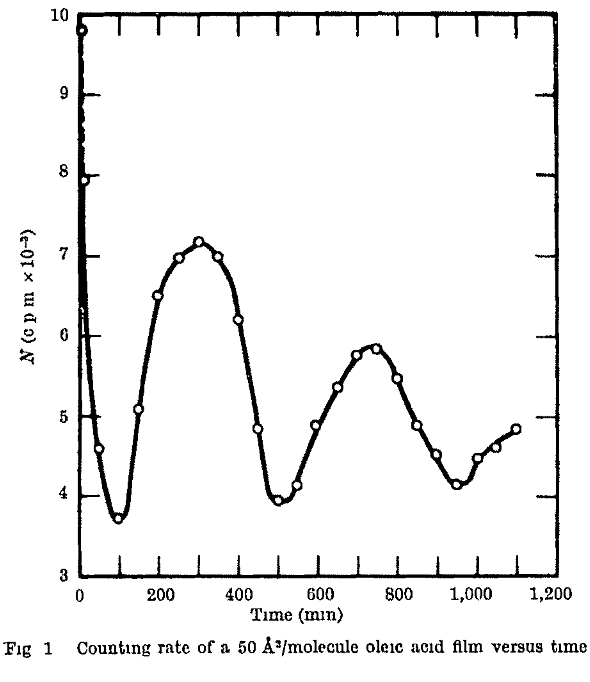

Fig 1 Counting rate of a 50 Å^2/molecule oleic acid film versus time

In many of the experiments, such as that shown in Fig 1, an oscillatory character was discovered in the counting rate The oscillations were attributed to an instability in the system termed the Marangoni effect[3] As the surface film diffuses into the water, the film remaining on the surface begins to form islands, a process which simultaneously gives rise to tension gradients engendered by the following mechanism

At equilibrium there would be islands but no surface tension gradients, because the islands would be surrounded by surface active molecules and smaller islands acting more or less individually, but nevertheless present in sufficient numbers so as to maintain a constant surface pressure over the entire surface Thus at the start of the experiment we have a picture of islands amid a distribution of smaller film segments without surface tension gradients If we permit molecular transfer by diffusion from the surface into the bulk, then it is easy to picture a situation in which the loss of a few molecules from an island into bulk will have little effect on the local surface pressure whereas the diffusion into the bulk of some of the individually acting molecules will result in a local increase in the surface tension, thus inducing a gradient Indeed, it is our opinion that the diffusion from the surface into the bulk of surface active material existing as a configuration of islands surrounded by molecules will be inherently unstable and always exhibit the Marangoni effect

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[1] Davies, J T , and Rideal, E K , in *Interfacial Phenomena*, second ed (Academic Press, New York, 1963)

[2] Ries, H E , *Proc Amer Petrol Inst* , Sect 3, 152 (1962)

[3] Sternling, C V , and Scriven, L E , *Amer Inst Chem Eng J* , 5, 514 (1959)

Transport Processes and the Chemical Potential

MELEHY[1,2] has proposed a theory of transport phenomena, based on a claim that the chemical potential for each constituent is not a constant everywhere in a system which is in thermodynamic equilibrium He argues that the usual proof of its constancy everywhere is invalid, and that, in any case, its consequences are contrary to experiment For example he states[1] that the net work in transferring a particle between two points, whatever the gravitational or electrostatic potential difference may be, would be zero on the usual theory, that is, the transfer would be at constant energy Some consequences would be the denial of the existence of such effects as the Peltier effect and the necessity in thermal equilibrium for the conduction bands to line up throughout a crystal containing a non-degenerate p–n junction

I criticized[3] his theory, using a new method to validate again the constancy of the chemical potential, $\mu_i+\varphi$, throughout a system at thermodynamic equilibrium, μ_i

being the intrinsic chemical potential and φ the potential energy per unit mass of the long range gravitational or electrostatic forces I also showed that, in considering the work done in transferring a particle from one point A in the system to another point B, Melehy had neglected the contribution to the work done in changing the volume occupied by the particle from that at A to that at B

In a further communication[4], Melehy has applied his theory to obtain a formula for the current–voltage relation at a *p–n* junction His formula undoubtedly gives good agreement with the experimental results quoted. His reply to my criticism of his theories however, consists of the statement that his theory does account for the work contribution mentioned earlier He gives two reasons for this, the first that it is accounted for in the "subtle arguments" used in developing his theory, the second that his formula gives good agreement with the experimental results used Both reasons clearly do not assist scientific discussion, especially as in his arguments he postulates an expression for the diffusion force (see equation (28) of ref 1)

No comment is made on my defence of the constancy of the chemical potential at equilibrium, which is basically important because his theory is explicitly based on a contradiction of this well known result

In section (6) of ref 2 the following statement is made "The conclusion that $d\mu \neq 0$ whenever $dP \neq 0$ in the two preceding examples is an inevitable consequence of equation (16)

$$\{d\mu = -s dT + (1/n) dP\}$$

It might be of historical interest to state that the example of an atmosphere in thermal equilibrium happens to have been treated by Gibbs[21], who also has concluded that for that case $d\mu \neq 0$ in thermal equilibrium Clearly, it may now be stated that, under thermal equilibrium conditions, $d\mu$ will never vanish in any system wherever $dP \neq 0$, that is wherever $dn \neq 0$ "

On further study of ref 2, and taking into account especially this passage and the two examples cited, it is certain that the principal error has been a confusion between what Gibbs[5] called the intrinsic chemical potential and the chemical potential of a system under long range forces, which, for electric forces, is known as the electro-chemical potential It is, of course, well known that this latter quantity $\mu_i + \varphi$ is a constant everywhere at equilibrium and that its constancy determines the lining up of Fermi-levels It is also well known (see, for example, ref 3) that in a quasi-static isothermal displacement $\delta \mathbf{r}$ of a mass element m the work done is

$$\begin{aligned} \delta W &= \delta \mathbf{r} \, \{\nabla(Pv) + \nabla\varphi\} m \\ &= \delta \mathbf{r} \, \{\nabla(Pv) - \nabla\mu_i\} m \\ &= -m \delta \mathbf{r} \, \nabla f_i \qquad (1) \end{aligned}$$

where μ_i, f_i are the intrinsic chemical potential and intrinsic Helmholtz free energy per unit mass

Thus at equilibrium

$$\nabla\mu_i = -\nabla\varphi \qquad (2)$$

and $-\nabla\mu_i$ may in some way be regarded as a fictional force balancing that caused by the long-range forces The work done in a quasi-static isothermal displacement however, is not solely that arising from these forces but is given by (1) If ∇f_i is regarded also as a fictional force it is, of course, irrotational

A further confusion occurs in section (8) of ref 2 concerning the thermodynamic pressure and the pressure obtained from the Virial theorem in liquids and vapours It is claimed that in liquids if P is calculated by the Virial theorem it could be magnitudes higher than in the vapour This is wrong, as reference to any good text (for example, Hirschfelder, Curtiss and Bird[6]) on liquid theory would show, in fact the pressures according to either kinetic theory or thermodynamics are the same and this does not conflict, as implied, with the fact that, in the classical case, Maxwell–Boltzmann statistics applied

The agreement with experiment of the formula obtained by Melehy for the current–voltage relation of a *p–n* junction is interesting and this communication is written in the hope that a valid explanation may be made of the new terms introduced in the formula

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[1] Melehy, M A , *Nature*, **205**, 456 (1965)

[2] Melehy, M A , *Nature*, **209**, 670 (1966)

[3] McLellan, A G , *Nature*, **211**, 359 (1966), ibid , **213**, 60 (1967)

[4] Melehy, M A , *Nature*, **215**, 1251 (1967)

[5] Gibbs, J W , in *Scientific Papers*, **1**, 147 (Longmans, Green and Co London, 1906)

[6] Hirschfelder, J O , Curtiss, C F , and Bird, R B , *Molecular Theory o Gases and Liquids*, 134 (Wiley and Sons, New York 1954)

CHEMISTRY

Simplification of the Synthesis of Tetramethyldipicrylamine

BECAUSE the antibacterial agent, tetramethyldipicrylamine, which we reported recently[1] has aroused wide interest we have developed a modification in the synthesis of the intermediate, 3,3′,5,5′-tetramethyldiphenylamine, which will make it possible for research workers to prepare experimental quantities of pure material in good yield without the necessity of elaborate equipment The methods reported previously involved heating 3,5-dimethylaniline and its hydrochloride in either a glass[2] or metal[3] autoclave at 235°–250° C or heating a mixture of 3,5-dimethylaniline and 3,5-dimethylphenol at 300°–400° C under 50 to 300 atmospheres pressure[4]

It was found that a sealed tube reaction was feasible when a slurry of equal amounts of 3,5-dimethylaniline and its hydrochloride, not exceeding a total of 7 g, was packed into a glass tube by means of a glass wool plug The tube, selected to have a volume of 3 ml /g of material, was sealed and heated in a constant temperature oven for 72 h at 230°–240° C A 74 per cent yield of good quality 3,3′,5,5′-tetramethyldiphenylamine was obtained This was washed with warm 7 per cent hydrochloric acid, extracted with either benzene or ether, dried over anhydrous sodium sulphate and nitrated without further purification to give a 72 per cent yield of the antibacterial agent, tetramethyldipicrylamine, melting point 232° C dec , reported 231° C dec We have extended the use of this simplified sealed tube method to the synthesis of 3,3′-dimethyldiphenylamine in 64 per cent yield and to 3,3′ *bis*- and 3,3′,5,5′-*tetrakis*-trifluoromethyldiphenylamine in lesser yields

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[1] Meyer T S , Moore, C E , and Frank, P F , *Nature*, **215**, 312 (1967)

[2] Hebky, J , Kejha, J , and Karasek M , *Collection Czechoslov Chem Commun* , **26** 1559 (1961), *Chem Abst* , **55**, 18732 (1961)

[3] Moore, C E , Meyer, T S , and Hudson, J W , *Talanta*, **13**, 171 (1966)

[4] Birch, F S , Fidler, F A , Hardy, D V N , and Molloy, E L , Brit. Pat No 619 877, *Chem Abst* , **43** 5799 (1949)

BIOCHEMISTRY

Action of Atomic Hydrogen on Aqueous Bacteriophage T_7

WE have examined the action of hydrogen atoms on bacteriophage T_7 in aqueous suspension, and found the mechanism of inactivation to be premature release of the DNA.

The experimental procedure for treating with hydrogen atoms, using simple compounds and enzymes, was described earlier[1-6]. Hydrogen atoms produced in a 30 Mcs electrodeless discharge in pure hydrogen gas at 27 mm of mercury were swept through the solution at 4° C. The hydrogen gas flow of about 50 l./min excluded oxygen from all experiments. Control experiments showed that bubbling hydrogen alone did not produce significant effects.

The dose rate of hydrogen atoms, of about $1·5 \times 10^{-4}$ moles/l. min, was exactly determined before and after each experiment using the ferricyanide dosimeter[7,8].

Bacteriophage are more sensitive to ionizing radiation when they are in dilute aqueous suspension than when protected by gelatine or broth from the action of radicals formed in the water[9]. The protective action of oxygen in these conditions suggests that the reducing species produced in water may be responsible for bacteriophage inactivation[10], and preliminary experiments by Shapira and Stein on the effect of hydrogen atoms on phage T_7 indicates that this is so.

We used bacteriophage T_7 because Freifelder[13] has already shown that the DNA extracted from this phage has an extremely uniform sedimentation coefficient. Damage to the DNA can easily be measured with an analytical ultracentrifuge.

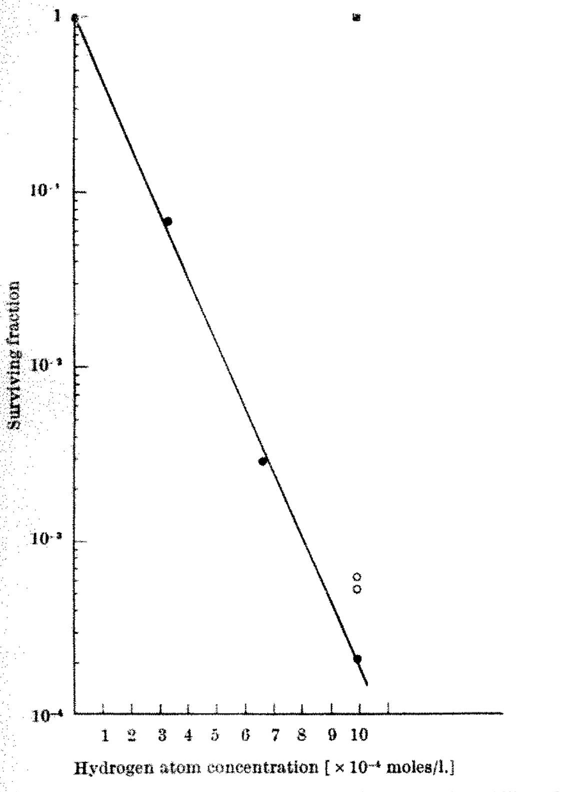

Fig. 1. The effect of hydrogen atoms on the plaque forming ability of phage T_7. Solid circles indicate the survival of phage suspensions given successive small doses of hydrogen atoms. Two large single doses are represented by hollow circles. The fact that these are above the line may be caused by small droplets thrown from the suspension by the gas bubbling and not falling back till after the irradiation. The lack of effect on phage in 1 per cent nutrient broth is indicated by the solid square.

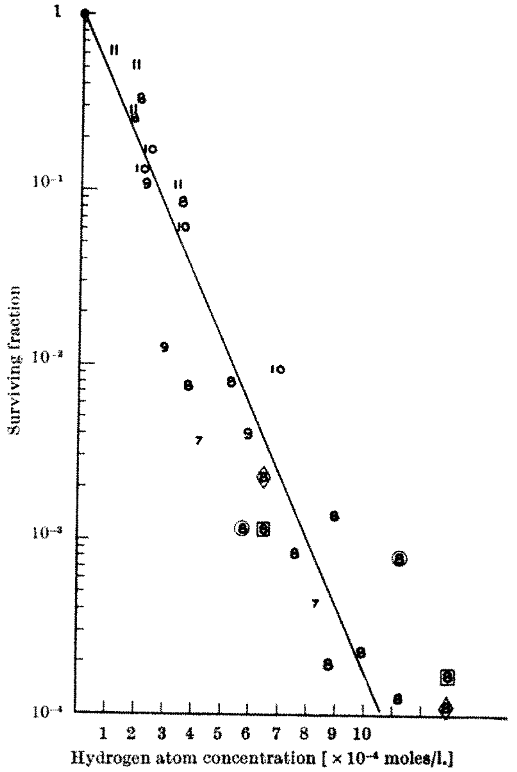

Fig. 2. Phage T_7 at various concentrations treated with hydrogen atoms. The numbers are the log concentration of phage/ml. For example, 8 represents the surviving fraction after treating a phage suspension containing 10^8 phage/ml. The enclosed numbers refer to treatment of the phage in the presence of added magnesium sulphate. ○, 10^{-4} Molar magnesiun. □, 10^{-3} molar magnesium; ◇, 2×10^{-2} molar magnesium. The line is taken from Fig. 1.

The experimental details of growing, purifying and assaying the phage on *E. coli B* have been described[11,12]. The bacterial debris was removed by centrifugation at 4,000*g* for 10 min. Phage was recovered by treatment at 75,000*g* for 2 h; the caesium chloride buoyant density purification procedure was carried out at 150,000*g* for 14 h.

Figs. 1 and 2 show the effects of atomic hydrogen on T_7 at various concentrations with and without 1 per cent broth and with and without various concentrations of added magnesium ions. It will be seen that phage T_7 in 0·01 molar phosphate buffer at *p*H 7·8 and 0·1 molar NaCl was inactivated in triple distilled water by atomic hydrogen with a strict exponential relationship between survival and dose. The results in Figs. 1 and 2 show clearly that there is no initial shoulder which has sometimes been observed with X-rays by others[13]. The absence of a shoulder is consistent with a mechanism of inactivation which involves only one crucial event per phage. This does not exclude the possibility that some of the hydrogen atoms reacted at a number of inessential sites on the phage.

The almost complete protection by broth (Fig. 1) is consistent with competition for the available atomic hydrogen. In our system, hydrogen atoms are introduced into the liquid from the gas phase and their reaction with phage (Ph) competes with their reactions with other substrates (S), and also with the recombination of hydrogen atoms

$$H\cdot + Ph \rightarrow HPh\cdot \rightarrow \text{unknown products}$$
$$H\cdot + S \rightarrow HS\cdot \rightarrow \text{unknown products}$$
$$H\cdot + H\cdot \rightarrow H_2$$

As shown in Fig. 2, the D_0 for the loss of plaque-forming ability is about $1·2 \times 10^{-4}$ moles of hydrogen atoms/l., and is independent of phage concentration between 10^7 and 10^{10} phage/ml., with possibly a very slight increase at 10^{11} phage/ml. We considered three

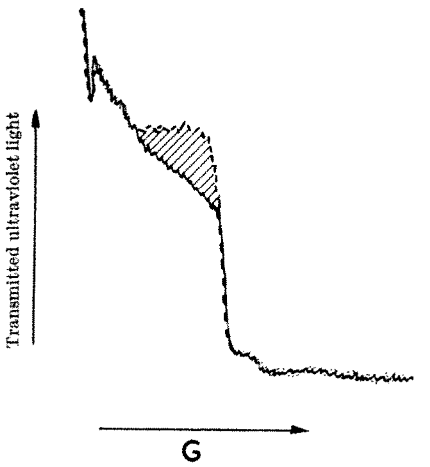

Fig. 3. Tracings of the ultraviolet absorption patterns of the DNA from phage T_7 in an analytical ultracentrifuge. The control DNA (dotted line) has been superimposed on the DNA from phage treated with hydrogen atoms (solid line) after suitable adjustment of magnification to allow for differences in concentration and differences in film development. The shaded area shows where the two lines diverge because of the slower sedimentation rate of some of the broken DNA released from phage treated with hydrogen atoms.

possible mechanisms of action of hydrogen atoms on phage. (*a*) Damage to the DNA. This assumes that atomic hydrogen penetrates the protein coat or that one electron equivalent is transferred to DNA from the protein after hydrogen atoms have been added to the protein coat. (*b*) Bond formation between protein and DNA. This might prevent efficient injection of DNA into the host bacterium. (*c*) Action on the protein coat only. This might either inhibit the injection mechanism or cause premature release of DNA in the absence of a host bacterium.

To distinguish between these possibilities we studied the sedimentation patterns of the DNA from T_7, using an ultracentrifuge. Phage T_7 was induced to release its DNA by heat treatment in the presence of 0·001 molar EDTA (ref. 14). Very sharp Schlieren boundaries were obtained and the microdensitometer traces of ultraviolet absorption photographs also indicated a single sharp moving boundary consistent with the presence of only one component.

By spinning down the sample which had been treated with hydrogen atoms and which had not received the heat release treatment, we found indications that material had been released from the phage. A very diffuse Schlieren picture was obtained, and the microdensitometer traces of the ultraviolet photographs were consistent with an inhomogeneous population of smaller molecular weight fragments, which might have resulted from the breaking up of released DNA. Finally, when samples which had been treated with hydrogen atoms were also heat-released in the absence of magnesium ions (to release any DNA still retained within the protein coat) a mixed population was obtained in the ultraviolet microdensitometer trace, indicating the presence of both intact and broken DNA. The ratio between intact T_7 DNA and broken fragments was approximately the same as that between surviving and inactivated T_7 in the particular treated sample (Fig. 3).

Further evidence to suggest a mechanism of DNA release actuated by hydrogen atoms was obtained by studying electron microscope photographs of untreated and hydrogen atom treated T_7 phage. The results, shown in Fig. 4, are consistent with the view that after treatment with hydrogen atoms we are observing hollow shells of the protein coat, from which DNA has been ejected into the solution in the absence of bacteria.

To correlate our results with the effect of ionizing radiations in similar conditions of water purity and absence of competing scavengers, we examined the action of caesium-137 gamma rays on T_7 phage. If gelatine or broth is present to remove the effects of radicals formed in the water by ionizing radiation, high doses are needed to inactivate phage T_7 (ref. 13). At the low doses used in these experiments the direct target type action is negligible. The results are shown in Fig. 5. The D_0 for the 0·6 MeV gamma rays is about 500 rad. Thus in our experimental conditions the sensitivity to radiation is higher than previous data in the literature[13]. For the radiation experiments, too, the strict exponential relationship holds and there is no shoulder.

The calculation of reaction velocities and rate constants of a very large molecule, such as T_7 phage, with hydrogen atoms in our experimental conditions cannot be justified, because of the many assumptions required. Qualitatively, however, the number of radicals needed to inactivate phage irradiated with gamma rays and with hydrogen atoms, respectively, are comparable, when allowance is made for the non-homogeneous kinetics in the case of hydrogen atoms[1].

There is a slow spontaneous release of DNA from phage T_7 which is prevented by the presence of magnesium ions[11]. To see whether the only action of hydrogen atoms is to speed up the rate of spontaneous release, we examined the effect of added magnesium ions on the effectiveness of hydrogen atoms. There was no effect of added magnesium, as Fig. 2 shows. Thus the role of hydrogen atoms in inactivating phage T_7 is not connected directly with magnesium ions as a stabilizing factor, although it may have to do with that part of the protein responsible for the binding of magnesium.

To determine the sensitivity of *E. coli* bacteria to treatment with hydrogen atoms, compared with the sensitivity of phage T_7, bacteria grown on Difco nutrient broth were twice centrifuged, washed with triple distilled water, and brought to a concentration of 10^5 bacteria/ml., in 0·01 molar phosphate buffer at *p*H 6·8. A dose of 6×10^{-4} moles/l. of hydrogen atoms gave no measurable effect. The same dose reduces phage T_7 to less than 1 per cent of its original value. This shows that phage may be selectively inactivated by a method which has almost no effect on *E. coli*.

Hydrogen atoms act in the systems now investigated

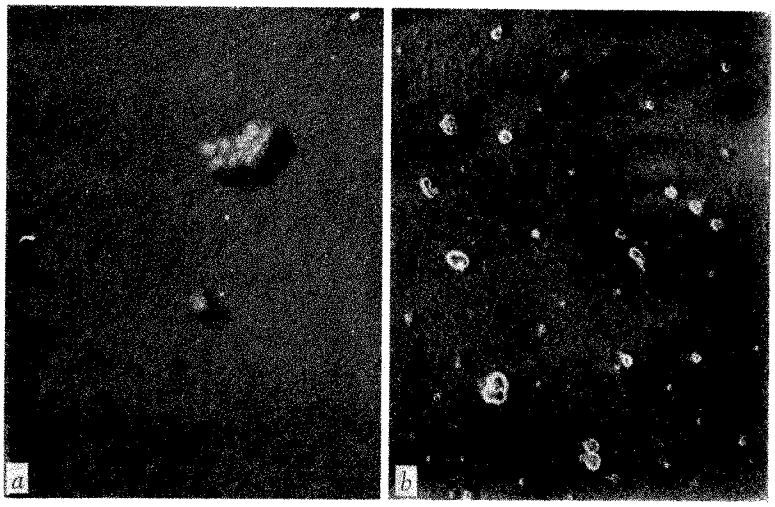

Fig. 4. Photographs of uranium shadowed phage T_7 taken on an RCA electron microscope at 100 kV. (× 47,600.) *a*, Control untreated phage; *b*, after treatment with hydrogen atoms ($3{\cdot}6 \times 10^{-4}$ moles/l.).

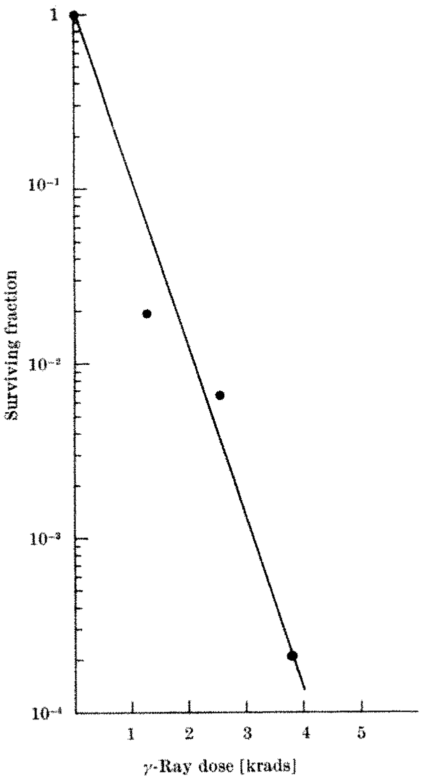

Fig. 5. Inactivation of phage T_7 by caesium-137 gamma rays. The phage were diluted to 10^8/ml. in phosphate buffer *p*H 7·8 using the same triple distilled water as was used for the hydrogen atom experiments. The samples were saturated with hydrogen gas before irradiation.

as simple one electron equivalent reagents. Other one electron equivalent chemical reagents as well as electrolytic reduction may be expected to behave in the same way. An advantage of hydrogen atom treatment is that at the end of the experiment no additional chemical other than inert hydrogen gas remains in the system.

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[1] Navon, G., and Stein, G., *J. Phys. Chem.*, **69**. 1384, 1389 (1965).
[2] Navon, G., and Stein, G., *Israel J. Chem.*, **2**, 151 (1964).
[3] Czapski, G., Frohwirth, N., and Stein, G., *Nature*, **207**, 1191 (1965).
[4] Mee, L. K., Navon, G., and Stein, G., *Nature*, **204**, 1056 (1954).
[5] Mee, L. K., Navon, G., and Stein, G., *Biochim. Biophys. Acta*, **104**, 151 (1965).
[6] Holmes, B. E., Navon, G., and Stein, G., *Nature*, **213**, 1087 (1967).
[7] Czapski, G., and Stein, G., *J. Phys. Chem.*, **64**, 219 (1960).
[8] Navon, G., and Stein, G., *J. Phys. Chem.*, **70**, 3630 (1966).
[9] Luria, S. E., and Exner, F. M., *Proc. US Nat. Acad. Sci.*, **27**, 370 (1941).
[10] Ebert, M., and Alper, T., *Nature*, **173**, 987 (1954).
[11] Davison, P. F., and Freifelder, D., *J. Mol. Biol.*, **5**, 635 (1962).
[12] Dewey, D. L., *Intern. J. Radiat. Biol.*, **12**, 497 (1967).
[13] Freifelder, D., *Proc. US Nat. Acad. Sci.*, **54**, 128 (1965).
[14] Davison, P. F., Freifelder, D., and Holloway, B. W., *J. Mol. Biol.*, **8**, 1 (1964).

Gene Conversion: Temperature Data from *Sordaria fimicola* on the Correction of Mis-paired Bases

CORRECTION of mis-paired bases in hybrid DNA is a basic postulate of current models of gene conversion and recombination[1-5]. Although some models invoke different origins of hybrid DNA, they involve similar repair processes. At each heterozygous site in a region of hybrid DNA, there are three possibilities: repair to wild type, repair to mutant and non-repair.

The temperature dependence of correction processes in gene conversion has been studied by obtaining data relating to these possibilities from visual scoring of asci with different ratios of wild type (+) to mutant (*m*) spores in crosses of the ascospore-pigmentation mutants grey (*g*) and hyaline (*h*) in *Sordaria fimicola*. These mutants were crossed with their respective wild types at different temperatures, at 2·5° C intervals, for the range 10°–31° C, using methods already described[6]. To test for phenocopies as a cause of apparent conversion, spores were isolated from crosses made at 10° C, 20° C and 30° C: *h* spores were inviable but none of about 200 *g*, g^+ and h^+ spores which germinated to produce fertile colonies were phenocopies. Large changes in total conversion frequency with temperature were observed but will be discussed elsewhere, for they are not directly relevant to the correction data.

Effects of temperature on the direction of correction (that is, whether correction is to wild type or to mutant) were determined from the observed frequencies of correction to wild type (one such event in 5+ : 3 *m* asci and two in 6+ : 2 *m* asci) and of correction to mutant (one such event in 3+ : 5 *m* asci, two in 2+ : 6 *m* asci). The relative frequencies of these two kinds of correction did not change significantly at any of the temperatures used for either locus, although the ratio differed for each locus (Table 1). The constancy of these relative frequencies indicates that the direction of correction of mis-pairing is independent of temperature. If there is one enzyme that can excise a base from any of the eight possible pairs of mis-paired bases, this evidence would suggest that the affinity of the excising enzyme for one base rather than another is unchanged by temperature; this applies both to conversion at the hyaline site, where correction in either direction was equally frequent (heterogeneity $\chi^2_1 = 0{\cdot}4$, $P = 0{\cdot}5$–$0{\cdot}7$), and to the grey site, where a preferential correction to wild type was found ($\chi^2_1 = 12{\cdot}5$, $P < 0{\cdot}01$). Alternatively, if there is more than one enzyme, each excising different bases, then the present data indicate that their relative effectivenesses—which would depend on their relative concentrations, rates of action and affinities—are unaffected by temperature. The one enzyme hypothesis seems more probable than the multienzyme hypothesis because the former requires fewer variables to be independent of temperature.

Table 1. EFFECT OF TEMPERATURE ON RATIOS OF ABERRANT ASCI IN CROSSES OF *Sordaria fimicola*

	Hyaline (*h*) locus				Grey (*g*) locus			
			Ratio of:				Ratio of:	
Temp. (° C)	Total asci	Asci with 5 : 3 or 6 : 2 ratios	Conversions to + : convs. to *h**	6 : 2 asci : 5 : 3 asci	Total asci	Asci with 5 : 3 or 6 : 2 ratios	Conversions to + : convs. to *g**	6 : 2 asci : 5 : 3 asci
31	5,022	45	1·0	1·4		Infertile		
30	8,381	112	0·9	1·3	4,267	30	1·3	0·6
27·5	7,375	75	0·8	1·7	6,887	73	1·5	1·0
25	7,932	64	1·2	1·9	6,079	44	1·5	0·5
22·5	7,063	52	1·0	2·1	5,932	41	1·3	0·9
20	8,150	44	0·8	2·1	8,229	34	1·5	0·7
17·5	7,245	36	0·8	2·3	8,033	27	1·6	0·8
15	7,659	27	1·1	2·0	7,126	12	1·0	0·5
12·5	8,286	50	0·9	1·8	9,076	9	1·3	1·3
10	8,158	74	1·1	1·3	13,014	12	1·6	1·0
Totals and averages	75,271	579	0·98	1·77	68,643	282	1·40	0·79

* The same trend is shown if this value is calculated separately for 5 : 3 and for 6 : 2 asci.

There are three frequencies pertinent to the efficiency of correction: that of no correction (giving post-meiotic segregation in 4 : 4 asci), that of correction in one chromatid (giving 5 : 3 asci) and that of correction in both chromatids (giving 4 : 4 and 6 : 2 asci). Post-meiotic segregation was not distinguishable from spindle overlap at the third division in the ascus, so the first of these frequencies could not be determined. The 4 : 4 class from correction in both chromatids is not distinguishable from other 4 : 4 asci, so the 6 : 2 class can only be used here as a measure of correction in both chromatids if the proportions of 6 : 2 and 4 : 4 asci from correction did not vary with temperature. If the direction of correction is independent in both chromatids, these proportions will depend solely on the relative frequencies of correction to wild type and to mutant, which were here independent of temperature. Changes in the relative frequencies of 6 : 2 and 5 : 3 ratios can therefore be used as a measure of changes in the relative frequencies of correction in both chromatids, or in only one chromatid, respectively. For the grey locus, no consistent change with temperature was observed for this value (Table 1) but at the hyaline locus it was highest over the range 17·5°–22·5° C and lowest at the two temperature extremes. Although the data are not heterogeneous as a whole for this value ($\chi^2_5 = 4{\cdot}9$, $P = 0{\cdot}8$–$0{\cdot}9$ for hyaline, $\chi^2_5 = 5{\cdot}0$, $P = 0{\cdot}7$–$0{\cdot}8$ for grey), hyaline values at high temperatures are significantly different from those at medium temperatures ($\chi^2_1 = 4{\cdot}0$, $P = 0{\cdot}02$–$0{\cdot}05$ for pooled values at 30° and 31° C versus pooled values for 17·5°, 20° and 22·5° C). These results suggest that correction at the hyaline site is impaired at extreme temperatures but a similar trend was not found for the grey site. The difference observed between loci could possibly result from the different genetic backgrounds in the two sets of crosses, or perhaps correction is not independent in both chromatids.

Another difference between conversion at the two sites is that for grey 5 : 3 asci are significantly more frequent than 6 : 2 asci ($\chi^2_1 = 5{\cdot}7$, $P = 0{\cdot}01$–$0{\cdot}02$), while the reverse is true for hyaline ($\chi^2_1 = 33{\cdot}4$, $P < 0{\cdot}01$). If allowance is made for undetected conversion resulting in asci with 4+ : 4 *m* ratios, correction in both chromatids is more frequent than correction in only one chromatid for grey as well as for hyaline, but the difference between the two sites in the relative frequency of the two kinds of correction becomes still greater. This suggests that correction is more efficient at the hyaline site than the grey site. Allowance for this undetected reciprocal conversion class would also result in values closer to unity for the ratio of correction to + : correction to *m*.

From these studies it is concluded that the direction of correction of base mis-pairing is independent of temperature; in other respects, different trends were observed for conversion at the grey and at the hyaline sites. These differences may result from different bases being mis-paired in hybrid DNA at each site but other explanations are possible.

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[1] Holliday, R., *Genet. Res.*, **5**, 282 (1964).
[2] Whitehouse, H. L. K., *Nature*, **199**, 1034 (1963).
[3] Whitehouse, H. L. K., and Hastings, P. J., *Genet. Res.*, **6**, 27 (1965).
[4] Emerson, S., *Genetics*, **53**, 475 (1966).
[5] Clark, A. J., and Margules, A. N., *Proc. US Nat. Acad. Sci.*, **53**, 451 (1965).
[6] Lamb, B. C., *Genet. Res.*, **7**, 325 (1966).

Indospicine, a New Hepatotoxic Amino-acid from *Indigofera spicata*

Indigofera spicata Forsk (previously *I. endecaphylla* Jacq.) would be a useful tropical pasture legume without its toxic properties (for review, see ref. 1). It has been shown to be hepatotoxic to sheep, cows and rabbits, and has caused abortion in pregnant animals[2]. Hutton *et al.*[3,4] showed that the leaf and seed were hepatotoxic to rabbits and mice and that β-nitropropionic acid, which occurs only in the leaf, did not account for these liver lesions. Britten *et al.*[5] found that β-nitropropionic acid or a compound closely associated with it in the leaf was toxic to chicks, which were not affected by the hepatotoxin(s) present in the seed.

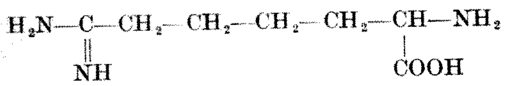
We report here the isolation from seed and leaf of a new amino-acid, designated indospicine, which produces characteristic liver lesions in mice. Indospicine is shown to be L-α-amino-ε-amidino caproic acid, corresponding to structure (I).

A rapid, semi-quantitative bioassay in mice was developed for testing the hepatotoxicity of the various extracts and fractions. It was based on the accumulation of fat in the liver produced by a single subcutaneous injection of a toxic aqueous extract of seed[6]. Further experiments were carried out in which groups of four female mice were injected with graded doses of the same extract and then killed at intervals. Frozen sections of the livers stained with sudan IV for fat were examined microscopically in random order and fat accumulation was graded from 0–4. Based on this method of grading, the maximum accumulation of fat occurred about 48 h after injection and was related to the amount of extract used. Repeated tests showed that the results were reproducible. This bioassay was then used to compare the hepatotoxicity of various extracts and fractions from extracts during the isolation

Earlier work[6] showed that the hepatotoxin behaved as a cation on ion-exchange resins, and further experiments indicated that the hepatotoxicity was associated with the strongly basic fraction of the seed. This fraction was isolated from the ground seed on a preparative scale by a modification of the adsorption-dialysis method[7,8] in which Zeokarb 226 (H) was used inside the dialysis tubes. The bases were eluted with 2 normal acetic acid and the extract concentrated to a small volume (1 ml. = 10 g of seed). Some of the bases present were tentatively identified by paper chromatography in several solvent systems and by the use of a wide range of detecting reagents[9]. Canavanine, arginine and several unidentified guanidino-compounds were present, but the principal ninhydrin-positive substance was an α-amino-acid with properties different from those of the known amino-acids. The ninhydrin-positive substances present in the extract were isolated by one-directional chromatography of aliquots of the extract (0·25 ml.) on 400 sheets of Whatman 3 MM paper. Isopropanol/2 normal hydrochloric acid (78 : 22 v/v) was used as the solvent. The various bands, located by ninhydrin treated test strips, were cut from each sheet; corresponding bands were bulked and extracted with water. The separate extracts were concentrated and excess hydrochloric acid was removed. Part of each extract was assayed for hepatotoxicity. The toxicity of the original extract was almost quantitatively located in one band which contained the new amino-acid, now named indospicine, and a small amount of arginine. Indospicine was isolated from the remainder of this extract as the sparingly soluble monoflavianate which, after repeated recrystallization from water, was converted to the hydrochloride which still contained a small amount of arginine. This was removed by ion-exchange chromatography[10]. Indospicine was also isolated from an aqueous extract of leaf by a modification of the method of Awapawa *et al.*[11]. The flavianate and hydrochloride were identical with the corresponding derivatives from seed.

Indospicine monohydrochloride crystallized from aqueous ethanol as white needle-like crystals of the monohydrate, melting point 131°–134° C, $(\alpha)_D^{22} + 18°$ in 5 normal hydrochloric acid (C, 1·1). (Found: C, 37·4; H, 8·1; N, 18·5; Cl, 16·0 per cent. Calculated for $C_7H_{15}O_2N_3.HCl.H_2O$: C, 37·0; H, 8·0; N, 18·5; Cl, 15·6 per cent). Analyses of the monoflavianate and monopicrate completely supported the empirical formula $C_7H_{15}O_2N_3$ for the free base. The picrate ion absorption at 380 mµ indicated a molecular weight of approximately 175 for indospicine. It gave a purple colour with ninhydrin on paper chromatograms and behaved as an α-amino-acid when treated successively with cupric acetate and ninhydrin. With the nitroprusside–alkaline ferricyanide reagent[9] it gave a yellow colour similar to that given by acetamidine and quite different from the red colours shown by mono-substituted guanidines. It moved as a positively charged ion on paper ionophoresis in the range *p*H 2–10·5 and decomposed at higher *p*Hs.

The free base, prepared by chromatography on a column of 'Dowex 2', was degraded to distilled water at 120° C or in 0·1 molar sodium carbonate at 40° C to ammonia (1 mole) and a neutral α-amino-acid $C_7H_{14}O_3N_2$. This compound was shown to be an amide by hydrolysis in 1 normal hydrochloric acid at 120° C to ammonia (1 mole) and L-α-amino-pimelic acid (APM). Indospicine was also degraded in 6 normal hydrochloric acid at 120° C to APM (1 mole) and ammonia (2 moles). APM and its amide were identified by melting point, optical rotation, elemental analysis, infrared, nuclear magnetic resonance and mass spectra. The chemical and spectroscopic evidence is in agreement with formula (I) for indospicine.



$$H_2N-\underset{\underset{NH}{\|}}{C}-CH_2-CH_2-CH_2-CH_2-\underset{\underset{COOH}{|}}{CH}-NH_2 \quad (I)$$

Indospicine hydrochloride produced liver lesions in mice identical with those produced by various crude extracts of the seed. Canavanine and arginine did not cause fat accumulation in the liver, nor did they interfere with the indospicine assay. Pearn[12] has recently demonstrated the presence of a new type of cleft palate teratogen in the same seed extract from which we isolated indospicine. The teratogenic activity of the extract is caused by indospicine (unpublished results of Pearn and Hegarty).

Indospicine is the first naturally occurring hepatotoxic amino-acid to be isolated. Compounds containing the simple amidine system have not previously been found in plants although the grouping occurs in the antibiotics congocidin and distamycin *A* (ref. 13).

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[1] Kingsbury, J. M., *Poisonous Plants of the United States and Canada*, 323 (Prentice-Hall, Englewood Cliffs, 1964).

[2] Norfeldt, S., Henke, L. A., Morita, K., Matsumoto, H., Takahashi, M., Younge, O. R., Willers, E. H., and Cross, R. F., *Hawaii Agr. Exp. Sta. Tech. Bull.*, 15 (1952).

[3] Hutton, E. M., Windrum, G. M., and Kratzing, C. C., *J. Nutrit.*, **64**, 321 (1958).

[4] Hutton, E. M., Windrum, G. M., and Kratzing, C. C., *J. Nutrit.*, **65**, 429 (1958).

[5] Britten, E. J., Palafox, A. L., Frodyma, M. M., and Lynd, F. T., *Crop Sci.*, **3**, 415 (1963).

[6] Coleman, R. G., Windrum, G. M., and Hutton, E. M., *J. Nutrit.*, **70**, 267 (1960).

[7] Hegarty, M. P., Schinckel, P. G., and Court, R. D., *Austral. J. Agric. Res.*, **15**, 153 (1964).

[8] Beyermann, H. C., Maat, L., and Hegarty, M. P., *Recl. Trav. Chim. Pays-Bas*, **83**, 1078 (1964).

[9] Smith, I., *Chromatographic and Electrophoretic Techniques*, **1** (Heinemann, London, 1960).

[10] Blau, K., *Biochem. J.*, **80**, 193 (1961).

[11] Awapawa, J., Davis, V. E., and Graham, O., *J. Chromatog.*, **3**, 11 (1960).

[12] Pearn, J. H., *Nature*, **215**, 980 (1967).

[13] Arcamone, F., Penco, S., Orezzi, P., Nicolella, V., and Pirelli, A., *Nature* **203**, 1064 (1964).

Relative Binding of Strontium and Calcium in Protein and Non-protein Fractions of Serum in the Rabbit

WE have reported a discrimination between the ratio of strontium to calcium in rabbit plasma and that in bone[1]. We reasoned that this discrimination could be the result of a difference in this ratio in the protein and non-protein fractions of the plasma. If the ratio of strontium to calcium in the non-protein fractions was lower than that in the plasma as a whole, then the ratio in the extracellular fluid might be expected to be lower and hence account for the lower ratio which has been observed in bone. To test this hypothesis a double tracer experiment using strontium-85 and calcium-47 was carried out in two rabbits which were of the same strain and age (7 months) as those used before. The protein and non-protein fractions were separated in an ultracentrifuge and the ratio of strontium-85 to calcium-47 was determined for these fractions as well as for the whole serum. These measurements showed that the ratio was the same for all fractions within the limits of experimental error.

Two rabbits were injected intravenously with 30 µc./kg of calcium-45 and 85 µc./kg of strontium-85 into the marginal vein of the ear and killed 24 h later, when about 40 ml. of blood was collected from each animal after severing the jugular vein. The blood was allowed to clot and the serum decanted off. A sample of serum (10 ml.) was spun at about 200,000*g* (40,000 r.p.m.) in an ultracentrifuge[2] to separate the protein and non-protein. A thin layer of liquid paraffin was floated on top of the serum to prevent changes in *p*H during centrifugation, because changes in *p*H have been shown to alter the proportion of calcium bound to protein[3]. The non-protein component, which appeared clear at the top of the centrifuge tube, was removed with a syringe in two fractions, the first fraction being just below the layer of paraffin; the second fraction, the bottom of which was in contact with the protein, was removed separately to prevent mixing during transfer. The protein component, which was a gelatinous semi-solid (yellowish at the top and merging into a reddish-brown colour at the bottom), was also removed with a syringe into a counting dish for radioactive assay.

Strontium-85 and calcium-45 were assayed on the same sample using a scintillation counter with a sodium iodide crystal. The energy spectra for the two isotopes standards were run independently to determine the optimum conditions for distinguishing between them. The discriminator bias was set so that only pulses from calcium-47 were counted. Strontium-85 was counted using a single channel at a lower discrimination level; the counts in this channel caused by calcium-47 were subtracted. The amount of this isotope injected was chosen to be about one-third of the amount of strontium-85, thereby reducing the inter-

Table 1. STRONTIUM-85 AND CALCIUM-47 IN DIFFERENT FRACTIONS OF SERUM 24 h AFTER INJECTION

Sample		Strontium-85 (per cent injected dose/ml.)	Calcium-47 (per cent injected dose/ml.)	Strontium-85 : calcium-47
Whole serum	1623	$2\cdot30\times10^{-3}$	$5\cdot69\times10^{-3}$	0·41
	1624	$2\cdot70\times10^{-3}$	$6\cdot12\times10^{-3}$	0·44
Fraction I	1623	$1\cdot58\times10^{-3}$	$3\cdot82\times10^{-3}$	0·41
	1624	—	—	—
Fraction II	1623	$1\cdot73\times10^{-3}$	$4\cdot08\times10^{-3}$	0·42
	1624	$2\cdot10\times10^{-3}$	$4\cdot80\times10^{-3}$	0·44
Fraction III (protein)	1623	$3\cdot01\times10^{-3}$	$7\cdot25\times10^{-3}$	0·42
	1624	$3\cdot76\times10^{-3}$	$8\cdot02\times10^{-3}$	0·47

ference of the calcium isotope in this channel. All the samples used for measuring radioactivity were made up to a standard volume for counting.

The results for the different fractions of serum (Table 1) show that for rabbit 1623 the ratio of strontium-85 to calcium-47 is the same to within 3 per cent in the whole serum as in both the protein-bound and the protein-free fractions. In rabbit 1624 the ratio is somewhat greater but is the same for all fractions within experimental error. It is therefore clear that the previously reported[1] discrimination of 1·6 against strontium in the transfer between blood and bone cannot be attributed to the difference in the ratio of strontium to calcium between the protein-free plasma and the plasma as a whole.

The discrimination might be explained by the fact that the measurements were made of naturally occurring levels of the stable isotopes when the animals were in a state of equilibrium, rather than of the radioisotopes shortly after administration. Recent measurements made at longer intervals after administration suggest that there may be a difference between the relative behaviour of strontium and calcium soon after administration and after a longer interval.

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[1] Kshirsagar, S. G., Lloyd, E., and Vaughan, J., *Brit. J. Radiol.*, **39**, 131 (1966).
[2] Breen, M., and Freeman, S., *Clin. Chim. Acta*, **6**, 181 (1961).
[3] Munday, K. A., and Mahy, B. W. J., *Clin. Chim. Acta*, **10**, 144 (1964).

Detection of Insulin Precipitating Antibodies

THERE are various immunological techniques for detecting antibodies to peptide antigens, but the detection of precipitating antibodies to small peptide hormones which are poor antigens may be difficult. Preparations of crystalline insulin from a variety of species are quite similar and only a few differences in amino-acid composition have been found, which in addition to the smaller molecular size may account for its poor antigenicity. Nevertheless, the Ouchterlony technique[1] has revealed precipitating antibodies to insulin (for example, refs. 2–5).

With guinea-pig insulin antisera, we were unable to see clear insulin–anti-insulin precipitates by the agar-gel immunoelectrophoretic technique[6,7] but have demonstrated several antigen–antibody precipitin lines by a modification of this procedure. A new immunological technique has been developed which involves (*a*) electrophoretic separation, (*b*) immunodiffusion and (*c*) exclusion of antigen–antibody complexes by the addition of the water-soluble polymer dextran.

Pork insulin (24 IU/1 mg) ten times recrystallized (gift from Novo) was used to immunize guinea-pigs. The hyperimmune sera obtained by cardiac puncture were a gift from Dr U. Lundkvist. Five different hyperimmune insulin antisera were tested and they gave identical results.

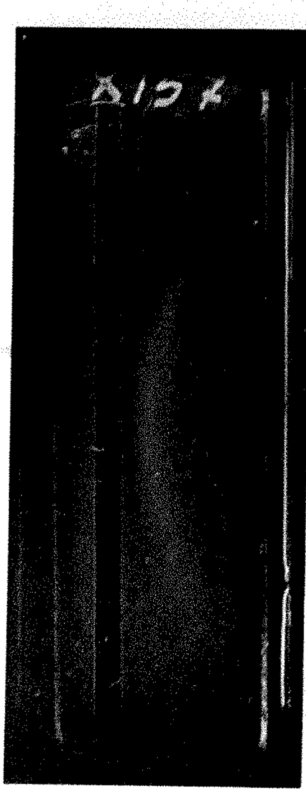

Fig. 1.

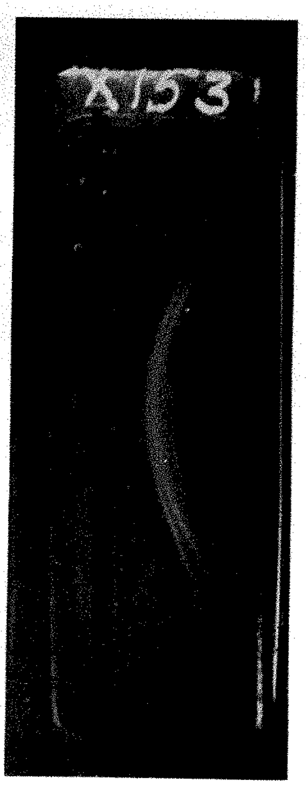

Fig. 2.

Fig. 1. Immunoelectrophoretic run of insulin and guinea-pig insulin antisera in agar medium. (Control experiment.)

Fig. 2. Immunoelectrophoretic run of insulin and guinea-pig insulin antisera in agar medium. (Infusion with dextran solution.)

Immunoelectrophoresis was carried out on microscope slides in LKB apparatus. The slides were coated with a 1·5 per cent agar solution (agar-noble, Difco) made up in veronal buffer (pH 8·6, ionic strength 0·03). The antiserum used was shown to contain insulin antibodies by the radioimmunoassay technique of Wide and Porath[8]. After antiserum had diffused into the agar gel, 'Dextran 10' (mean molecular weight 10,000, Pharmacia AB) was poured into the trough and allowed to diffuse (infusion procedure). The dextran used in these experiments was dissolved in a veronal buffer solution (pH 8·6 and ionic strength 0·1). The final concentration of 'Dextran 10' was 8 per cent (w/v).

The microscope slides were then placed in a moist chamber and incubated for 2 days at 37° C in an air-incubator. During incubation the dextran solution was placed three times (at equal time intervals) into troughs (altogether 0·75 ml. of 8 per cent 'Dextran 10' per slide or per 1·5 ml. of agar was added). After 2 days of incubation at 37° C, the slides were placed in a cold room at 4° C for 3 days.

After 13 h of incubation at 37° C, antigen–antibody precipitates could be seen in agar–dextran media, while in the control experiment (only agar) a very faint and broad, cloudy precipitate was just detectable. In agar–dextran medium a sharp and distinct precipitin line was seen on the side nearer the well, while the second precipitin line underneath (nearer the trough) was rather diffused, but easily seen. Both lines showed the same anodic movement. After 40 h of incubation at 37° C the precipitin bands sharpened and a new and sharp precipitin band appeared below the broad cloudy precipitate. After a further 3 days of incubation at 4° C, the immunoprecipitates were observed again and photographed. Results of the control experiment are shown in Fig. 1, while Fig. 2 shows the precipitin lines of antigen–antibody in agar medium which was infused with dextran solution.

The time sequence of the appearance of precipitin lines was as follows: first, the less diffusible antigen(s) precipitated out, while lastly the most diffusible antigen(s) formed a precipitate. Possibly the aggregated part of insulin, if any, was precipitated first, while the precipitin reaction between less aggregated insulin molecules and the antibody took longer.

It has been suggested[9-13] that polysaccharides may exclude some proteins from solution rather than interacting with them. Dextran seems to decrease the solubility of immuno-complexes in agar gel medium and helps to exclude them.

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[1] Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, **25**, 186 (1948).

[2] Moloney, P. J., and Aprile, M. A., *Canad. J. Biochem. Physiol.*, **37**, 793 (1959).

[3] Jones, V. E., and Cunliffe, A. C., *Nature*, **192**, 136 (1961).

[4] Steigerwald, H., Spielman, W., Fries, H., and Grebe, S. T., *Klin. Woch.*, **38**, 973 (1960).

[5] Birkinshaw, V. J., Randall, S. S., and Risdall, P. C., *Nature*, **193**, 1089 (1962).

[6] Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, **10**, 193 (1953).

[7] Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, **17**, 67 (1955).

[8] Wide, L., and Porath, J., *Biochim. Biophys. Acta*, **130**, 257 (1966).

[9] Ogston, A. G., and Phelps, C. F., *Biochem. J.*, **78**, 827 (1961).

[10] Laurent, T. C., *Biochem. J.*, **89**, 253 (1963).

[11] Laurent, T. C., *Acta Chem. Scand.*, **17**, 2664 (1963).

[12] Hellsing, K., and Laurent, T. C., *Acta Chem. Scand.*, **18**, 1303 (1964).

[13] Iverius, P. H., and Laurent, T. C., *Biochim. Biophys. Acta*, **133**, 371 (1967).

Occurrence of Haemocoelic Erythrocytes containing Haemoglobin in a Wood Boring Mollusc

DURING recent studies of the rock and wood boring molluscs of the super-family Adesmacea (Mollusca : Bivalvia) the occurrence of haemocoelic erythrocytes in the blood of *Xylophaga dorsalis* Turton was noticed while examining serial sections. The erythrocytes are slightly flattened disks of diameter $10–12\mu$, each containing a prominent nucleus (Fig. 1).

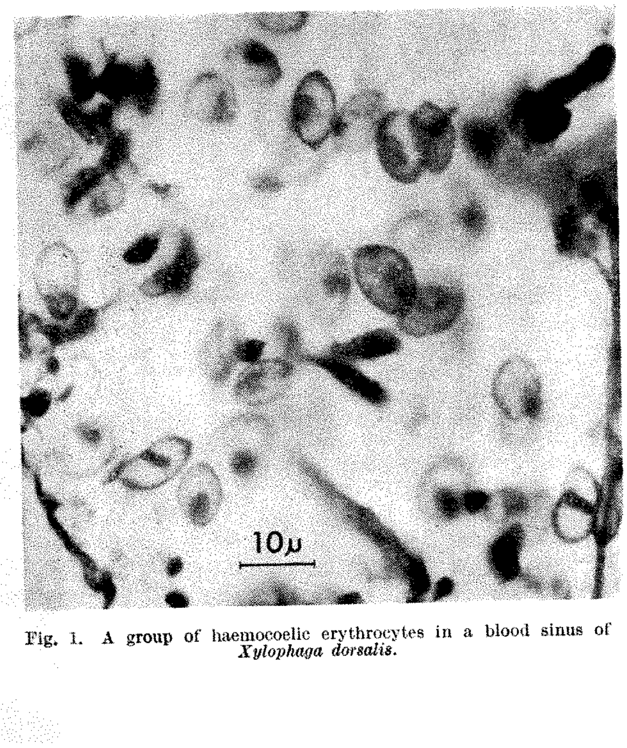

Fig. 1. A group of haemocoelic erythrocytes in a blood sinus of *Xylophaga dorsalis*.

The blood of this species possesses a light pinkish colour, and our observations suggest that this tint is a consequence of the presence of haemoglobin in the erythrocytes. Dried smears of the blood gave a positive result to the benzidine reaction[1] which stained only the erythrocytes dark blue, while a dilute solution of the pigment in distilled water showed a major absorption peak at 415 $m\mu$, and indications of α and β peaks at 578 $m\mu$ and 540 $m\mu$. Treatment with carbon monoxide caused a shift in the major absorption peak to 420 $m\mu$ and of the α peak to 568 $m\mu$. These changes are consistent with the transformation of oxy-haemoglobin to carboxy-haemoglobin and with previous knowledge of absorption spectra of molluscan haemoglobins[2], and are therefore taken as confirmation that the pigment in *Xylophaga* is a haemoglobin.

The possession of haemoglobin by a member of the Adesmacea has not been reported before, although myoglobin occurs in the adductor muscle of species of *Bankia* and *Teredo*[3]. The isolated instances of the occurrence of haemoglobin in solution in the haemolymph, in the ctenidium, and in haemocoelic erythrocytes elsewhere in the Bivalvia, have been reviewed recently[4]. Interpretation of the function of the haemoglobin of *Xylophaga*, a species which is usually found attacking submerged waterlogged timber[5], must await more detailed biochemical and physiological investigations of its properties.

This work was carried out during the tenure of a Royal Society and Nuffield Foundation Commonwealth bursary held by one of us (N. B. N.).

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[1] Pearse, A. G. E., *Histochemistry, Theoretical and Applied*, second ed. (J. and A. Churchill, Ltd., London, 1960).

[2] Prosser, C. L., and Brown, jun., F. A., *Comparative Animal Physiology* (Saunders, Philadelphia, Pennsylvania, 1961).

[3] Manwell, C., *Comp. Biochem. Physiol.*, **8**, 209 (1963).

[4] Read, K. R. H., in *Physiology of Mollusca* (edit. by Wilbur, K. M., and Yonge, C. M.), **2** (Academic Press, New York, 1966).

[5] Tebble, N., *British Bivalve Seashells* (British Museum [Natural History], London, 1966).

Specific Method for Estimating Cell Numbers in Tendons

STUDIES of growth, ageing and functional adaptation of dense regular connective tissue of tendons and ligaments are limited because of the lack of a simple accurate method for the estimation of the cell populations involved[1]. Standard histometric techniques for cell counting are especially difficult to apply to tough intractable, fibrous tissues, and there is an obvious need for a simpler quantitative method which is accurate, reproducible and rapid.

If the amount of DNA in the somatic diploid cell nucleus is known, and if total tissue DNA can be extracted, cell numbers can be readily calculated, for it has been shown that the diploid amount of DNA in the cell nuclei is constant for a particular species[2]. This fact is the basis of a number of growth and cell population studies involving whole animals and tissues[3]. These methods have, however, never been applied to dense, fibrous, relatively acellular tissues such as tendons and ligaments.

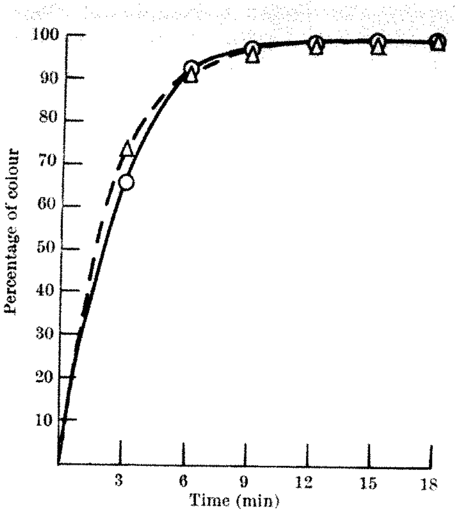

Fig. 1. Rate of development of colour at 100° C. Equal parts of 0·04 per cent indole-HCl reagent and sample were used. ○, Tendon organ extract-indole; △, DNA-indole. Read at 490 mμ. Cell length was 1 cm. The concentration of the sample was 30 μg/ml.

This communication describes a specific method, and its use in determining cell numbers in rabbit tendons. First, the diploid amount of rabbit nuclear DNA was established by counting and analysing three morphologically distinct populations of rabbit cells, namely, peritoneal leucocytes, thymocytes and nuclei extracted from kidney cortex. Cells and nuclei were suspended in physiological medium and each population was counted in a haemocytometer. Aliquots of the same individual cell suspensions were homogenized and analysed for DNA content using a modification of the Schmidt–Tannhauser procedure[4]. The desoxyribose fraction was measured in a Unicam *SP*500 spectrophotometer using the modified reaction with diphenylamine[5] against a standard calibration curve prepared from serial dilutions of a pure solution of desoxyribose treated in the same way. (The curve was linear up to a sample concentration of 0·1 μmoles/ml., giving a useful range over 0·02–0·1 μmoles/ml.)

The DNA content was then calculated on the basis that the purine/pyrimidine base ratio is unity, that the diphenylamine reacts with the sugar residues originally combined with purines in the DNA[6], and that the DNA–P content is 9·4 per cent.

Table 1 shows the range of rabbit diploid nuclear DNA values measured in pg (1 pg$=10^{-12}$ g) obtained from the three cell populations examined.

Fresh portions of adult rabbit tibialis anterior and peroneus longus tendons, freed from their sheaths, were finely sliced and submitted to the fractionation procedure outlined by Munro and Fleck[7]. DNA content was determined using the indole reaction[8] and a standard calibration curve prepared from serial dilutions of a solution of purified calf thymus DNA reacted with indole (a linear curve for the range 10–80 μg/ml.).

The specificity of the method was tested by comparing the absorption spectra of the tendon extract–indole compound with a pure DNA–indole compound[9]. Both wave forms showed the characteristic flattening at 460 mμ with a maximum peak absorption at 490 mμ, suggesting that the compounds formed in each case were identical. Further confirmation of this was obtained when rates of colour development were compared (Fig. 1). Table 2 gives results of recovery experiments with digests of tendon. Measured amounts of DNA were added to tendon extract solutions of known DNA concentration and the total recoveries were measured after the reaction with indole.

Although a number of factors will govern the choice of a particular method for cell population determination in a given tissue, the method described here is probably the most satisfactory one available for measuring cell numbers in tendon and other forms of dense, regular, connective tissues such as ligaments or aponeuroses.

Table 1

Peritoneal leucocytes	Gross % error	Peritoneal leucocytes	Gross % error
6·38	6·9	5·78	3·2
5·57	6·7	Thymocytes	
5·91	1·0	5·9	1·2
5·78	3·2	5·61	6·0
6·38	6·9	5·56	6·9
5·98	0·3	6·17	3·4
6·19	3·7	Kidney cortex nuclei	
6·28	5·2	6·03	1·0
		6·07	1·7

Mean is 5·97; mean gross error 3·8 per cent; standard deviation 5·97 ± 0·28; error 4·7 per cent.

Table 2. RECOVERY OF DNA FROM TENDON ORGAN EXTRACT BY INDOLE REACTION DNA OF TENDON EXTRACTS

	DNA added (μg/ml.)	Total DNA (μg/ml.)	Recovery (μg/ml.)	% Recovery (μg/ml.)
(1)	17·2	41·2	41	99·9
	34·5	58·5	60	102·5
(2)	17·2	54·8	57	102
	34·5	72·1	70	97

(1) Concentration 24 μg/ml.; (2) concentration 37·6 μg/ml.

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[1] Viidik, A., in *Studies on Anatomy and Function of Bones and Joints* (edit. by Gaynor Evans, F.) (Springer Verlag, 1966).

[2] Boivin, A., Vendrely, R., and Vendrely, C., *CR Acad. Sci.*, **226**, 1061 (1948).

[3] Leblond, C. P., *J. Nat. Cancer Inst.*, **14**, 119 (1964).

[4] Schmidt, G., and Tannhauser, S. J., *J. Biol. Chem.*, **161**, 83 (1945).

[5] Burton, K., *Biochem. J.*, **62**, 315 (1956).

[6] Chargaff, E., in *The Nucleic Acids* (edit. by Chargaff, E., and Davidson, J. N.) (Academic Press, Inc., New York, 1955).

[7] Munro, H. N., and Fleck, A., *Analyst*, **91**, 78 (1966).

[8] Ceriotti, G., *J. Biol. Chem.*, **214**, 59 (1955).

[9] Ceriotti, G., *J. Biol. Chem.*, **198**, 297 (1952).

IMMUNOLOGY

Gamma Globulin Production and Antibody Synthesis in Chickens bursectomized as Embryos

DEVELOPMENT of the immunoglobulin producing system of cells is controlled by the avian bursa of Fabricius[1,2]. Elucidation of the role of the bursa in developing immunological potential began with the discovery that surgical removal of this hindgut lymphoid organ in the newly hatched chicken prevents later development of normal antibody response[3]. Depression of antibody response is greatest when the bursa is removed immediately after hatching. After 8–10 weeks, bursectomy alone no longer has a demonstrable effect on antibody response[4]. It was discovered later that normal embryonic development of the bursa could be inhibited by testosterone or other androgens[5]. Chickens which are given testosterone during embryonic life are often even more immunologically deficient than chickens which are subjected to bursal removal at hatching. Chickens which are surgically or hormonally bursectomized have reduced numbers of plasma cells and decreased levels of circulating gamma globulin. Near lethal whole body irradiation of newly hatched chicks in addition to bursectomy completely prevents later development of germinal centres and plasma cells and such chickens are agammaglobulinaemic[6,7]. Germinal centres, plasma cells and immunoglobulin synthesis can be restored in bursectomized-irradiated chicks by injection of their unirradiated bursal lymphocytes[8]. These observations suggest that the bursa could be the only source of the germinal centre and plasma cell line but that some radiosensitive bursal cells leave the bursa before hatching.

In these experiments with embryonic bursectomy, we show that the role of the bursa in development of a competent immunoglobulin-producing cell system is a sequential process in a qualitative way: bursal support of development of capacity for 7*S* immunoglobulin synthesis occurs after the integrity of the 19*S* immunoglobulin synthesis system has been established.

All experimental chickens were from a single hatch of Ghostly Pearl White Leghorn eggs. The bursa of Fabricius was surgically removed on the nineteenth day of incubation in one group of birds and on the day of hatching in another. Birds in another group were subjected to a sham bursectomy procedure on the nineteenth day of incubation. The rest of the hatched chickens were kept as an unoperated control group.

Bursectomy of the 19 day old embryo was performed by cutting a 1·5 × 2 cm hole in the egg shell overlying the tail; the tail was pulled out through a small opening in the vascular chorioallantoic membrane. Through a lateral incision, dorsal to the cloacal vent, the bursa was dissected and removed by cutting the base of its stalk attachment to the posterior cloacal wall. Following bursectomy the embryo was replaced in the egg, the opening was sealed with transparent tape and the egg was returned to the incubator. In sham bursectomies, the entire procedure was carried out except for cutting the bursal stalk. All procedures were performed aseptically.

Of thirty-six embryos which were bursectomized on the nineteenth day of incubation, twenty-five hatched; one of these was destroyed because of a herniated gut. All the twelve embryos which were subjected to sham bursectomy hatched as normal chicks. All groups of birds survived equally well until the fifth week after hatching (Fig. 1). By this time, most of the maternal 7*S* immunoglobulins derived from the yolk had been catabolized. After the sixth week, there was a sharp decline in survival of both groups of bursectomized chickens.

Chickens surviving at 4 weeks were weighed, bled and injected intramuscularly with 10^9 washed sheep erythrocytes. One week later the chickens were again bled and injected with 10^9 sheep erythrocytes. Bleeding was repeated on days 14 and 17 after the initial injection. The sera which were collected at these times were tested for the ability to agglutinate sheep erythrocytes by a microagglutination technique[9] and were assayed for 19*S* and 7*S* immunoglobulins by immunoelectrophoretic analysis using specific antiserum to chicken 19*S* and 7*S* gamma globulins prepared in goats[7].

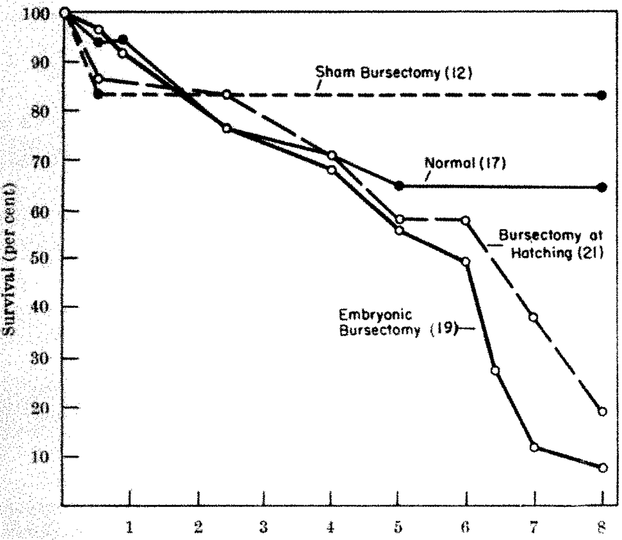

Fig. 1. The effect of embryonic (19 days) and post-hatching (21 days) bursectomy on survival of chickens.

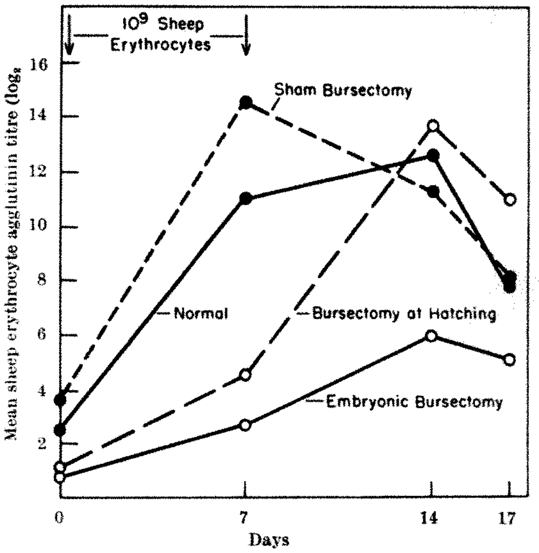

Fig. 2. Antibody titres before and after immunization of 4 week old chickens with sheep erythrocytes on day 0 and day 7. The curves show the mean agglutinin titres for the chickens which were bursectomized as embryos (19 days), bursectomized post-hatching, sham bursectomy, and normals.

The agglutinating antibody responses of the experimental chickens are shown in Fig. 2. Birds which were subjected to bursectomy at hatching had depressed antibody responses after primary immunization with sheep erythrocytes but, as others have found[10,11], they responded as well as controls did to secondary immunization. Birds which were deprived of their bursae at day 19 of embryonic life had barely detectable primary antibody responses and had feeble responses even after secondary immunization. The mean levels of antibodies in this group of birds were significantly lower than those of all other groups of birds after both primary and secondary immunizations.

Analysis of circulating immunoglobulins showed a striking reduction of 7*S* immunoglobulin content in each bird which was subjected to bursectomy at the nineteenth day of embryonic life. Even in this group of birds, however, 19*S* immunoglobulin levels were normal. A typical immunoelectrophoretic pattern of the serum of one of these dysgammaglobulinaemic birds is shown in Fig. 3. By contrast, less than 30 per cent of the birds from this strain which were bursectomized at hatching had this serum pattern of low 7*S* but normal 19*S* immunoglobulins (Table 1).

Table 1. GAMMA GLOBULIN PATTERNS AS EVALUATED BY IMMUNOELECTROPHORESIS OF SERUM FROM BURSECTOMIZED CHICKENS DURING THE SIXTH WEEK AFTER HATCHING

Group	Normal 19*S* low 7*S*	Normal 19*S* and 7*S*
Bursectomy at 19 days	10*	0
Bursectomy at 21 days	4	10
Sham	0	10
Normal	0	11

* No. of sera tested.

Tissue sections of the spleens and caecal tonsils from birds which died or which were killed on the conclusion of these studies were histologically examined with particular reference to germinal centre and plasma cell development. The number of germinal centres, and probably plasma cells, were reduced in the tissues of both groups of bursectomized animals. Both germinal centres and mature plasma cells were present in tissues from all groups of experimental animals, however, and the cells seemed morphologically normal.

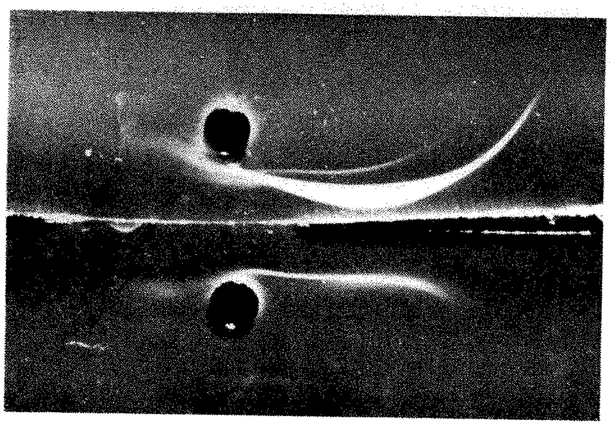

Fig. 3. Immunoelectrophoresis pattern of normal chicken serum (top) and typical embryonic bursectomized chicken serum (bottom) at 6 weeks of age. The amount of $7S$ gamma globulin in the embryonic bursectomized chicken was only 15 per cent of that found in normal chickens. Goat antiserum to chicken gamma globulin in the trough.

Severe depression of $7S$ immunoglobulin contents in birds showing intact $19S$ immunoglobulin synthesis has occurred after surgical bursectomy at hatching[6,7,12], after hormonal bursectomy[13], and rarely as a genetically determined spontaneous event[14]. Over the past several years in studies involving many hundreds of bursectomized chickens in our laboratory, we have found that approximately 20 per cent of birds which are subjected to bursectomy as newly hatched chicks, develop this type of experimental dysgammaglobulinaemia, but the incidence has varied from 5 per cent to 50 per cent in different experimental groups of birds. In the present experiments, we consistently produced this dysgammaglobulinaemic pattern by removing the bursa 2 days before hatching whereas bursectomy immediately after hatching led only to a 30 per cent incidence. The observations indicate a bursa-controlled sequential maturation of the immunoglobulin-producing system of cells somewhat analogous to that observed in differentiation of the haemoglobulin-producing system of cells[15-17].

A central question raised here is how the bursa regulates this stepwise differentiation pattern. One possibility is that $19S$ immunoglobulin-synthesizing cells arise from a source other than the bursa. Against this interpretation, the developing immunoglobulin-producing system vigorously springs back after heavy total body irradiation but fails completely after bursectomy and irradiation[6,7]. Furthermore, unirradiated bursal lymphoid cells from newly hatched chicks are capable of restoring the immunoglobulin-producing cell system in bursectomized-irradiated birds[8]. Chickens restored in this manner synthesize both $19S$ and $7S$ immunoglobulins strongly suggesting that, by the time of hatching, bursal cells are capable of differentiating into both $19S$ and $7S$ immunoglobulin producers. Another possibility, which seems unlikely to us, is that the bursa secretes a hormone which regulates a differentiation of lymphoid cells resulting in the shift from only $19S$ to both $19S$ and $7S$ immunoglobulin synthesis. Evidence favouring a hormonal function for the bursa has been presented[18], but we have recently shown that the proposed bursa hormonal action might be attributable to bacterial contamination of bursa explants by means of endotoxin enhancement of the remaining potential of recipient bursectomized animals[19].

Two remaining alternatives seem attractive. The first is that the difference in immunoglobulin synthesizing capability after bursectomy at 19 compared with 21 days merely reflects the numbers of stem cells which were seeded from the bursa before its removal. The second alternative is that the earliest stem cells seeded from the bursa are qualitatively different in that they are only capable of differentiation toward IgM synthesis whereas stem cells residing within the bursa for a longer period of time can give rise to an ancestral line of cells capable of either $19S$ or $7S$ immunoglobulin synthesis.

Theoretical support for the first alternative might be provided by Sterzl's contention that supersaturation of antigen-committable immunoglobulin-producing cells by antigen promotes differentiation to $19S$ antibody synthesis while suppressing the cell division necessary for the activation of $7S$ antibody synthesis[20,21]. According to this view, the small number of stem cells seeding from the bursa by the nineteenth day might easily be saturated by environmental antigens with preferential enhancement of $19S$ immunoglobulin synthesis. This interpretation would suggest that administration of large amounts of antigens beginning at hatching would increase the incidence of dysgammaglobulinaemia in birds which were bursectomized at 21 days; we have not, however, been able to alter the incidence of dysgammaglobulinaemia by such treatment.

In support of the latter alternative we have found that there is a difference in nucleic acid ratios of bursal lymphoid cells at different stages of embryonic life. The ratio of RNA to DNA in these cells drops sharply on about the nineteenth day[1]. Further evidence has been provided by Thorbecke and Warner[2], who have found that bursal fragments of 19 day old embryos produced only $19S$ immunoglobulins in short term tissue culture whereas small amounts of both $19S$ and $7S$ were produced by bursal explants obtained from chickens later in life.

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[1] Cooper, M. D., Gabrielsen, A. E., and Good, R. A., *Ann. Rev. Med.*, **18**, 113 (1967).

[2] Warner, N. L., *Folia Biol.*, **13**, 1 (1967).

[3] Glick, B., Chang, T. S., and Japp, R. G., *Poultry Sci.*, **35**, 224 (1956).

[4] Chang, T. S., Rheins, M. S., and Winter, A. R., *Poultry Sci.*, **36**, 735 (1957).

[5] Meyer, R. K., Rao, M. A., and Aspenall, R. A., *Endocrinology*, **64**, 890 (1959).

[6] Cooper, M. D., Peterson, R. D. A., and Good, R. A., *Nature*, **205**, 143 (1965).

[7] Cooper, M. D., Peterson, R. D. A., South, M. A., and Good, R. A., *J. Exp. Med.*, **123**, 75 (1966).

[8] Cooper, M. D., Schwartz, M. L., and Good, R. A., *Science*, **151**. 471 (1966).

[9] Wegmann, T. G., and Smithies, O., *Transfusion*, **6**, 67 (1966).

[10] Claflen, A. J., Smithies, O., and Meyer, R. K., *J. Immunol.*, **97**, 693 (1966).

[11] Janković, B. D., and Isaković, K., *Nature*, **211**, 202 (1966).

[12] Ortega, L. G., and Der, B. K., *Fed. Proc.*, **24**, 160 (1965).

[13] Pierce, A. E., Chubb, R. C., and Long, P. L., *Immunology*, **10**, 321 (1966).

[14] Brüggemann, J., Merkenschlager, M., Kirchner, B., and Lösch, U., *Naturwissenschaften*, **54**, 97 (1967).

[15] Fennell, R. A., *J. Agric. Res.*, **74**, 217 (1947).

[16] Sandreuter, A., *Acta Anat.*, **11** (Suppl. 14), 1 (1951).

[17] Romanoff, A. L., *The Avian Egg* (Macmillan Co., New York, 1960).

[18] St. Pierre, R. L., and Ackerman, G. A., *Science*, **147**, 1307 (1965).

[19] Dent, P. B., and Peterson, R. D. A., *Fed. Proc.*, **26**, 621 (1967).

[20] Sterzl, J., *Nature*, **209**, 416 (1966).

[21] Sterzl, J., and Silverstein, A. M., in *Advances in Immunology* (edit. by Dixon, jun., F. J., and Humphrey, J. H.), 337 (Academic Press, New York, 1967).

PATHOLOGY

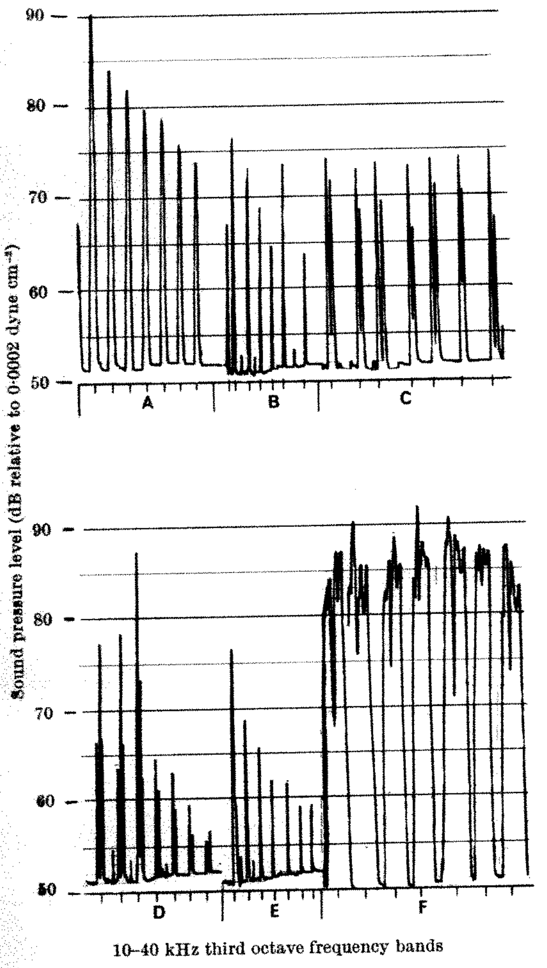
Asthma and the High Frequency Sound Environment

ASTHMA is a disturbance of the respiratory mechanism involving reversible, partial obstruction of the airway. One symptom is a raised sensitivity in human relationships. In a previous communication it has been shown that sufferers from asthma are unusually sensitive to high frequency sound waves between 10 and 30 kHz[1]. In this communication, an investigation of the high frequency sound environment is described, from which it may be inferred that these frequencies play a part in human communications.

The instruments which were used were the Holgate ultrasonic detector, which transduces high frequency sound to more audible low frequency signals, and the Bruel and Kjaer sound spectrometer, which was used to record the root mean square of the amplitude of the sounds which were tested. The microphone (a 0·5 in. condenser) was suspended in an anechoic chamber, some 55 cm from the sound source, and the amplitude of the sound was measured at 10, 12·5, 16, 20, 25, 31·5 and 40 kHz.

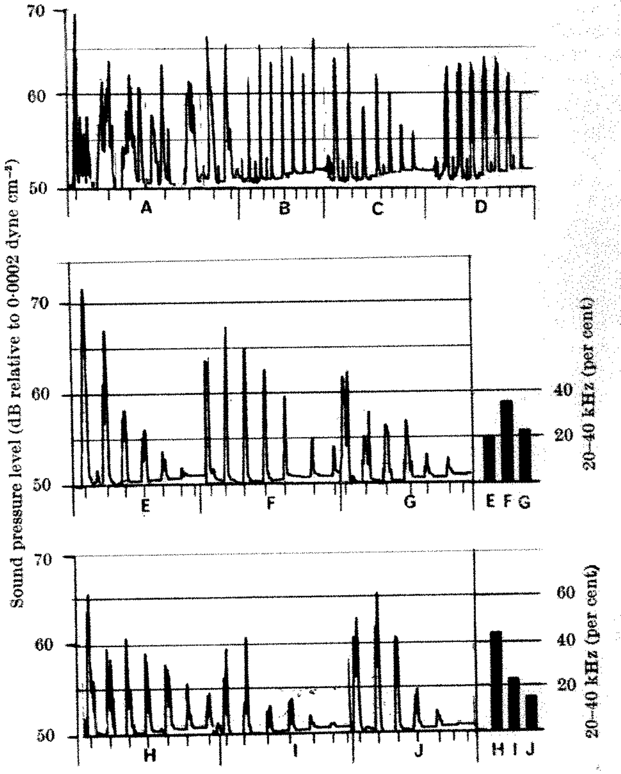
Initially, it was found that high frequency sound formed an important component of sounds which were associated with disturbances of an arresting or irritating nature, for example, the rustling of paper, the ringing of alarm bells or the sudden opening of metal catches. Certain children's toys, such as baby rattles or toy xylophones, also produced significant amounts of high frequency sounds. Various human activities were also found to involve the production of high frequency sound, such as eating an apple or lettuce, snapping the fingers, coughing, scratching the skin and, particularly, shouting and screaming of children. Examples of spectrograms of some of these sounds are shown (Figs. 1 and 2).

The voices of a few volunteer subjects were then tested to find short words of definite emotional content which could be analysed by the spectrometer. The words chosen were "mummy", "daddy", Christian names, "yes", "no", "please", "stop it", and, in addition, it was thought that the letters of the English alphabet covered most of the sounds which were used in lettered speech and, being well known, could be easily recited even by young children.



10–40 kHz third octave frequency bands

Fig. 1. The sound spectrograms of: *A*, a child screaming; *B*, forced breathing; *C*, coughing; *D*, baby's rattle; *E*, toy xylophone; *F*, alarm bell, all recorded at 10, 12·5, 16, 20, 25, 31·5 and 40 kHz.



10–40 kHz third octave frequency bands

Fig. 2. The sound spectrograms of: *A*, eating an apple; *B*, scratching the skin; *C*, snapping the fingers; *D*, rustling paper; *E*, yes; *F*, no; *G*, please; *H*, stop it; *I*, mummy; *J*, daddy, all recorded as in Fig. 1. *E* to *J* are test words and the equivalent percentage of ultrasonics (dB) are shown as histograms (right hand ordinate).

From the preliminary results which were obtained, an experiment was designed to contrast the high frequency content of the speech of normal and asthmatic families.

An asthmatic family was defined as one possessing one or more members who had, at one time or another, exhibited asthmatic attacks. The control families had no such members. The asthmatic attacks were not all of the same severity, persistence and frequency, and some asthmatic subjects had not had attacks for many months. The children in both groups were classified as first born, second born, third born, and so on, but their age and sex were not taken into account. The youngest child it was possible to test was 4 yr old, and the oldest adolescent which was classified as a child was 18 yr old.

All the members of any family were asked to say into the microphone the words and letters suggested. The parents also said their own and each other's Christian names. It was then possible to contrast not only the way in which a parent in either group of families spoke the same test words and letters, but also the way in which succeeding children in a family were addressed. This was done because of the odd frequency distribution of asthmatics in the test families: 48 per cent first born, 35 per cent second born, 13 per cent third born, 4 per cent fourth born, 0 per cent fifth born; a circumstance which, although it could result from chance, aroused suspicions ($\chi^2 = 7{\cdot}4$, $n=4$, $P=0{\cdot}12$).

The subjects were seated so that they spoke some 55 cm from the microphone at the same height; they were told to speak directly into it and not to drop their heads while they were speaking: this is important, as high frequency sound is very directional. They were tested as far as possible in the presence of the rest of the family, and in a random order. Each word had to be spoken seven times so that the seven frequency bands which were tested between 10 and 40 kHz could be analysed by the spectrometer. Seven times was found to be the limit a short word could be repetitively and consistently spoken. With the apparatus used it was not possible to analyse all seven frequency bands simultaneously. The letters of the alphabet were spoken once only and were analysed at the single frequency of 20 kHz because repetition would cause tiredness and loss of interest by the subject.

In all, 106 subjects were tested and comparisons were made on the basis of the percentage of sound (dB) above 20 kHz in each spectrogram, that is between the relative amount of ultrasonics produced by each subject when saying the test words.

An example of the conversion of six test word spectrograms into the equivalent histograms of the ultrasonic percentage is shown in Fig. 2 In order to reduce the influence of particular letters, the results from saying "yes", "please", "no" and "stop it" were summed. In each group, the mean histograms of each parent, both parents together, and the four classes of children are plotted in Fig. 3. With the aid of a digital computer, the significance of the differences between the histograms of the different classes was investigated and it was found that with respect to the control group, the following differences in the asthmatic group were statistically significant.

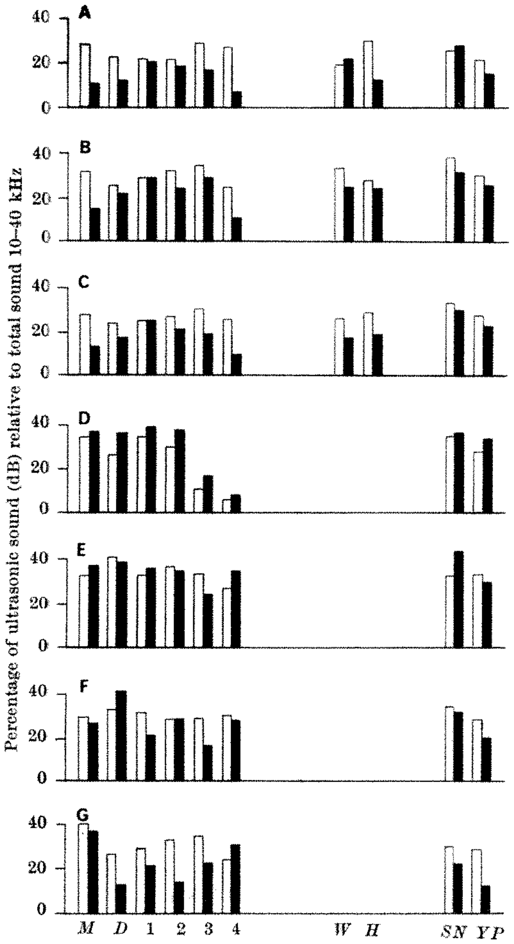


Fig. 3. These histograms represent the relative percentages of ultrasonics (dB) which were produced when the following were spoken: *M*, mummy; *D*, daddy; the Christian names of (1) the first born child; (2) the second born child; (3) the third born child; (4) the fourth born child; *W*, mother; *H*, father; *SN*, average of stop it and no; *YP*, average of yes and please. White columns refer to the asthmatic group and black columns to the control group of subjects. Class *A* consists of the mean histograms of seventeen asthmatics and ten control mothers; class *B* consists of fourteen asthmatic and ten control fathers; class *C* represents the mean of the subjects in (*A*) and (*B*) combined into one class as parents; class *D* is the mean of thirteen asthmatic and nine control first born children; class *E* is the mean of thirteen asthmatic and seven control second born children; class *F* is the mean of eight asthmatic and two control third born children; class *G* is the mean of two asthmatic fourth born children and the histogram of one fourth born child.

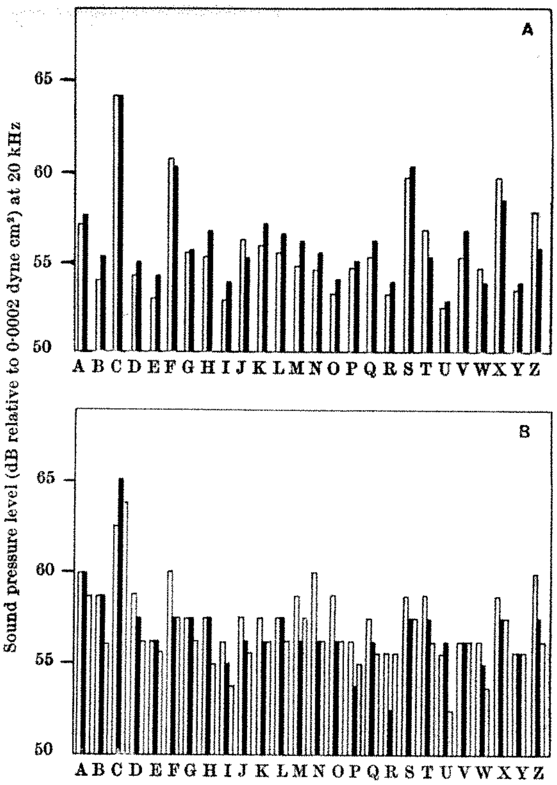


Fig. 4. The histograms of sound pressure levels which were produced when pronouncing the letters of the English alphabet recorded with a third octave band analyser at 20 kHz. *A*, White columns represent mean of twenty-nine asthmatic group parents, and black columns represent the mean of twenty control group parents. *B*, Left hand white column represents the mean of the seventeen children in the asthmatic group who have had asthmatic attacks, and the centre black column represents the mean of the fifteen children in the asthmatic group who have not had asthma. The right hand white column represents the mean of the twenty-two children in the control group.

In class *A* (Fig. 3) the mothers produced more ultrasonics than controls did when saying: (1) mummy ($t=3{\cdot}3$, $n=25$, $P=0{\cdot}01$); (2) daddy ($t=2{\cdot}26$, $n=25$, $P=0{\cdot}04$); (3) the Christian name of the father ($t=3{\cdot}85$, $n=20$, $P=0{\cdot}001$).

In class *B* (Fig. 3) the fathers produced more ultrasonics than controls when saying: (1) mummy ($t=2{\cdot}2$, $n=22$, $P=0{\cdot}05$); (2) the Christian names of the third child ($t=3{\cdot}27$, $n=10$, $P=0{\cdot}01$); (3) the Christian name of the fourth child ($t=5{\cdot}19$, $n=4$, $P=0{\cdot}01$).

In class *C* (Fig. 3) both parents together produced more ultrasonics than controls did for the words : (1) mummy ($t=3{\cdot}6$, $n=50$, $P=0{\cdot}001$) ; (2) the Christian name of the third child ($t=3{\cdot}8$, $n=24$, $P=0{\cdot}01$) ; (3) the Christian name of the fourth child ($t=3{\cdot}4$, $n=11$, $P=0{\cdot}01$); (4) the Christian name of the father ($t=2{\cdot}9$, $n=44$, $P=0{\cdot}01$).

In classes *D* (Fig. 3) to *G* no significant differences were found between the children of the asthmatic and control groups. Mean histograms of the sound pressure levels of letters of the alphabet, recorded with a third octave band analyser, at 20 kHz are plotted in Fig. 4. The only difference which occurs is between children of the asthmatic group who have had asthma and children of the normal group and this is for the two letters Q and Z ($t=2{\cdot}34$, $n=27$, $P=0{\cdot}04$; $t=2{\cdot}25$, $n=27$, $P=0{\cdot}04$) (Fig. 4): otherwise there were no significant differences between the groups.

The results were obtained by testing subjects for a short time with a few words and letters in the somewhat artificial conditions of the laboratory. Although the circumstances surrounding the test were made as routine as possible, the procedure is in practice not very easy, particularly for children. Thus the conclusions to be drawn can only be tentative.

In my opinion, the results which I obtained support the clinical impression that the voices of parents in the asthmatic group sound different to the ears of the observer from the voices of the parents in the control group, and they retain a childlike quality. If the very young asthmatic child with its unusual sensitivity to high frequency sound could instinctively perceive this difference, it might explain some of its behavioural characteristics. Much information could be obtained by the very young child, if he or she noted the frequency at which sound waves from a particular source were no longer perceived, that is if it followed the upper limit of the total band width. Thus it is possible that the high frequency content of speech, hitherto supposed to be of negligible importance[3], might be of use, particularly to children, in the communication of emotional states as observed by Sewell in the rat[2].

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[1] Mason, R. K., *Nature*, **214**, 99 (1967).
[2] Sewell, G. D., *Nature*, **215**, 512 (1967).
[3] Luchsinger, R., and Arnold, G. E., *Voice–Speech–Language*, 99 (Constable and Company Limited, London, 1965).

Lack of Complement Fixing Antigens in DMBA-induced Leukaemia of Rats

MOUSE leukaemia viruses have not induced overt cytopathic effects in tissue cultures and so the complement fixation technique has been used to detect virus antigens in tissue cultures[1]. The technique involved sera of rats which were carrying transplanted tumours originally induced by leukaemogenic virus, and extracts of rat tumours. Germ-free and normal rats developed lesions of myelogenous leukaemia after repeated intraperitoneal administrations of sterile 7,12-dimethylbenz(a)anthracene (DMBA) in fat[2,3]. Viral flora could not be detected in their tissues by electron microscopy, nor by other extensive examinations[4]. This communication describes the use of the complement fixation technique in a search for disease-related antigens in rats with DMBA-induced leukaemia.

Nineteen Lobund germ-free rats with lesions of primary leukaemia which was induced by DMBA, and twenty normal rats with transplanted leukaemia induced by DMBA, were examined. The rats were of three strains: Sprague–Dawley, Wistar and Fischer. Blood, leukaemic tumour and infiltrated liver and spleen tissues were collected from each rat. Serum was separated, inactivated at 56° C for 30 min and stored at $-20°$ C. Tumours and other organs were homogenized in Eagle's medium with penicillin and streptomycin to a concentration of 10 per cent, centrifuged at 2,500 r.p.m. for 30 min and the supernatant fluids were stored at $-70°$ C until they were used.

Germ-free Wistar rat embryos were dispersed with trypsin at 15 days of age and confluent cell sheets of primary or secondary cultures were used for production of antigens. Rat embryo tissue cultures in 32 ounce bottles were seeded with 2 c.c. of organ and tumour extracts, and Eagle's medium with streptomycin and 10 per cent heat-inactivated calf serum was added as nutrient medium. The seeded and control rat embryo tissue cultures were incubated at 37° C for 21 days without changing the nutrient fluid, but the *p*H was adjusted by adding a 5 per cent solution of sodium bicarbonate. At the end of the incubation period, three test antigens were prepared from each tissue culture[5]: (*a*) nutrient fluid; (*b*) ten-fold concentrated nutrient fluid; and (*c*) cells scraped from the glass and re-suspended in 2 ml. of nutrient fluid. The preparations were frozen and thawed three times, centrifuged at 2,500 r.p.m. for 30 min, and stored at $-70°$ C until they were used.

A suspension of thymoma cells from a Sprague–Dawley rat which was infected with Gross leukaemia virus was injected subcutaneously into conventional weanling rats of the same strain. Tumours appeared at the site of injection after one week, and 6 weeks later the rats were bled and sera were collected. Each serum was examined by complement fixation test according to the micro-titre technique[6], with antigen prepared from a 10 per cent extract of the tumour. All the sera were positive and the titres ranged from 1 : 16 to 1 : 64. Sera were pooled accordingly and stored at $-70°$ C for use as reference anti-Gross leukaemia sera.

The four preparations from the organs and the tumours of DMBA and of rats inoculated with Gross virus were tested as antigens with antiserum which was collected from rats with (*a*) primary leukaemia which was induced by DMBA, (*b*) transplanted leukaemia which was induced by DMBA and (*c*) lymphoma induced by Gross virus (Table 1).

Table 1. COMPLEMENT FIXATION TESTS WITH VIRUS AND WITH DMBA-INDUCED LEUKAEMIA OF RATS

Antigens	Complement fixation antibody in rats with Primary DMBA tumour	Transplanted DMBA tumour	Gross virus lymphoma
Organ extracts			
(1) Primary	–	–	–
(2) Transplanted	–	–	–
(3) Gross lymphoma	–	–	+
Tissue culture preparations			
(1) Primary	–	–	–
(2) Transplanted	–	–	–
(3) Gross lymphoma	–	–	+

Examinations of antigens from Gross lymphoma and antiserum from rats with Gross lymphoma were the only combinations in which complement was fixed. Antigens which were prepared from leukaemia induced by DMBA did not fix complement with sera collected from homologous rats nor from rats with Gross lymphoma. Furthermore, antigens prepared from Gross lymphomas did not fix complement when examined with sera from rats with DMBA-induced leukaemia. Sera from twenty normal germ-free rats were examined for complement fixing antibodies against Gross lymphoma antigen, and four gave reactions of ± at 1 : 2 dilution of serum. Similar results were reported to us by Dr Wallace P. Rowe of Bethesda. We cannot explain the presence or the nature of the antibodies to Gross virus in the germ-free rats. Virus has not been detected in the rats and the latter did not develop lymphatic leukaemia unless they were inoculated at birth with Gross leukaemia virus[4].

Gross and FMR complement fixing antigens have been detected in tissue cultures by Hartley and Rowe[1,7]. Complement fixing antigens and antibodies from Gross virus-inoculated rats were shown by us and were used as positive reference reagents for the tests of the unknown sera and antigens. Sera and antigens from DMBA-leukaemic rats were examined with Gross virus reference reagents, but complement fixation was not observed. Negative results in the complement fixation tests on the experimental rats would indicate that they did not produce antibodies or antigens to Gross virus. Furthermore, negative results were obtained when homologous DMBA related antigens and antibodies were examined, which suggested that they did not produce complement fixing antigens at all. These results support the concept[4] that the myelogenous leukaemia which is induced by DMBA in rats bears no relation to Gross leukaemia virus or to other antigenic viruses and can be considered as virus-free.

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[1] Hartley, J. W., *Proc. US Nat. Acad. Sci.*, **53**, 931 (1965).
[2] Huggins, C. B., and Sugiyama, T., *Proc. US Nat. Acad. Sci.*, **55**, 74 (1966).
[3] Pollard, M., and Kajima, M., *J. Nat. Cancer Inst.*, **39**, 135 (1967).
[4] Pollard, M., and Kajima, M., *Proc. Soc. Exp. Biol. and Med.*, **121**, 585 (1966).
[5] Sever, J. L., *Science*, **148**, 385 (1965).
[6] Sever, J. L., *Amer. J. Clin. Pathol.*, **41**, 162 (1964).
[7] Rowe, W. P., Hartley, J. W., and Capps, W. I., *Conf. on Murine Leukemia. US Nat. Cancer Inst.*, Monograph No. 22, 15 (1966).

Transport Piece in the Urines of Premature Infants

IN 1965 Tomasi *et al.*[1] presented evidence that the IgA in saliva and colostrum are similar but differ from serum IgA in that they have a higher sedimentation coefficient and an additional antigenic determinant. Later, South *et al.*[2] confirmed and extended the discoveries of Tomasi *et al.* and observed that IgA can be selectively transported from serum into saliva and that salivary IgA can be dissociated into two antigenically distinct components by reduction with 2-mercaptoethanol. One of the components was immunochemically similar to serum IgA, and the other immunochemically identical to a protein, the transport piece demonstrable in saliva of patients lacking both serum and salivary IgA. They noted that transport piece is present in an unbound form in the saliva of most children and some adults. During the course of a longitudinal study carried out to determine the nature of proteins in urine of premature and full-term infants (our unpublished work) the experiments described here were carried out to determine if transport piece is present in the urine of premature infants.

Twenty-four hour urine samples were collected into a bottle packed in ice from premature infants in the Premature Research Center at the Palo Alto–Stanford Hospital and from full-term infants in the regular nursery. Bladder urine was also collected immediately after birth from ten full-term infants. All urines were dialysed against deionized water at 4° C for 24–48 h and then lyophilized. The resulting dry powders were reconstituted with 0·15 molar sodium chloride to effect a 200–400-fold concentration of the original urine sample.

Antiserum against human serum IgA was purchased from Hyland Laboratories, Los Angeles, California, and adsorbed with serum from an individual lacking serum IgA. When reacted against normal human serum, saliva, colostrum, urine, nasal secretion or sweat in immunoelectrophoresis or gel diffusion, this antiserum gave a single precipitin arc and a reaction of identity between the precipitin arc of serum and those of the various body secretions. An antiserum against secretory IgA was produced in rabbits using IgA isolated by chromatography of normal human colostrum. The antiserum was adsorbed with human serum devoid of IgA. In gel diffusion experiments this antiserum gave a single precipitin band with normal human serum, a reaction of identity between the precipitin arcs of colostrum, saliva, nasal secretions and spurring of the precipitin lines obtained with each of these secretions over the serum IgA precipitin arc.

Table 1. PRESENCE OF URINARY TRANSPORT PIECE AND SECRETORY IgA IN INFANTS

Group	Weight at time of urine collection	No. of infants*	Days after birth urine collected	Free transport piece	Secretory IgA
Premature infants*	860–1,100	5	8–17	+	0
	1,101–1,500	12	6–42	+	0
	1,501–2,000	16	5–56	+	0
	2,001–2,500	13	28–77	+	+
	2,501–2,800	3	43–93	+	+
	4,850–6,500	5	111–195	+	+
Full-term infants	2,948–3,827	5	6	+	0
Bladder urine of newborn infants	2,951–3,632	10	1	+	+ †
Older children	—	9	8–12 yr	+	+

* Figures represent the number of infants in the designated weight group whose 24 h urine samples were pooled.

† See text.

An antiserum specific for transport piece was made by adsorbing the antisecretory IgA antiserum with serum IgA; similar antiserum was supplied by Dr Richard Hong of the University of Minnesota School of Medicine. These antisera gave no precipitin arcs when reacted by double diffusion in agar with normal human serum, 1 per cent serum IgG or 1 per cent serum IgA.

Table 1 summarizes data from pooled urine obtained from premature and full-term infants. The weight groups represent data from infants tested once and from infants who were studied repeatedly as they grew and gained weight in the nursery. Transport piece was present in urines collected from premature infants in each of the six weight divisions as well as from full-term infants and older children. Secretory IgA was demonstrable in the pools of concentrated urines and in a number of individual urines of premature infants weighing more than 2,000 g and of older children. Only one of ten individual bladder urines from full-term infants revealed IgA, but each contained transport piece. Examination of individual and pooled urines of premature infants in the first three weight groups did not reveal IgA. When urines of individual infants weighing 860–1,500 g were reacted with the antibody against purified colostral IgA, a single precipitin band formed (transport piece) which gave a reaction of complete non-identity with serum IgA and of partial identity with colostral and salivary IgA. Urines containing IgA often showed two precipitin bands with this antiserum, one of which gave a reaction of identity with colostral IgA and the other (nearest the antiserum well) a reaction of identity with the transport piece precipitin arc of urines without demonstrable IgA. Thus free transport piece was demonstrable in urines which contained secretory IgA, a finding similar to that described by South *et al.*[2] in saliva of newborn babies. Using the antiserum against colostral IgA and this same antiserum after it had been adsorbed with serum IgA, the transport piece in these urines was seen to migrate in immunoelectrophoretic patterns with the electrophoretic mobility of a fast gamma globulin.

Longitudinal studies were carried out in the five infants weighing between 860 and 1,100 g to determine whether transport piece persists in the urine and to determine the onset of appearance of secretory IgA. At birth, this group weighed between 947 and 1,134 g, representing gestational ages of between 25 and 31 weeks, based on the weight estimates of Lubchenco *et al.*[3]. Representative data in two of these infants (the lightest and heaviest in this weight group) and in one infant in the third weight group are shown in Table 2. The appearance of IgA in the urine of these infants did not appear to be related so much to the number of days after birth as to the weight of the infant.

Amniotic fluid was obtained, free of contamination by

Table 2. LONGITUDINAL STUDIES OF URINARY TRANSPORT PIECE AND IgA IN PREMATURE INFANTS

Patient 1	Weight†	860	1,220	1,540	2,018	2,230	2,497
BW, 947 g	Age‡	8	26	40	54	61	195
(25–29 weeks)*	Transport piece	+	+	+	+	+	+
	IgA	0	0	0	+	+	+
Patient 2	Weight†	1,100	1,380	1,640	2,200	2,300	2,724
BW, 1,134 g	Age‡	13	27	42	54	65	194
(26–31 weeks)*	Transport piece	+	+	+	+	+	+
	IgA	0	0	0	0	+	+
Patient 3	Weight†	1,980	2,220	2,644	2.790		
BW, 1,588 g	Age‡	25	33	43	49		
(29–35 weeks)*	Transport piece	+	+	+	+		
	IgA	0	+	+	+		

BW, Birth weight.

* Figures in parentheses are the range of gestational ages based on the weight estimates of Lubchenco *et al.*[3].

† Weight at time urine collected.

‡ Age in days after birth at time of urine collection.

maternal blood, from seven pregnancies, by amniocentesis. Approximate gestational ages of the foetuses at the time of amniocentesis and volumes of fluid obtained were 14 weeks (150 ml. and 48 ml.), 17 weeks (141 ml.), 19 weeks (135 ml. and 74 ml.) and 40 weeks (134 ml. and 9 ml.). The fluid was dialysed for 24 h, lyophilized and reconstituted in 0·15 molar sodium chloride to obtain a concentration approximately 100 times the original. Transport piece was not demonstrable in the amniotic fluid obtained from the five foetuses less than 19 weeks old but was found in the two fluids from the 40 week old foetuses.

Individual serum samples from four infants in each of the premature weight groups were tested for the presence of IgA, which was not demonstrable in the serum of infants weighing 860–1,100 g but was present in the serum of two of the four infants weighing 1,101–1,500 g and in all of the sera tested from the infants in the other four weight groups.

The studies described here demonstrate the presence of transport piece in the urine of premature infants and suggest that transport piece is produced *in utero*. These data do not define the origin of the transport piece demonstrable in these urines which may have originated in either the infant's urinary tract or serum. Because transport piece has been described in the saliva of older infants[2], it may be that this protein is cleared by the kidneys after its passage into the blood stream from multiple sites (such as nose, gut and salivary glands).

Infants weighing as little as 2,001–2,500 g had secretory IgA in their urine, and IgA was also detectable in their sera. The inability to detect IgA in urine of infants of lower weight despite the presence of IgA in their sera may have been a result of the smaller volumes of urine available from these infants for concentration.

South *et al.*[2] stated that newborn babies, although they have no IgA in saliva, have transport piece. The present studies support their findings of the presence in newborns of transport piece and suggest that transport piece is produced *in utero*, probably at least as early as the sixth to seventh month of gestation.

It is interesting to note that a transport protein was present in utero, of certain of these infants and foetuses in the absence of the material to be transported—IgA. We wonder if the presence of transport piece is a remnant of a phylogenetically important protein and represents a mechanism of host resistance to early invasion by pathogens. *In utero* infection can stimulate *in utero* production of IgA[4–6] which might then be transported to the infants' secretions by means of transport piece already present. It would thus be interesting to know whether high concentrations of secretory IgA are present in the saliva or urine of newborn infants who had been exposed to *in utero* infection when localized IgA response in the secretory glands could be very strong. That infection may be necessary for this to occur, if it does at all, is suggested by the findings of Haworth and Dilling[7]. They noted the presence of IgA in the sera, but not saliva, of seven infants who had received exchange transfusions at birth. IgA appeared in the saliva of these infants at the same age as in infants who did not have transfusions.

During this study, Bienenstock and Tomasi[8] reported the presence of secretory IgA in human urine and found it to be identical to the IgA of saliva and colostrum. Free "secretory piece" was found in all normal urines tested including agammaglobulinaemic urine.

If transport piece is produced during gestation and excreted into the urine, it might be expected in amniotic fluid[9]. Unfortunately, amniotic fluid was not available from foetuses in the 6–8 month gestational age but rather from younger or older foetuses. Transport piece was demonstrable only in the amniotic fluid obtained from the two 40 week old foetuses. The origin of transport piece in the premature infants remains to be defined. There are no published data on foetal synthesis of transport piece, on placental transport of maternal transport piece or on placental production of this protein.

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[1] Tomasi, T., Tan, E. M., Solomon, A., and Prendergast, R. A., *J. Exp. Med.*, **121**, 101 (1965).

[2] South, M. A., Cooper, M. D., Wollheim, F. A., Hong, R., and Good, R. A., *J. Exp. Med.*, **123**, 615 (1966).

[3] Lubchenco, L. O., Hansman, C., Dressler, M., and Boyd, E., *Pediatrics*, **32**, 793 (1963).

[4] van Furth, R., Schuit, H. R., and Hymans, W., *J. Exp. Med.*, **122**, 1173 (1965).

[5] McCracken, G. H., and Shinefield, H. R., *Pediatrics*, **36**, 933 (1965).

[6] Miller, M. J., Seaman, E., and Remington, J. S., *J. Pediat.*, **70**, 714 (1967).

[7] Haworth, J. C., and Dilling, L., *J. Lab. Clin. Med.*, **67**, 922 (1966).

[8] Bienenstock, J., and Tomasi, T. B., *Clin. Res.*, **15**, 292 (1967).

[9] Schultze, H. E., and Heremans, J. F., *Molecular Biology of Human Proteins*, **1**, 547 (Elsevier, 1966).

β-Alaninuria in Patients with Tuberculosis

A HIGH incidence of a high voltage electrophoretic band which is visualized with ninhydrin was noticed in the urine of patients with pulmonary tuberculosis during a study of the excretion of β-aminoisobutyric acid by patients with various pathological conditions. The mobility of the band was identical to that of β-alanine, and the identity was confirmed by paper chromatography and isolation.

Early morning urine was collected and a sample containing 1 mg of creatinine was evaporated in a vacuum desiccator over sulphuric acid and potassium hydroxide. One tenth of the solution of the residue was subjected to paper electrophoresis at a voltage gradient of 100 V/cm for 30 min at pH 3·4 (pyridine : acetic acid : water, 1 : 10 : 189). As standards, 2, 5 and 10 μg of β-alanine were run together with urine specimens. The dried strip was immersed in 0·25 per cent ninhydrin in a mixture of acetone, pyridine and acetic acid (17 : 1 : 2) and heated at 90° C for 5 min. A bluish purple band of β-alanine was eluted with 4 ml. of 50 per cent aqueous ethanol, and the eluate of the ninhydrin negative area was taken as a blank. The optical density was read at 590 mμ. An amount of less than 2 μg/mg creatinine was below the sensitivity of the method of determination and was expressed as zero in the tables. Recovery for 20 μg of β-alanine added to urine was 100 ± 5 and 102 ± 4 per cent in five urine specimens from patients with tuberculosis and fifteen specimens from normal subjects, respectively. The eluate of the band corresponding to β-alanine contained no ninhydrin positive substance other than β-alanine when urine specimens from tuberculous and normal subjects were examined by paper chromatograms, but urine specimens from patients with liver diseases occasionally contained another ninhydrin positive substance as well as β-alanine. The contaminated substance in the urine of patients with hepatic dysfunction could be removed by the adsorption of the urine on a column of 'Amberlite *IR*-120', H^+ form, followed by elution with 1 molar pyridine; the dried residue was used for the determination. The recovery of known amounts of β-alanine added to urine was 98 ± 3 per cent in fifteen estimations. The amounts of β-alanine in some urine specimens were determined simultaneously with an amino-acid analyser[1]. The amounts in six urine specimens were 0, 5, 0, 50, 55 and 50 μg/mg of creatinine with the electrophoretic method which are compared with 2·5, 4·0, 3·0, 48·3, 55·1 and 48·1 μg, respectively, found with the analyser. A reasonable agreement of the values obtained with the two methods provides support for the validity of the method of determination with electrophoresis.

Patients with pulmonary tuberculosis excreted, on average, more β-alanine than normal healthy subjects, and the concentrations of the amino-acid were beyond the normal range in two thirds of urine specimens of the patients. There was no significant difference in the concentration of creatinine in urine from patients with pulmonary tuberculosis and normal subjects. The increase in the amount of β-alanine in the patients was not related to drug treatment. The concentrations of β-alanine in the urine of patients who had not received any treatment were as great as those of the patients receiving antituberculous treatments, and there was no correlation between the amounts and kinds of drugs. In addition three patients with lung cancer who had received antituberculous drugs after the mistaken diagnosis of tuberculosis excreted less than 5 μg of β-alanine/mg of creatinine.

The identity of β-alanine was confirmed by the isolation of the amino-acid from 7 l. of pooled urine from patients with pulmonary tuberculosis. The urine was passed through a 6 × 45 cm column of 'Amberlite *IR*-120 × 8', H^+ form, and the resin was washed with 3·5 l. of water. The adsorbed amino-acids were eluted with 1 molar pyridine. A fraction of the eluate between 2·3 and 3·7 l. was evaporated to dryness *in vacuo* and the residue was applied to a 6 × 35 cm column of 'Amberlite *IR*-120', pyridine form. Each 1 l. of water and 0·2 molar pyridine was passed through the column and a fraction of the eluate (between 900 and 1,720 ml.) was collected and evaporated to a dried residue which contained β-alanine and β-aminoisobutyric acid. The residue was applied to a 6 × 75 cm column of 'Amberlite *IR*-120', equilibrated with a mixture of pyridine, acetic acid and water (2:8:123) and chromatographed with 10·5 l. of the same buffer followed by a mixture of pyridine, acetic acid and water (14 : 40 : 615). The eluate (between 12 and 13·2 l.) was evaporated to dryness *in vacuo*, and the residue was crystallized from aqueous ethanol to give 157 mg of a crude product.

It was further treated with charcoal and recrystallized twice from aqueous ethanol to obtain 93 mg of colourless plates, melting point 189°–192° C (synthetic 191°–192·5° C).

Found C: 40·35%, H: 8·04%, N: 15·33%
Calculated for $C_3H_7NO_2$ C: 40·44%, H: 7·92%, N: 15·72%

The results summarized in Table 1 suggest that β-alaninuria is a common phenomenon among patients suffering from tuberculosis of various tissues and is not specifically related to affections of pulmonary tissue. Other amino-acids were measured with an amino-acid analyser in urine specimens from three patients with lung tuberculosis and three normal subjects. β-Aminoisobutyric acid (two-fold) and glycine (two-fold) also increased in addition to β-alanine (ten-fold), but amounts of other amino-acids remained normal. The increase in the first two amino-acids cannot be taken as significant because only a small number of specimens were examined and because the concentration of these two amino-acids in human urine varies widely between individuals because of genetic influences.

Table 1. CONCENTRATION OF β-ALANINE IN THE URINE OF PATIENTS WITH VARIOUS DISEASES

Subject	No. of cases	Age	Amount of β-alanine (μg/mg of creatinine) Mean value	Range	No. of cases of more than 8 μg/mg creatinine
Normal	51	14–72	1·4	0–8	0
Pulmonary tuberculosis	141	16–72	22	0–130	94
Tuberculous arthritis and ostitis	16	13–67	19	0–49	13
Tuberculosis of cervical lymph nodes	17	14–65	11	0–38	4
Pulmonary cancer	32	37–73	1·1	0–8	0
Bronchiectasis and pneumonias	15	17–52	0·7	0–5	0
Liver diseases	12	18–40	0·8	0–5	0
Renal diseases	11	7–62	2·2	0–8	0
Others	26	8–72	0·9	0–8	0

Table 2. CONCENTRATION OF β-ALANINE IN URINE AND PLASMA

Subject	β-Alanine in urine (μg/mg of creatinine)	β-Alanine in plasma (μg/ml.)
Normal	1	0·27
	3	0·45
	2	0·40
Pulmonary tuberculosis	20	0·67
	21	0·78

Table 3. CONCENTRATION OF β-ALANINE IN LUNG TISSUE

Subject	β-Alanine in urine (μg/mg of creatinine)	Pathological tissue (μg/g of wet tissue)	Normal tissue (μg/g of wet tissue)
Pulmonary tuberculosis	49	9	14
Pulmonary tuberculosis	35	11	—
Bronchiectasis (not tuberculous)	1	14	14

Concentration of β-alanine in plasma was determined with an amino-acid analyser after passing an extract of plasma with trichloroacetic acid through a column of 'Amberlite *IR*-120', H^+ form, followed by washing with water and elution with 1 molar pyridine. The result is shown in Table 2, which shows an increased concentration of β-alanine in plasma from patients with β-alaninuria. Concentrations of β-alanine in extracts with trichloroacetic acid of the lung tissue were low both in apparently normal and pathological tissues as Table 3 shows, suggesting that β-alaninuria in patients with tuberculosis is not the result of increased production of β-alanine in the pathological lesion of the lung. To determine the extent of breakdown of β-alanine, 500 mg of β-alanine was administered orally to two patients with pulmonary tuberculosis who had excreted moderately large amounts of β-alanine and two normal subjects. Urine specimens were collected every hour for 12 h. When administered to the patients the amount of β-alanine in urine increased significantly and returned to the original concentration in 8 h. β-Alanine increased during this period by 60 and 100 mg in the two patients, and in normal subjects by 18 and 10 mg, respectively. This suggests a disturbed metabolism of β-alanine in the patients with tuberculosis.

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[1] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).

Immune Response of Rabbits during Short Term Exposure to High Altitude

THERE is evidence that both a hypoxic stimulus, such as high altitude exposure, and an immunogenic stimulus act by inducing cell differentiation of some pluripotential stem cell[1,2]. It may or may not be the same pluripotential stem cell on which these stimuli act, the former causing differentiation into erythrocytes, the latter into lymphoid cells which produce antibody (Ab). Recent results have shown that a hypoxic stimulus also increases the immune response of animals exposed to chronic high altitude[3-5]. This suggests that perhaps there is a synergistic action between the two stimuli on a common target cell or organ. If cell differentiation is involved in the synergistic action, then two simultaneous stimuli of equal duration applied to undifferentiated stem cells would be expected to be more effective in producing a demonstrable increase in the immune response than separate, successive applications of the two stimuli.

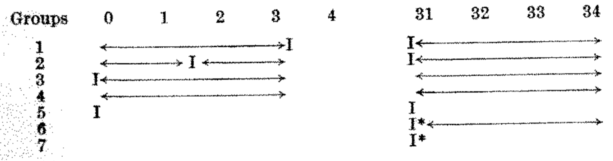
To test this assumption we subjected rabbits to a short time high altitude stress (72–82 h, which approximately corresponded with the time needed for the expression of the primary immune response) and to an immunogenic stimulus simultaneously or successively. The conditions of these experiments were very different from those involving exposure to chronic high altitude[3-5], and consequently their interpretation may be different.

New Zealand white male rabbits, weighing between 2·1 and 2·6 kg, were used in the experiments. The rabbits were immunized with crystalline ovotransferrin (Sigma). One mg of this protein in 0·1 ml. of saline, emulsified with an equal volume of Freund's complete adjuvant, was injected into each foot pad of the rabbits, and another 1 mg was injected intraperitoneally. After 31 days, 0·5 mg of ovotransferrin, emulsified with Freund's adjuvant, was injected into the left hind foot pad of the rabbit. Some rabbits were immunized with a single 0·2 ml. injection of packed sheep erythrocytes into their right foot pads and with ovotransferrin, as described, into their left foot pads. These rabbits were used in determining the number of Ab forming cells by the haemolytic plaque technique. In these conditions of immunization the popliteal lymph node is stimulated primarily[6].

The rabbits were exposed to a simulated 20,000 ft. (350 mm of mercury) altitude in a 30 ft. long, 10 ft. diameter, double pressure lock chamber (Pittsburgh DesMoines Steel Company). The pressure was maintained within ± 20 mm of mercury throughout the experiments. The temperature was kept at a constant 74° F, and the relative humidity between 45 and 60 per cent. (During the early stages of this work the malfunctioning of the air conditioning system caused a rise in temperature up to 82° F and in humidity up to 92–96 per cent.) The experimental design is shown in Table 1.

Serum antibody titre was determined by the passive haemagglutination test of Stavitsky and Arquilla[7]. Ovotransferrin (2 mg) in 0·2 ml. of 0·1 molar phosphate buffer, *p*H 7·3, was first added to 0·05 ml. of packed sheep erythrocytes, then 0·5 ml. of bis-diazotized benzidine (BDB, 0·36 mg/ml.) diluted fifteen times was added for optimal conjugation. Less ovotransferrin resulted in spontaneous haemagglutination, whereas less BDB decreased the specific haemagglutination titre.

Table 1. EXPERIMENTAL DESIGN



I, Time of injection with ovotransferrin as described in the text.

I*, Immunization with both ovotransferrin and sheep erythrocytes. The horizontal arrows indicate the duration of exposure to simulated altitude of 20,000 ft. in the chamber. Groups 1–5 contained seven rabbits each, groups 6 and 7 contained four each. The rabbits were always immunized outside the chamber either immediately preceding or immediately following exposure in the chamber.

The haemolytic plaque test was carried out as described by Jerne and Nordin[8]. The right popliteal lymph node was removed from the rabbits 5 days after immunization with sheep erythrocytes. The haemolytic plaque forming lymph node cells were observed and counted under a Bausch and Lomb stereoscopic microscope.

Haematocrit and haemoglobin assays were taken throughout the experiments to act as a routine check on the effect of high altitude on the number of erythrocytes in the circulating blood. To have some measure of erythropoietic activity we also measured the activity of delta-amino-laevulinic acid dehydrase (DALAD) in the spleen and in the femoral bone marrow by the technique of Mauzerall and Granick[13]. DALAD is the key enzyme in haemoglobin biosynthesis and it may be an excellent indicator of erythropoietic stimulus and erythropoietic activity[14].

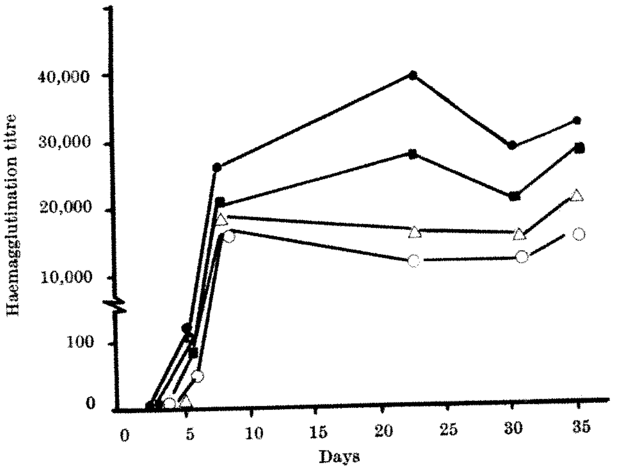


Fig. 1. Haemagglutination mean titre in immunized rabbits and rabbits exposed to altitude. The mean titres were determined from three assays for each rabbit (3 × 7 = 21 samples). ○, Group 1; ■, group 2; ●, group 3; △, group 5.

We have found that the humoral antibody production was stimulated significantly in the altitude stressed rabbits only if the immunogenic stimulus coincided with the altitude stress (Fig. 1). Only group 3 rabbits had a significantly increased haemagglutinin titre compared with the ground control (group 5). If immunization took place 40 h after exposure to altitude (group 2), there was a slight increase in the haemagglutination titre, but if immunization took place after 82 h altitude exposure (group 1) the immune response was essentially the same as in the ground control immunized rabbits.

The cellular antibody response corroborated these findings. Only rabbits immunized and exposed to altitude simultaneously (group 6) had an elevated haemolytic plaque count, as is shown in Table 2. The rabbits in groups 6 and 7 were immunized with two immunogens, sheep erythrocytes and ovotransferrin. The good correspondence between the haemolytic plaque count (indicating antibody producing cells against sheep erythro-

Table 2. EARLY ANTIBODY RESPONSE OF ALTITUDE STRESSED AND NORMAL RABBITS IMMUNIZED WITH SHEEP ERYTHROCYTES AND OVOTRANSFERRIN

Groups	No. of antibody forming cells in 0·2 ml. of popliteal lymph node suspension*	Haemagglutinating titres against ovotransferrin at 6 days
6	$\bar{x} = 216 \pm 24{\cdot}0$	$\bar{x} = 120$
7	$\bar{x} = 141 \pm 19{\cdot}2$	$\bar{x} = 40$

* The weight of the popliteal lymph nodes was approximately equal in both groups, 0·18–0·20 g, almost twice that of the lymph nodes from non-immunized rabbits (0·09–0·12 g).

cytes) and the serum haemagglutin titre against ovotransferrin suggest that the observed effect of high altitude is independent of the immunogen used.

It appears that the increased immune response was compensated by a decrease in haematocrit and haemoglobin levels and in the activity of DALAD in the spleen and bone marrow. As Fig. 2 shows, the haematocrit and haemoglobin levels of group 3 rabbits (first immunized, then exposed immediately to altitude) did not rise as much as those of altitude control group 4. When the rabbits were exposed again to altitude without re-immunization about a month after the first exposure, all groups showed an approximately equal erythropoietic response. This observation shows that the immunogenic stimulus was responsible for the altered erythropoietic response during the first exposure of the rabbits in group 3. It is interesting to note in Fig. 2 that the increase in haemoglobin and haematocrit levels was significantly smaller in the second short term exposure to high altitude than in the first one.

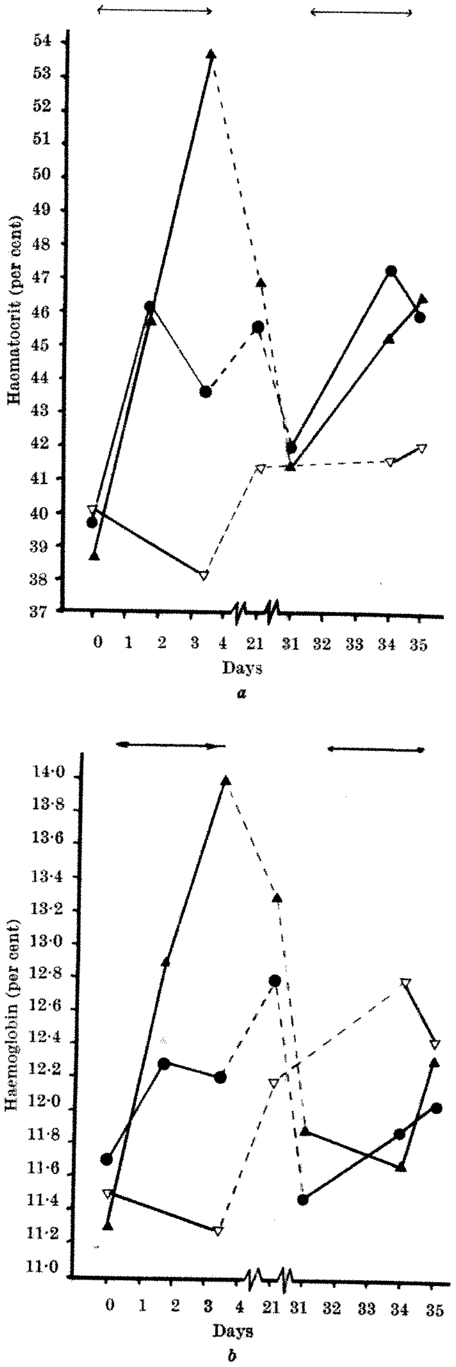


Fig. 2. Haematocrit (*a*) and haemoglobin (*b*) level of altitude stressed rabbits with simultaneous immunization. The horizontal bars indicate the time the rabbits spent in the chamber. The data shown in this and subsequent figures are mean values. The standard deviation varied between ±0·2–2·4, P ≥0·05. For experimental group description refer to Table 1. ●, Group 3; ▲, group 4; ▽, group 5.

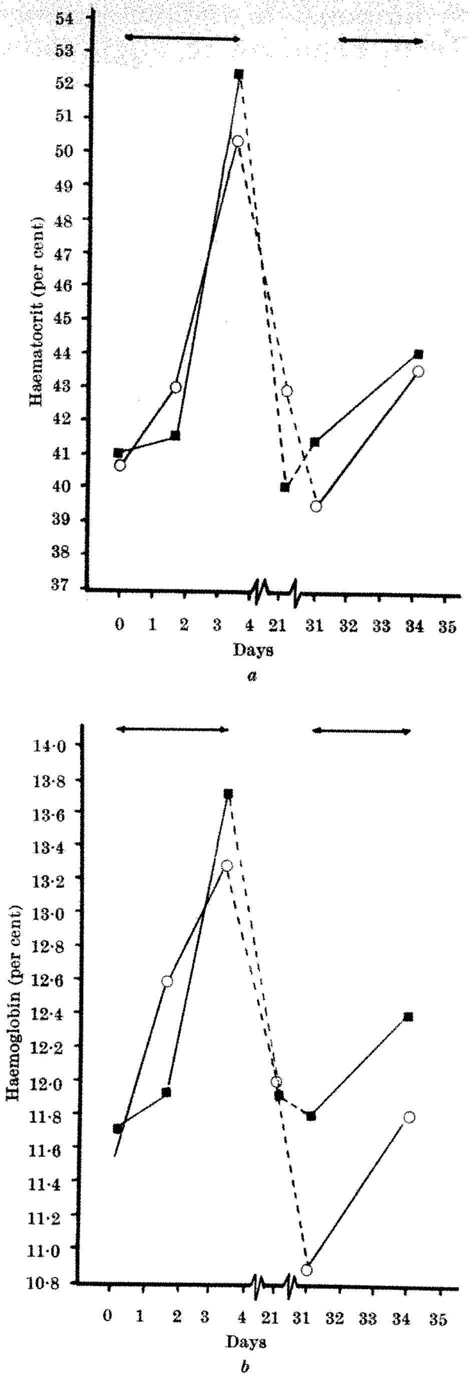


Fig. 3. Haematocrit (*a*) and haemoglobin (*b*) values of altitude stressed rabbits with delayed immunization. ○, Group 1; ■, group 2.

When the two stimuli were applied successively as in groups 1 and 2, the haematocrit and haemoglobin levels were essentially the same as in altitude control group 4 (Fig. 3). The immunogenic stimulus alone (ground control, group 5) did not have any effect on these parameters.

The activity of DALAD in the spleen was significantly lower in group 3 than in the other altitude stressed groups (groups 1, 2 and 4). The same but less pronounced results were obtained with bone marrow (Table 3).

Table 3. ACTIVITY OF DALAD IN THE SPLEEN AND FEMORAL MARROW OF IMMUNIZED AND ALTITUDE EXPOSED RABBITS

Group	Activity of DALAD Spleen	 Bone marrow
1	0·71 ± 0·05	0·56 ± 0·05
2	0·68 ± 0·007	0·49 ± 0·05
3	0·57 ± 0·03	0·46 ± 0·05
4	0·73 ± 0·04	0·55 ± 0·04
5	0·45 ± 0·04	0·38 ± 0·04

Activity of DALAD is expressed as porphobilinogen formed in μmole/g of wet tissue in 1·5 h of incubation. For this assay three extra rabbits were included in groups 1–5; these rabbits were killed on day 5 and the spleens or femoral marrow were assayed after thorough homogenization.

The results are consistent with the assumption that the synergistic effect of the hypoxic and immunogenic stimuli is caused by their simultaneous action on a common target cell, which may be a pluripotential stem cell. When the altitude stress was applied before immunization, the immune response followed the same pattern as if the two stimuli were applied separately. Our data indicate that once the triggering event of the cell differentiation has taken place (in our case there seems to be a critical time within approximately 40 h) the subsequent new stimulus acts independently of the first one. The increased number of Ab producing cells found in group 6 rabbits strongly supports the assumption that the synergistic action of the two stimuli was an increase in the rate of cell differentiation in the lymphoid line.

It is interesting to note that the secondary, anamnestic, immune response was not influenced by high altitude exposure, even if the two stimuli were applied simultaneously (Fig. 1). This observation is in agreement with the often expressed but widely debated opinion that the secondary immune response acts on already differentiated cells, but does not cause stem cell differentiation[12].

The behaviour of the haematocrit and haemoglobin levels and especially the activity of DALAD indicates that a carefully controlled homoeostatic balance must be maintained in the system. The increase in the immune response took place at the expense of processes controlling the level of erythrocytes in the circulating blood. The decrease in the activity of DALAD in the spleen and bone marrow at the same time as the number of Ab producing cells increased in the lymph node suggests that perhaps the cell differentiation of a common pluripotential stem cell was directed into lymphoid cell production. If such a stem cell would have the potential of differentiating into either erythrocytes or lymphocytes, and we could assume that it is available in a limited supply, it would be understandable that when a large proportion of these stem cells is committed for lymphocytes a smaller proportion would be available for differentiation into erythrocytes. A tentative model for this hypothesis is offered in Fig. 4, which may be in accordance with present theories on stem cell differentiation[9–11]. This model may be a useful starting point for detailed investigations of processes of cell differentiation involved in erythropoietic and immunogenic stimuli. The possibility that the hypoxic and immunogenic stimuli may affect the same pluripotential stem cell makes the simultaneous study of altitude stress and immune response particularly interesting.

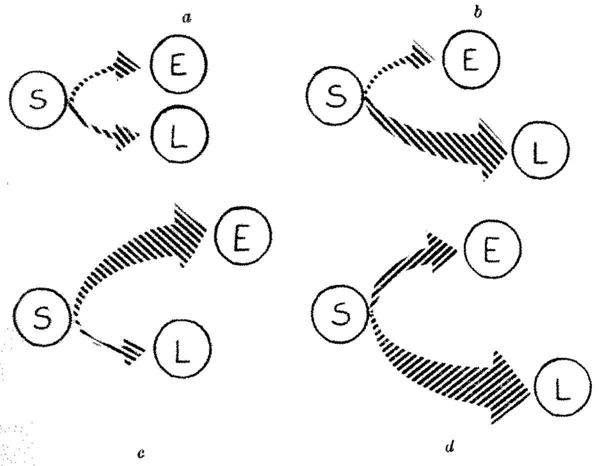


Fig. 4. Scheme for cell differentiation in altitude stressed and immunized rabbits. *S*, Stem cell; *E*, erythroid cell line; and *L*, lymphoid cell line. The arrows illustrate the rate of cell differentiation in the indicated direction. *a*, Normal control; *b*, immunized; *c*, altitude stressed; *d*, immunized and altitude stressed.

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[1] Van Liere, E. J., and Stickney, J. C., *Hypoxia* (The University of Chicago Press, Chicago, 1963).
[2] Reynatarje, C., in *The Physiological Effects of High Altitude* (edit. by Weihe, W. H.), 73 (Macmillan, New York, 1964).
[3] Trapani, I. L., in *The Physiological Effects of High Altitude* (edit. by Weihe, W. H.), 167 (Macmillan, New York, 1964).
[4] Altland, P. D., Highman, B., and Smith, F., *J. Infect. Dis.*, **113**, 228 (1963).
[5] Trapani, I. L., *Fed. Proc.*, **25**, 1254 (1966).
[6] Carpenter, P. L., *Immunology and Serology*, 125 (W. B. Saunders, Philadelphia, 1965).
[7] Stavitsky, A. B., and Arquilla, E. R., *J. Immunol.*, **74**, 306 (1955).
[8] Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).
[9] Sainte Marie, G., *J. Cell. Physiol.*, **67**, suppl. 1, part II, 109 (1966).
[10] Lajtha, L. G., *J. Cell. Physiol.*, **67**, suppl. 1, part II, 133 (1966).
[11] Lajtha, L. G., in *Erythropoiesis* (edit. by Jacobson, L. O., and Doyle, M.), 140 (Grune and Stratton, New York, 1962).
[12] Stavitsky, A. B., in *Reticulo Endothelial Structure and Function* (edit. by Heller, J. H.), 91 (The Ronald Press, New York, 1960).
[13] Mauzerall, D., and Granick, S., *J. Biol. Chem.*, **219**, 435 (1956).
[14] Tengerdy, R. P., *Cancer Res.*, **26**, part I, 312 (1966).

CYTOLOGY

Growth and Morphogenesis of Asparagus Cells cultured *in vitro*

SINCE Steward[1] demonstrated the totipotency of plant cells the morphogenesis of plant tissues cultured *in vitro* has often been investigated. So far, embryoid formation has been found only in dicotyledons. We report here the production of embryoids and their subsequent development into complete plants in a culture of a monocotyledon, *Asparagus officinalis* L.

Asparagus callus, derived from hypocotyls of sterile seedlings, was grown on the basal medium developed by Linsmaier and Skoog[2], with the addition of 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin. The cultures were kept in a growth room maintained at 25° ± 2° C under continuous illumination from Philips TL 4010/33 tubes. The light intensity at the level of the culture vessels was about 40 mcal/cm^2/sec.

In a number of growth experiments on solid agar media, the callus was found to grow most rapidly with hormone concentrations of 1 mg 2,4-D/l. and 0·315 mg kinetin/l. The green callus proliferated vigorously on this medium with occasional organ formation. When both growth substances were omitted from the medium, large numbers of shoots formed with few or no roots. Both growth substances, when added either singly or in combination with a relatively low concentration of the other substance, were able to suppress organ formation without seriously impairing callus growth.

For a more detailed investigation we decided to use suspension cultures. The tissue was multiplied in 100 ml. Erlenmeyer flasks rotated at 100 r.p.m. After about

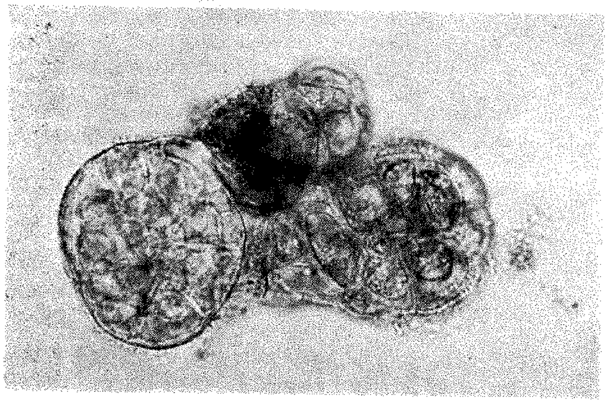

Fig. 1. Globular asparagus embryoids grown in suspension culture (× 480).

2 weeks of growth the larger clumps were removed by sieving through a 2 mm stainless steel mesh. After centrifugation at 2,000 r.p.m. the old medium was discarded and the pellet resuspended in fresh basal medium. The resulting cell suspension was then equally divided into 35 ml. aliquots in 100 ml. flasks by means of a dispensing apparatus fitted with a magnetic stirrer. Finally, the test compounds were added with a sterile syringe. The wet weight increase and morphogenetic behaviour of each culture were then recorded over a growth period of about two weeks.

Several such experiments were carried out, and the effect on cell behaviour of 2,4-D concentrations of 0, 0·01, 0·1, 0·315, 1 and 3·15 mg/l. in combination with 0, 0·1, 0·315 and 1 mg/l. of kinetin was studied. As with the solid culture, a combination of 0·315 mg of kinetin and 1 mg of 2,4-D/l. of medium was found to be optimal for callus growth. The average length of the cells was about 100μ.

Although, initially, the callus cells proliferated well in this medium, the growth pattern changed after a short time. Large numbers of small, round, densely plasmatic cells appeared, and these showed a marked tendency to aggregate. Simultaneously, small nodules, resembling young globular embryoids varying in size from 50 to 200μ, appeared in the cultures.

Several experiments showed that with decreasing concentrations of 2,4-D, callus growth in the form of large vacuolated cells was reduced, with a simultaneous reduction in average cell size. Cell clumping and the formation of globular embryoids increased with decreasing 2,4-D in the media. Furthermore, the embryoid, once formed, grew faster with smaller quantities of 2,4-D.

Although kinetin was beneficial for embryogenesis and growth of young embryoids, the best result was obtained with a low concentration (0·1 mg/l.). Larger amounts frequently induced dedifferentiation of the embryoids.

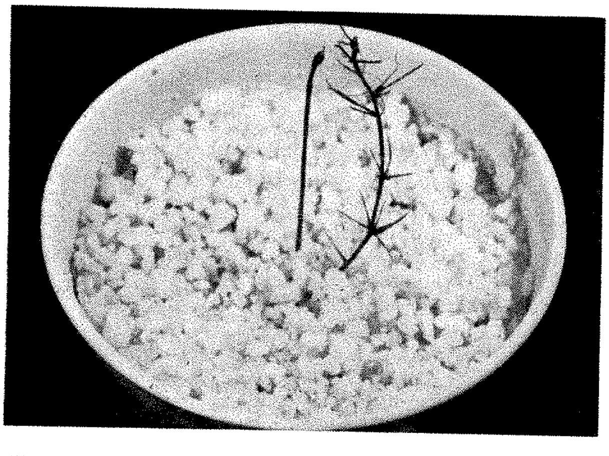

Fig. 2. Young asparagus plantlets grown from callus culture. Stem length about 4 cm.

Once globular embryoids had formed, they developed according to the pattern of normal embryos *in vivo*. They elongated to form a banana-shaped structure, up to 2 mm in length. The first sign of polarity consisted of a one-sided increase in chlorophyll content and gradually the other end elongated to form a radicle.

A striking difference found between normal embryos formed *in vivo* and embryoids formed *in vitro* was the often disproportionate elongation of the stem and radicle ends in comparison with the relatively slow development of the growing points. In order to investigate whether an increase in the osmotic value of the medium would delay "germination" and allow the embryoids to develop more slowly into more balanced seedlings, some experiments were carried out with sucrose concentrations of 2, 4, 6, 8 and 10 per cent. Raising the osmotic value did reduce premature germination, but the percentage of abnormal embryoids increased with increasing amounts of sucrose.

Only small embryoids (not more than about 2 mm in length) grew into normal plantlets after transfer to solid media. When left in the suspension culture beyond the 2 mm stage, the embryoids dedifferentiated at the apex and remained abnormal after transfer. The best initial growth of the transferred embryoids was obtained on a solid medium of the same composition as that of the suspension culture. When transferred at the right stage of development to solid media, the embryoids soon attained geotropic orientation.

When left on this medium, however, the plantlets often dedifferentiated again. Transfer to a simpler medium (Difco orchid agar) within 1 week was therefore necessary. After some weeks the plantlets could be transferred to vermiculite or soil. With this succession of media we were able to grow completely healthy normal plants from undifferentiated callus cells. Among these plantlets were normal diploids as well as some tetraploids. These results indicate that in monocotyledons as well as in dicotyledons differentiation is a natural property of the cells, which cannot manifest itself in callus growth.

We have carried out similar experiments with tissue of the wild carrot *Daucus carota*, and have been able to confirm the results reported by Halperin[3,4] for this tissue. So far, the differences in reaction to environmental conditions with respect to morphogenesis have been largely quantitative for the two species.

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Received December 6, 1967.

[1] Steward, F. C., Maples, M. O., and Mears, K., *Amer. J. Bot.*, **45**, 705 (1958).
[2] Linsmaier, E. M., and Skoog, F., *Physiol. Plant.*, **18**, 100 (1965).
[3] Halperin, W., and Wetherell, D. F., *Amer. J. Bot.*, **51**, 274 (1964).
[4] Halperin, W., *Amer. J. Bot.*, **53**, 443 (1966).

GENETICS

Absence of Thymus in a Mouse Mutant

THE mouse mutant "nude" has been described by Flanagan[1]. It is an autosomal recessive. The homozygotes, *nu nu*, are hairless and their growth is retarded. More than half die before weaning and none survives for as long as 25 weeks. Other parts of the syndrome were: sulphydryl group deficiency and abnormal keratinization of hair follicles, and necrosis of the liver, associated with *Toxoplasma gondii* infection. No single primary defect of development responsible for these pleiotropic effects could be suggested.

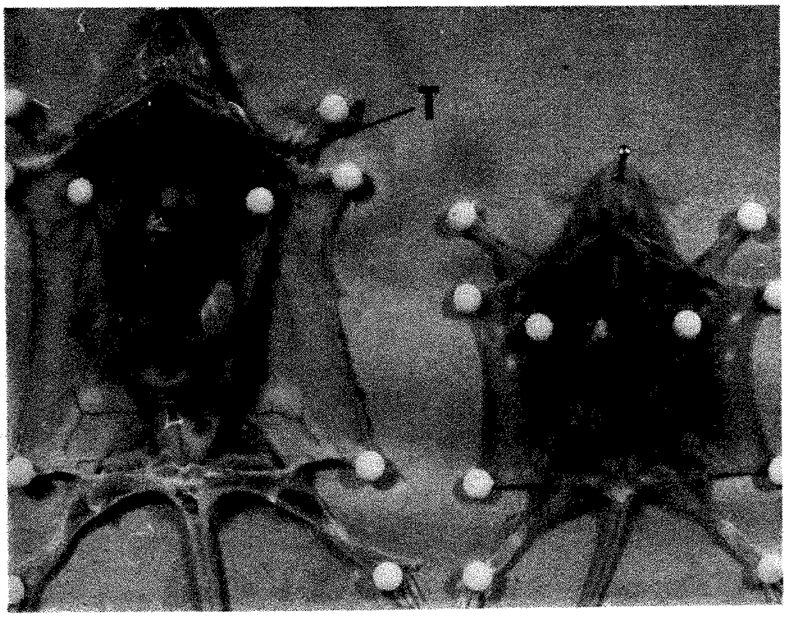

Fig. 1. Dissected normal (left) and homozygote nude (right) sibs. Note the absence of the thymus (T) from the latter.

A re-investigation of the mutation has yielded the following results. No thymus can be found in the homozygotes, while in all normal phenotypes (homozygote wild type or heterozygotes) the organ is normally developed (Fig. 1). The absence of the thymus was confirmed for both sexes and for all ages.

Counts of blood leucocytes were made on groups of various ages (Table 1). In homozygote *nu nu*, counts are extremely low. In normal phenotypes, counts are either about four times greater, or as low as in the nude phenotypes. The low counts are given by the heterozygotes, as confirmed by those cases where the samples were collected from known (genetically tested) heterozygotes. Thus leucocyte counts can serve as a test for the detection of heterozygote carriers.

Necrosis of the liver was not observed in my stock carrying the mutation. Adult homozygotes often showed yellow spots or areas on the liver surface. Histologically, some of these livers exhibited foci of leucocyte infiltration. In some cases, these foci were very extensive, and the organ appeared as a chimaera of liver parenchyma and lymph node (Fig. 2), suggesting virus infection.

It is possible that the lack, or agenesia, of thymus may be the primary defect of development in this mutation. The exact chain of causation leading to other defects remains to be investigated.

I thank Dr D. S. Falconer and Dr S. P. Flanagan for a breeding nucleus of the mutant and for helpful discussions.

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Received December 22, 1967.

[1] Flanagan, S. P., *Genet. Res.*, 8, 295 (1966).

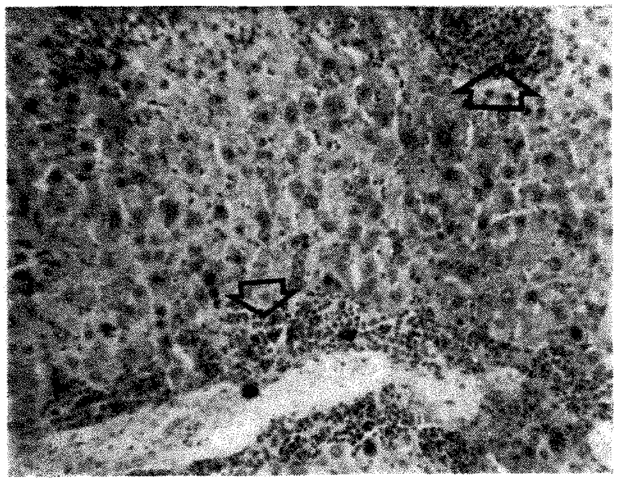

Fig. 2. Section of liver of an adult homozygote nude showing extensive areas packed with leucocytes (arrows).

Table 1. LEUCOCYTE COUNTS (PER MM^3) OF NUDE MICE AND THEIR SIBS

Mouse	Phenotype	Age	Sex	Weight	Leucocytes	Definite or likely genotype
2	Normal	12 weeks	F	22·0	7,300	+ +
3	Nude	12 weeks	M	26·0	3,480	*nu nu*
4	Nude	12 weeks	F	14·0	2,150	*nu nu*
6	Normal	Adult	M	19·5	5,235	+ +
7	Normal	Adult	M	19·0	2,502	+ *nu*
8	Nude	Adult	M	19·0	2,305	*nu nu*
9	Nude	Adult	M	22·0	1,320	*nu nu*
10	Nude	Adult	M	20·0	1,658	*nu nu*
11	Nude	Adult	F	23·0	2,435	*nu nu*
12	Normal + nu	Adult	M		3,720	+ *nu*
14	Normal + nu	Adult	F		4,330	+ *nu*
15	Normal	Adult	M		3,300	+ *nu*
16	Normal	Adult	M		8,375	+ +
18	Normal	Adult	F		7,820	+ +
19	Normal	Adult	F		9,970	+ +
20	Normal + nu	Adult	F		3,900	+ *nu*
21	Nude	17 days	F	5·5	2,780	*nu nu*
22	Normal	17 days	M	9·0	805	*nu* +
23	Nude	16 days	M	4·5	815	*nu nu*
24	Normal	16 days	M	9·5	510	*nu* +
25	Two nudes pooled	11 days	F	3·7	1,501	*nu nu*
26	Normal	11 days	F	7·0	2,220	+ +
27	Normal	11 days	M	7·0	2,500	+ +

PHYSIOLOGY

Drinking Rates and Gill Ionic Turnover in relation to External Salinities in the Eel

SMITH[1] has shown that seawater teleosts maintain their water balance by drinking water and eliminating the divalent ions renally and the monovalent ions extrarenally. Keys[2] demonstrated that the extra-renal route of excretion is most probably the gills. On the other hand, Smith[3] and Krogh[4] say that "the freshwater teleost apparently does not drink water but absorbs it through the oral membranes or perhaps through the gills". This statement has been challenged by several authors[5,6], but no quantitative evaluation of the drinking rate was attempted.

Recently, the use of radioactive tracers which make possible dynamic studies of salt balance has shown that the salt fluxes were much greater in seawater than in brackish or fresh water[7-15]. Motais and Maetz[11,12] showed by simultaneous measurements of the drinking rates and ionic turnover of *Platichthys* and *Serranus*, that only 10–20 per cent of the influx could be accounted for by salt swallowed. The rest enters by the body surface, presumably through the gills. Similar observations were made on *Fundulus heteroclitus*[13,14] and *Xiphister atropurpureus*[15]. Furthermore, for these species, drinking was confirmed and measured quantitatively in the freshwater and brackish water individuals.

In our investigation of *Anguilla anguilla*, ^{131}I-phenol red permitted the evaluation of the drinking rates in animals adapted either to freshwater, seawater or double strength seawater. Fifty yellow eels were used (body weight, 80–225 g; mean 150 g). The animals were adapted to the external media[16] for at least a week at an uncontrolled temperature (16°–24° C). Drinking rates were measured at 20° C using fishes which had been kept for at least 3 days before the experiment in constant temperature close-circuit tanks. This was to avoid shock effects. A sample (1 ml.) of an aqueous phenol red solution labelled with iodine-131 (3 mg and 0·6 mc./ml.) was added to the 15 l. tank. After 2 h, the fish were rapidly transferred into an inactive medium containing anaesthetic (MS 222, Sandoz). A blood sample was drawn from the caudal vein, the peritoneal cavity was opened and the gut removed, care being taken not to lose any gut fluid. The total radioactivity of the gut (R_g in c.p.m.) was measured in a well-type γ-counter and compared with duplicate samples of external fluid (r_e in c.p.m./ml.) and plasma (r_p in c.p.m./ml.). After these short-term experiments, the hind gut contains relatively much less radioactivity than the stomach and anterior intestine. Error caused by anal loss of gut fluid can therefore be neglected and tying off the anus as recommended by Smith[1] is unnecessary. The drinking rate (ml./h/100 g) was calculated from the following equation

$$\text{drinking rate} = \frac{(R_g - C)100}{r_e . W_f . t}$$

W_f is the body weight of the animal in grams, and t is the duration of the experiment in hours—that is, 2 h. C is a correction factor (in c.p.m.) taking into account the radioactivity of the extracellular fluids contained in the gut and estimated to be 30 per cent of the gut volume (W_g in ml.). This correction was necessary because a certain amount of radioactivity was found in the plasma which could not possibly have originated from a leakage of phenol red through the gut wall. C was estimated to be equal to $0{\cdot}3\ W_g . r_p$. In any case, the correction amounts to less than 5 per cent of R_g. Several control experiments were carried out to detect the nature and origin of this radioactivity. The possibility of any marked release of iodide caused by radiolysis could be excluded, because electrophoresis of the phenol red solution produced no iodide spot. Furthermore, no accumulation of radioactivity was observed in the thyroid tissue of the fish. On the other hand, control experiments were run by the *in vivo* gut perfusion technique described previously[16]. They showed that the amount of phenol red passing through the gut wall amounts to a maximum of 2 per cent of the dye content of the gut each hour (unpublished work of E. Skadhauge).

The drinking rates observed (right side of Fig. 1) were 135 ± 27·7 µl./h/100 g (mean and standard error of the mean for $n = 17$) for the freshwater eels, 325 ± 33·5 ($n = 12$) for the seawater animals and 802 ± 182 ($n = 5$) for the fishes adapted to double strength seawater. The increase in the amount of water swallowed with increasing external salinities is very significant ($P < 0{\cdot}01$). Drinking is therefore confirmed for the freshwater eel, which drinks much less than *Fundulus*[14] and much more than *Xiphister*[15]. The drinking rates found for the seawater eels agree well with the data of Smith[1], recalculated as 277 ± 72·8 µl./100 g/h ($n = 5$).

Sodium turnover rates were measured at 17° C in a close-circuit system after intraperitoneal injection of an isotonic solution of ^{24}NaCl or ^{22}NaCl. The adaptation media were either half strength seawater, seawater (Na concentration, 525 mequiv/l.) or double strength seawater (Na concentration 1,040 mequiv.). On the left of Fig. 1 the calculated values of the fraction of exchangeable sodium renewed in percentage/h are summarized. For the freshwater eels, we used the data of Garcia Romeu and Motais[17]; the observed fluxes for an external sodium concentration of 0·5 mequiv. represent 0·5–1 per cent of the exchangeable sodium each hour. For half strength seawater, the turnover rate was found to be 6·6 ± 0·36 ($n = 5$). The rate of sodium exchange was 27·2 ± 3·54 ($n = 5$) and 61·2 ± 7·92 ($n = 4$) for the eels adapted to seawater and double strength seawater, respectively. This highly significant increase ($P < 0{\cdot}01$) in turnover rate in relation to increasing external salinity confirms and extends to hypersaline media previous observations obtained with other teleosts[18,19].

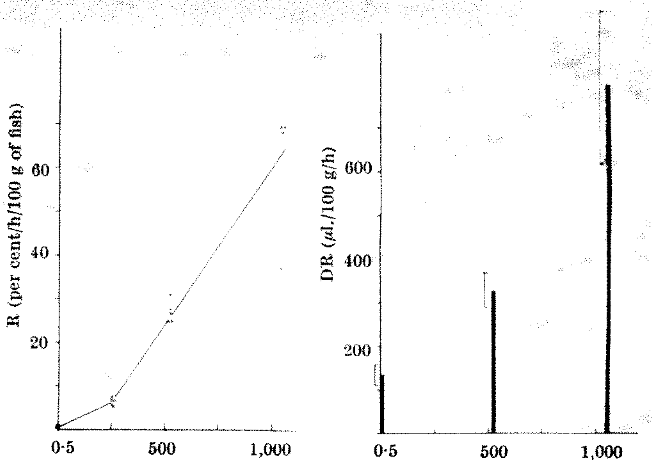

Fig. 1. Left hand side: sodium turnover rates (R) as a function of external concentration of sodium. Right hand side: drinking rate (DR) as a function of external concentration of sodium (standard error of the mean is indicated by brackets).

Both ourselves[16] and Utida *et al.*[20], independently, have shown for the eel that the permeability of the gut to water as well as the ionic absorption capacity of the gut increases with increasing external salinities. It is interesting to compare this capacity with the amount of salt swallowed (or gut influx) and with the total ionic influx as measured isotopically. Such a comparison is given in Table 1 for the sodium ion. It can be seen that the gut absorption capacity exceeds the amount of sodium swallowed except in the animals adapted to double strength seawater. In all media, the gut sodium influx is much less than the total sodium influx.

As expected, with increasing external salinity and an increased osmotic gradient between external and internal media, the rate of drinking increases and compensates for the increased water loss through the body surface. Consequently, the amount of salt absorbed increases and, because the animals remain more or less in a steady state with respect to their sodium balance, more salt is excreted

Table 1. COMPARISON OF TOTAL SODIUM INFLUX, GUT SODIUM ENTRY AND MAXIMAL GUT ABSORPTION CAPACITY FOR EELS ADAPTED TO VARIOUS SALINITIES

Adaptation medium	Total influx	Gut sodium influx	F	Gut absorption capacity	Plasma sodium
Fresh water	21	0·065	0·3	151	130·2 ± 2·43 (25)
Salt water	1,320	170	13·0	332	146·9 ± 2·34 (25)
Double strength seawater	3,550	835	23·5	669	176·0 ± 3·84 (11)

Total influx of fresh water is given in ref. 17. Total influx in seawater and in double strength seawater was calculated from the turnover values and the amount of the exchangeable internal sodium, which is the product of the sodium plasma concentration and the sodium space considered as being 33 per cent of the body weight in all external salinities, as in the flounder[11,12]. Gut absorption capacity is as given in ref. 16. Plasma sodium from gut sodium influx, the amount of sodium swallowed each hour per 100 g is the product of the drinking rate and the external sodium concentration (0·5, 525 and 1,040 mequiv./l. for fresh water, seawater and double strength seawater. All fluxes are given in µequiv./h/100 g. F is the fraction (in per cent) of the gut sodium influx to the total influx. Plasma sodium is given in mequiv./l. (Mean ± *S.E.*, number of experiments given in parentheses.) Some of the data correspond to animals used in gut perfusion experiments.

through the gills. The observed increased turnover rate reflects the increased transport activity of the gills.

One last point needs to be emphasized. While the influx of sodium into the gut is negligible in the freshwater animals, the amount of water swallowed (135 µl./h), which is presumably identical to the amount of water absorbed through the gut, is of the same order of magnitude as the urine flow, 200 µl. according to Butler[21] or 450 µl. according to Sharratt *et al.*[22]. This indicates that the gill osmotic permeability is probably less than previously suspected[23].

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[1] Smith, H. W., *Amer. J. Physiol.*, **93**, 480 (1930).
[2] Keys, A. B., *Z. Vergl. Physiol.*, **15**, 364 (1931).
[3] Smith, H. W., *Quart. Rev. Biol.*, **7**, 1 (1932).
[4] Krogh, A., *Osmotic Regulation in Aquatic Animals* (Cambridge University Press).
[5] Allee, W. C., and Frank, P., *Physiol. Zool.*, **21**, 381 (1948).
[6] Bergeron, J. A., *Doc. Diss. Series*, No. 19, 154 (Cornell University, 1956).
[7] Mullins, L. J., *Acta Physiol. Scand.*, **21**, 303 (1950).
[8] Motais, R., *CR Acad. Sci.*, **253**, 724 (1961).
[9] House, C. R., *J. Exp. Biol.*, **40**, 87 (1963).
[10] Gordon, M. S., *Biol. Bull.*, **124**, 45 (1963).
[11] Motais, R., and Maetz, J., *Gen. Comp. Endocrinol.*, **4**, 210 (1964).
[12] Motais, R., and Maetz, J., *CR Acad. Sci.*, **261**, 532 (1965).
[13] Potts, W. T. W., and Evans, D. H., *Biol. Bull.*, **131**, 363 (1966).
[14] Potts, W. T. W., and Evans, D. H., *Biol. Bull.*, **133**, 411 (1967).
[15] Evans, D. H., *J. Exp. Biol.*, **47**, 525 (1967).
[16] Skadhauge, E., and Maetz, J., *CR Acad. Sci.*, **265**, 247 (1967); ibid., **265**, 923 (1967).
[17] Garcia Romeu, F., and Motais, R., *Comp. Biochem. Physiol.*, **17**, 1201 (1966).
[18] Motais, R., Garcia Romeu, F., and Maetz, J., *J. Gen. Physiol.*, **50**, 391 (1966).
[19] Motais, R., *Ann. Inst. Oceanog. Monaco*, **45**, 1 (1967).
[20] Utida, S., Isono, N., and Hirano, T., *Zool. Mag.*, **76**, 203 (1967).
[21] Butler, D. G., *Comp. Biochem. Physiol.*, **18**, 773 (1966).
[22] Sharratt, B. M., Chester Jones, I., and Bellamy, D., *Comp. Biochem. Physiol.*, **11**, 9 (1964).
[23] Maetz, J., and Morel, F., *Arch. Anat. Microsc. Morphol. Exp.*, **54**, 515 (1965).

Effect of Bromide Ions on Junctional Transmission

The efficacy of bromides as hypnotics or sedatives has long been recognized, but their mode of action is unknown. Many drugs which affect the central nervous system, alcohols and barbiturates, for example, have been shown to influence synaptic activity[1–3] and it seemed likely that bromides might act similarly. The effect of bromide ions on transmission at the neuromuscular junction has therefore been investigated.

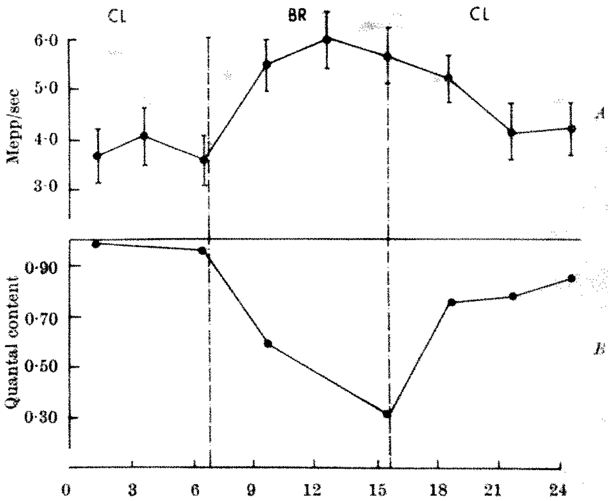

Fig. 1. The effect of bromide ions on neuromuscular transmission. The vertical broken lines denote changes from chloride solution (CL) to bromide solution (BR) and back. *A*, The effect of bromide ions on the frequency of miniature end-plate potentials (mepp/sec; ordinate). Solutions contained 10 mequiv./l. of potassium ions. *B*, The effect of bromide ions on the quantal content of end-plate potentials (for details, see text). Solutions contained 0·7 mequiv./l. of calcium ions, and 6·0 mequiv./l. of magnesium ions.

We have made conventional intracellular recordings at end-plate regions of frog sartorius muscles (*Rana pipiens*). Solutions containing anions other than chloride were made by substituting the sodium salt for sodium chloride in the Ringer solution which contained (mmoles/l.) sodium chloride, 115; potassium chloride, 2·5; calcium chloride, 1·8; and a phosphate buffer (*p*H 7·2). When sodium bromide was substituted for sodium chloride, the frequency and amplitude of miniature end-plate potentials (mepps) increased. The increase in mepp frequency caused by bromide and the decrease in frequency on return to normal Ringer are shown in Fig. 1*A*. In this experiment, solutions contained 10 mequiv./l. of potassium ions to increase the level of mepp frequency for accurate counting. The vertical bars show ± 1 *SEM*. No change in the membrane potential of the muscle was observed during the changes in the concentration of external chloride. The stability of the membrane potential and the increase in the amplitude of the mepp when bromide was substituted for chloride together indicate that bromide decreases the chloride permeability of muscle membrane as has been shown before[4,5]. Increased mepp frequency could be caused by depolarization of the presynaptic membrane from net outward movement of chloride ions which might occur if the nerve terminal were significantly permeable to chloride ions. This possibility was tested by substituting another anion, methylsulphate, for chloride. In such experiments, no increase in the frequency of mepps was seen. Thus bromide ions must have a specific effect on the presynaptic membrane independent of the reduction in the concentration of extracellular chloride. Furthermore, the nerve terminal membrane must have a low permeability to chloride, for large changes in the chloride equilibrium potential apparently do not depolarize the membrane.

The specific effect of bromide on the mechanism of transmitter release is unlike that of calcium or sodium, the ions of which both have greater effects at higher concentrations of potassium[6–8]. When bromide ions were substituted for chloride in solutions containing different concentrations of potassium, the percentage increase in the frequency of mepps was independent of the concentration of potassium. Thus bromide seems to shift to the left the curve relating the frequency of mepps to the concentration of potassium. A similar effect of ions of the lyotropic series on the relationship between tension in frog muscle fibres and potassium concentration has been reported by Hodgkin and Horowicz[9], who suggested that these ions exert their effects by adsorbing onto the outer surface of the muscle membrane and so altering the electric field across it. Because of this analogy another anion of the lyotropic series, nitrate, was substituted for chloride in several experiments. In nitrate solution there was an increase in the frequency and amplitude of mepps similar to that caused by bromide. It was noticed that the muscle membrane was hyperpolarized some 7–9 mV shortly after changing to the nitrate solution and it is possible that in these conditions the muscle membrane was more permeable to nitrate than chloride so that there was a net influx of nitrate causing hyperpolarization.

If bromide and nitrate ions do adsorb to the outside of the nerve terminal the effect on transmitter release should be similar to that caused by membrane depolarization: the frequency of mepps should increase whereas the amount of transmitter released by an action potential should decrease[6,8,10,11]. The effect of these anions on the number of quanta contained in an average end-plate potential (epp quantal content) was therefore investigated. The result of one of these experiments is shown in Fig. 1*B*. The solution contained 0·7 mequiv./l. of calcium and 6 mequiv./l. of magnesium to allow accurate determination of mean quantal contents which were calculated from the formula $\bar{m}$ = ln No. of stimuli/No. of failures (ref. 12). The quantal content in a solution containing 115 mmolar sodium chloride was between 0·9 and 1·0 (Fig. 1*B*). When sodium bromide was substituted for sodium chloride, there was a large decrease in quantal content to approximately 0·3 and the effect can be seen to be almost completely reversible when the preparation was returned to the chloride solution. In solutions containing more calcium and less magnesium, the quantal content of epps, determined from the ratio of the mean amplitudes of epps and mepps, was similarly reduced. A complicating factor in experiments in which the quantal content was derived from measurements of the size of postsynaptic potentials was the slow increase in the resistance of the postsynaptic membrane caused by bromide or nitrate. At junctions blocked by curare the increase in membrane resistance often overwhelmed the fall in quantal content so that epps increased in size. For this reason the "failure" method described here was the method chosen to determine the effect of bromide and nitrate on transmitter release. The potentiating effect of bromide caused by the large increase in "input" resistance of the muscle membrane would not occur at central synapses if, as is generally believed, the postsynaptic membrane has a low chloride permeability. In several experiments, it was found that a bromide concentration as low as 20 mequiv./l. caused a detectable reduction of quantal content.

The effects of bromide and nitrate ions in increasing the frequency of mepps and reducing the quantal content of epps are similar to the effects of presynaptic membrane depolarization[6–8,10,11]. Reduction of the extracellular chloride concentration (methylsulphate substitution), however, does not cause an increase in the frequency of mepps, which indicates that the presynaptic membrane is not significantly depolarized. The effects of bromide and nitrate are not therefore related to changes in the concentration of chloride. The results are consistent with the hypothesis that these anions adsorb to the outer surface of the presynaptic membrane and change the electric field across it in such a way that the efficacy of synaptic transmission is reduced. It seems very likely that the hypnotic and sedative actions of bromides are also related to a pharmacological "presynaptic inhibition" at central synapses.

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[1] Gage, P. W., *J. Pharmacol.*, **150**, 236 (1966).
[2] Thesleff, S., *Acta Physiol. Scand.*, **37**, 335 (1956).
[3] Løyning, Y., thesis, Australian Nat. Univ. (1966).
[4] Harris, E. J., *J. Physiol.*, **141**, 351 (1958).
[5] Hutter, O. F., and Padsha, S. M., *J. Physiol.*, **146**, 117 (1959).
[6] Del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 586 (1954).
[7] Liley, A. W., *J. Physiol.*, **134**, 427 (1956).
[8] Gage, P. W., and Quastel, D. M. J., *J. Physiol.*, **185**, 95 (1966).
[9] Hodgkin, A. L., and Horowicz, P., *J. Physiol.*, **153**, 404 (1960).
[10] Hubbard, J. I., and Willis, W. D., *Nature*, **193**, 1294 (1962).
[11] Bloedel, J., Gage, P. W., Llinás, R., and Quastel, D. M. J., *Nature*, **212**, 49 (1966).
[12] Del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 560 (1954).

Physico-chemical Interaction between Adenosine Phosphates and Fibrinogen as a Cause of Platelet Aggregation

Platelets in a citrated or heparinized plasma system adhere to each other to form aggregates in the presence of low concentrations of adenosine diphosphate (ADP)[1]. Adenosine monophosphate (AMP) and adenosine are ineffective in this respect and indeed act as inhibitors of aggregation by ADP[2]. The degree of aggregation depends on the rate of stirring and, consequently, on the collision frequency of the cells, which is an indication that the process causing aggregation occurs at or very close to the platelet surface. Born and Cross[3] and Cross[4] showed that fibrinogen acts as a co-factor for aggregation, and Deykin *et al.*[5] suggested that another plasma protein may also be required. Although Born[6] and Hampton and Mitchell[7] found that ADP is taken up immediately by specific receptors at the surface of the platelets, the mechanism of this type of aggregation is still obscure. Born concluded that "platelet aggregation by ADP comes about through a reaction in which each ADP molecule is briefly involved to produce an alteration in another component of the system, for example in the receptor or in the protein co-factor, without necessarily being altered itself".

Direct cinematographic observation of the influence of ADP upon platelet-rich plasma through a phase-contrast microscope showed that the moment a crystal of ADP was added to the plasma, many fibrin-like particles formed. These particles stuck together and accumulated around platelets and leucocytes. Similar events were seen when ADP was replaced by ATP and AMP. The explanation of these findings is that the solubility of plasma proteins is changed by the addition of AMP, ADP and ATP.

The precipitation of proteins by the addition of salts, which is often accompanied by irreversible denaturation, is a physico-chemical phenomenon and is influenced by the concentration and valency of the added anions. Usually, the higher their valency the lower the concentration at which the protein precipitates. From their molecular structure, the precipitation capacity of AMP, ADP and ATP should be high and should increase in that order. The adenosine phosphates, however, are acidic and their addition to protein solutions also causes a decrease in the *p*H of the solution. At the iso-electric point of a protein its solubility is very low. For nearly all plasma proteins this point lies between *p*H 5·8 and *p*H 3·5, and these values are easily reached in a 10^{-3} molar solution of ADP.

The critical value at which precipitates form was determined for fibrinogen (human and bovine), gamma globulin (human), alpha globulin (human), and albumin (human), by measuring the optical density at 360 mμ of solutions of each of these proteins after the addition of increasing amounts of adenosine phosphates. As soon as a critical concentration was reached there was a sharp rise in the optical density. All proteins were freeze-dried fractions dissolved in a 0·9 per cent NaCl solution. To remove traces of trisodium citrate and other salts, the solutions were dialysed for 24 h against a 0·9 per cent NaCl solution. The protein concentration was made equal to that of normal plasma. The results are shown in Table 1.

It is clear that, if the four proteins, fibrinogen was the most sensitive to low concentrations of AMP, ADP and ATP. The *p*H at the onset of precipitation was 5·0 for all the adenosine phosphates. Proteins with a low iso-electric point (pI) needed higher concentrations of adenosine phosphates, ATP being the most effective. Because of their buffering capacity, compounds such as EDTA and trisodium citrate, which are inhibitors of platelet aggregation, increased the concentration at which precipitates were formed.

We therefore conclude that precipitation was caused chiefly by lowering of the *p*H. The precipitates consisted

Table 1. PRECIPITATION OF PLASMA PROTEINS BY ADENOSINE PHOSPHATES WITH AND WITHOUT ADDITION OF TRISODIUM CITRATE AND EDTA

Protein	Critical concentration AMP (mmoles/l.)	ADP (mmoles/l.)	ATP (mmoles/l.)	pI
Fibrinogen (human)	1·2	1·0	1·3	5·8
Fibrinogen (bovine)	1·1	1·0	1·3	5·8
Gamma globulin (human)				
Alpha globulin (human)				
Albumin (human)	—	24·2	16·8	4·7
Fibrinogen + citrate				
4·4 mmoles/l.	—	4·5	3·5	
14·6 mmoles/l.	—	7·2	5·8	
24·8 mmoles/l.	—	11·2	10·4	
Fibrinogen + EDTA				
7·2 mmoles/l.	—	7·6	9·6	
13·7 mmoles/l.	—	15·5	18·5	
20·2 mmoles/l.	—	31·9	28·5	

* Exceeds the solubility of AMP in 0·9 per cent NaCl.

of fine particles which stuck together to form aggregates (Fig. 1), and it might be supposed that proteins adsorbed at the platelet cell surface would cause aggregation of the cells themselves if precipitated in the presence of adenosine phosphates. Two arguments seem to conflict with this idea: first, the concentrations of ADP and ATP at which fibrinogen precipitated where higher than those causing platelet aggregation, and AMP does not cause any platelet aggregation at all; and second, calcium or magnesium is required for aggregation.

Concerning the first point, it should be remembered that through their specific adsorption, the "concentration" of nucleotides at the platelet surface may be much greater than that in the solution. Born[6] provided evidence that each platelet possesses about 2×10^5 receptors for adenosine-like molecules, but that the rate of adsorption of adenosine is very different from that of ADP, which is adsorbed immediately. In slow adsorption only a part of the receptors will be occupied. If, however, all receptors are occupied and we also assume that they are equally distributed over the whole surface of the platelet, which has a diameter of 2 to 3μ, the "concentration" at the cell surface would correspond to a concentration in the solution of 5×10^{-3} mole/l. This "surface concentration" is high enough to induce the precipitation of all fibrinogen adsorbed at[8,9] or present near the cell surface.

Concerning the second point, although the role of divalent cations in platelet aggregation is still obscure, it is known that ADP and ATP can bind these ions in equimolar quantities. We have found that precipitates of $CaHPO_4$, $CaCO_3$ and $MgHPO_4$ are rapidly dissolved by the addition of ADP, ATP or EDTA or, less effectively, AMP. This indicates that ADP and ATP formed soluble calcium complexes, and that calcium is chiefly bound by the pyrophosphate groups. There is little reason why these complexes should not be formed in plasma when free calcium ions are available before these are adsorbed by the platelets. This is in accordance with the experiments of Naets[10], who showed that adenosine diphosphate was only adsorbed by the platelet when free calcium was present. It seems justifiable therefore to conclude that the binding of calcium ions to the adenosine phosphates facilitates their adsorption at the negatively charged platelet surface by diminishing the negative charges of the phosphate group without changing the acidic character of the complex.

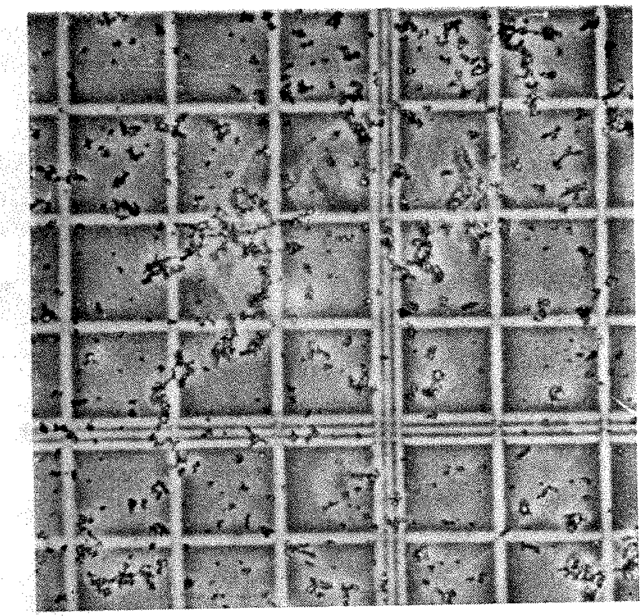

Fig. 1. Precipitation of fibrinogen by $2 \cdot 0 \times 10^{-3}$ molar ADP (phase-contrast photograph).

In summary, we propose that platelet aggregation may be caused by the specific adsorption of an acidic calcium–adenosine phosphate complex causing a lowering of the *p*H near the surface, which is followed by precipitation of proteins, chiefly fibrinogen, giving rise to the formation of bridges between the platelets. This proposal represents an extension of Born's hypothesis. This work forms part of an investigation into chemical aspects of human atherosclerosis and thrombosis, carried out by a research team of the Netherlands Organization of Health Research TNO.

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[1] Gaarder, A., *Nature*, **192**, 531 (1961).

[2] Born, G. V. R., and Cross, M. J., *J. Physiol.*, **168**, 178 (1963).

[3] Born, G. V. R., and Cross, M. J., *J. Physiol.*, **170**, 397 (1964).

[4] Cross, M. J., *Thromb. Diath. Haem.*, **12**, 524 (1964).

[5] Deykin, D., Pritzker, C. R., and Scolnick, E. M., *Nature*, **208**, 296 (1965).

[6] Born, G. V. R., *Nature*, **206**, 1121 (1965).

[7] Hampton, J. R., and Mitchell, J. R. A., *Brit. Med. J.*, i, 1074 (1966).

[8] Ganguly, P., and Moore, R., *Clin. Chim. Acta*, **17**, 153 (1967).

[9] Morse, E. E., Jackson, D. P., and Conley, C. L., *J. Clin. Invest.*, **44**, 809 (1965).

[10] Naets, J. P., and Witlek, M., *Nature*, **206**, 727 (1965).

Fibrin Substrate free from both Plasminogen and Fibrin Stabilizing Factor

IN biological systems, the differentiation of plasmin activity from plasminogen activator activity can only be made in a substrate free of plasminogen. Fibrin is probably the natural substrate of plasmin and almost certainly the most sensitive, but it is usually contaminated with plasminogen[1]. Several methods for removing plasminogen from fibrinogen have been described, but in practice these are usually tedious and not always reproducible[2,3].

Most fibrinogen preparations are also contaminated with fibrin stabilizing factor (FSF or factor XIII)[4] and are thus not a suitable substrate for the assay of factor XIII in plasma samples. Methods for removing FSF from fibrinogen preparations are also laborious and not entirely reliable[5,6].

The preparation described and tested here is simple to make, does not require any special apparatus or equipment, is reproducible and seems to be virtually free of both plasminogen and factor XIII.

During an electron microscopic study of the structure of fibrin recently reported by Kay and Cuddigan[7], it was noted that fibrin prepared from a fibrin monomer solu-

Table 1. MONOCHLORACETIC ACID SOLUBILITY OF FIBRIN CLOTS PREPARED BY DILUTION OF FIBRIN MONOMER

0·2 ml. of fibrin monomer (pH 4·8) were added to 0·4 ml. of buffer solution, pH 7·65, containing

1/40 plasma in 0·1 molar borate	0·1 molar borate	1/40 plasma in 0·1 molar borate and 0·025 molar $CaCl_2$	0·1 molar borate and 0·025 molar $CaCl_2$	1/40 plasma in 0·1 molar borate 0·025 molar $CaCl_2$ 0·05 molar cysteine	0·1 molar borate 0·025 molar $CaCl_2$ 0·05 molar cysteine
Soluble	Soluble	Insoluble	Soluble	Insoluble	Soluble

Clots were incubated at 37° C for 18 h before addition of 3 ml. of 1 per cent monochloracetic acid. "Soluble" indicates that the clot dissolved within 15 min of adding solvent; "insoluble" indicates that clot did not dissolve within 2 h of adding solvent.

Table 2. EFFECT OF PLASMINOGEN ACTIVATORS AND PLASMIN ON FIBRIN CLOTS PREPARED BY DILUTION OF FIBRIN MONOMER (LYSIS TIMES)

		Fibrin prepared by:		
		(a) Adding	(b) Adding	(c) Adding
	Streptokinase (Kabi) (U/ml.)	0·2 ml. of fibrin monomer (pH 4·8) to 0·4 ml. of 0·1 molar borate (pH 7·65)	0·2 ml. of fibrin monomer (pH 4·8) to 0·4 ml. of 1/50 plasma in 0·1 molar borate (pH 7·65)	0·1 ml. of bovine thrombin to 0·5 ml. of whole fibrinogen solution in 0·1 molar borate (pH 7·65)
0·1 ml. of plasminogen activator or plasmin added after incubation of the fibrin at 37° C for 15 min	500	No lysis (37° C for 40 h)	Less than 40 h	—
	50	No lysis (37° C for 40 h)	Less than 12 h	—
	5	No lysis (37° C for 40 h)	Less than 12 h	—
	0·5	No lysis (37° C for 40 h)	Partial lysis (40 h)	—
	Urokinase (Leo) (U/ml.)			
	100	No lysis (37° C for 40 h)	Less than 12 h	—
	10	No lysis (37° C for 40 h)	Less than 40 h	—
	Plasmin (Kabi) (casein U/ml.)			
	2·0	35 min	—	45 min
	1·0	85 min	—	75 min
	0·5	120 min	—	135 min

tion gave a much more clearly defined ultrastructure than did fibrin prepared by directly clotting fibrinogen with thrombin (personal communication from B. J. Cuddigan). This suggested that the fibrin monomer contained less contaminating protein than did the fibrinogen solution. Subsequent studies with fibrin monomer prepared as described here have proved this to be so.

Kabi fibrinogen (human grade L) was dissolved in distilled water to a concentration of 10 mg/ml. This was diluted fifteen- to twenty-fold with 0·85 per cent NaCl, and clotted by the addition of 5 U of bovine thrombin/100 mg of protein. The fibrin was collected onto a glass rod after 90 min of incubation at 37° C and washed in large volumes of 0·85 per cent NaCl, drying carefully with clean filter paper after each wash. After the third wash the fibrin was dissolved in a small quantity of 5 molar urea (1 ml./50 mg of starting material). Complete solution was obtained in 20–30 min and the material could then be reclotted by ten- to fifteen-fold dilution with 0·85 per cent NaCl. This process of clot collection, washing, drying and redissolving was repeated three times until the final urea solution was diluted with an equal volume of 0·05 molar acetate buffer, pH 4·8. The urea was removed by overnight dialysis at 4° C against two changes of acetate buffer. A sample of the final solution (0·1 ml.) clots instantaneously when diluted three- to five-fold with 0·1 molar borate buffer, pH 7·65.

Fibrin monomer prepared in this way was allowed to clot in the presence and absence of calcium, with and without cysteine and with and without added plasma (Table 1). After incubation at 37° C for 18 h, the fibrin in each tube was tested for stabilization by the addition of 1 per cent monochloracetic acid. Clots formed in the presence of plasma and calcium were insoluble in the monochloracetic acid. All the fibrin formed in the absence of plasma and calcium was rapidly soluble in the monochloracetic acid. Cysteine with or without calcium had no effect on solubility.

In another experiment, fibrin formed in the presence and absence of dilute plasma (as a source of plasminogen) was treated with varying concentrations of streptokinase and urokinase (Table 2). Only the clots formed in the presence of plasma lysed during the period of observation.

It seems that fibrin substrate prepared by this method could have considerable advantages when used in assay systems for components of the fibrinolytic mechanism or for the assay of factor XIII. Studies directed towards this end are proceeding and will be published elsewhere.

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[1] Sherry, S., Fletcher, A. P., and Alkjaersig, N., *Physiol. Rev.*, **39**, 343 (1959).
[2] Mosesson, M. W., *Biochim. Biophys. Acta*, **57**, 204 (1962).
[3] Berg, W., and Korsan-Bengtsen, K., *Thromb. Diath. Haemorrh.*, **9**, 151 (1963).
[4] Loewy, A. G., Dunathan, K., Kriel, R., and Wolfinger, H. L., *J. Biol. Chem.*, **236**, 2625 (1961).
[5] Sigg, P., *Thromb. Diath. Haemorrh.*, **15**, 238 (1966).
[6] Tyler, H. M., and Lack, C. H., *Clin. Sci.*, **28**, 527 (1965).
[7] Kay, D., and Cuddigan, B. J., *Brit. J. Haemat.*, **13**, 341 (1967).

Inhibition by Adenosine Diphosphate of the Thrombocytopenia induced in Rabbits by Collagen or Thrombin

IT has been reported[1] that in rabbits intravenous infusions or intramuscular injections of adenosine diphosphate (ADP) or adenosine triphosphate (ATP) reduce platelet adhesiveness as measured by glass bead columns. Because collagen and thrombin are implicated in thrombogenesis we thought it worth while to study their thrombocytopenic activities and any changes produced in them by exogenous ADP. In the experiments reported here, ADP markedly inhibited the thrombocytopenia induced by collagen or thrombin.

The rabbits (New Zealand white strain) in these experiments weighed 2·5 to 5·0 kg. They received neither anaesthetics nor anticoagulants.

Collagen extract was prepared as follows. One gram of collagen (tendon-Sigma) was ground by hand for about 20 min in a tissue grinder with three lots of 10 ml. of sterile saline, free from pyrogen. The bulked extracts were centrifuged at about 1,500g for 30 min, when the supernatant was decanted. Additional 1 g lots were processed and all the extracts were combined. The volume was adjusted to give a concentration equivalent to 30 mg of collagen/ml., and the preparation was dispensed in 10 ml. lots into polythene vials and stored at −20° C. Three batches of extract were made, and each was used within

3 weeks. The curves relating dose and thrombocytopenic response, and the effects of ADP on it, were determined for each batch: all three gave similar results, which we have combined in this report.

Thrombin ('Pure', Koch Light) was dissolved in saline to give a solution containing 20 NIH units/ml.

ADP (50 mg/ml. saline) was administered in a dose volume of 1 ml./kg.

Platelet counts were determined electronically[2] on duplicate samples of blood drawn from an ear artery through disposable needles into polystyrene tubes containing dipotassium ethylenediamine tetraacetate.

In the collagen experiments, all the rabbits were given single intravenous injections of extract in doses equivalent to 5, 10, 20, 40 or 60 mg of collagen/kg, and some were injected intramuscularly with ADP (50 mg/kg) 30 min before receiving the collagen. Platelet counts were determined immediately before and 5, 10, 20 and 60 min after administration of collagen. The results for collagen alone are given in Fig. 1. The thrombocytopenia is dose dependent, maximal at about 5 min, and long lasting compared with that after intravenous ADP[1]. The inhibition by ADP of the thrombocytopenia after an injection of 20 mg of collagen/kg is shown in Fig. 2. The antagonism by ADP (measured 5 min after the administration of collagen) to various doses of collagen is indicated in Fig. 3. Clearly ADP markedly inhibits the thrombocytopenic activity of collagen.

The experiment relating to Fig. 2 was repeated with an equimolar dose (60 mg/kg) of ATP. The inhibitory effects of the two nucleotides were almost identical.

We have previously reported that intramuscular administration of ADP (50 mg/kg) to rabbits reduces platelet adhesiveness measured by glass bead columns, the reduction being maximal at 30 min and negligible at 4 h. We thought that it would be of interest in these new experiments to investigate the antagonism by ADP on the thrombocytopenic activity of collagen at various times after the administration of the nucleotide. Pairs of rabbits were injected intramuscularly with ADP (50 mg/kg) and after 0·5, 1, 2 or 4 h they received collagen (40 mg/kg) intravenously. A further pair of rabbits served as controls and received only collagen. Platelet counts were determined immediately before and 5 min after administration of the collagen. The results shown in Fig. 4 indicate parallelism between the reduction in platelet adhesiveness to glass by ADP and its ability to inhibit the effects of collagen *in vivo*.

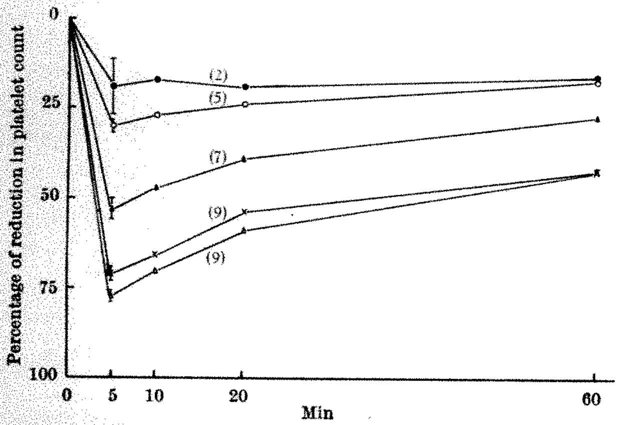

Fig. 1. Reductions in platelet count in rabbits given single intravenous injections of collagen extract. ●, 5 mg/kg; ○, 10 mg/kg; ▲, 20 mg/kg; ×, 40 mg/kg; △, 60 mg/kg. The numbers in brackets refer to the number of rabbits per dose. Vertical lines indicate standard errors.

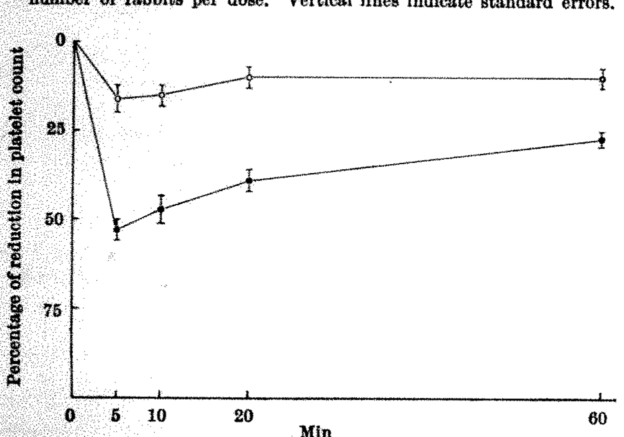

Fig. 2. Inhibition by ADP of the thrombocytopenia induced by collagen in rabbits (seven per group). ●, Collagen 20 mg/kg intravenously; ○, collagen 20 mg/kg intravenously, 30 min after ADP (50 mg/kg intramuscularly). Vertical lines indicate standard errors.

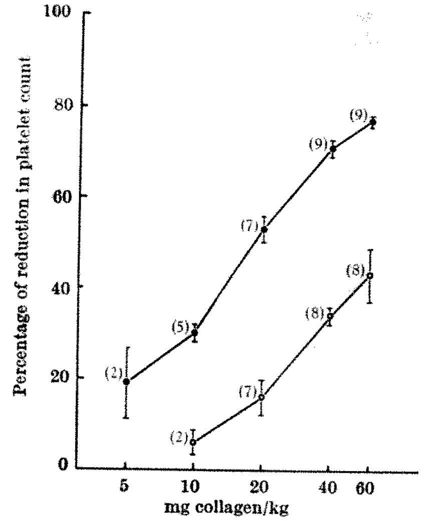

Fig. 3. Inhibition by ADP of the thrombocytopenia measured 5 min after intravenous injections of collagen in rabbits. ●, Collagen only; ○, collagen 30 min after ADP (50 mg/kg intramuscularly). Numbers in brackets refer to the number of experiments. Vertical lines indicate standard errors.

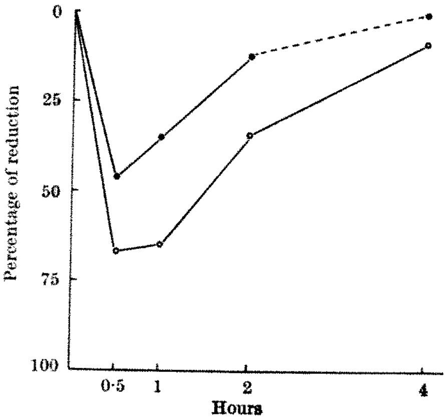

Fig. 4. Reductions by ADP of platelet adhesiveness to glass (○) and of the thrombocytopenic activity of collagen (●). Thrombocytopenia measured 5 min after collagen (40 mg/kg intravenously).

In the thrombin experiments a syringe needle was inserted into an ear artery and about twelve samples of blood were collected with the same needle in the first 2 min after the injection. Thereafter sampling was conducted more leisurely.

The thrombocytopenia after a single intravenous injection of thrombin was pronounced and quick in onset, while the recovery rate was only slightly less than that after ADP. Administering single intravenous injections (20 U/kg) at 10 a.m., 11.30 a.m. and 3 p.m. shows that thrombocytopenia in the same rabbit is reproducible. The three curves relating platelet count with time were almost identical, and the maximum percentage reductions in count were 67, 66 and 61.

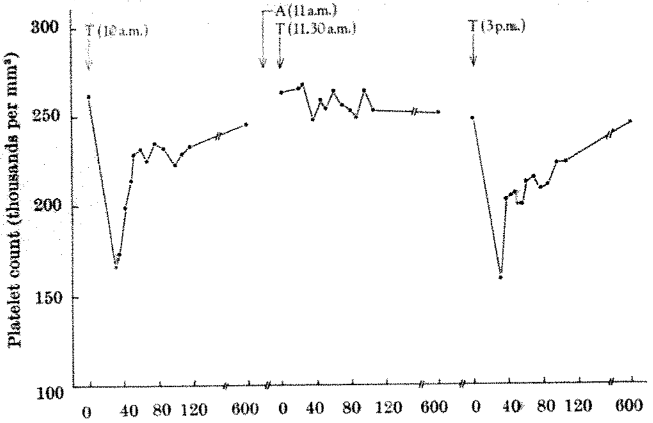

Fig. 5. Thrombocytopenic activities in the same rabbit of thrombin alone or thrombin in the presence of ADP. T, Thrombin 15 U/kg intravenously; A, ADP 50 mg/kg intramuscularly.

The preliminary results with thrombin alone indicated that the response depended on the dose but that the effort necessary to establish a satisfactory dose-response curve would be considerable. It was therefore decided to study possible antagonism by ADP to thrombin using only one dose (15 U/kg) of thrombin.

Antagonism by ADP was studied by giving each of five rabbits single intravenous injections of thrombin (15 U/kg) at 10 a.m., 11.30 a.m. and 3 p.m., and one intramuscular injection of ADP (50 mg/kg) at 11 a.m. A typical result is shown in Fig. 5. The group mean maximum percentage reductions in platelet count after the three doses of thrombin are 43 ± 8, 14 ± 2 and 41 ± 7.

Our earlier experiments indicated that platelets in blood from rabbits injected with ADP had a lowered affinity for glass, and we suggested that if this result truly reflected a decreased tendency for platelets to adhere to damaged intima, then circulating ADP might inhibit thrombogenesis. Our new finding that ADP antagonizes the thrombocytopenia after collagen or thrombin, and the parallelism of this effect with the reduced adhesiveness to glass, strengthen our belief in the relevance of the glass bead test and prompt us to repeat our earlier suggestion of a possible role for ADP or its metabolites in inhibiting thrombogenesis.

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[1] Busfield, Dorothy, and Tomich, E. G., *Nature*, **214**, 1360 (1967).
[2] Williamson, J. R., *Prog. Med. Lab. Tech.* (edit. by Baker, S.), **41** (1967).

Free Fatty Acid Patterns of Human Maternal Plasma, Perfused Placenta and Umbilical Cord Plasma

CHEN *et al.*[1] have shown that there is a variation in the amount of individual free fatty acids in maternal and cord plasma, suggesting that the foetal free fatty acids do not arise solely from the maternal circulation. The placenta is known to synthesize fatty acids[2] and to use free fatty acids in the formation of phospholipids[3] and cholesterol esters (unpublished work of A. Robertson, H. Sprecher and C. Dobbs). To investigate the inter-relationships of maternal, placental and newborn free fatty acids, we have analysed the free fatty acids from these sources in normal pregnancies.

Maternal and umbilical cord blood was collected at the time of delivery into tubes containing ethylenediaminetetraacetate (EDTA) as an anticoagulant. The placenta was perfused as previously described[3]. Lipids were extracted from the tissues by repeated homogenization with a solution of 2 : 1 chloroform and methanol. The neutral lipids were separated by thin-layer chromatography with a solvent system of petroleum ether, diethyl ether and acetic acid in a ratio of 90 : 20 : 2. The lipid fractions were located by spraying with dichlorofluoroscein and identified by comparison with standards. The free fatty acid band was scraped into glass tubes where the methyl esters were formed by refluxing with 5 per cent H_2SO_4 in methanol. The methyl esters were then extracted with petroleum ether and analysed on an F and M model 810 gas chromatograph[4].

The results of the fatty acid analyses are presented in Table 1. We did not determine the absolute concentrations of the free fatty acids and so we cannot speculate about correlation between the concentrations of maternal and foetal individual free fatty acids. Chen *et al.*[1] suggested that the foetal free fatty acids arise from placental transfer and foetal synthesis. The surprising result in this study is the percentage of arachidonic acid (20 : 4w6) in the placental free fatty acids. This elevation of the relative percentage of arachidonic acid (20 : 4w6) is also reported by Renkonen[5] in his analysis of a single placenta. This may be a result of specific placental transport or metabolism or of foetal influences. In one reported case, that of one liveborn and one stillborn twin, the free fatty acid concentrations of the two distinct placentas were similar[6], suggesting that the placental free fatty acid pool is not derived solely from the foeto-placental circulation. That maternal arachidonic acid (perhaps in the free fatty acid form) may be very specifically transported into the placenta is untested so far, but is suggested by the fact that following delivery the maternal arachidonic acid percentage (of total lipid fatty acids) rises[7]. The fact that this rise does not occur within 2 days of delivery weakens this argument. A valuable study would be the serial measurement of maternal arachidonic acid in the free fatty acid fraction before and after delivery.

Table 1. PERCENTAGES OF FREE FATTY ACIDS

Component	Maternal plasma (6)	Perfused placenta (6)	Umbilical cord plasma (5)
12:0	0·7 + 0·7	1·0 + 0·5	1·3 + 0·6
14:0	5·1 + 2·9	2·0 + 1·1	6·9 + 3·3
16:0	28·4 + 5·7	28·8 + 2·4	37·6 + 2·8
16:1w7	3·7 + 0·6	1·7 + 0·4	3·4 + 1·2
18:0	12·3 + 2·6	20·6 + 2·1	15·8 + 7·3
18:1	38·6 + 7·1	13·4 + 0·6	23·2 + 5·4
18:2	8·0 + 2·6	7·7 + 1·6	7·8 + 3·7
18:3w3	0·8 + 0·9	0·5 + 0·4	0·2 + 0·5
20:3w6	0·8 + 1·0	3·0 + 0·9	0·7 + 0·8
20:4w6	1·5 + 1·1	21·4 + 3·1	2·9 + 1·6

Components are identified by their number of carbon atoms. The number of unsaturated bonds is given after the colon in the first column. Following the w is the carbon number of the first unsaturated bond counting from the methyl end of the molecule. Figures in parentheses are the number of samples. Values are the mean percentage weight plus one standard deviation.

If the placenta gives rise to the accumulation of arachidonic acid within itself, how does this occur ? Arachidonic acid is present in fairly large amounts in lecithin[4], cephalin[4] and cholesterol esters (unpublished work of A. Robertson, H. Sprecher and C. Dobbs) of placental tissue. These materials, however, are either not hydrolysed or hydrolysed at a very low rate by placental tissue[8]. Possibly the placental tissue converts linoleic acid to arachidonic acid. This reaction has not been demonstrated in placental tissue although Kleine[9], from his work on placental triglyceride synthesis, suggests, without proof, that this occurs.

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[1] Chen, C. H., Adam, P. A. G., Laskowski, D. E., McCann, M. L., and Schwartz, R., *Pediatrics*, **36**, 843 (1965).
[2] Hosoya, N., Hagerman, D., and Villee, C., *Biochem. J.*, **76**, 297 (1960).
[3] Robertson, A., and Sprecher, H., *Pediatrics*, **38**, 1028 (1966).
[4] Sprecher, H., and Robertson, A., *J. Lab. Clin. Med.*, **70**, 389 (1967).
[5] Renkonen, O. V., *Ann. Med. Exp. Fenn.*, **44**, Suppl. 1 (1966).
[6] Nelson, G., Zuspan, F., and Mulligan, L., *Amer. J. Obstet. Gynec.*, **91**, 949 (1965).
[7] Hansen, A. E., Wiese, H. F., Adam, D. J. D., Boelsche, A. N., Haggard, M. E., Davis, H., Newsom, W. T., and Pesut, L., *Amer. J. Clin. Nutrit.*, **15**, 11 (1964).
[8] Robertson, A., and Sprecher, H., *Lipids*, **2**, 403 (1967).
[9] Kleine, U., *Clin. Chim. Acta*, **17**, 95 (1967).

Structure and Function of the Extraneural Sheath in Insects

Treherne and Maddrell[1,2] have described a continuous sheath of tracheal and fat-body cells around the nerve cord of the Phasmid *Carausius*. They have also obtained measurements showing that the fluid between these structures was some 20 mV positive with respect to the haemolymph, seemingly because of a chloride diffusion potential. Their investigation was related to the specialized ionic composition of the haemolymph in *Carausius*, but they concluded that the sheath was not obviously related to the functioning of the nervous system in this environment.

Their conclusion is supported by our observations of a similar sheath around the thoracic nerve cords of *Schistocerca gregaria* (Orthoptera, Acridoidea), *Homorocoryphus nitidulus* (Orthoptera, Tettigoniidae) and *Sphodromantis* sp. (Dictyoptera, Mantoidea) (Fig. 1). In *Schistocerca* the sheath closely resembles that in *Carausius*; in *Sphodromantis* the sheath is very thin and tenuous including only tracheal cells and no fat-body cells. In *Homorocoryphus* intermediate structures occur. This species is found in two forms; one is large, fatty, edible, seasonal and swarm-forming, and the other has opposite characteristics. The extraneural sheaths of these two forms differ in the number and size of fat-body cells, the former having more.

In *Schistocerca*, as in *Carausius*, the sheath is always cellular. In the other two species, the sheath is partially cellular but also has areas which consist of only a very thin basement membrane. This membrane is often so closely appressed to the neural lamella that the boundary between the two can be distinguished only in electron micrographs, but it seems to be continuous in all the connectives that we have seen (Fig. 2). The continuous cellular sheath of *Schistocerca* could presumably function as an active ionic regulator, as postulated for *Carausius*. The mantid and tettigoniid sheaths, on the other hand, have large areas devoid of cytoplasm, and even if the membrane has specific ionic permeabilities, it could function at best as a passive element in ionic regulation, though possibly supplemented by active processes in its cellular portion.

The ionic composition of the haemolymph of these three insects differs widely from each other and from that of *Carausius*. Insects vary widely in this respect; sodium/potassium ratios range from 0·04 to 32·0, nearly three orders of magnitude[3]. *Carausius* (0·8) falls in the middle of this range[2,4,5], *Schistocerca* (6–20, mean 11) near one extreme[6]. *Homorocoryphus* is unlikely to be different from the closely similar *Tettigonia viridissima* with a sodium/potassium ratio of 1·6 (ref. 4). We know of no determinations on mantises, but as carnivores they are likely to equal or exceed *Schistocerca* in the sodium/potassium ratio.

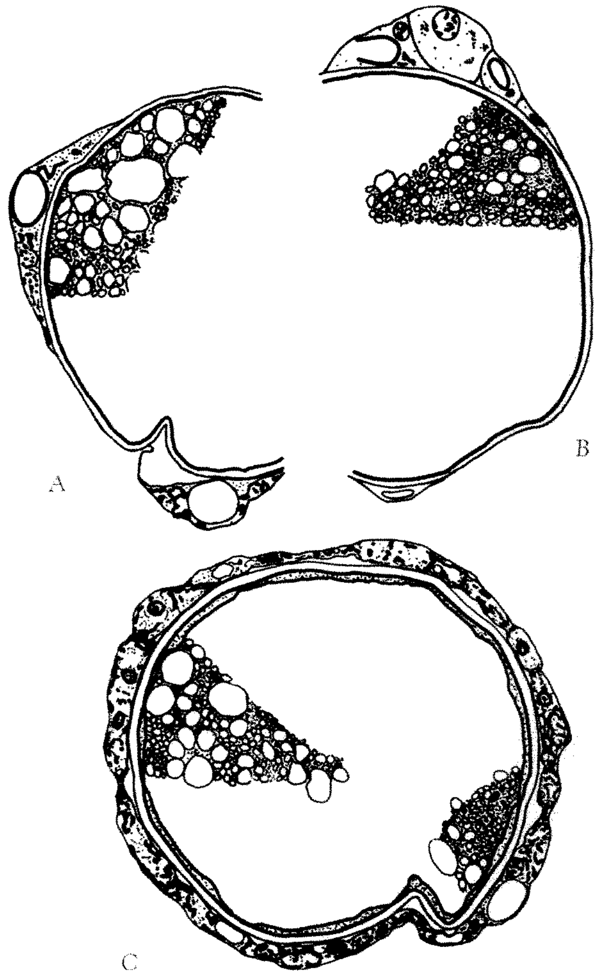

Fig. 1. Partial tracings of sections of nerve cords embedded in 'Araldite' and stained with toluidine blue. Pro-mesothoracic connectives throughout. *A*, *Homorocoryphus*; *B*, *Sphodromantis*; *C*, *Schistocerca*.

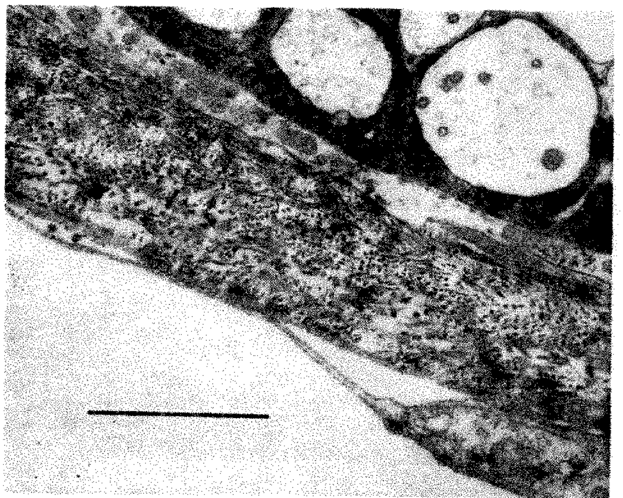

Fig. 2. Electron micrograph of *Homorocoryphus* pro-mesothoracic nerve cord. The neural lamella encloses a very thin perineurium and the axons. Lying on the neural lamella is the end of a fat-body cell, with its membrane prolonged into a thin sheet closely appressed to the surface of the lamella; this sheet is continuous around the connective. Material fixed in buffered osmium tetroxide, stained with uranyl acetate. Scale mark 1μ.

In summary, seemingly homologous sheaths exist around the nerve cords of the orders so far investigated, and vary greatly in morphology. These variations do not correlate with systematic position nor with any obvious feature of the ionic composition of the haemolymph.

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[1] Maddrell, S. H. P., and Treherne, J. E., *Nature*, **211**, 215 (1966).
[2] Treherne, J. E., and Maddrell, S. H. P., *J. Exp. Biol.*, **46**, 413 (1967).
[3] Narahashi, T., *Adv. Insect Physiol.*, **1**, 175 (1963).
[4] Boné, G. J., *Ann. Soc. Zool. Belg.*, **75**, 123 (1944).
[5] Ramsay, J. A., *J. Exp. Biol.*, **32**, 183 (1955).
[6] Philips, J. E., *J. Exp. Biol.*, **41**, 39 (1964).

BIOLOGY

Detachment of Sea Anemones by Commensal Hermit Crabs and by Mechanical and Electrical Stimuli

The ability of some hermit crabs to detach commensal sea anemones and place them on their shells has interested ecologists and ethologists for many years[1]. Anemones belonging to the genus *Calliactis* are perhaps best known in this respect. Excellent accounts of the behaviour of the Mediterranean hermit crab *Dardanus* (= *Pagurus*) *arrosor* Herbst, towards *C. parasitica* (Couch) have long been available[2,3]. Ross[4] showed that *Pagurus bernhardus* (L.) which lives with *C. parasitica* in the English Channel has no behaviour pattern in relation to the anemone. *C. parasitica* has its own behaviour pattern triggered by a chemical factor in the gastropod shell occupied by the pagurid and the anemone is able to transfer to the shell without help from the crab. Ross and Sutton[5] have also shown that the success of *D. arrosor* in transferring *Calliactis* to its shells depends on the simultaneous occurrence of the anemone's behaviour pattern in response to the shell; they emphasized the co-operative roles of both partners in establishing the association.

Recently we have been able to study the associations between *C. tricolor* Lesueur and *D. venosus* (H. M. Edwards) in the Caribbean, and *C. polypus* Forskal and *D. gemmatus* (H. M. Edwards) at Hawaii. *C. tricolor* showed a response to shells that was similar to *C. parasitica*—that is, it could transfer to shells from other surfaces without the help of *D. venosus*, though the crab was usually an active participant. *C. polypus*, however, seemed to lack the response to shells observed in the other two species. In our experiments, it responded to shells only when its pedal disk was already detached and free from the substratum, though Dr C. E. Cutress reports (personal communication) that he has seen *C. polypus* detaching itself from wood or slate to climb on passing gastropods or empty shells.

In these associations with *C. tricolor* and *C. polypus* it was clear that the behaviour patterns of the crab evoked responses from the anemones which facilitated its detachment from the surface on which it was settled. This happened even when there was no apparent contact with the shell. By moving their appendages on the surface of the anemones, the crabs were able to open up closed *Calliactis* and to induce in them a state of complete relaxation so that the anemone could be lifted or pulled off quite easily.

After studying the behaviour of these pagurids as they detached *Calliactis*, it was possible to simulate the movements of the crabs and to bring about the relaxation and detachment of the anemones without difficulty. Thus tapping the margin of the base of *C. tricolor* with plastic rods (*D. venosus* usually began its activity by using its walking legs and claws in this way) brought about the complete relaxation of the anemone in about 1–2 min and its detachment within 3 min. The most conspicuous feature of the behaviour of the Hawaiian pagurid, *D. gemmatus*, towards *C. polypus* was a gentle prodding of the column with the walking legs and chelipeds. After limited success with metal and plastic rods, we found that the activity of this crab could be mimicked better by applying pressure gently and rhythmically around the column of the anemone with pipe-cleaners attached to the second and third fingers on each hand. These "pseudo-legs" produced a state of complete relaxation in *Calliactis* so that the pedal disk could be freed from a stone or plastic surface (Fig. 1). By this method we were able also to detach *Calliactis* from shells, as *Dardanus* does when it "steals" anemones from other members of its own species.

We noted that these two crabs were applying tactile stimuli to *C. tricolor* and to *C. polypus* at a frequency of about one contact/sec. We therefore also tested the effect of electrical stimuli on *C. polypus* at a frequency of one contact/sec. At the temperatures of the tropical waters in which these anemones live (26°–30° C), stimuli at this frequency do not produce the quick retraction reflex of the staircase type. This occurs when stimuli are applied at frequencies of three contacts/sec and higher[6]. When stimuli were applied and continued at a frequency of one contact/sec, an occasional stimulus at the beginning sometimes caused retraction but these closing movements were short-lived. Within 20–30 sec the anemone usually opened up actively and became relaxed completely in exactly the same way as it did when subjected to the crab's palpations or to the simulated crab behaviour as described. Shortly after *C. polypus* went into this state of relaxation, and with the electrical stimuli continuing, the edge of the pedal disk began to move inwards and within a few seconds the pedal disk became completely detached from the substratum. The time from the beginning of stimulation to the detachment of the anemone rarely exceeded 1 min, which is more rapid than the detachment achieved by the most active crabs.

It is known that stimuli at frequencies below those which cause retraction evoke slow contractions of the column musculature in *C. parasitica*[7]. Such stimuli applied to an isolated basal ring were shown by Ewer[8] to have inhibitory effects and were also shown to cause a local contraction of the pedal edge after a long latent period. Although detachment and relaxation to electric stimuli have been demonstrated only in *C. polypus*, it

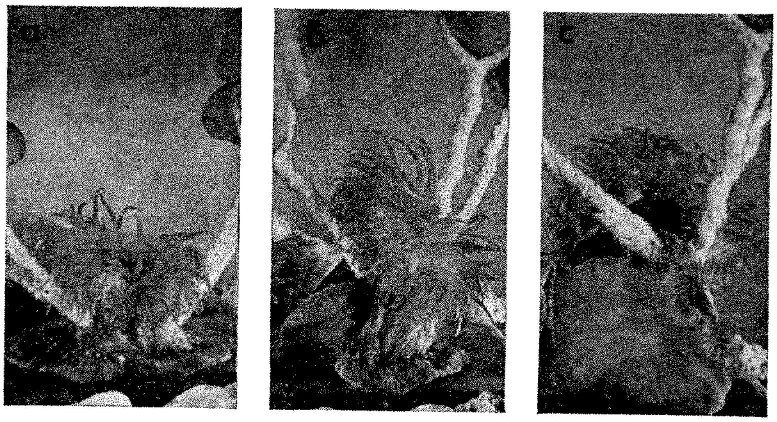

Fig. 1. Release of pedal disk of sea anemone *Calliactis polypus* by mechanical stimuli mimicking stimuli applied by the commensal hermit-crab *Dardanus gemmatus* (pipe-cleaners on fingers used as "pseudo-legs"). *a*, Anemone open and relaxed, stimuli being applied to column, at beginning of treatment; *b*, pedal disk loosening and being lifted by "pseudo-legs"; *c*, exposed pedal disk lifted free after release. (Time between *a* and *c*, 4·5 min.)

would be surprising if *C. tricolor* and *C. parasitica* do not respond in the same way, because the behaviour seems to be based on a direct motor response.

These observations demonstrate new aspects of the mutual interdependence of the behaviour patterns of both partners in the associations between *Calliactis* spp. and *Dardanus* spp. In the association between *D. gemmatus* and *C. polypus* the behaviour of the crab seems to resemble a basic motor mechanism which produces a general inhibition throughout the animal and then induces a local contraction at the base.

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[1] Caullery, M., *Parasitism and Symbiosis* (Sidgwick and Jackson, London, 1952).
[2] Brunelli, G., *Zool. Jb. (Abt. Zool.)*, **34**, 1 (1913).
[3] Faurot, L., *Arch. Zool. Exp. Gén.* (5), **5**, 421 (1910).
[4] Ross, D. M., *Proc. Zool. Soc.*, **134**, 43 (1960).
[5] Ross, D. M., and Sutton, L., *Proc. Roy. Soc.*, B, **155**, 282 (1961).
[6] Josephson, R. K., *J. Exp. Biol.*, **45**, 305 (1966).
[7] Ross, D. M., *J. Exp. Biol.*, **37**, 732 (1960).
[8] Ewer, D. W., *J. Exp. Biol.*, **37**, 812 (1960).

Effects of Cercarial Extracts of *Schistosoma mansoni* on HeLa Cells

For many years investigators have suggested that parasite toxins play a part in the pathology of schistosomiasis[1-3]. Unfortunately, no attempts seem to have been made to evaluate the cytotoxic potential of schistosomes *in vitro* until recently. In 1936 and 1937 cercarial extracts of the trematodes *Diplostomum flexicaudum* and *Cryptocotyle lingua* were reported to produce cytopathology in excised tissue from lower vertebrates[4,5]. Not until 1965, however, was there any report of the toxic effects of schistosomes on mammalian cells in culture[6]. It is now suggested that abnormal changes occur in monkey heart cell cultures to which viable eggs or egg extracts of *Schistosoma mansoni* have been added. Some of the effects include: breakdown of the monolayer growth pattern, loss of selective permeability against eosin Y, and metabolic inhibition (that is, no drop in *p*H) of treated cultures.

Compared with the eggs of *S. mansoni*, invading cercariae produce minor effects on host cells *in vivo*. The potential toxicity of cercariae, however, is interesting because they, like the egg stage, seem to depolymerize non-cellular tissue barriers but with little effect on mammalian cells[7,8].

Cercariae of *S. mansoni* were obtained when they emerged from infected *Australorbis glabratus*. The larvae were filtered through a 100 mesh stainless steel screen, refrigerated, and allowed to settle overnight in Imhoff cones. The sediment was washed by cold centrifugation in three changes of 0·9 per cent NaCl and stored at −30° C for up to 2 weeks.

Cercarial extracts were prepared by grinding the frozen larvae in sterile sand and adding enough cold, sterile, Earle's balanced salt solution to permit pouring of the material after standing for 4 h at 4° C. The Earle's solution contained 100 U of penicillin and 50 μg of streptomycin/ml. Solid debris was removed by cold centrifugation (1,200*g*) for 20 min, and the supernatant fluid was dialysed overnight at 4° C against 100 volumes of Earle's solution.

HeLa cells were cultivated on coverslips in Leighton tubes with bicarbonate-buffered medium 199 and 10 per cent calf serum. Antibiotics were included as described for Earle's solution. Warm, dialysed cercarial extract or Earle's solution, *p*H 7·4, was added to cultures previously rinsed with three changes of sterile balanced salt solution. A portion of the extract was heated in a water bath at 60° C for 1 h to test for thermolability. Gravimetric or colorimetric protein determinations were made from aliquots precipitated with two volumes of 20 per cent trichloroacetic acid[9,10]. Most extracts contained approximately 0·5 mg of precipitable protein/ml. Cultures were incubated at 37° C, examined every 30 min during the first 8 h, and again after 18–24 h. Parallel examinations were made of live as well as methanol-fixed cells stained with Giemsa.

The appearance of cells in a typical control culture held in Earle's solution is shown in Fig. 1; these cells retained their normal contours, had pale basophilic cytoplasm, and possessed open faced or vesicular nuclei. Their appearance was identical to that of cells treated with previously heated cercarial extract.

HeLa cells in cultures containing unheated extracts became refractile in 3–5 h and at the same time the cytoplasm became more basophilic than usual and there was a further intensification of basophilic staining as cells displayed cytoplasmic shrinkage.

Changes in the staining reaction suggested an initial concentration of basophilic components accompanying leakage and cytoplasmic retraction. This interpretation was supported by two observations: (*a*) that HeLa cells lost all but a few adhering shreds of cytoplasm as shown in Fig. 2, and (*b*) the packed cell volume of equal numbers of treated and control cultures was compared after pooling and centrifugation; the treated cells lost about 95 per cent of the packed volumes observed for control cultures. After cytoplasmic retraction, cells detached from the glass and the monolayer eventually disintegrated. After 18–24 h only free-floating, deeply basophilic, shrunken nuclei remained.

The suppressive effect of the antibiotics was checked by a visual search for bacteria in or on Giemsa- or Gram-stained cells, and by overnight incubation of the culture fluids in thioglycollate medium. The fluids remained bacteriologically "sterile" and it was concluded that only unheated cercarial extracts had caused the cellular damage during the previous period of observation.

Unsuccessful attempts were made to introduce live cercariae into HeLa cultures: before transferring recently emerged live larvae into cultures, it was necessary to deplete and replace the original water with fluid isotonic

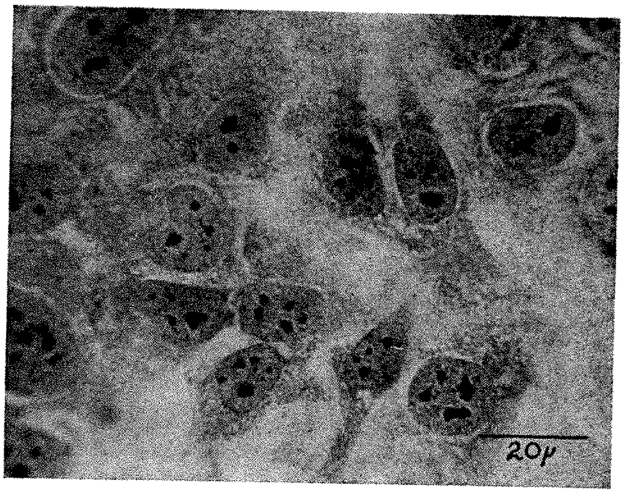

Fig. 1. HeLa cells after incubation in Earle's solution for 18 h at 37° C. (Giemsa stain.)

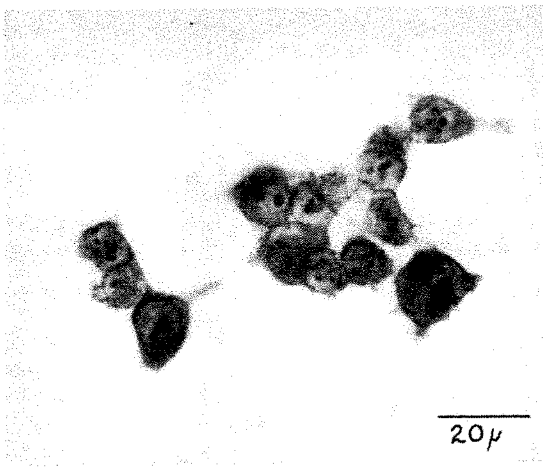

Fig. 2. HeLa cells after 7 h incubation with cercarial extract. The normal contour of the cells was obliterated leaving basophilic nuclei with narrow margins of basophilic cytoplasm.

to mammalian cells. The larvae were concentrated to a small volume on a sterile fritted glass filter, dialysed against sterile Earle's solution for several hours at room temperature, and inoculated into monolayer cultures. Practically all the larvae were dead after 18 h at 37° C, and the HeLa cells seemed to be normal.

If Medium 199 containing 10 per cent serum was used as the solvent for cercarial extracts, no cytopathogenic changes occurred. This was observed after testing 10 per cent calf, rat, mouse, or any of several different species of monkey sera. The results agree to some extent with the fact that normal animal sera also contain substances inhibitory to some cercarial enzymes[11].

In evaluating the cytotoxic potential of *S. mansoni*, or any schistosome, the approach of Gutekunst, Browne and Meyers[6] should be favoured. These workers showed that the intact, viable eggs of the parasite were toxic for mammalian cells *in vitro*, suggesting that cells succumbed to exotoxic products from miracidia. I was unable to keep cercariae alive in HeLa cultures, and so it is possible that the lysosomal or other abnormally released materials contributed to the apparent toxicity of disrupted cercariae.

The alterations produced by cercarial extracts were in the nature of an "all or none" response in the absence of serum. It seems, however, that viable eggs of *S. mansoni* can exert their effects despite the presence of serum in conditions necessary for normal growth of mammalian cells. It is concluded that experimental cell damage requiring disrupted cercariae and the exclusion of serum suggests a low cytotoxic potential for this stage of *S. mansoni*.

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[1] Fairley, N. H., *J. Pathol. Bact.*, **23**, 289 (1920).

[2] Girges, R., *Schistosomiasis* (*Bilharziasis*) (John Bale, Sons and Danielson, Ltd., London, 1934).

[3] Meleney, H. H., Sandground, J. H., Moore, D. V., Most, H., and Carney, B. H., *Amer. J. Trop. Med. Hyg.*, **2**, 883 (1953).

[4] Davis, D. J., *J. Parasitol.*, **22**, 108 (1936).

[5] Hunter, G. W., and Hunter, W. S., *J. Parasitol.*, **23**, 64 (1937).

[6] Gutekunst, R. R., Browne, H. G., and Meyers, D. M., *Exp. Parasitol.*, **17**, 194 (1965).

[7] Lewert, R. M., and Lee, C. L., *J. Infect. Dis.*, **95**, 13 (1954).

[8] Lewert, R. M., *Rice Inst. Pam.*, **45**, 97 (1958).

[9] Hoch, F. L., and Vallee, B. L., *Anal. Chem.*, **25**, 317 (1953).

[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[11] Lewert, R. M., Lee, C. L., Mandlowitz, S., and Dusanic, D., *J. Infect. Dis.*, **105**, 180 (1959).

Homologous Alkaline Phosphatases and Homologous Loci in Two Sibling Species of European Anopheline Mosquitoes

Anopheles atroparvus and *Anopheles labranchiae* are two sibling species belonging to the maculipennis group of European anopheline mosquitoes. They may be considered the most closely related species of the group; their gene pools are probably the most similar. By the definition of Lorković they could be termed "semispecies", showing some of the characteristics of the species and some of subspecies[1]. Individuals of *A. atroparvus* and *A. labranchiae* are not clearly distinguishable by cytogenetic analysis of the banding pattern of the salivary chromosomes. They can be distinguished on the basis of slight differences in the micromorphology of the eggs, and also on the basis of hybridization data: the interspecific hybrid males are sterile, but introgressive hybridization is possible, for the females can be backcrossed to *A. atroparvus* and *A. labranchiae* males, and again produce normal females and several normal males.

Individuals of *A. atroparvus* and *A. labranchiae* can easily be distinguished at the fourth larval stage by comparing the relative electrophoretic mobility of two specific non-polymorphic bands showing alkaline phosphatase activity, arbitrarily designated Aphl (ref. 2).

If amino-acid sequences in proteins are colinear with the nucleotide sequences of the DNA subunits which code for them, and if the different relative electrophoretic mobilities of homologous proteins are an expression of their different molecular structure, it can be concluded generically that there is no identity between the structural gene or genes controlling the synthesis of the proteins examined in both species. Such conclusions raise the following questions. (*a*) Are these catalytically homologous proteins also homologous molecules? (*b*) Are they phenotypic expressions of the activity of a single genetic locus in both species? (*c*) If the answer to question (*b*) is affirmative, are then the specific loci under examination also homologous?

To answer these questions, I used the following experimental results. (1) Hybrids at the fourth larval stage obtained from the crosses: *atroparvus* males × *labranchiae* females (pair matings) and *labranchiae* males × *atroparvus* females (forced copulation) have given Aphl bands which always show intermediate electrophoretic mobility compared with Aphl of the parental species. (2) The same type of intermediate band has also been obtained *in vitro*, by mixing *atroparvus* and *labranchiae* homogenates, freezing them in the presence of 1 molar NaCl and then thawing, and applying them to a gel for electrophoresis (Fig. 1). (3) The same results were also obtained when *A. atroparvus* and *A. labranchiae* were simply ground together and the homogenates maintained at room temperature for a few minutes before electrophoresis.

It was not possible to obtain typical electrophoretic patterns showing three distinct bands, which heterozygous variant forms of dimeric proteins usually produce[3]. In this case, the indication is that the phenomenon occurs when two alleles producing different polypeptide subunits are brought together in a hybrid.

In spite of this gap, I think that these results could still be interpreted as representing the phenomenon already mentioned. The differences in relative mobility between Aphl of *A. atroparvus* and *A. labranchiae* were very small, and the hybrid pattern constantly appeared as a zone of intermediate mobility, in which the bands were also thicker and less well defined than those of Aphl of the parental species. It could therefore be possible to conclude, according to Coates[4], that this pattern is caused by the recombination of polypeptide chains, where two different bands, and another with intermediate mobility, are overlapping. Alternatively, the intermediate band of the hybrid larvae could represent

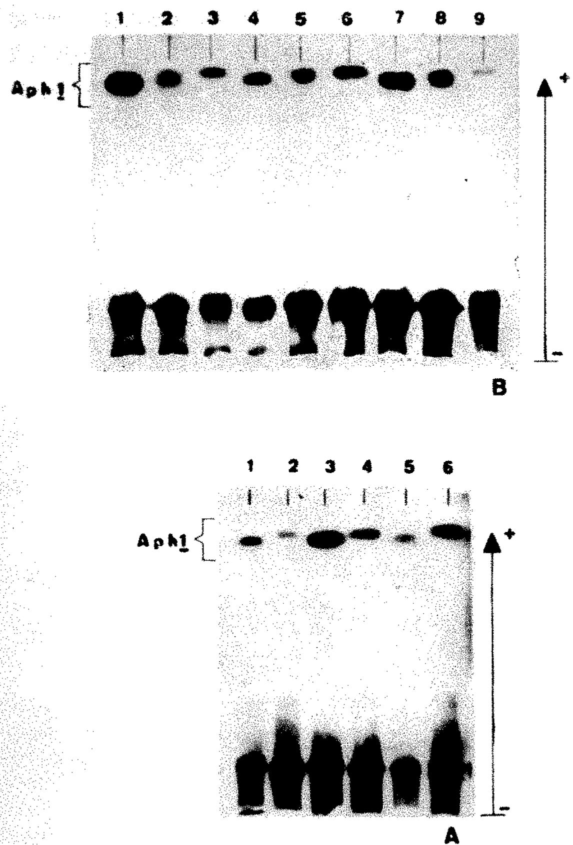

Fig. 1. Zymograms of single larva homogenates illustrating the electrophoretic relative mobility of Aph1. *A*, 1, 3, 5, *atroparvus*; 2, 4, 6, *labranchiae*. *B*, 1, 4, 7, *atroparvus*; 3, 6, 9, *labranchiae*; 2, hybrid (*atroparvus* male × *labranchiae* female); 8 hybrid (*labranchiae* male × *atroparvus* female); 5, hybrid band obtained *in vitro*. Methods for starch gel electrophoresis and staining for phosphatase were described earlier[2].

principally a hybrid enzyme molecular form. In other words, the fact that the hybrid larval pattern has appeared as a thicker band of intermediate mobility could be considered significant in suggesting that the hypothesis stating that no molecular hybridization takes place between *A. atroparvus* and *A. labranchiae* Aph1 subunits is invalid.

On the other hand, the following genetic data fit this consideration. (*a*) Individuals at the fourth larval stage, obtained from the backcross *A. atroparvus* males × hybrid females (introgressive hybridization by pair matings), have given the typical band of *A. atroparvus* and the typical band of the hybrids, according to the Mendelian ratio 1 : 1. (*b*) Individuals at the fourth larval stage, obtained from the backcross *A. labranchiae* males × hybrid females (introgressive hybridization by forced copulation), have also given alternatively the band typical of *A. labranchiae* and the band typical of the hybrids, according to the Mendelian ratio 1 : 1 (Table 1).

These results suggest that both the larval alkaline phosphatases of *A. atroparvus* and *A. labranchiae* are controlled by autosomal genes, specifying the primary molecular structure of the polypeptide subunits which form the catalytically active proteins. On the basis of these data the hypothesis that the enzymes Aph1 of the two sibling species are homologous molecules with two associating subunits, which are identical at the intraspecific level and non-identical, but homologous at the interspecific level, seems to be consistent. In other words, the sibling species *A. atroparvus* and *A. labranchiae* should possess electrophoretically pure enzymes, not identical but homologous, each possessing two identical subunits, while their hybrid offspring should possess hybrid enzymes with two different subunits.

Table 1

	Total No. of examined individuals	*Slow Aph*1 deduced genotype $Aph1^s/Aph1^s$	*Intermediate Aph*1 deduced genotype $Aph1^s/Aph1^f$	*Fast Aph*1 deduced genotype $Aph1^f/Aph1^f$
A. atroparvus	250	250	0	0
A. labranchiae	250	0	0	250
Hybrids (*at.* ♂ × *lab.* ♀)	50	0	50	0
Hybrids (*lab.* ♂ × *at.* ♀)	50	0	50	0
Backcross (*at.* ♂ × hybrid ♀)	60	28	32	0
Backcross (*lab.* ♂ × hybrid ♀)	81	0	37	44

I would like to point out, however, that this biochemical interpretation can be considered reasonably correct, but not sufficiently proved.

On the contrary, it is well established that the differences in electrophoretic pattern of the two species are phenotypic expressions of the activity of a single locus. Although these genetic data do not contradict the hypothesis that the two genes coding for *Aph*1 do not occupy the same position in the genotype of the two species, it seems more correct and more feasible to assume that the genes in question are homologous. In this hypothesis, the structural genes carrying the genetic information for *Aph*1 in *A. atroparvus* and *A. labranchiae* are viewed as alleles. One allele, which according to Beckman and Johnson[5] can be written $Aph1^f$, controls the synthesis of the fast moving enzyme of *A. labranchiae*, while the other allele ($Aph1^s$) specifies the structure of the slow moving enzyme of *A. atroparvus*.

It has been impossible to find electrophoretic variants either in *A. atroparvus* or in *A. labranchiae*, and the offspring obtained by backcrossing the hybrid females to the males of the parental species have segregated according to the ratio 1 : 1, and so I think that the *Aph*1 loci of both species can be considered to be homozygous and therefore monomorphic loci. Thus the *Aph*1 locus of *A. atroparvus* may be written $Aph1^s/Aph1^s$, that of *A. labranchiae* $Aph1^f/Aph1^f$, and finally that of the hybrids $Aph1^s/Aph1^f$ (Table 1).

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[1] Lorković, Z., in *Animal Species and Evolution* (edit. by Mayr, E.) (Harvard University Press, Cambridge, Massachusetts, 1963).

[2] Bianchi, U., *Atti Ass. Genet. It.*, **11**, 276 (1966).

[3] Shaw, C. R., *Science*, **149**, 936 (1965).

[4] Coates, M., *Evolution*, **21**, 130 (1967).

[5] Beckman, L., and Johnson, F. M., *Genetics*, **49**, 829 (1964).

Introduction of Yellow Rust Resistance of *Aegilops comosa* into Wheat by Genetically Induced Homoeologous Recombination

YELLOW rust (*Puccinia striiformis*) is a hazard to the cultivation of wheat (*Triticum aestivum* $2n = 6x = 42$) in many temperate regions. The most economic method of limiting losses of yield caused by the disease lies in the cultivation of varieties with genetically determined resistance to infection. Most current varieties owe their resistance to the activities of a few major genes which they carry in various combinations. New sources of resistance, however, must be sought continually because mutation or somatic recombination in the pathogen can give rise to genetic variants against which particular resistance genes or combination of genes, in current use, cease to be effective.

In the search for new forms of disease resistance for use in wheat improvement, attention has often been turned to related species, some of which display outstanding levels of resistance. For example, the wild annual grass

Aegilops comosa ($2n = 14$), which is distributed around the Aegean Sea in Greece, Turkey and Crete, has especially good resistance to yellow rust[1]. Like all species in the genus *Aegilops*, *Ae. comosa* is closely related to wheat in an evolutionary sense and *T. aestivum–Ae. comosa* hybrids can be produced by hand pollination and hybrid–embryo culture. In our work we have incorporated the yellow rust resistance of *Ae. comosa* in wheat by interference with the genetic regulation of meiotic recombination. Such procedures have never previously been used in crop improvement.

Riley, Chapman and Macer[2] initiated a backcrossing programme from 28-chromosome hybrids derived from the cross *T. aestivum* 'Chinese Spring' × *Ae. comosa*, using 'Chinese Spring' as the recurrent parent. After the second backcross, selection was practised for resistance to yellow rust. The result of this programme was the isolation of a 44-chromosome addition line that had the forty-two chromosomes of 'Chinese Spring' and the pair of chromosomes of *Ae. comosa* determining rust resistance. It was further shown that this pair of *Aegilops* chromosomes would substitute for chromosomes $2A$, $2B$ and $2D$ of the wheat complement, but for no other chromosome. The *Aegilops* chromosome, determining rust resistance, is therefore closely related genetically to the chromosomes of wheat homoeologous group 2. Indeed, it can be regarded as homoeologous with these chromosomes, and designated $2M$ because *Ae. comosa* carries the *M* genome.

In normal circumstances, however, chromosome $2M$ does not pair and recombine with any wheat chromosome[2]. Consequently, the single gene on chromosome $2M$, which is presumed to be responsible for rust resistance, cannot be incorporated in a wheat chromosome without further manipulation. Nevertheless, it seemed likely that chromosome $2M$ would recombine, at meiosis, with its wheat homoeologues if the genetic suppression of homoeologous recombination normally exercised by chromosome $5B$ were removed[3,4].

To achieve this condition, use was made of another *Aegilops* species, *Ae. speltoides* ($2n = 14$), which has the property of inhibiting the $5B$ activity in wheat hybrids[5–7]. Thus in *T. aestivum* × *Ae. speltoides* hybrids there is homoeologous chromosome pairing and recombination. In the present work, therefore, the line with chromosome $2M$ added to the normal complement of 'Chinese Spring' was crossed with *Ae. speltoides*. The resulting 29-chromosome hybrids had twenty-one chromosomes of *T. aestivum*, seven chromosomes of *Ae. speltoides* and chromosome $2M$ of *Ae. comosa*. Because of the inhibition of the $5B$ activity, recombination could take place at meiosis in these hybrids between chromosome $2M$ and its homoeologues in the wheat complement.

A backcrossing programme was initiated from these 29-chromosome hybrids using *T. aestivum* 'Chinese Spring' as the recurrent parent. After the first backcross, selection was practised for rust resistance. In the third backcross generation a 42-chromosome, rust-resistant plant was isolated that formed twenty-one bivalents at meiosis. This plant was heterozygous for a dominant rust resistance condition and homozygotes were isolated in its progeny.

A line homozygous for rust resistance was thus established which, when crossed with standard wheat varieties, gives hybrids that form twenty-one bivalents at meiosis. In the F_2 generation, derived from hybrids between 'Chinese Spring' and this rust resistant line, segregation fits the ratio three resistant : one susceptible, so that the resistance introduced from *Ae. comosa* is inherited as though determined by the dominant allele at a single locus; designated Yr_8. The resistant line has been called 'Compair' to indicate that it has a pair of genes for yellow rust resistance derived from *Ae. comosa*.

The study of meiotic pairing configurations in cytologically marked hybrids, and of the disturbance of segregation ratios in the F_2 generations derived from monosomic hybrids, shows that, in the origin of 'Compair', homoeologous recombination took place in the long arm of chromosome $2M$ and the right arm of chromosome $2D$. The recombined chromosome, that is homozygous in 'Compair', has a distal segment of the right arm of $2D$ and a proximal segment of the long arm of $2M$; while the centromere and the entire short arm are derived from $2M$. This is the first example of the introduction of useful alien genetic variation into wheat by genetic interference with the regulation of recombination in the manner originally proposed by Riley and Chapman[3].

The *Ae. comosa* form of yellow rust resistance, carried by 'Compair', is effective against races $2B$, 3/55 (Opal-attacking), $8B$, 54 and 60; giving 00-0 reactions in every case. Indeed, this form of resistance is effective against all variants of the pathogen to which it has so far been exposed.

'Chinese Spring' was the recurrent wheat parent in the breeding programme because its use simplifies cytogenetic procedures and allows a rapid turnover of generations. Of course, the incorporation of the alien yellow rust resistance in a genotype which is close to that of 'Chinese Spring' means that further work must be carried out to establish it in an agriculturally acceptable genotype. This presents no further cytogenetic problems, however, for the recombined $2M$–$2D$ chromosome pairs at meiosis in intervarietal heterozygotes with chromosome $2D$ and segregates normally, apparently with unimpaired transmission through both pollen and eggs. Consequently, 'Compair' can be treated as a normal wheat variety in planning breeding programmes and breeders need have no particular concern for chromosome behaviour in the derivatives of hybrids of which it was a parent.

In phenotype 'Compair' is close to 'Chinese Spring' but with longer spikelets, florets, glumes and grains. These are attributes that may result from the activities of *Ae. comosa* genes linked to Yr_8—the rust resistance gene. In 'Compair' the development of awns is very variable because on the same plant some spikes may have short scurs while others are fully awned.

Seeds of 'Compair' will be supplied to all breeders and pathologists who would like to use them.

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[1] Riley, R., and Bell, G. D. H., *Proc. First Intern. Wheat Genet. Symp.*, 161 (1959).

[2] Riley, R., Chapman, V., and Macer, R. C. F., *Canad. J. Genet. Cytol.*, **8**, 616 (1966).

[3] Riley, R., and Chapman, V., *Nature*, **182**, 713 (1958).

[4] Riley, R., *Heredity*, **15**, 407 (1960).

[5] Riley, R., and Chapman, V., *Nature*, **203**, 156 (1964).

[6] Riley, R., and Chapman, V., in *Chromosome Manipulations and Plant Genetics* (edit. by Riley, R., and Lewis, K. R.), 46 (Oliver and Boyd, Edinburgh, 1966).

[7] Johnson, R., and Kimber, G., *Genet. Res. Camb.*, **10**, 63 (1967).

Determining Sizes and Distribution of Sizes of Spherical Bodies such as Chromaffin Granules in Tissue Sections

QUANTITATIVE microscopy is difficult at the ultrastructural level because many organelles and inclusions have irregular shapes and the images represent chance sections through bodies of unknown orientation. Size determination for chromaffin granules is easier than for many other inclusions or organelles because their profiles are usually rounded in section and in isolated granule preparations (Fig. 1) implying a spherical form and so orientation presents no problem.

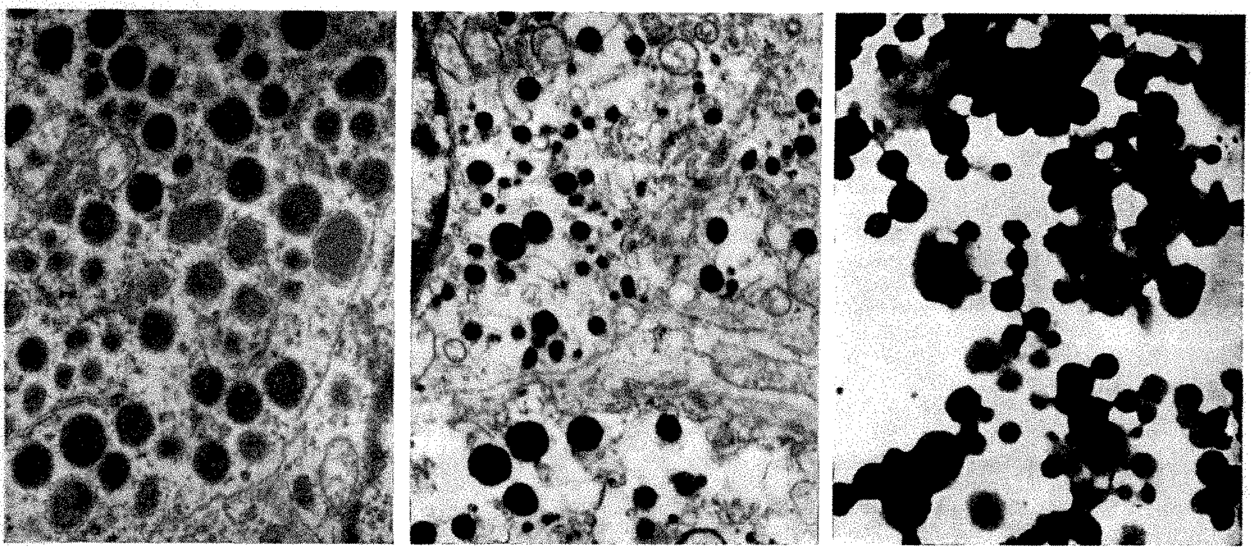
A *B* *C*

Fig. 1. Electron micrographs of bovine *A*-cell granules (*A*), *N*-cell granules (*B*) and isolated granules (*C*). Fixed initially in buffered glutaraldehyde (× 12,000).

After fixation with the glutaraldehyde/osmium tetroxide sequence and staining[1] the membrane-bound adrenaline-storage (*A*) and noradrenaline-storage (*N*) granules can be identified by their moderate or high electron-density.

Uncorrected measurements of granule profiles are not a true picture of the distribution of sizes of chromaffin granules within the tissues, for they ignore factors such as planes of sections, relative size of granules and section thickness. I have now applied methods which correct for these factors to test systems and electron micrographs. The correction is carried out in two stages.

(1) *The conversion of profile numbers to granule numbers.* This correction makes use of the fact that when size-distribution curves are plotted for cellular inclusions these are grouped into a series of size ranges.

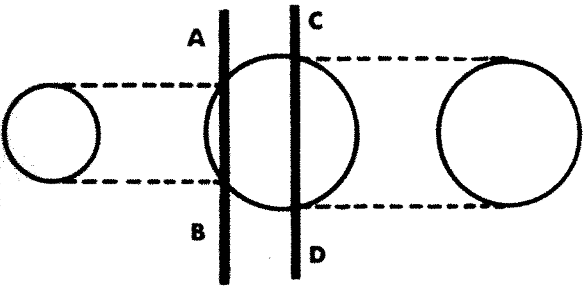

Fig. 2. Variations in profile diameter with plane of section (*A–B* and *C–D*) through a spherical body (centre).

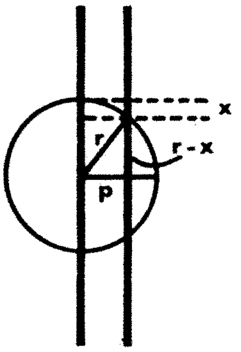

Fig. 3. Circle represents a spherical body and the two vertical lines planes of sections. The proportion (y) of the body falling within profile diameters $2r$ to $2r-2x$ may be calculated for different values of r and x. $y = p/r \times 100$ per cent.

Most single membrane-bound chromaffin granules are spherical, and so sections through them appear as circular profiles, with diameter increasing towards the centre of the granule (Fig. 2). Profiles can be grouped into size ranges 0 to x, x to $2x$, $2x$ to $3x$ units, etc.

With a granule $2r$ units in diameter (r = radius) profile diameters between $2r$ and $2r-x$ can conveniently be counted. Next smallest groups are, in order, $2r-x$ to $2r-2x$, etc. The interval x can be selected for a particular problem and magnification. For a plane of section giving a profile diameter of less than $2r$ the proportion of the granule available (y) for section within a particular range may be calculated (Fig. 3),

$$\text{that is, } y = \frac{p}{r} \times 100 \text{ per cent}$$

while in this case $p = r\cos\theta$ or $p = \sqrt{(2xr - x^2)}$.

If sufficient profiles can be measured, y per cent of all cuts will fall within the range $2r$ to $2r-x$ and $100-y$ per cent will fall within the lower ranges. Thus the number n of granules which fall within the range $2r$ to $2r-x$ corresponds to y per cent of all granules sectioned and the total number of granules present

$$n_t = n \times \frac{100}{y}$$

Table 1. PROPORTIONS (EXPRESSED AS PERCENTAGES) OF SPHERES OF VARIOUS SIZES FALLING WITHIN DIFFERENT PROFILE DIAMETERS

r	r-0·5	r-1	r-1·5	r-2	r-2·5	r-3	r-3·5	r-4	r-4·5	r-5	r-5·5	r-6	r-7	r-8
1	86	100	—	—	—	—	—	—	—	—	—	—	—	—
1·5	76	93	100	—	—	—	—	—	—	—	—	—	—	—
2	66	86	97	100	—	—	—	—	—	—	—	—	—	—
2·5	61	80	92	98	100	—	—	—	—	—	—	—	—	—
3	55	74	86	94	98	100	—	—	—	—	—	—	—	—
3·5	52	70	82	91	95	99	100	—	—	—	—	—	—	—
4	48	66	78	87	92	97	99	100	—	—	—	—	—	—
4·5	46	63	75	84	89	94	97	99	100	—	—	—	—	—
5	43	60	71	81	86	91	95	98	99	100	—	—	—	—
5·5	41	58	69	78	84	89	93	96	98	99	100	—	—	—
6	39	55	66	75	81	86	91	94	97	99	99	100	—	—
6·5	38	53	64	73	79	84	88	92	95	98	99	99	—	—
7	37	51	62	70	76	82	87	90	93	96	98	99	100	—
7·5	36	50	60	68	74	80	85	89	92	95	97	98	99	—
8	35	48	58	66	72	78	83	87	90	93	95	97	99	100
8·5	34	47	57	65	71	76	81	85	89	92	94	96	98	99
9	33	46	55	63	69	74	79	83	87	90	92	94	97	99
9·5	32	45	54	62	68	73	78	82	86	89	91	93	96	98
10	31	44	53	60	66	71	76	80	84	87	90	92	95	98
10·5	30	43	52	59	65	70	75	79	83	86	89	91	94	97
11	29	42	50	57	63	68	73	77	81	84	87	89	93	96
11·5	28	41	49	56	62	67	72	76	80	83	86	88	92	95
12	27	40	48	55	61	66	71	74	78	81	84	86	91	94

r = 0·5 maximum profile diameter, r-0·5, etc. = 0·5 lesser profile diameter (expressed in units). Percentages calculated to nearest whole numbers.

Table 2. MEASUREMENT OF AREA (CORRESPONDING TO GRANULE PROFILES) OF FOUR SIZES OF CIRCLES IN MODEL SYSTEM

Counts	Size range (mm) 0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13
Initial (arcs)	125	46	170	50	78	185	23	26	30	42	50	77	200
No. of circles	106	3	184	4	0	300	−2	0	−2	−12	−8	−2	528
Relative numbers of circles (corrected for size)	1,380		800			650							528
Relative numbers of circles adjusted to 13 mm = 100	262		152			123							100
True relative proportions	265		159			112							100

If granules are initially in a series of specific sizes equal to the maximum for each range (this becomes more correct as size range is reduced), and if the largest granules are considered first, the previous statements can be applied to a mixed population of granules. Given an adequate granule profile count the number (n_1) of granule profiles of the largest size range ($2r_1$ to $2r_1-x$) represents y_1 per cent of all granules (size $2r_1$) present in the section(s) and the total number (n_{1t}) of these is

$$n_{1t} = \frac{n_1 \times 100}{y_1}$$

n_{1t} exceeds n_1 and the difference $n_{1t}-n_1$ represents granules of this size which have been sectioned further from the centre; these will have been counted within the ranges $2r_1-x$ to $2r_1-2x$, $2r_1-2x$ to $2r_1-3x$, etc. The number in each of these ranges can be calculated, that is y_1 per cent of granules, within the range $2r_1$ to $2r_1-x$, y_2 per cent of granules within the range $2r_1$ to r_1-2x, y_3 per cent within $2r_1$ to $2r-3x$. Hence y_2-y_1 per cent of granules must lie within the range $2r_1-x$ to $2r_1-2x$ and y_3-y_2 per cent of granules lie within the range $2r_1-2x$ to $2r_1-3x$ and so on.

Once all granules of size $2r_1$ to $2r_1-x$ have been accounted for and the number present in each other size range deducted from the initial counts, that is the number n_2 in the second largest column becomes

$$n_2 - \left(n_{1t} \times \frac{y_2-y_1}{100}\right)$$

the same method can be applied to the next largest group until all have been corrected.

This correction ideally requires an infinitely thin section, but permissible thickness varies with size ranges selected, size of granules and minimum thickness of granule (considered to be 100 Å) which allows identification. In this work, using the parameters indicated later for electron micrographs, errors arising from thickness are significant only in the four smaller size ranges and no extra correction has been introduced for this factor or for the tangential slope of the margin of sections through the edge of granules. Table 1 shows variations in y with r and x; this can be extended to cover other profile diameters and ranges.

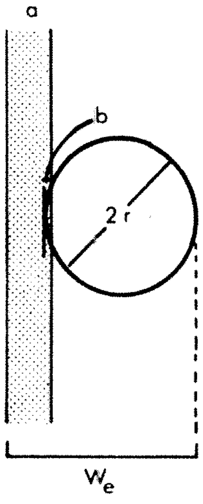

Fig. 4. Circle represents a spherical body. The effective thickness of the body in tissue, $W_e = 2r + a - b$, where a is the thickness of the section and b is the depth of the body which must be sectioned in order to allow recognition in optical system.

(2) *The correction for granule size and section thickness.* In a block of tissue containing randomly distributed granules of different sizes, the likelihood of an infinitely thin section passing through a particular size of granule will depend on the relative sizes of the granules and the number of granules of each size. The latter has been dealt with in correction 1. In considering the relative sizes of granules, section thickness (a) must also be taken into account because the effective width (W_e) of a particular granule is increased by the section thickness (Fig. 4) and becomes $W_e = 2r + a - b$ where b is the minimum thickness of granule allowing identification. The chance of a section passing through a particular granule is proportional to the effective width of the section, and in any section the total number of granules of each size range should be corrected for this factor, namely n_{1t} requires no correction, n_{2t} becomes

$$n_{2t} \times \frac{W_{e_1}}{We_2}, \; n_{3t} \text{ becomes } n_{3t} \times \frac{W_{e_1}}{W_{e_3}}$$

(n_{1t} = number of largest granules after correction 1, n_{2t} next largest column and so on).

Using a model system containing 864 randomly distributed circles 1, 3, 6 and 13 mm in diameter on which randomly directed and positioned lines are superimposed, arcs may be measured (Table 2) to assess the method for profile-to-granule number conversion (correction 1). Numbers of circles of various sizes intersected by lines or bands (representing different section thicknesses) were also determined (Table 3) to assess correction 2, using the formula $2r + a - b$ where $b = 0$.

Table 3. MODEL SYSTEMS

Scan	Circle diameters (mm) 1	3	6	13
Single line—uncorrected	82	142	220	384
Single line—corrected for size of circles	1,050	615	475	384
Single line—corrected and adjusted to 13 mm = 100	274	160	123	100
8 mm bandscan	101	73	63	86
8 mm bandscan—corrected for size and band width	236	140	95	86
8 mm bandscan—corrected and adjusted to 13 mm = 100	275	162	110	100
16 mm bandscan	186	125	106	120
16 mm bandscan—corrected for size and band width	316	191	140	120
16 mm bandscan—corrected and adjusted to 13 mm = 100	264	158	116	100
True relative proportions	265	159	112	100

Correction for size of circles (representing inclusions) and intersecting line/band width which represents different thicknesses of sections.

The results (Tables 2 and 3) agree well with true proportions. The small negative and positive counts for certain size ranges following correction 1 may result from inaccurate measurement (difficult with tangential sections) or from a population sample which is too small.

This method has been illustrated with electron micrographs. Initial magnifications were × 6–10,000; final print magnification × 24,000 so that 1 mm = 420 Å. Granule profile diameters were measured within the ranges 0–1, 1–2, 2–3 mm, etc. Diameters of the electron-dense contents were measured, for the membranes may be distorted.

Table 4 records profile diameters and initial correction for extra-adrenal (N) granules of a 9 day old rabbit. Granules in column F have a maximum diameter of 6 mm; hence $r_F = 3$. Range of column F is therefore r_F to r_F-

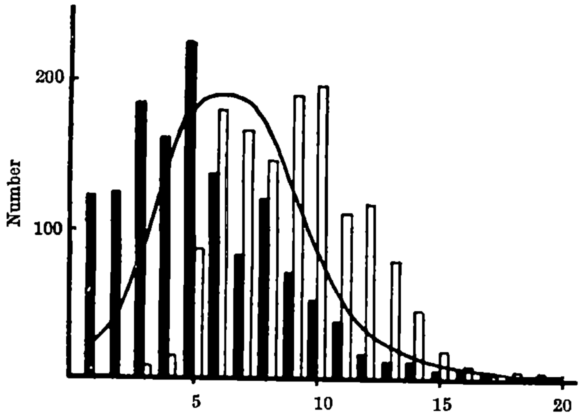

Fig 5 Histogram showing distribution of sizes of *A* and *N* granules of bovine adrenal slices with superimposed curve showing distribution of sizes of granules in an isolated granule preparation ■, *N* granules, □, *A* granules Scale, 1 mm = 420 Å

0 5 From Table 1, 55 per cent of profiles of granules size $r_F = 3$ would fall within this range, so that the total number (n_{Ft}) of granules ($r_F = 3$) in the sample,

$$n_{Ft} = \frac{4 \times 100}{55} = 7 \text{ (to the nearest whole number)}$$

Three of these will be present as smaller profiles in other columns From Table 1, 74 per cent of the profiles of these granules will be found for the range r_F to $r_F - 1$, that is in columns *E* and *F*, thus column *E* contains 19 per cent of these granules, that is

$$\frac{7 \times 19}{100} = 1 \text{ (to the nearest whole number)}$$

For the range r_F to $r_F - 1\ 5$ (columns $D+E+F$) 86 per cent of the granule profiles are found so that the number in

$$D = \frac{7 \times 12}{100} = 1$$

For the range r_F to $r_F - 2$ (columns $C + D + E + F$) 97 per cent of the granule profiles will be found, hence *C* contains

$$\frac{7 \times 11}{100} = 1$$

These counts relating to the 5–6 mm granules are now subtracted from (Table 5) the original counts of columns *C*, *D* and *E*

The same correction is then applied to the next largest group, that is those in column *E* The maximum radius of these profiles is 2 5 mm and column *E* corresponds to the range r_E to $r_E - 0\ 5$ From Table 1, 61 per cent of granules radius = 2 5 mm fall within this range, hence the total present in the section is

$$\frac{31 \times 100}{61} = 51$$

For the range r_E to $r_E - 1$ (columns $E+D$) 80 per cent of the profiles will occur, thus 19 per cent of these will appear in column *D*, that is

$$\frac{51 \times 19}{100} = 10$$

Thus the number of profiles listed under *D* must be further adjusted to 184 − 10 = 174 and so on throughout the series The corrected counts are indicated in Table 4

Table 4 NUMBER OF GRANULE PROFILES AND CHROMAFFIN GRANULES IN TISSUE SECTIONS, AND DISTRIBUTION OF SIZES OF CHROMAFFIN GRANULES IN A 9 DAY OLD RABBIT

Column	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
Size range (mm)	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
Extra-adrenal *N* cells										
No of profiles in section(s)	108	293	373	185	32	4				
No of granules in section(s)	40	220	412	264	51	7				
Size distribution of granules	156	520	742	372	59	7				
Adrenal *A* cells										
No. of profiles in section(s)	0	53	123	177	136	58	46	13	2	0
No of granules in section(s)	< 0	< 0	128	186	182	73	79	23	4	0
Size distribution of granules			335	384	309	106	100	26	4	0

Scale, 1 mm = 420 Å

With highly electron dense *N* granules no negative counts are obtained (Tables 4 and 6) With the less electron dense *A* granules of adrenal medulla, however, some negative figures are obtained for lower size ranges Population samples of similar sizes (> 800) were used for both *A*- and *N*-granule counts, and so this phenomenon is unlikely to be a result of sampling It may, however, result from the difficulty and near impossibility of satisfactorily identifying tangential sections through the less dense (*A*) granules or from a virtual absence of small *A* granules possibly because of a different method of synthesis or rate of turnover of *N* granules With *N* granules, correction 1 serves as a check on the accuracy of measurement and adequacy of population sample

Table 5 PARTIALLY CORRECTED PROFILE AND GRANULE COUNTS OF EXTRA-ADRENAL CHROMAFFIN CELLS OF 9 DAY OLD RABBIT

Column	*A*	*B*	*C*	*D*	*E*	*F*
Size range (mm)	0–1	1–2	2–3	3–4	4–5	5–6
No of profiles in section(s)	108	293	373–1 = 372	185–1 = 184	32–1 = 31	3
No of granules in sections						7

Scale, 1 mm = 420 Å

After the profile-to-granule-number correction, correction 2 is applied Effective thickness = $2r + a - b$, $a = 400$ Å, $b = 100$ Å, hence

$$\text{for column } E = 51 \times \frac{2r_F + 300}{2r_E + 300} = 51 \times \frac{2{,}520 + 300}{2{,}100 + 300}$$

$$\text{for}' \text{column } D = 264 \times \frac{2r_F + 300}{2r_D + 300} = 264 \times \frac{2{,}520 + 300}{1{,}680 + 300}$$

Table 6 NUMBER AND DISTRIBUTION OF SIZES OF BOVINE ADRENAL CHROMAFFIN GRANULES IN TISSUE SECTIONS AND IN ISOLATED GRANULE PREPARATIONS

Range (mm)	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20
Ox adrenal *N* granules																				
No of profiles in section(s)	50	88	135	139	162	115	93	98	65	50	34	18	13	10	6	6	5	2	1	
No of granules in section(s)	27	43	89	101	168	120	84	137	89	74	56	26	18	19	8	11	12	5	3	
Distribution of sizes of granules	310	312	472	422	580	350	214	310	180	136	94	40	26	25	10	13	13	5	3	
Ox adrenal *A* granules																				
No of profiles in section(s)	0	13	34	54	81	106	109	105	115	104	68	62	40	23	9	5	2	2	1	
No of granules in section(s)	< 0	< 0	2	5	35	86	89	90	132	151	93	107	76	51	18	12	3	5	3	
Distribution of sizes of granules			11	21	120	252	228	202	266	276	156	165	109	64	23	14	3	5	3	
Isolated chromaffin granules																				
Distribution of sizes	21	49	71	107	193	189	186	167	126	112	39	41	23	15	15	8	7	6	4	4

Scale, 1 mm = 420 Å

There is a general shift to the right of the counts after correction 1 because many small profiles are sections through peripheral zones of large granules After correction 2 there is a marked increase in the relative numbers of the smaller granules

Only sections showing a mixed population randomly scattered were included in this analysis, avoiding regions such as the Golgi zone, where a variation in size of granules is common[2]

Table 6 shows the results of applying the same corrections to bovine *A* and *N* cells and also indicates that the size distribution of isolated granules is similar to those in sections of adrenal medulla allowing for the relative proportions of the two amines in the bovine adrenal medulla (*A* . *N* = 75 · 25) (ref 3) The histogram (Fig 5) clearly illustrates that bovine *A* granules have a larger mean diameter than *N* granules

These methods are applicable at both the ultra-structural level and in light microscopy if an appropriate section thickness relative to object size and size ranges are selected, that is using the light microscope for nuclear size 0 5 – 1μ sections would be used

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[1] Coupland, R E , Pyper, A S , and Hopwood, D , *Nature*, **201**, 1240 (1964)
[2] Coupland, R E , *J Anat* , **99**, 231 (1965)
[3] Euler, U S von, *Noradrenaline* (Thomas, Springfield, Ill , 1955)

GENERAL

Neutron Activation Analysis of Medieval Ceramics

An increasing awareness of the number of British medieval pottery kilns working at any one time has thrown into sharp perspective the problem of establishing local ceramic sequences by programmes of kiln excavation Traditional methods and residual magnetic dating techniques often enable reasonably precise dates to be given to kilns and thus their products It remains, then, to establish a technique whereby material excavated from sites other than kilns can be identified back to its source of production, and thus to make use of scientifically established dates Recently neutron activation analysis has been used with some success to distinguish between specimens of ancient pottery[1–5] and glass[6]. Differences in concentrations of trace elements in samples have been noted A programme of work has therefore been initiated, using lithium-drifted germanium detectors, to examine the trace element constituents of pots from some eighty British kiln sites

Preliminary experiments have been carried out on shards from Olney Hyde, Buckinghamshire, and Holme-on-Spalding-Moor, Yorkshire Fragments of approximately 50 mg of materials were irradiated in a thermal neutron flux of 10^{12} n cm^{-2} sec^{-1} in the BEPO reactor, AERE, Harwell, for 100 h Gamma ray spectra were then recorded at intervals during 6 weeks using a lithium–germanium gamma ray detector of sensitive volume approximately 7 c c , which was coupled through appropriate amplifiers to a 1024 channel pulse-height analyser A typical spectrum is shown in Fig 1 Peak intensities were ascertained from the digital output of the analyser and corrected for background by averaging counts in channels on either side of individual peaks The following active isotopes were identified in each sample sodium-24, scandium-46, iron-59, cobalt-60, caesium-134, lanthanum-140, cerium-141, europium-152, samarium-153, ytterbium-169, thulium(?)-170, ytterbium-175, lutetium-177, hafnium-181 Not all of these, however, were amenable to quantitative analysis In this preliminary experiment, all activities in a given sample were compared with that of the scandium 890 keV peak, when extrapolated to a fixed time after irradiation Ratios thus obtained give an arbitrary indication of the trace concentrations, relative to scandium, in a particular specimen These ratios for nine isotopes are given in Table 1 Very significant differences are apparent in the cases of lanthanum-140, europium-152 and samarium-153, the large differences in sodium-24 may be fortuitous in that no prominent photopeak of sodium was examined

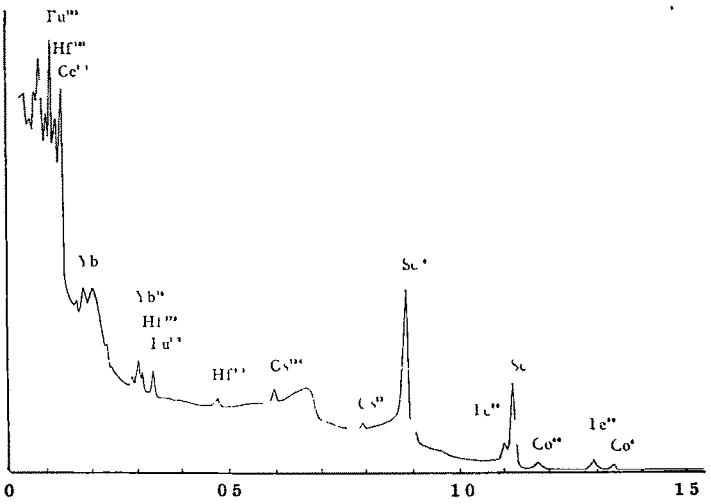

Fig 1 A gamma ray spectrum for a shard from Holme on-Spalding-Moor, Yorkshire, 100 h after irradiation

This investigation of shards from two kilns allows us to assume with fair certainty that fragments from one of the pots may be differentiated from those of the other, and a full programme of examination of kiln product groups has now commenced

Table 1 RATIO OF RATE X TO RATE SCANDIUM-46 AT 1600 h ON MARCH 14, 1967

Isotope X	^{46}Sc	^{24}Na	^{59}Fe	^{60}Co	^{134}Cs	^{140}La	^{141}Ce	^{152}Eu	^{153}Sm	^{181}Hf
Specimen 1	1	0 7	0 112	0 038	0 12	5 0	0 85	0 32	12 1	0 066
Standard deviation		±0 1	±0 003	±0 003	±0 02	±0 2	±0 04	±0 02	±0 3	±0 006
Specimen 2	1	1 4	0 099	0 046	0 09	2 0	0 69	0 22	4 8	0 067
Standard deviation		±0 3	±0 003	±0 003	±0 01	±0 1	±0 05	±0 02	±0 2	±0 008

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[1] Sayre, E V , and Dodson, R W , *Amer J Archeol* , **61**, 35 (1957)
[2] Sayre, E V , Murrenhoff, A , and Weick, C F , *Brookhaven Nat Lab Rep* , *BNL*508(*T*–122) (1958)
[3] Emeleus, V M , *Archaeometry*, **1**, 6 (1958)
[4] Emeleus, V M , *Archaeometry*, **3**, 16 (1960)
[5] Emeleus, V M , and Simpson, G , *Nature*, **185**, 196 (1960)
[6] Sayre, E V , *Proc Seventh Intern Conf Glass, Brussels* (1966)

BOOK REVIEWS

ASTRONOMY IN A NUTSHELL

Astronomy Old and New

By Ernest Agar Beet Pp x+189+16 plates (London G Bell and Sons, Ltd , 1967) 25*s* net

ASTRONOMY is probably the only major science in which the number of books for the tiro, the dedicated amateur and the layman with a passing interest exceeds the number written for the professional The general standard is correspondingly good, and the difficulty of saying anything new is correspondingly great Mr Beet's new book, continuing a personal tradition begun by his *The Sky and its Mysteries* (1952), is nothing if not up to date This applies to the plates, which include among several of the old favourites some of the Ranger 9 pictures

The book is not only new in the sense of cramming in last-minute information It is unusual in the attention given to the astronomer's equipment, which must now be taken to include satellites and space probes It is unusual in its good bibliographical supplements to individual chapters All told, it will be found useful for sixth form teaching, and for supplementing courses in astronomy in such institutions as our colleges of technology It is essentially a book requiring its own supplementation, however It is short, and yet the new has not so much squeezed out the old as compressed it, to the point of mild obscurity in places Thus the second chapter, nineteen pages long, is a rapid conspectus of four thousand years of astronomical history The relevance to the book of this chapter would have been suspect even had it not contained many small errors of fact (The most misleading is perhaps that relating to the period of Mars in its epicycle, on page 15 wrongly stated to be equal to the period in the deferent Babylonian and Egyptian astronomy are, moreover, sadly confused) The accuracy of the work is not so much in question as the need for such excessive brevity The last seven pages, for example, covering modern cosmology, are like a mirage to a man lost in a desert of understanding His temperament is what will determine whether he should give up in despair, or turn to more substantial works on the same subject On the whole, the second reaction seems the more probable, and this is ample justification for a work of this kind

J D **NORTH**

CHANCE CHANGES LITTLE

The Doctrine of Chances

Or A Method of Calculating the Probabilities of Events in Play By A de Moivre Pp xxvi+257 (London Frank Cass and Co , Ltd , 1967 First published 1718) 105*s*

IT is perhaps not usual for this journal to review a reprint of the second edition of a work which has already proceeded to its third edition, but none of the three editions were reviewed here when they came out, for unavoidable reasons, and so some notice seems deserved De Moivre's famous work was first published in 1718 This is an exact reproduction of the second (1738) edition, with an index and table of contents added The third edition appeared in 1756, I do not know to what extent it differed from the second

Two hundred years have dated the book very little, and it makes most refreshing reading For historians of science its re-issue must be a great blessing, but statisticians even without an historical sense could not fail to find it of great interest This is because, whether de Moivre is writing about the "meaning" of a "chance", or about the interpretation of data, he is concerned with old questions still full of perplexity and still liable to arouse controversy The applications discussed in the book are, as might be expected, to gambling and to actuarial science Those who wish to compare this book with a review of the present state of those two applications may wish to refer to L E Dubins and L J Savage, *How to Gamble if You Must* (McGraw-Hill, 1965), and to K Borch's article "The Theory of Risk" and the accompanying discussion in the *Journal of the Royal Statistical Society*, Series B (29, 432, 1967) For modern developments of de Moivre's limit theorem a good reference would be the second edition of W Feller's *Introduction to Probability* (Volume 1, Wiley, 1957), and for the latest developments of the combinatorial approach to which de Moivre made great contributions a useful reference is L Takács, *Combinatorial Methods in the Theory of Stochastic Processes* (Wiley, 1967)

The general editor (Dr L L Laudan) and the publishers of the Cass Library of Science Classics are very much to be congratulated on making this important work once more generally available D G **KENDALL**

METAL STRUCTURE

Structure of Metals

Crystallographic Methods, Principles, and Data Third edition By Charles S Barrett and T B Massalski (McGraw-Hill Series in Materials Science and Engineering) Pp xvii+654 (London and New York McGraw-Hill Publishing Company, 1966) 126*s* , $15 75

THIS book presents a new edition of the original classic text first published by C S Barrett in 1943 and revised in 1952 Now under dual authorship the contents remain, nevertheless, in general, much as before without any noticeable change in philosophy or presentation

The first four chapters discuss the fundamentals of crystal lattices, crystal nomenclature and standard projections, together with the basic principles of the reciprocal lattice and diffraction of X-rays by crystals Then follows, in Chapters 5 to 9, a descriptive treatment of the various experimental X-ray methods, that is, Laue, rotating crystal, powder method, crystal structure determination and texture methods The other common experimental methods of studying the structure of metals and alloys are treated later in the book with X-ray and electron microscopy in the fifteenth chapter, stress measurement by X-rays in Chapter 17, ending with electron and neutron diffraction in Chapters 22 and 23 Between the two treatments of methods, the authors present the crystal, electronic and defect structure of metals and alloys including superlattices together with the relevant modern theories (Chapters 10 to 14) The remainder of the book is taken up in discussing some selected research results where X-ray methods seem to have been of value, such as Fourier analysis of particle size, strain or stacking fault broadening in Chapter 16, solid state phase transformations in Chapter 18 and preferred orientations after various treatments in Chapters 19, 20 and 21

The material and coverage of topics in this text remain as broad as in the previous editions with much revision, expansion and modernization This has been possible only at the expense of the more elementary sections, which the authors justify on the grounds that such elementary material is adequately covered in other books and is already familiar to most readers The book therefore presents a strange mixture of the elementary, intermediate and advanced, and as a consequence is unsuitable in treatment for all but a few undergraduate and graduate students There is too much emphasis on research for undergraduates to buy the book, which is too broad to be of real use to the graduate student and researcher Even the detailed treatments such as preferred orientation, and microscopy involving X-ray and electron diffraction, are of limited value because the researcher invariably consults the more detailed up to date work of, for example, the A S M book on *Recrystallization, Grain Growth and Textures* and *Electron Microscopy of Thin Crystals*, respectively Thus although such a book as *Structure of Metals* was invaluable up to the early sixties, its usefulness today is much more limited Nevertheless, a tremendous amount of work has been put into the present edition and no doubt the book will still be useful as a quick reference to a wide variety of topics, without being the classic of the earlier years

R E. SMALLMAN

FLAME PHOTOMETRY

Flame Photometry Theory

By E. Pungor Translation edited by R A Chalmers (The Van Nostrand Series in Analytical Chemistry) Pp x+200 (London D Van Nostrand Company, Ltd ; Princeton, N J. D Van Nostrand Company, Inc , 1967) 60*s*

THIS is a very worthwhile book, collecting together a wealth of scattered information on flame processes in the broadest sense of the term Flame photometry is now a widely used analytical tool, the ease and rapidity with which it produces data engender a temptation to relegate the underlying principles to a subsidiary role There are few areas in analytical chemistry where the relationship between theory and practice is as tenuous as it is in emission spectrography. The book is written primarily for analysts and it will certainly be welcomed by them. It serves to underline the fact that the apparently simple and rugged technique of flame photometry is based on complex interrelationships of chemical and thermal processes It can be recommended to a wider readership as an informative text

Considerably more than half of the book is devoted to two important chapters The first is a survey of flame processes, covering the basic parameters for evaluating flame performance together with the experimental techniques involved The various theories of flame propagation are also discussed The second chapter is concerned with radical equilibria in flames and again a substantial amount of experimental information is given The treatment is largely concerned with hydrogen/oxygen flames, but the literature on hydrocarbon flames is adequately covered The remainder of the book is made up of brief chapters on flame temperature and its measurement, important flames used in flame photometry, theory of atomization and spray collection (based largely on the author's own work) and flame emission and atomic absorption spectroscopy It is well produced and reasonably priced.

J. K FOREMAN

AUSTRALIAN LANDFORMS

Landform Studies from Australia and New Guinea

Edited by J. N Jennings and J A Mabbutt Pp xxiii+434 (London Cambridge University Press, 1967) 105*s* ; $19.50

THIS is a very Australian book It is a collection of essays dealing with landforms in Australia and New Guinea The three essays concerned with New Guinea are almost exclusively confined to the highlands The other fourteen essays cover many characteristic aspects of Australian landform study, and although the book is not intended to be a regional geomorphology almost every principal region is represented It is the Australian-ness that gives the main unifying theme to the book because the essays are of very different types and many aspects of landform study are included Therefore, although the reader is dealing with seventeen different authors and their different approaches, the collection is remarkably successful Most of the essays are between about 10,000 and 16,000 words long, one or two are longer, and the shortest is an interesting little essay by B P Ruxton on "Slopewash under Mature Primary Rainforest in Northern Papua".

The studies are very variable, in that some contain results of new research, while others are more like review articles Moreover, the whole range of disciplines concerned with landform study is dealt with Modern statistical and computer techniques have been used by J G Speight in "Spectral Analysis of Meanders of some Australasian Rivers", and by D S Simonett in his discussion of landslide distribution and its relationship to earthquakes in the Bewani and Torricelli mountains in New Guinea More conventional field geomorphology is represented by C. R Twidale's work on the "Hillslopes and Pediments on the Flinders Ranges" and by R W Galloway's discussion of the pre-, sub- and post-basalt surfaces of the Hunter valley in New South Wales Edmund Gill uses the methods of the Quaternary geologist to unravel the problems of the evolution of the coastline between Warrnambool and Port Fairy in western Victoria

To make any really constructive criticism of the evidence and the conclusions presented in these essays, one would need to know Australia extremely well and to have been working most recently in the areas concerned. Although I am familiar with certain parts of Australia, it is only possible to make general comments on them. As would be expected from the remarks already made, the most interesting and perhaps the most successful essays are those dealing essentially with Australian rather than with more general problems These include particularly the studies of the geomorphological history of the Lake Eyre basin by H. Wopfner and C R Twidale, those dealing with landforms and laterites in both north and south-western Australia (by J Hays and by M J Mulcahy), and that discussing soil periodicity in relation to landform development in south-eastern Australia by B Butler It may be that these studies evoke more especially the nostalgia that affects all who have lived in Australia which is referred to by Professor Hills in his foreword

But it does seem to me that, in the essays where there is a considerable amount of European literature (and American) available, the collection is rather less successful It seems to indicate that the study of landforms in Australia has had to rid itself of geomorphological ideas inherited from other continents before it could really progress This is illustrated by the development of ideas on the glaciation and peri-glaciation in Tasmania, which is the subject of a beautifully clear and succinct contribution by J L Davies The survival of outmoded concepts is also indicated at the end of J. Mabbutt's interesting work on the "Denudation Chronology in Central Australia", in the comment on the "usefulness of the concept of the arid cycle" (page 179); this seems to a European as unnecessary, as the concept of the arid cycle left us long ago Survival is also faintly perceptible in my colleague J N Jennings's persistence in discussing the karst areas of Australia in purely morpho-climatic terms, at a stage when there is a great emphasis, in eastern Europe at any rate, on lithological studies in limestone terrains Nevertheless, it is very good to see the work of the Australian speleologists progressing so well and so much work being done in the Australian karst areas

The two final essays are more in the nature of reviews The first attempts to discuss the factors responsible for the formation of the coastal lagoons in south-east Australia, this work suffers from a certain diffuseness, and it may be, as E. C. F. Bird has himself said, that an attempt at this kind of essay is a little premature in the present state of knowledge. The second of these essays, on the coral reefs of the Australian region by R. W. Fairbridge, is in many ways the most brilliant in the book. Professor Fairbridge's knowledge of coral reefs and of coral limestones is unrivalled, and his application of this to the Australian reefs makes interesting and instructive reading.

Because all seventeen essays are by different people they are in varied styles, but this does not impede the reader greatly. But like "American English", "Australian English" is now written by people whose original language was not English, this leads to much writing in the passive rather than in an active sense, and occasionally to somewhat obscurely written passages like those contained in the interesting work by M. J. Bik on the morpho-climatic zonation in the central highlands of New Guinea.

This collection of essays brings together scientists of many different disciplines—geomorphologists, geologists and pedologists all make contributions. In particular much of the work on Australian landforms stems from the resource surveys of the CSIRO Division of Land Research, illustrating how applied geomorphology has helped the work of the more academic side of the subject and also how Australia has applied landform knowledge to practical ends.

The volume shows signs of having been edited hastily, for there is a long list of corrigenda both to the text and to the diagrams. It was probably better, however, to get the work published rather than delay it too long. It is well illustrated by thirty-six pages of half-tones, each essay containing at least one of them. The diagrams are plentiful and on the whole uniformly drawn. It would have been helpful to have had some of the maps on folding sheets, so that they could be referred to more easily when reading the text.

The book marks a distinct stage and a great development in the knowledge of Australian landforms. It not only introduces the results of new work, but the many references will make the problems of Australian landforms much more familiar to all students of the subject.

M. M. SWEETING

PROTEIN MODELS

Poly-α-Amino Acids

Protein Models for Conformational Studies. Edited by Gerald D. Fasman. (Biological Macromolecules: a Series of Monographs.) Pp. xv+764. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1967.) 275s.

OCCASIONALLY on examining edited books of review or general research articles by, as this dust-cover proclaims, leading authorities, I find that either the authorities are far from leading their fields or they are almost unknown. It is probably the first duty of an editor of such a volume to ensure that the writers are known and respected in their various fields of activity, and in assembling the contributors to this volume Dr Fasman has successfully discharged this duty.

This is the first edited volume of a series on biological macromolecules, the purpose of which, according to the introduction, is a "periodic presentation of critical evaluations of various aspects of a general field". In this case the general field being evaluated concerns the application of physico-chemical techniques to conformational studies of poly-α-amino-acids. Of the thirteen articles presented, one by Silman and Sela on the "Biological Properties of Poly-α-Amino Acids" falls outside the general scope of the volume but nevertheless gives a full and interesting account of the use of synthetic polypeptides in studies of such topics as the action of proteolytic enzymes, the molecular basis of immunogenicity and antigenic specificity and the interaction of poly-α-amino-acids with biological macromolecular systems such as nucleic acids, proteins, viruses and bacteria. Nine of the other twelve articles are by specialists in the various techniques used, one by Mandelkern is a review of the physical studies of poly-L-proline and the remaining two articles are concerned with the factors involved in the conformations and stabilities of polypeptide chains. Both of the last two articles are very long, about 100 pages, the one by Poland and Sheraga is theoretical and applies statistical mechanics to conformational analysis, discussing in particular the non-covalent factors involved in the determination of polypeptide conformations and also the mechanics of the helix→coil transition. The article by Fasman is an exhaustive review of the considerable body of experimental data, as attested by the 457 references quoted, which now exist on the factors (for example, nature of sidechain, length of main chain and solvent system) which determine the conformation of the polypeptide backbone.

Leading research workers have contributed the articles on the specialist techniques and so it need scarcely be said that they are authoritative accounts of the current situation in the various fields. The topics covered are high angle X-ray diffraction of polypeptides (A. Elliott) and low angle diffraction of the different phases in polypeptide/solvent systems (P. Saludjian and V. Luzzati), the usual spectroscopic techniques of the infrared (T. Miyazawa), ultraviolet (W. B. Gratzer), optical rotatory dispersion (J. T. Yang) and circular dichroism (S. Beychok) and the additional fields of light scattering and hydrodynamics (H. Benoit, L. Freund and G. Spach), hydrogen exchange (S. W. Englander) and the dielectric properties of polypeptides in solution (A. Wada). Although the preface states that the "considerable amount of exciting work" forthcoming since 1961 was responsible for the publication of this volume, not all the fields have made significant advances since then and some of them add little new to what was known in 1962. If any of these articles were to be specially mentioned, one might select that by Gratzer on ultraviolet studies of polypeptides which includes not only the basic theory, application and results but also an extremely useful section on the experimental aspects and difficulties of measurements in the far ultraviolet, and the articles by Miyazawa and Englander, who have endeavoured not to re-review work but to present a more critical account of the application of their techniques.

There is little doubt that this volume will be a very useful source of reference to the work completed on poly-α-amino-acids up to the beginning of 1966. There are two criticisms: one is the usual complaint of the time delay, in this case almost two years, which has elapsed since the completion of the articles and their publication. The second criticism is the extremely high price.

E. M. BRADBURY

BIOLOGICAL ULTRASTRUCTURE

Engstrom–Finean Biological Ultrastructure

Second edition. By J. B. Finean. Pp. viii+384. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 120s.

I THINK it would have been wiser, despite Finean's declared attachment to it, if the book had not attempted in a section of forty-seven pages to give before the ultrastructural details themselves a summary of the methods used in their elucidation. To deal adequately in such a short compass with the principles of the operation of the electron microscope and its application in biology, with high- and low-angle X-ray diffraction both as applied to solids and solutions, besides various optical and spectroscopic methods, is impossible A surprisingly balanced introduction to electron microscopy is achieved, but in the section dealing with X-ray diffraction the author tends to set and fall into traps caused by over-brevity There is not the space to distinguish between unit cell and Fourier transforms, it is misleading in relation to diffraction by helical structures to dismiss more complicated diffraction than that from a single helix by "Double and triple chain helices show simple variations of such (single helix) characteristics" Multiple helix systems (for example, α-helix, collagen) usually take the form of coiled-coils and the relationships of their diffraction patterns to simple ones can be complex. Guinier's relationship is quoted for the determination of the radius of gyration of a particle from the X-ray scattering of solutions, but no indication is given of the angular range of its application In a similar manner, in the chapter dealing with principles of molecular structure, I question the value of two pages on quantum mechanics. It is as if the author has tried for the understanding of his readers in the manifold "tricks of the trade" used in ultrastructure determination, I believe instead he should have given sufficient only for the reader to follow the arguments with detailed references to the specialized texts for the details

The book is successful in presenting biological phenomena as "patterns of physical and chemical events organized in space and time" There are sections on proteins, nucleic acids, lipids, carbohydrates, the role of mineral salts and the role of ultrastructure in biology and medicine. In general the structural interpretations are up to date and embrace references until 1966 Occasionally over-brevity gives a slightly misleading impression, for example, the higher order low-angle meridional X-ray diffractions from all dry collagens do not fan out, and besides the order–disorder explanation of Bear for the fanning there is the fibril-shearing mechanism proposed by Tomlin and Ericson Occasionally additional references would be valuable; for example, in connexion with the movement of a cilium or flagellum the author whets the appetite by remarking that plausible theories have been proposed for the control of the movement and its energy requirement, but gives no lead in following it up. There are a few misprints but none to deceive—I wonder whether the reference (page 105) to "Caltech" was intended, but perhaps today everyone knows what it means.

The section of the book dealing with the role of lipids is really a valuable review of the field to which Finean has himself contributed greatly Isolated lipids, lipid interactions and natural lipoproteins are treated most comprehensively and this section merits the space it has been given—the same as the proteins, amino-acid sequences and complete structure determinations notwithstanding

As a broad introduction to molecular biology and ultrastructure research I believe this book to be valuable both at the undergraduate level and especially to the graduate physicist or chemist "converting" to molecular biology I hope that in future editions the space now occupied by "methods" will be given to further structural detail

R E Burge

GENETICS AND BEHAVIOUR

The Genetic Analysis of Behaviour

By P. A Parsons (Methuen's Monographs on Biological Subjects) Pp. viii+174. (London Methuen and Co, Ltd, 1967) 30*s*.

As the author of this biological monograph points out, behavioural traits, when under some degree of genetic control, have a highly significant role in initiating evolutionary change There is also a compelling urge for knowledge in this field as regards our own species, despite the well recognized difficulties

Much of the book is devoted to an exposition of how a quantitative behavioural trait can be analysed from a genetic point of view This includes a scheme of analysis of variance for separating out genotypic and various environmental and interactional effects in a set of inbred strains for a series of environments, this has apparently not been carried out before There is also reference to recent attempts at "locating the polygenes", and a chapter on the evolutionary consequences of variations in mating behaviour. The behaviour that is discussed in greatest detail is that of *Drosophila*, the organism with which the author is most familiar As he mentions, conclusions about mating preferences, the speed with which mating is attempted and the duration of copulation which are derived from laboratory studies may not apply in wild populations of *Drosophila* Still less can findings about the extent and nature of the genetic control of such traits in *Drosophila* be extrapolated to man Yet the difficulties of research with the fruit-fly and the methods that have been devised to overcome them suggest ideas for the genetic study of rodent behaviour, and in turn the analysis of rodent behaviour can be helpful to the human psychologist While the author's approach to man through work on experimental organisms helps to highlight difficulties such as inadequate control of the environment, he also points out the advantages of the more sophisticated methods of biochemical, physiological and psychological investigation that can be applied in man

When he comes to describe and discuss work on rodents and man, Professor Parsons has to rely more heavily on secondary sources, and he does not claim to be anything but extremely selective in his approach to behaviour in man. In some respects, such as the genetics of normal vision and ideas about the evolution of colour-blindness, knowledge may not be as limited as he suspects And despite the difficulties of making any sort of exact statement, there have been continuous attempts in the past few years to apply some of the principles described in the monograph. One thinks of heritabilities calculated for defined behavioural traits such as intelligence, extraversion, drinking habits and schizophrenia, some of them based on natural "wild" populations, and of renewed efforts at assessing environmental variables by means of discordant twins, twins reared apart and fostered or adopted children. This merely shows the potential fruitfulness of a genetic approach in psychology.

The present monograph with its clear exposition of methods, its awareness of the difficulties and its broadly based "attempt at applying evolutionary principles to behaviour" should serve as a useful introduction to its subject.

James Shields

UNRESPONSIVENESS

Natural and Acquired Immunologic Unresponsiveness

By William O. Weigle (Monographs in Microbiology) Pp. x+180 (Cleveland and New York: The World Publishing Company, 1967) $8.

Immunological unresponsiveness can result from the contact of a potential antigen with the immature lymphoid cells of an animal in the perinatal period The concept that this mechanism allows the development of a natural immunological tolerance to autologous body constituents was generated by the observations of Owen on the chimaerism of dizygotic cattle twins; by the speculations of Burnet and Fenner, and by the experiments of Billingham, Brent and Medawar and of Hasek on the consequences of perinatal contact with transplantation antigens Our understanding of the cellular events underlying the induction of immunological unresponsiveness is still uncertain and the importance of the work of Dixon and his colleague Weigle lies essentially in the contributions they made using single protein antigens. This led to a clearer definition of the experimental circumstances involved in the induction, maintenance and termination of the unresponsive state not only in the neonate but also in adult animals The account of this work given by Weigle in his monograph will not provide easy reading for the general immunologist owing to the excessive wealth of experimental detail Nonetheless, the specialist in this field will find it of value to have the data collected in one volume

The author's important studies on the termination of natural or artificially acquired unresponsiveness by cross-reacting antigens feature prominently, his results indicate that antibodies to the tolerance-inducing antigen evoked in this way react only with determinants similar to those present on the cross-reacting antigen but not with determinants to which tolerance has already been established This conclusion has since been elegantly supported by the findings of Paul, Siskind and Benacerraf (*Immunology*, 13, 147, 1967), who studied the termination of unresponsiveness to human serum albumin (HSA) by injection of dinitrophenyl–HSA, the antibodies purified by adsorption to and elution from HSA had a much higher affinity for dinitrophenyl–HSA than for the unconjugated albumin Weigle's caution in referring to "termination of the unresponsive state" rather than "abrogation of tolerance" is thus entirely justified. Demonstration of the latter phenomenon, by providing evidence for the survival of tolerant cells, would have helped to clarify the fundamental question of whether unresponsiveness involves merely the inhibition of lymphoid cells or their elimination

The author clearly considers that in the present state of knowledge extensive theoretical considerations are not warranted, but nevertheless some useful generalizations are made He stresses the need for adequate equilibration of antigen between intra- and extra-vascular spaces for the successful induction of tolerance and directs attention to the fact that all manipulations which facilitate induction of unresponsiveness such as neonatal injection or X-irradiation also provide conditions in which the initial antibody response is depressed, he suggests that antigen can thereby persist at concentrations adequate to inhibit or eliminate the appropriate lymphoid cells The interesting conclusion is drawn that the inhibitory effect of simultaneous injection of the tolerogenic antigen on the termination of unresponsiveness by cross-reacting antigen provides a mechanism by which the body is protected from the formation of autoantibodies by cross-reacting microbial antigens. Where the concentration of a body constituent in the circulation is low, one could expect, and indeed may find, formation of autoantibodies in response to such exogenous antigens Weigle speculates that this may be one of the ways in which autoimmune diseases arise, but could have been more explicit in relating this hypothesis to specific clinical disorders The relevance of tolerance induction in the adult to problems of transplantation should perhaps have been discussed in a book on this subject, and in this context the possible implications of Mitchison's observations on "low dose tolerance" would seem to merit greater emphasis I M Roitt

INSECT PHYSIOLOGY

Insects and Physiology

Edited by J W L. Beament and J E Treherne. Pp viii + 378 + 34 plates (Edinburgh and London : Oliver and Boyd, Ltd., 1967) 130*s*

The greatness of a man of science is not measured by the number of his pupils, the weight of his publications or even the number of times his name appears in textbooks Professor V B Wigglesworth would qualify on any of these counts, but none would do him justice The true measure of greatness is the extent to which others have been able to build, on foundations securely laid, a structure of greater beauty than the founder himself could have created

This book is a collection of articles specially written on the main theme of Wigglesworth's 1948 Croonian Lecture—the suitability of insects for the study of physiology The authors are all people who have worked at least for a time in his unit in Cambridge As is almost inevitable in such a publication, the standard varies widely and it is depressing to note that the main flowering of ideas seems at present to be taking place on the other side of the Atlantic. The four essays from Western Reserve University are all extremely stimulating and so are five others from elsewhere in the United States and Canada, many of these record important new experimental results By contrast (and apart from those by Maddrell and Weis-Fogh), the articles from Cambridge are mainly historical or repeat discussions already published elsewhere. Migration appears to be desirable for the health of this species, though the quality of the writing by Miller shows that it need not be to another country

Wigglesworth has worked on insects for most of his life, but he has always tried to relate his discoveries to the rest of physiology Another distinction can be made between the different articles in this volume by the extent to which the authors follow their mentor's example Most of the Americans show a clear appreciation of the fact that insects are merely one of the types of animal and thus strengthen their advocacy of this particular biological material for the solution of their problems. Some of the local products seem to be entomologists first and physiologists second, and a few do not appear even to be familiar with the recent entomological literature

The main subjects discussed are morphogenesis, neurosecretion, the growth and organization of the sensory and central nervous systems and the structure and permeability of the cuticle. The foundations for each of these branches of insect physiology were laid by Wigglesworth. The reviews by Weis-Fogh on flight metabolism, by Berridge on ion and water transport and by Davey on reproductive physiology are also important, though drawing only general inspiration from his writings, these three bring out very well the value of studies on insects for the general physiologist Although, as Miller says, "reviews and hypotheses, like nappies, must be freely disposable", many of these articles will certainly remain in use for some time and many of the suggestions made by the authors will justify several passages through the clarifying laundries of the scientific world I fear that because the word "insects" is printed large and the word "physiology" small on the dust cover and spine, many physiologists will miss the opportunity offered to them, for the crusade launched by Wigglesworth in 1948 still hardly affects the security of the great para-medical laboratories Perhaps David Smith puts his finger on the reason for this when he writes that, for all the many advantages of insects, "their small size hampers correlated structural and biochemical studies involving the separation of subcellular fractions" Certainly the attempts made in this volume to jump on the DNA/RNA band-wagon reveal a singular lack of secure footholds

I must regretfully note certain signs of carelessness in printing and editing There are minor misprints in nearly every article and in several places the initial letter of a line has fallen out, once the initial words on two adjacent lines are interchanged And surely one contributor should not be allowed to mis-spell the name of another in his text and list of references! For all this, the volume is a worthy tribute to a great man and one may hope that this evidence of the progress made by some of his pupils and collaborators will be to him both a source of satisfaction and an encouragement to continue his own researches, which are by no means at an end

J W S Pringle

Announcements

Applications are now invited for the 1968 Drummond Prize, an annual prize of £250 awarded for research in the field of nutrition. The prize, which is for research undertaken by persons normally resident in Great Britain, is open to those who have not reached the age of 35 by the closing date Further information can be obtained from Mr D S Miller, Honorary Secretary. Nutrition Society, Queen Elizabeth College, London W8

The American Microchemical Society has created a fund to commemorate the work of the late Dr A A Benedetti-Pichler, and an award will be made to an individual who has made outstanding contributions to the practice or teaching of microtechniques Nominations are now being sought and further information can be obtained from Dr Michael Cefola, Department of Chemistry, Fordham University, New York, NY 10458

Applications are now invited for the 1968 Langley Memorial Prize, founded to commemorate the work of Dr W H Langley, principal medical officer, southern Nigeria The prize, value £21, is awarded triennially for the best paper on one of the following subjects Tropical Medicine or Surgery, Tropical Hygiene and Sanitation, Tropical Entomology and Parasitology Further information can be obtained from the Dean, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street London WC1

Meetings

Modern Chemistry in Industry, March 11–14, Eastbourne (Honorary Secretary, 14 Belgrave Square, London SW1)

Brain Research and Human Behaviour, March 11–15, Unesco House (P Dell, Ibro/Unesco, Place de Fontenoy Paris 7^e)

The Council of the European Organization for Nuclear Research has planned four sessions during 1968 to be held on the following dates March 14, June 19–20, September 25–26, December 18–19

Physical Properties of Liquids and Gases for Plant and Process Design, March 20–21, Ministry of Technology's National Engineering Laboratory, East Kilbride, Glasgow (Mr S W Turner, BP Chemicals (UK) Ltd , Bo'ness Road, Grangemouth, Stirlingshire)

Optimization, March 25–28, University of Keele (The Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex).

Mechanical and Thermal Properties of Ceramics, April 1–2, US National Bureau of Standards (J B Wachtman, Jun , Rm A359, Materials Bldg , National Bureau of Standards, Washington, DC 20234)

Wind Effects on Buildings and Structure, April 2–4, University of Technology, Loughborough (Mr J Charles Department of Transport Technology, University of Technology, Loughborough, Leicestershire)

Erratum In the article by Professor Herbert Dingle in the issue of January 6 (*Nature*, 217, 19, 1968), one paragraph was unfortunately garbled in transmission by telephone The first complete paragraph in the right-hand column of page 20 should read as follows

The letters of Dr Barrett[6] and Mr Fullerton[7] reveal elementary misconceptions Barrett writes "for *A*, these two clocks are not synchronized" Synchronized clocks are not synchronized for *A* or for *B*, they are simply synchronized The process is experimental if the readings satisfy the condition $T_3=2T_2-T_1$, given in my article, the clocks are synchronized absolutely Barrett confuses clock-readings with the times at which the clocks have those readings the same reading is correct, fast or slow, according to the co-ordinate system chosen, but the reading is absolute Clock-readings alone are involved in my disproof of special relativity That is why Einstein rightly concluded that his theory required one clock to work absolutely more slowly than the other Fullerton refers to an observer who "synchronizes *A*, *B* and *H* to zero but not *N*" Clocks are not synchronized to one reading, synchronized clocks give a common reading for every event *B* cannot be synchronized with *A* and *H*, for it moves with respect to them If Fullerton means only that these clocks read zero at the event E_0, then so does *N*, but the fact is trivial

Erratum In the communication "Semi-annual Variation in Thermospheric Density" by Reginald E Newell (*Nature*, 217, 150, 1968) in the twenty-second line of the third paragraph $K_p \leqslant 5_0$ should read $K_p \geqslant 5_0$

Erratum In the communication "Interaction between Model Compounds of Actinomycin *D* and DNA Physicochemical Studies" by F Ascoli, M Savino and A M Liquori (*Nature*, 217, 162, 1968), the last sentence of the fifth paragraph should read "The values so obtained are 1 125 and 1 350 which are of the same order of magnitude as those obtained for 3,4-benzpyrene"

Erratum The bibliographical details of the review of the books by G K Batchelor and Frank Chorlton (*Nature*, 217, 199, 1968) should have been as follows

An Introduction to Fluid Dynamics

By G K Batchelor Pp xviii + 615 (London Cambridge University Press, 1967) 75*s* net, $13 50

Textbook of Fluid Dynamics

By Frank Chorlton Student's Paperback edition Pp xiv + 399 (London D Van Nostrand Company, Ltd , Princeton, N J D Van Nostrand Company, Inc , 1967) 35*s*

On page 204 of the same issue the ninth of the reports and publications should be Freedom of Action in a Mechanistic Universe

CORRESPONDENCE

New Era for Salk Institute?

Sir,—There is one factual error in the article about the institute in the December 9, 1967, issue of *Nature* (216, 961, 1967), and one passage that deserves comment

The physical plant of the institute has not cost twenty million pounds, as the article states, but approximately twenty million dollars

The statements in the article which refer to the "past two years" might be interpreted as a pejorative personal reference to the past President, Dr Augustus B Kinzel whose long-time performance in many positions has earned him a reputation as a very strong administrator Your readers may therefore be interested to know that the trustees of the institute approved a resolution which contained the following remarks

" Dr Kinzel contributed importantly to the administration of the Institute during a period when there were complex matters of concern to the Fellows and the Trustees During his tenure, the scientific work of the Institute has made notable progress and the broad interests of the Institute have been clearly advanced

"Steady perseverance and resourcefulness have characterized Dr Kinzel's work for the Institute . "

Yours faithfully,

Jonas Salk

Salk Institute for Biological Studies,
San Diego

FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, January 29

ROYAL INSTITUTION (at 21 Albemarle Street London, W1), at 5 30 p m —Professor Julius Sumner Miller "What is Physics?" (Lecture for Fourth orm Boys and Girls from Schools in London and the Home Counties To e repeated on January 30, 31 and February 1)

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, JOINT IEE/IERE EDICAL AND BIOLOGICAL ELECTRONICS GROUP (at the Institution of Elecical Engineers, Savoy Place, London, WC2), at 6 p m —Colloquium on Diathermy"

INSTITUTION OF MECHANICAL ENGINEERS, MEDICAL ENGINEERING WORKNG PARTY (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m — Discussion Meeting on "The Physics and Medical Applications of Lasers"

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompon Hospital, London, SW3), at 6 15 p m —Dr J R May "The Chemoherapy of Non-Tuberculous Respiratory Infections" *

Tuesday, January 30

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 1 20 p m —Mr E W Foster "The ennine Way" *

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2) t 5 30 p m —Discussion Meeting on "Electrical Breakdown in Magnetic ields" opened by Mr L King, Mr N L Allen and Mr K A Bunting

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the Institution of Mechanical Engineers, at Savoy Place, London, WC2), at 5 30 p m —Mr H Nicholson "Computer Simulation of Boilers and Turbo alternators"

UNIVERSITY OF LONDON (at Chelsea College of Science and Technology, Manresa Road, London, SW3), at 5 30 p m —Mr H G Gilson "The Biological Problems of Estuary Barrage Reservoirs" *

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Dr G F M Russell "Anorexia Nervosa Psychological, Endocrine and Nutritional Aspects" (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by The British Postgraduate Medical Federation)*

INSTITUTION OF MECHANICAL ENGINEERS, RAILWAY ENGINEERING GROUP (at 1 Birdcage Walk, Westminster London, SW1), at 6 p m —Discussion Meeting on "U I C Standards and Design Limitations which would Result from the Channel Tunnel"

INSTITUTION OF THE RUBBER INDUSTRY LONDON SECTION (at the Eccleston Hotel, Victoria, London, SW1), at 6 p m —Mr H Walton "Critical Path Analyses", "Ozone Resistance of Elastomers" by a speaker from Dupont

Wednesday, January 31

ROYAL INSTITUTION, HISTORY OF SCIENCE DISCUSSION GROUP (at 21 Albemarle Street, London, W1), at 1 p m —Mr H G Frost "The Nineteenth Century Science Text Book Its Role in the Application of Science" *

UNIVERSITY OF LONDON (at the Royal Postgraduate Medical School, Du Cane Road, London, W12), at 2 p m —Dr M S Dunnill "Measurement in Histology" *

SOCIETY FOR ELECTROCHEMISTRY (at the Royal School of Mines (Room 53), Imperial College of Science and Technology, London, SW7), at 2 30 p m — Lord Wynne-Jones "Chemistry at Electrodes" (Inaugural Address)

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W1), at 5 p m —Mr N E Butcher "The Earliest Known English Colour-Printed Geological Maps" (Demonstration), Mr P Lambort "Reproduction of Old Geological Maps Produced by Colour Photography" (Demonstration), Dr A C Bishop, Dr J D Bradshaw, Dr J T Renouf and Mr R T Taylor "The Stratigraphy and Structure of Part of West Finistère, France" (Paper)

UNIVERSITY OF LONDON (in the Physiology Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Dr E J Furshpan (Harvard Medical School) "Low-Resistance Intercellular Junctions" *

Thursday, February 1

ROYAL SOCIETY (at 6 Carlton House Terrace, London, SW1) at 4 30 p m —Professor D Gabor, FRS, and Mr G W Stroke "Theory of Deep Holograms", Mr J H Thomson and Mr J E B Ponsonby "A Method of Obtaining High Resolution Radar Maps of the Moon by Two Dimensional Aperture Synthesis Using a Single Aerial"

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi London, WC2), at 5 15 p m —Mr Keith R Harding "Mining Development in Australia" (Henry Morley Lecture)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Optimisation of Traffic Throughput" opened by Mr D J Lyons.

UNIVERSITY OF LONDON (at the Institute of Child Health, Guilford Street London, WC1), at 5 30 p m —Professor R W Gilliatt "Experimental Peripheral Neuropathy" (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

UNIVERSITY OF LONDON (at the Royal College of Surgeons Lincoln's Inn Fields, London, WC2), at 5 30 p m —Dr A R de C Dencock "Medical Problems of Underwater Diving" *

INSTITUTE OF REFRIGERATION (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, SE1) at 6 p m —Mr G Mann "Methods of Regulating the Carbon Dioxide and Oxygen Concentrations in Controlled Atmosphere Stores for Fresh Produce"

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, EDUCATION GROUP (at 8–9 Bedford Square, London, WC1), at 6 p m —Mr C F Davidson "Satellite Communication Systems and the Diverse Engineering Techniques Which They Require"

INSTITUTION OF MECHANICAL ENGINEERS, TRIBOLOGY GROUP, and the INSTITUTION OF METALLURGISTS (at the INSTITUTION OF MECHANICAL ENGINEERS, 1 Birdcage Walk, Westminster, London SW1), at 6 p m — Discussion Meeting on "Metallurgy is the Most Important Discipline in Tribology"

Friday, February 2

ROYAL INSTITUTION, PHOTOCHEMISTRY DISCUSSION GROUP (at 21 Albemarle Street London, W1) at 1 p m —Dr J B Birks "The Pyrene Excimer" *

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS DIVISION (at Savoy Place, London, WC2), at 2 30 p m —Colloquium on "M O S T 's for Analogue Switching"

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (at Savoy Place, London, WC2), at 5 30 p m —Mr V S Ramsden and Mr R R Booth "The Digital Computer Representation of Induction Machine Transient Performance"

Saturday, February 3

INNER LONDON EDUCATION AUTHORITY (at the Horniman Museum London Road, Forest Hill, London, SE23), at 3 30 p m —Mr K M Harris "Living in Darkness—Biology of Caves" *

Monday, February 5

INSTITUTION OF ELECTRICAL ENGINEERS ELECTRONICS DIVISION (at Savoy Place, London, WC2), at 5 30 p m —Mr F E Williams, Mr F A Wilson and Mr K A T Knox "Future Trends in the Design of Subscribers' Telephone Instruments"

INSTITUTION OF ELECTRICAL ENGINEERS, JOINT PROFESSIONAL GROUP (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Specification of the British Electrical Indicating Instrument" opened by Mr L B S Golds

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Fine Chemicals Group, at 14 Belgrave Square, London, SW1), at 6 30 p m —Professor R C Cookson "Some Aspects of Photochemistry"

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

ASSISTANT LECTURERS or LECTURERS (2) in the DEPARTMENT OF PHYSIOLOGY—The Registrar, University College, Cathays Park, Cardiff (January 31)

RESEARCH ASSISTANTS (2) IN GEOLOGY to complete the team of six engaged in the resurvey of the Teignmouth One inch Geological Map (Geological Survey Sheet 339) under a contract with the National Environment Research Council—Professor S Simpson, Department of Geology, The University, North Park Road, Exeter, Devonshire (February 2)

HARRIS CHAIR OF PHYSICS—The Secretary, The University, Dundee Scotland (February 10)

LECTURER or ASSISTANT LECTURER (mathematician with research interests in any branch of pure mathematics or mathematical statistics) in the DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Swansea, Singleton Park, Swansea (February 10)

UNIVERSITY LECTURER in either SOCIAL PSYCHOLOGY or SOCIOLOGY—The Secretary of Faculties, University Registry, Oxford (February 10)

LECTURER in APPLIED MATHEMATICS—The Secretary, Trinity College, University of Dublin, Dublin 2, Republic of Ireland (February 12)

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF METALLURGY for duties in electron micro-probe analysis, mainly for work on meteorites—The Registrar, The University of Manchester, Manchester, 13, quoting Ref 2/68Na (February 14)

EXPERIMENTAL OFFICER (preferably with experience with X-ray equipment, high vacuum equipment or electronic instrumentation of any kind) in the DEPARTMENT OF GEOLOGY for the maintenance and operation of a geoscope electron probe microanalyser and an electron microscope—The Registrar, The University, Manchester 13, quoting Ref 8/68/Na (February 14)

LECTURER (with medical qualifications registrable in the United Kingdom) IN ANATOMY—The Registrar, The University, Manchester, 13, quoting Ref 3/68 (February 14)

ASSISTANT LECTURER/LECTURER (with an interest in animal behaviour) IN PSYCHOLOGY—The Secretary (NAT), University of Stirling, Stirling Scotland (February 15)

SENIOR RESEARCH OFFICER (with a degree in agricultural science, rural science or equivalent, and several years of postgraduate experience in animal husbandry research) at the Agricultural Research Institute, Wagga Wagga, New South Wales, Australia, to formulate and supervise animal husbandry research programmes in the southern wheat belt and southern tablelands of New South Wales—The Recruitment Section, New South Wales Government Offices, 56 Strand, London, WC2 (February 16)

LECTURER IN NEUROSURGERY at the Institute of Neurological Sciences, Glasgow—Secretary to the University Court, The University, Glasgow (February 17)

LECTURER (suitable medical or science graduate) in the DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY at the Western Infirmary—The Secretary of the University Court, The University, Glasgow (February 17)

ASSISTANT LECTURER or LECTURER (with interests in either fluid dynamics or computation) in the DEPARTMENT OF MATHEMATICS—The Deputy Secretary, The University, Southampton (February 23)

RESEARCH FELLOW in the DEPARTMENT OF ELECTRICAL ENGINEERING for research into cryogenic cable systems—The Deputy Secretary, The University, Southampton (February 23)

CHAIR OF PSYCHOLOGY at Bedford College—The Academic Registrar (A) University of London, Senate House, London, WC1 (February 29)

LECTURER or ASSISTANT LECTURER (preferably with a special interest in immunology) in the DEPARTMENT OF BACTERIOLOGY AND VIROLOGY—The Registrar, The University, Manchester, 13, quoting Ref 4/68

POSTDOCTORAL FELLOW IN CHEMISTRY for research on Lewis acid-base reactions in the gas phase—Professor A F Trotman-Dickenson, University College of Wales, Aberystwyth

RESEARCH ASSISTANT in the DEPARTMENT OF FORENSIC MEDICINE to assist in a programme of research into the development of rapid methods of toxicological analysis—Dr H V Street, Department of Forensic Medicine, University Medical Buildings, Teviot Place, Edinburgh, 8, Scotland

SCIENTIFIC INFORMATION OFFICER (preferably with a botanical or agricultural degree, or knowledge of or aptitude for foreign languages, and ability to write good English) for duties which include the preparation and editing of abstracts for the journal *Plant Breeding Abstracts*, the classification of biological literature, and dealing with scientific enquiries—The Director, Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

University Grants Committee First Employment of University Graduates, 1965–66 Pp 63 (London H M Stationery Office, 1967) 5*s* 9*d*. net [2011

Bulletin of the British Museum (Natural History) Zoology Vol 15, No 7 Polyzoa (Bryozoa) from West Africa The Pseudostega, The Cribrimorpha and some Ascophora Imperfecta By Patricia L Cook Pp 321–351+2 plates (London British Museum (Natural History), 1967) 13*s* [2011

Ministry of Technology Torry Research Station Annual Report 1966 on the Handling and Preservation of Fish and Fish Products (Report of the Torry Research Station Steering Committee and Report of the Director of the Torry Research Station) Pp viii+70+4 plates (Edinburgh and London H M Stationery Office, 1967) 7*s* 9*d* net [2011

University of Nottingham Report of the School of Agriculture, 1966–1967 Pp 183 (Sutton Bonington, Loughborough University of Nottingham, School of Agriculture, 1967) 10*s* [2011

Institution of Gas Engineers Communication 753 Marketing Total Energy By C J Gauthier Pp 11 4*s* 6*d* Communication 754 The Philadelphia Story. By Charles G Simpson Pp 16 4*s* 6*d* Communication 756 Modified P V C Pipes Assessment for the Transmission of Present and Future Town Gas Supply By C A Brighton and J L Benton Pp 16 4*s* 6*d* Communication 757 Summer Hot Water from Central Heating Boilers By J Emerson and J P Roberts Pp 16 Communication 758 The Scientific Approach to the Design of Sectional Boilers By E E Blandon Pp 9 4*s* 6*d* (London The Institution of Gas Engineers, 1967) [2111

The Gas Council Research Communications GC138 Engineering Research for Transmission and Distribution By J L Van der Post Pp. 9 4*s* 6*d* GC139 Control Units for Automatic Burners By P G Atkinson, R N Grimsey and R A Hancock Pp 13 4*s*. 6*d* GC140 Operational Research in the Gas Industry By E F Mellen Pp 8 4*s* 6*d* GC141 A Unifying Theory for the Blow-Off of Aerated Burner Flames By Stuart B Reed Pp 12 4*s* 6*d* GC 142 Combustion Characteristics of Natural Gas and Manufactured Substitutes By J A Harris and D E Lovelace Pp 17 4*s* 6*d* GC143 Approval of Natural-Gas Appliances By E W B Dunning and R P Wakefield Pp 17 4*s* 6*d* GC144 58th Report of the Joint Refractories Research Committee, 1966–67 Pp 16 4*s* 6*d* GC145 Systems Engineering and the Advent of the Digital Computer By R J High and R Robson Pp 16 4*s* 6*d* GC146 Ignition of Domestic and Commercial Gas-Fired Appliances By B Ekins and Dr A M Brown Pp 19 4*s* 6*d* GC147 The Ignition of Industrial Burners By P G Atkinson, M R Marshall and D J Moppett Pp 9 4*s* 6*d* (London The Gas Council, 1967. Obtainable from the Institution of Gas Engineers) [2111

Other Countries

U S Department of Commerce National Bureau of Standards NBS Monograph 32 Supplement Revision of the NBS Tables of Spectral-Line Intensities below 2450 Å By Charles H Corliss Pp iii+33 (Washington, D C Government Printing Office, 1967) $03.0 [1511

Molecular Biology, Vol 1, No 1 (January–February 1967) Translated from the Russian Pp 1–151 Subscription (6 issues) $95, Single issue $30 (New York Consultants Bureau, 1967) [1511

Bulletin of the American Museum of Natural History, Vol 138, Article 1 Comparative Histological Studies on Duvernoy's Gland of Colubrid Snakes By Aaron M Taub Pp 1–50+8 plates (New York American Museum of Natural History, 1967) $2 50 [1711

Australian Mineral Development Laboratories Seventh Annual Report, 1966-67 Pp 64 (Adelaide Australian Mineral Development Laboratories, 1967) [1711

United States Department of the Interior Fish and Wildlife Service Resource Publication No. 44 Underwater and Floating-Leaved Plants of the United States and Canada By Neil Hotchkiss Pp vii+124 (Washington, D C Government Printing Office, 1967) $0 65 [1711

The Heredity of Normal and Defective Colour Vision. By Georg H M Waaler Pp 25 (Oslo Universitetsforlaget, 1967) [2011

Comité International des Poids et Mesures Procés-Verbaux des Séances 2e Série—Tome 34, 55e, Session—1966, 4–6 Octobre Pp 94 (Paris Gauthier-Villars, 1967) [2011

Colony of Mauritius Annual Report of the Meteorological Department, 1966 Pp 14 (Port Louis Government Printer, 1967) Rs 2 50 [2011

Koninklijk Nederlands Meteorologisch Instituut Mededelingen en Verhandelingen No 87 Climatology of Amsterdam Airport (Schiphol) Pp 145. F 16.50 No 88 The Southern Oscillation and World Weather By Dr H P Berlage Pp 152 F 15 50 No 89 Observation and Analysis of the Surface Wind Over the Ocean By G Verploegh Pp 67 No 90 Wind and Wave Data of Netherlands Light-vessels Since 1949 By R Dorrestein. Pp 123 (De Bilt Koninklijk Nederlands Meteorologisch Instituut, 1966 and 1967) [2011

Guide to Ship Sanitation By Vincent B Lamoureux Pp 119 (Geneva World Health Organization, London H M Stationery Office, 1967) 12 Sw francs, 20*s* $4 [2111

International Atomic Energy Agency Safety Series No 23 Radiation Protection Standards for Radioluminous Timepieces (Recommendations Drawn up by a Joint Group of Experts of the European Nuclear Energy Agency and the International Atomic Energy Agency) Pp 37 (Vienna International Atomic Energy Agency, London H M. Stationery Office, 1967) 26 schillings, 7*s* 2*d*, $1 [2211

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Union Radio Scientifique Internationale (URSI) XVth General Assembly, Munich, September 5–15, 1966 Volume XIV—5, Commission V—Radio Astronomy Pp 133 (Bruxelles Union Radio Scientifique Internationale, 1967) [2411

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Remote Sensing of Environment

AN INTERDISCIPLINARY JOURNAL

Published quarterly Volume 1, Number 1, Summer 1968

Editor-in-Chief. DAVID S. SIMONETT

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NATURE Volume 217
FEBRUARY 3, 1968

Hornet's Nest for Coal

THE continuing pantomime of public declarations about the fuel to be used for the aluminium smelting plant which Alcan wishes to build in the United Kingdom has brought to the surface a great many important issues of principle in the conduct of the business of the nationalized industries and a great many anomalies in the British Government's attempt to organize a co-ordinated fuel policy (see page 398). There seems to be no doubt that Alcan has offered to build an aluminium smelter based on coal rather than electricity from the national grid because the National Coal Board has been able to offer it cheap coal and stable prices. The immediate issue is whether this offer is realistic.

From the way in which Sir Ronald Edwards has now informed the coal board that he does not wish to accept the offer of cheap coal at 3.25*d* a therm for the new electricity generating station at Seaton Carew in north-east England, it is plain that the coal board's offers are not universally beguiling. Quite properly, what the Central Electricity Generating Board is anxious about is the prospect that coal prices will steadily increase during the lifetime of a power station built now to use coal as fuel. Evidently the coal board has not felt able to give the electricity board the kind of assurance which Alcan appears to have got. Nobody should be surprised that both the steel and electricity industries are up in arms at what seems to them to be unfair discrimination.

In practice, of course, it is only possible for the National Coal Board to sell coal on long-term contracts at prices less than 4*d*. or even 5*d*. a therm by counting on the elasticity afforded within its price structure by the other large customers for coal and by the fact that in the last resort the Government will have to bail it out of trouble. If, for example, the coal board were to guarantee a fixed low price for coal for an aluminium smelter, it would have to seek to recover the increased costs of mining that coal from its other customers, chiefly the electricity and steel industries. But is it proper for a nationalized industry to decide for itself which customers should pay what? Is it proper that it should first of all persuade the Government that the electricity industry should burn more coal than it wishes to do and then, without prior consultation, arrange things in such a way that coal will cost more than it should?

The trouble, of course, is that in a situation in which one large organization sells to a handful of other large organizations, fixing the proper selling price is a process which has more in common with the negotiation of a diplomatic treaty than the processes by which other market prices are fixed. The difficulty is exacerbated in the present circumstances by the common understanding, no doubt as vivid among the officials of the coal board as anywhere else, that the British coal industry is already supported to a very large extent by the determination of the British Government to see that it shall not collapse too quickly. If the coal board were to sign a contract with a would-be aluminium smelter which turned out to be an embarrassment in the years ahead, it is unthinkable that it would be allowed to default on the obligations it had undertaken. The Government, in the last resort, would be compelled to step in.

What, in these circumstances, should the Government do? Perhaps the most simple prescription for action in the months immediately ahead is that the Ministry of Power should make sure that the freedom which the National Coal Board is anxious to enjoy should somehow be accompanied by a full share of responsibility. The trouble in present circumstances is that the coal board—and many of the other nationalized industries as well—is able to pretend to commercial freedom in some of its activities while relying on an implicit guarantee that the Government will step in if commercial policies are wrong. Where the coal board is concerned, the Government has been unreasonably indulgent in recent years. For example, at a time when the coal board has been producing more coal than it could sell, the Government has provided the funds with which to finance an increased accumulation of coal stocks. By the same test, the rate at which the coal board is expected to earn a surplus on the capital employed in this industry is less than that of the other nationalized industries. It would obviously be an enormous benefit if the Ministry of Power could now return to its excited talk of only a few months ago and somehow arrange that the coal board were exposed to some of the harsher realities of the world in which it is seeking to live so freely. For one thing, it would obviously help enormously to create a sense of realism among the officials at the coal board if the industry were required to raise at least a part of the money needed to finance its development in the public market and not from the British Treasury. Where customers are concerned, it would obviously help if the large industries which fear from time to time that they are being asked to pay more for coal than is economically possible were allowed once more to import this from abroad.

But if this prescription is likely to help the coal board to put its house in order and cease being an embarrassment to the other nationalized industries and the Government as well, is there not a case for asking that similar rules should apply across the board? Certainly, the electricity industry itself would probably welcome a chance to finance some of its undertakings from sources other than the British taxpayer—at the very least this would provide a degree of flexibility which is at present lacking By the same test, the electricity industry should no longer be required to negotiate with the Government before deciding to build a power station of one kind or another

Smelting at Home

The British Government wants to save imports by establishing an aluminium smelting industry in Britain So much is clear But the idea is now so complicated by wrangling that it seems unlikely to be carried through without ill-will Internationally, the running has been made by Mr Kare Willoch, the Norwegian Minister of Commerce, who said that the move to establish aluminium smelting in Britain was both illegal and immoral and defied both the EFTA convention and the spirit of free trade In Britain, the objections are likely to be less easy to brush aside

The trouble is that Alcan, one of the aluminium companies which wants to build in Britain, has allowed itself to get involved in a long-standing row between the electricity generating boards and the National Coal Board The coal board, which claims that it is still possible to generate electricity as cheaply by burning coal as uranium, has persuaded Alcan to base its tender on a coal-fired generating station Although it would clearly be more attractive to Alcan to have the whole smelter complex under its own control (rather than sharing the output of a large nuclear station, which was the original intention), it has been generally assumed that the most compelling argument has been a promise by the coal board to supply coal at knock-down prices

Both the coal board and Alcan have refused to reveal the price that has been agreed for the coal It is, however, quite possible to calculate this Alcan has agreed to take 1 million tons of coal a year from the north-east coal-field, where the cost of coal in 1967 was an average of £5 32 a ton, according to the annual report of the coal board At this price, Alcan's annual bill would be £5 3 million, even if transport costs were ignored But Mr John Elton, managing director of Alcan in Britain, has said that power costs amount to no more than 15 per cent of production costs Aluminium ingots sell at about £228 per ton and, to make the investment worthwhile, Mr Elton will have to produce ingots at about £200 per ton For a 120,000 ton a year plant, this means annual production worth £24 million Fifteen per cent of £24 million is £3 6 million, far less even than the cost of fuel if Mr Elton were buying at the going rate It is clear that he can be paying no more than £2 8 to £3 million a year for coal, equivalent to 3·1*d* per therm at the smelter This is very markedly less than the 5*d* per therm paid by the generating boards, or the 4 6*d* per therm paid by the National Steel Corporation, both of which are much larger customers

Even if the deal is approved, Invergordon seems an odd place to choose for the smelter, particularly as it will involve the transport of coal by sea from the north-east of England Invergordon, it can be argued, is a development area and is thus a candidate for development grants, but so is the north-east In principle it would be most sensible of all to build the smelters where the ore, bauxite, is mined—in Jamaica, among other places At present bauxite is converted to alumina in plants in Jamaica and is then carried by sea to the smelters elsewhere in the world.

Slower Growth for Universities

The publication last week by the University Grants Committee of the grants to individual universities enables a preliminary assessment to be made of the way in which universities are likely to develop over the next five years Fig 1 shows the total support provided by the University Grants Committee to the universities in the years between 1957 and 1972 Fig 2, which takes the University of Birmingham as a particular example, shows the same pattern What the curves do is to confirm an impression that the years of really rapid growth lie between 1963 and 1968, and are now coming to an end Beyond 1968 there is evidence of a slackening of support, and it looks as if the growth will return to much the same rate as prevailed before 1963 On this rather pessimistic argument, the stimulation of support for universities which was achieved by the publication of the Robbins Report was little more than a temporary phenomenon

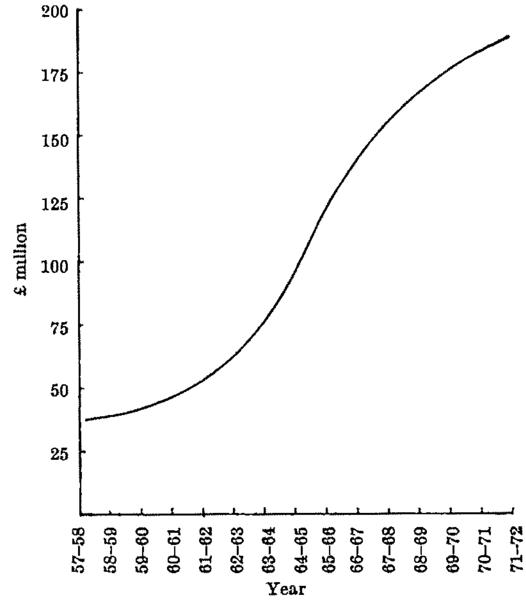


Fig 1 Support by UGC to British universities, 1957–72.

What is potentially more alarming—to scientists, at least—is that of the more modest increases which are promised to the universities in the next five years, the greater part will be devoted to subjects other than science and technology In this, as in other things, the UGC has been swept along by the tide Although Sir John Wolfenden is no Canute, he and his committee do seem to have made some efforts to resist—they provided for a small expansion in science and technology, although the evidence is that the total numbers of students coming forward will actually decline for the next few years The UGC is clearly terrified that if the swing away from science should reverse itself, and the attractions of social science become less compelling to schoolchildren, it is going to be left high and dry This is why it has provided for an increase in science places, although the evidence is that some of them will be left empty, or filled by students less able than their contemporaries in other departments.

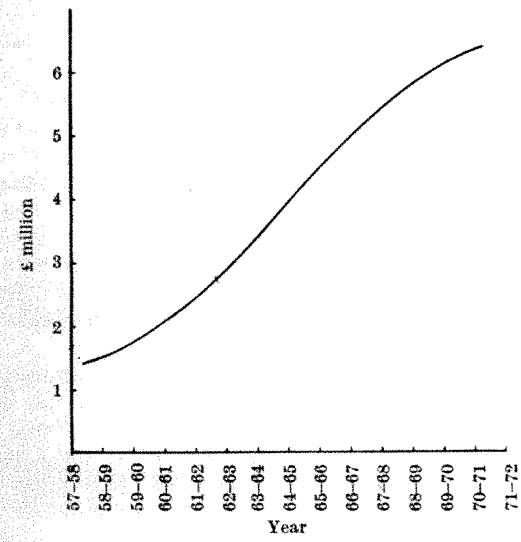


Fig. 2. Support by UGC to Birmingham University, 1957–72.

For the first time, the UGC report includes an interesting survey of the costs of producing graduates in a variety of disciplines. The figures, reproduced below, show how much it costs to produce a graduate in each of the disciplines listed, but do not include the costs of administration, libraries and maintenance of buildings. These costs add up to an additional £260 per student.

ACADEMIC YEAR, 1966–67

	Average Unit Cost	*Average Staff/ Student Ratio*
	£	1 :
Arts	276	9·8
Social Studies	255	11·3
Education	261	12·5
Biological Sciences	518	8·7
Physical Sciences	376	11·3
Technology	425	11·3
Medicine		
(*a*) pre-clinical	647	8·2
(*b*) clinical (excluding postgraduate institutes)	790	5·9
Dentistry	778	5·6
Veterinary Science	1,136	4·6
Agriculture	497	7·8

Sonic Booms and Superbooms

A PROGRESS report on research into sonic booms has been carried out by a sub-committee of the Committee of the US National Academy of Sciences on SST–Sonic Boom (*Generation and Propagation of Sonic Boom*, National Academy of Sciences. Obtainable in Britain from Universal Subscription Service, Ltd., 4 Footscray Road, London, SE9. 25*s*.). The sub-committee, which was chaired by Dr R. L. Bisplinghoff, professor of aeronautics at MIT, held out no hope for an immediate or dramatic improvement in noise generation.

Progress in supersonic aerodynamics has been such that sonic boom overpressures on the ground can now be accurately predicted to within margins of 10 per cent. But this is only for aircraft flying at constant speed, height and direction and over a quiescent atmosphere. Atmospheric inhomogeneities, such as those caused by temperature variations, wind shears and turbulence, exert a complex effect on the propagation of shock waves which at present is poorly understood. Topographic features and seasonal variations in atmospheric turbulence also exert profound influences on the sonic boom waves that reach the ground, and here again further research is needed.

The phenomenon of "superboom" occurs when shock waves from more than one point of the flight path reach the observer at the same time. This can happen when a supersonic aircraft changes speed or direction, with the result that propagated shock waves are momentarily focused at a single point. Current theory can predict the location of a superboom on the ground but not the size of the pressure wave. The sub-committee is dissatisfied with current investigation into superboom effects, and it considers that more flight and laboratory tests are necessary.

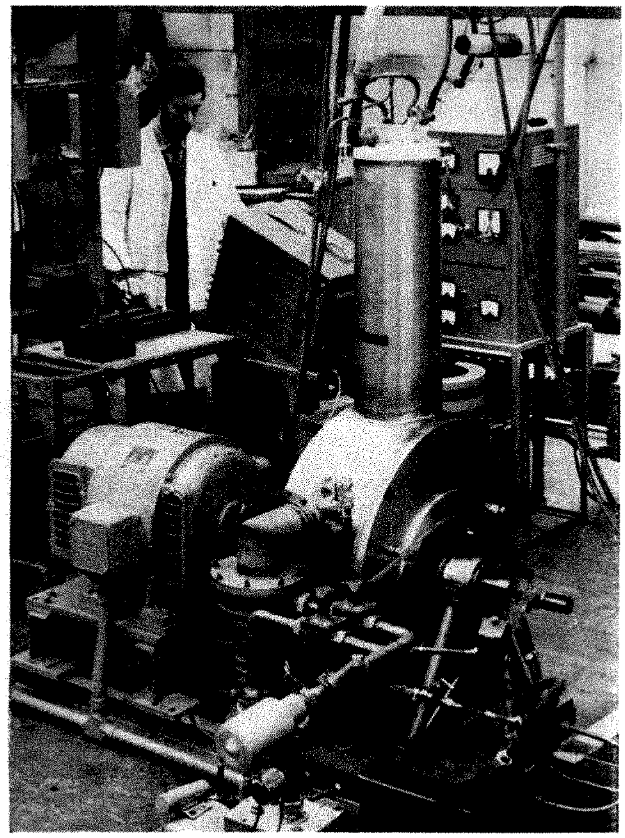
Studies of aircraft design aimed at minimizing sonic boom effects are of prime importance, but the designs so far tested have been relatively conventional. The sub-committee urges that less conventional configuration studies be undertaken in the future. The better radiation characteristics of such fuels as liquid methane or hydrogen hold the possibility of increased propulsion efficiency and possible reduction in sonic boom levels; this is another topic that would merit further attention.

At ground level, little is known about the exact features of the sonic boom which make it undesirable both to animals and to the stability of buildings. The record of the pressure wave as a sonic boom hits the ground, known as its signature, varies both with the type of aircraft and the prevailing conditions. Further research needs to be devoted to determining which parameters of the signature are responsible for damage to human sensibilities on the one hand and to buildings on the other.

It is clear that a large number of problems remain to be solved before the generation and behaviour of sonic booms are thoroughly understood. As far as the Concorde is concerned, the Royal Aircraft Establishment at Farnborough is confident that knowledge of the phenomenon, if not complete, is at least sufficient to allow Concorde flights to proceed. Unforeseen turbulence or topographic effects may perhaps cause prolongation or reverberation of the boom, but they are unlikely to affect the initial sound which is the main cause of annoyance. Unlike military aircraft, which can cause superbooms by abrupt turns or accelerations, the Concorde is expected to produce a superboom only at the period after take-off when its speed becomes supersonic. It is hoped that with suitable planning this superboom can be dumped in the sea.

Industrial Superconductivity

INTERNATIONAL Research and Development Ltd., a sponsored research organization based in Newcastle upon Tyne, announced this week that it has successfully designed and built an electric motor based on superconducting coils. This has been a dream of engineers since superconductivity—the phenomenon in which metals cooled to very low temperatures lose all electrical resistance—was first discovered by the Dutch physicist Kamerlingh Onnes in 1911. But it was not until the development of high-field superconductors and alloys capable of handling very large currents that it became clear that industrial development of superconductivity was worthwhile.



A 50 h.p. superconducting motor developed at International Research and Development Ltd. in Newcastle.

The project at IRD began as a feasibility study for the Ministry of Defence, and showed that a large superconducting motor was possible. Initially a small motor of only 2 h.p. was envisaged, but after some work it was realized that it would be possible to make a much more ambitious motor, producing 50 h.p. and running at 2,000 r.p.m. When this was built, further financial support was sought from the National Research Development Corporation, which appointed a working party to examine the proposal. Although the working party recommended that a 1,000 h.p. motor should first be constructed, NRDC decided to be bold and to go straight to a motor of 3,000 h.p. and 200 r.p.m., the size at which it is estimated superconducting motors will break even on cost with conventional motors. The large motor is to be tested at Fawley power station, by agreement with the Central Electricity Generating Board. The water cooling pumps at Fawley are rated at 3,250 h.p. and 200 r.p.m., designed to be driven by a conventional a.c. motor through reduction gearing. Although the conventional motor runs at 900 r.p.m. to produce the necessary power, the superconducting motor will be able to do it at 200 r.p.m. The stator for the Fawley motor will use a fully stabilized titanium/copper composite superconductor, cooled by a closed circuit helium refrigerator supplied by British Oxygen Cryoproducts Division. The rotor, which will not be superconducting, will be water cooled.

As well as using superconductivity, the engineers at Newcastle, led by Mr A. D. Appleton, have improved the design of the simple homopolar motor, first devised by Faraday. This type of motor, although very simple in design, has been slow to develop because it calls for a high current and low voltage supply. At IRD a system has been devised which uses a segmented disk —the rotor of the homopolar system—to overcome this difficulty, and Mr Appleton thinks that the new design may have potential uses in electric transport, though not in a superconducting form. Homopolar motors would have the distinct advantage that they could be used to regenerate the batteries while braking the vehicle.

The other advantage of the superconducting motor is a remarkable saving in weight. IRD estimates that with an 8,000 h.p. motor the saving in cost would be £70,000 (£150,000 instead of £220,000) and the superconducting version would weigh only one-ninth as much as the conventional motor. These advantages, IRD believes, will soon make the adoption of superconducting motors inevitable.

Cutting off Europe's Nose

TECHNOLOGICAL progress in Europe is being hampered by Britain's exclusion from the EEC. In an address to the Council of Europe on January 29, Mr Anthony Wedgwood Benn, Minister of Technology, reaffirmed the British Government's belief that technical collaboration "within an enlarged common market" is the only way Europe can hope to catch up and keep pace with the United States. Industrial and economic integration will be necessary for this, and European-based international companies must be created if industry is to operate on an adequate scale.

Despite the French veto, which "weakens European economic power in the face of transatlantic pressures", Britain is determined to pursue her policy of collaboration with the EEC and is prepared to consult with any country if mutual benefit will result. Mr Wedgwood Benn said that the decision to devalue the pound, which was taken in the hope of strengthening the economy, has turned Britain "decisively and irrevocably in the direction of Europe". Rationalization of industry within the country has been going on, notably in vehicle manufacturing and the electrical industries, and research projects are being examined for possible areas of collaboration with European countries. Computers, electronics and civil applications of atomic energy are three that have been mentioned already, but there are likely to be many more.

British collaboration is not, however, likely to be limited to Europe. Contact with the United States will be continued, and the technological agreements that have been signed with Poland, Hungary, Rumania and the Soviet Union will mean that planning techniques and long-term research will be discussed. Mr Wedgwood Benn stated his belief that "technological collaboration cannot come to full fruition except within the confines of a single industrial and trading market", and, although Britain's entry into the Common Market has been delayed, it is not too soon for a start to be made on collaboration in such fields as industrial standards, company law and patents which the Council of Europe has begun to examine. The European Institute of Technology proposed by the Prime Minister in November was described as an "organization that would provide an independent centre of analysis available to governments and industries on industrial structures, problems and future developments". It would provide a forum for technological exchanges, and by working in close collaboration with the EEC commission it could "play a major role in accelerating the achievement of a European technological community—a community which is essential if Europe's potentials are to be realized". But those who hoped that Mr Benn would finally disclose exactly what is in the Prime Minister's mind were disappointed. The European Institute of Technology remains a nebulous idea, splendid for injecting life into otherwise familiar speeches, but not apparently the subject of much serious study.

Unequal Trade

With the worsening of relations between rich and poor countries as a result of wars in the Middle East and in Vietnam and racial tension in many countries, the meeting of the United Nations Conference on Trade and Development (UNCTAD) which is taking place in New Delhi will not be all plain sailing. The last meeting in Geneva in 1964 was an acknowledged flop, and hopes for the New Delhi meeting are not high. Too many countries, Britain included, have gone to the meeting without a clear policy.

UNCTAD—defined in the most recent annual review of the Overseas Development Institute (ODI, 12*s*. 6*d*.) as "a forum for redressing the balance of the world's economic system in favour of the developing countries"—has not been a triumphant success in the past few years. Although one of its aims is to increase the production and value of commodities on which most developing countries rely, the price of commodities continues to fall. Increases in production or in productivity tend inevitably to be reflected by a decline in the prices paid for the commodities, so that the processes by which developed countries advance themselves tend to do the opposite for underdeveloped countries. UNCTAD also cherishes a hope of establishing aid to developing countries at the rate of 1 per cent of the GNP of developed countries, and of promoting tourism and regional co-operation between developing countries. But the chances of increasing aid at the moment are slim indeed—the British aid programme is to be fixed at the present ceiling of £205 million per year, despite devaluation, which means that the real value of the aid which Britain dispenses has fallen by between 8 and 11 per cent. This has happened after an actual cut of £20 million announced at the end of 1966, so that the value of British aid to developing countries has fallen steadily for the past two years. In the circumstances, it would perhaps have been unreasonable to expect anything else, but it is hard for developing countries to feel that Britain's economic difficulties, alarming as they are, are anything like as serious as their own.

The annual review from the ODI suggests ways in which developing countries can make the best of a bad situation. It would not be helpful, the report argues, if the developing countries use the present UNCTAD meeting to attempt to force concessions from developed countries. UNCTAD, the authors of the report say, must work through consensus—if the proceedings are based on bargaining from strength, it will be impossible to get the system changed for the simple reason that bargaining from strength has produced the very system which is under attack. The UNCTAD secretariat, headed by Dr Rául Prebish, has prepared a detailed analysis of the present situation, together with a series of proposals for action, but these have been treated with scepticism by some of the developed countries. The ODI report suggests that detailed argument about the way the case has been presented should not be allowed to obscure the basic issue—that the present system for operating world trade is biased against the developing countries. UNCTAD, the report concludes, must devote itself to changing the economic system, not to trying to secure concessions within the existing system.

Whether the developing countries accept this timely advice remains to be seen. There is little doubt, however, that without a new stimulus the world aid programme runs the risk of coming to a halt through political apathy in the developed countries. This is depressing, and in this context the occasional success stories are very refreshing. Pakistan and Kenya are likely to be quoted frequently at the UNCTAD meeting as examples of what can be done.

Reducing the Risks

The opening of the advanced Nuffield Transplantation Surgery Unit on January 31 by Sir Peter Medawar marks an important step forward in this extremely complex field. The Nuffield Foundation provided £230,000 towards the cost of the unit, which is at the Western General Hospital in Edinburgh.

According to Professor M. F. A. Woodruff, who is the director, the new unit has been designed for the treatment of patients who run a particular risk of infection because of having received immuno-suppressive agents. Three main categories of patients will be admitted; first, those patients receiving organ transplants and who are receiving immuno-suppressive drugs; second, patients with auto-immune diseases also receiving these drugs; and, third, certain types of cancer patients who have reduced resistance to infectious agents. The unit will, however, also be available to patients who have been accidentally exposed to radiation.

There are six patient rooms and two operating theatres in the unit. These work on a complicated system of ventilation with sterilized air under pressure which will prevent any cross-infection. All materials entering the unit will be sterile and wrapped, and communication between patients and visitors will be through glass and using microphones. The first patients will be those receiving kidney grafts.

Waiting, Not Yet Seeing

The meeting last week in Geneva of the Council of the European Molecular Biology Organization (EMBO) seems to have been a mixed success. The proposal for a central laboratory was presented by EMBO but not formally discussed—that will not happen until later this year. Nevertheless, a second firm offer of a site for the laboratory has now been made. As well as the offer of a site near Nice by the French Government last year, there is now the prospect of an Italian site near Rome.

At this stage, however, it seems that enthusiasm among some governments is more than counterbalanced by the scepticism of others. The West German Government, for example, seems to take the view that at this stage any attempt by EMBO to force the pace of a decision on the laboratory would invite refusal. Other governments, the British among them, take the line that the first step should be to make a decision about collaboration and continuing financing of the programme of fellowships and exchange visits on which EMBO has already embarked, chiefly with money from the Volkswagen Foundation. From this point of view, the laboratory is likely to become a live issue only towards the end of 1968, although there may be some discussion of it at the next governmental meeting arranged for April.

From EMBO's point of view, however, it is somewhat disconcerting that even the less ambitious set of proposals for fellowships and exchanges may not command full support by the next meeting. Although several delegations last week are known to have come out in favour of the fellowships, both the British and Belgian Governments—for different reasons—took a more negative line.

The British view seems to have been determined by recognition, rather late in the day, that even the proposal to finance EMBO fellowships on a continuing basis raises awkward issues of principle which have not yet been given the attention they deserve. There is, for example, the issue why molecular biology should be singled out for special treatment. There is also a fear that massive support for EMBO would serve unhealthily to institutionalize molecular biology. In more practical terms, although the amounts of money involved in financing the fellowships (as distinct from the laboratory) are comparatively small, it is inevitable, now that forward planning within a fixed budget has become everybody's watchword, that funds for EMBO will have to come out of somebody else's budget.

The difficulty here is that the Medical Research Council—one obvious source of finance—is likely to decide that it knows of better ways of spending its own money. At the same time, it is unlikely that the Royal Society would take kindly to a government instruction that a large part of the £200,000 a year which it has been allotted for European fellowships in all fields should in future be earmarked for EMBO. The result, in Britain, is most probably that the government will try to resolve its difficulties by a process of enquiry and consultation within the research councils and the Council for Scientific Policy. There may be some interest to see whether it can clear up its genuine difficulties without seeming to use consultation as a means of procrastination.

Franco-Soviet Space Agreement

SOME details have now been made available of the Franco-Soviet space agreement signed in Moscow on December 9. The agreement calls for the launching of a satellite containing an experiment of French design to make a spectroscopic analysis of the Moon's atmosphere, if it has one. The satellite will be launched at the end of this year or early in 1969.

Observations made during eclipses have given grounds for believing that the Moon may contain a thin atmosphere, produced, it is surmised, by volcanic eruption of gases or by radioactive decay of surface rocks, for example, of potassium into argon. Another possibility is that the high energy particles in solar winds might erode the lunar surface to the extent of releasing hydrogen and helium atoms; these might be retained by the Moon's gravitational field for brief periods. Any lunar atmosphere will probably be so tenuous that the exhaust gases from lunar landings could radically alter its composition; the experiment must therefore be performed as quickly as possible.

Another project outlined in the Franco-Soviet agreement is a study of the magnetic fields of the Moon. It has not yet been confirmed whether the USSR will make use of the French base at Kourou in Guyana for the launching of a manned satellite.

New Nature Reserve

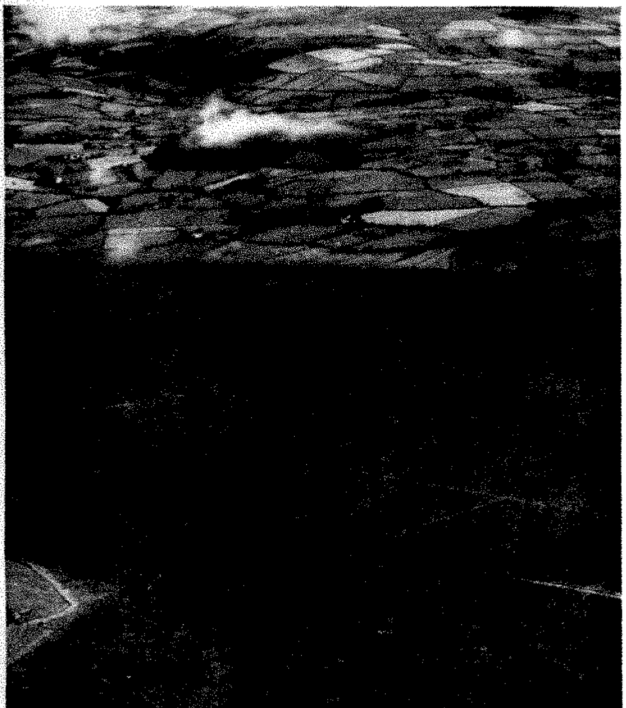
THE decision of the Natural Environment Research Council to establish a National Nature Reserve on about 182 acres of Cors Fochno, otherwise known as Borth Bog, in Cardiganshire, will be good news to many ecologists. There can now be no danger that draining will destroy a fine example of a relatively undisturbed raised bog.

Borth Bog is on the southern side of the estuary of the River Dovey and is an extensive area of acid peat which is in some places as much as twenty-three feet deep. The vegetation is very rich in mosses, and especially the genus *Sphagnum*—also known as bog moss. Among the species present is *Sphagnum imbricatum*, a peat-forming moss which is now quite rare in Britain. One of the problems now puzzling botanists is why this moss, which must have been the commonest when peat formation was progressing rapidly, has now become almost extinct.

Among the flowering plants there are no great rarities, but there are some interesting species. *Andromeda polifolia*, or bog rosemary, the pink flowered low woody plant, which is found on Borth Bog, is now decreasing on the bogs in the north and west of Britain. All three species of *Drosera*, the plant which catches insects on its sticky leaves, are found in close proximity at Borth, and this is a very rare occurrence. The fauna also has its interest; polecats, the stoat-like carnivores which are now rare except in central Wales, are numerous, and seventy-four species of butterflies and moths and twenty-two species of breeding birds have been identified.

As well as the distribution of the vegetation on the surface of the bog, and the effect on it of changes in the habitat, such as water movement, there is great interest in the history of the bog. Analysis of borings into the peat has revealed that the bog developed from fenland, later to be covered by forest, dominated by a sequence of different trees. The base of the bog is clay, containing Foraminifera—the fossil Protozoa which usually had a hard outer coat. Peat samples from 24 cm above the base of the bog contain less pollen from trees than from other plants, mainly monocotyledons, which suggests that this was fenland. The trees were elm, pine, oak and hazel. Samples from 34–54 cm above the base of the bog contain more pollen from trees than from other plants, with a marked preponderance of alder and birch pollen. The fen was evidently covered by forest, first dominated by alder, and later by birch. At 100–180 cm above the base the proportion of tree pollen decreases and there are many spores of the moss *Sphagnum*, and pollen of the small aromatic plant *Myrica gale*, also known as sweet gale or bog myrtle. The transition from fenwoods to raised bog—the peculiarity of a raised bog is that it has developed from the less acid fen—appears to be about 100 cm from the base of the profile.

This sequence resembles closely that found in the submerged forest which stretched intermittently between high and low tide marks for two miles along the coast between the villages of Borth and Ynyslas to the north. On the beach at Ynyslas there are exposed remains of tree trunks which were part of this forest.



Borth Bog, Cardiganshire, looking south-east. The Nature Reserve is shown in the middle distance. (Crown copyright; Dr J. K. S. St. Joseph.)

There is still much to be learnt about the historical aspect of Borth Bog. Dr Peter Moore of King's College, London, has recently made a series of borings in bogs, including Borth, and built up a historical picture of the human influence on the vegetation of central Wales. Pollen analysis can show, by such signs as the presence of a high proportion of weed pollen in peat samples, that man has been active in an area. There is other work in progress which is concerned with similar aspects of the history of Borth Bog, as well as various ecological studies on the surface, despite the impression given by the apparent paucity of published results. It will all be able to continue unhampered when the Nature Reserve is set up.

Documentation for Planning

HOUSING, building and planning are subjects which cut right across the conventional disciplines. As a result, the literature is diffuse and it is also inundated with trade material, good and bad, in enormous quantities. These problems make the proper organization of literature in the fields of housing, building and planning complicated, to say the least. There is not too much wrong with the two-way flow of information between North America and the countries of Western Europe, but it is a different story with Eastern Europe, Asia and Africa. There are the usual language difficulties, and most developing countries have far from sophisticated library and information services. It is hoped, however, that a new organization, an International Institute for Documentation of Housing, Building and Planning, will help. A talk about this was given recently at Aslib headquarters by Mr Agard Evans, former Chief Librarian, Ministry of Public Building and Works.

There are already in existence several organizations concerned with building and planning on an international scale—the International Council for Building Research, Studies and Documentation (CIB), and the International Federation for Housing and Planning, for example. None of the international bodies, however, appears to have the terms of reference, the funds or the facilities to cover documentation in building and planning on a world-wide scale. This is why it is proposed to set up a new institute which would be able to channel all its energies and resources into the problems of documentation. The Economic and Social Council of the United Nations has had the institute in mind for some time. Before giving final approval to the project, the council asked that an expert group of four from India, Czechoslovakia, France and Britain should undertake a feasibility study. The group, of which Mr Evans was the British member, met twice last summer and presented a unanimous report to the council in September. A draft resolution was approved and this is to be put before the full council meeting in May this year.

In outline, the proposal is to establish the headquarters of the institute in New Delhi. This will be supported by national documentation centres in each member country. Staff in New Delhi, documentalists, translators and others, will disseminate information on housing, building and planning in various ways—through "digests" (extended summaries of a selected number of primary documents, 100 per month); annotated references; abstracts (selected from those already published in the field); and "syntheses" (reviews of the subjects from primary and secondary sources). These services would be in four languages—English, French, Russian and Spanish. The national centres would assist in all this by making the information available to practitioners in their country. The institute in New Delhi would also act as an information centre for queries which cannot be answered from existing national sources. If necessary, queries could be channelled to the other international centres. Secondary functions of the institute could include standardization, classification and giving advice on any aspect of documentation in the fields covered. But it would not undertake full-length translations, critical appraisals of literature, the publishing of textbooks and manuals, nor anything falling outside the boundaries of documentation.

There is now a working party busy with all the details. If approved, the new institute should be fully operational within about 15 months. There remain some big questions. The institute will have to rely on voluntary support from governments. Will enough money be found for the capital costs and running expenses? The Indian Government is helping with staff and buildings. Are other countries going to follow? How will the institute select material? How will it be staffed? Documentalists are already hard to find.

Welsh Primary Schools

All primary schools in Wales should eventually become bilingual. With this aim as the chief recommendation in its newly published report, the Central Advisory Council for Education (Wales) must have gone a long way towards satisfying the Welsh Nationalists. The second recommendation, more the concern of educationists, calls for increased attention to in-service training and advisory services for teachers. One week's study leave on full pay for each year of service is recommended for all teachers.

A considerable amount of reorganization in rural areas is called for, so that the smallest schools will have a minimum of 50–60 pupils and not less than three teachers. Area schools in key villages are suggested for such areas, with the limit of 45 minutes being placed on the travelling time of the children. The committee comes out in favour of all-through schools for 5 to 12 year olds, rather than a two-tier system with a move at the age of 8. It is hoped that the schools can be connected with community centres dealing with social and welfare services and those which provide for leisure and further education. Liaison between these different groups in community life should be encouraged and teachers could help in this respect, particularly if, during their training, more emphasis is placed on this aspect of their work. The pattern of teacher training courses was given some attention, but the council believes that a full enquiry into the system is necessary.

The council was set up in April 1964, under the chairmanship of Professor C. E. Gittins, Professor of Education at University College, Swansea. This was some months after the English equivalent, under Lady Plowden, but the terms of reference given to both bodies "to consider primary education in all its aspects and the transition to secondary education" were identical. Professor Gittins and Miss S. E. Grey, Organizer for Infant Education for Glamorgan, served on both councils, and thus maintained close contact between the two groups. In general, the recommendations of the Gittins Report agree with those put forward by the Plowden Council, but additions are made wherever matters of special concern to Wales are mentioned.

Religious education was investigated by the council, and it was suggested that compulsory religious instruction be left to the discretion of head teachers. Professor H. D. Lewis, Professor of History and Philosophy of Religion at King's College, London, did not agree with the recommendations of the council. He believes that religious education should be compulsory, and did not sign the report. Professor D. C. Marsh, Professor of Applied Social Science at the University of Nottingham, did not support the recommendations for a fully bilingual primary education in Wales.

Action of Drugs

Professor M. J. H. Smith is the director of a new research department of biochemical pharmacology which has been instituted at King's College Hospital Medical School, Denmark Hill. The department, which consists of 2,000 sq. ft. of laboratory space, was made possible by a grant of £24,000 from the Nuffield Foundation and a subsequent grant from the University of London.

The research work in the new department will attempt to gain an understanding of the way in which drugs affect normal and diseased people; in particular the effects of anti-inflammatory drugs such as aspirin, phenylbutazone, indomethacin and various steroids used to treat rheumatism will be studied. This will involve investigating their effects on metabolic processes and tissue enzymes and using the results to explain known clinical and toxic effects of the drugs.

The cost of equipment, which includes a computer, radioactive counting material and a refrigerated respirometer, is being met by part of the £9,000 grant from the University of London, and also by a grant from King's College Hospital and Medical School Joint Research Committee. In addition, the Nuffield Foundation has made a three-year grant of £11,537 which includes a fellowship, and the Arthritis and Rheumatism Council is allocating £32,000 over the next five years.

Value from Scrap

Anything to reduce the British import bill is welcome these days, so that the work of the Warren Spring Laboratory at Stevenage in the recovery of valuable metals from scrap residues and waste is likely to be enthusiastically taken up. This particular research, which uses chemical processing techniques, is helpful to the non-ferrous metallurgical industry which depends heavily on imported raw materials. New techniques are also being developed for the extraction of rarer metals needed for high-duty alloys and for electronic devices, and for the finer purification of primary metals. The mineral industry is also likely to benefit from the new methods of dry separation of minerals which are being developed. Two separators using air fluidization which could be used in regions of the world where water, the normal medium, is scarce, are described in the laboratory's annual report for 1966, just published (HMSO, 8*s*. 6*d*.). Work in the mineral processing field accounted for some 40 per cent of the total expenditure of the laboratory.

An important 35 per cent of the expenditure was spent in the chemical engineering division. Some of the most significant experiments being done are those on the computer control of complex chemical processes. A pilot plant which produces acetone by the dehydrogenation of iso-propanol is being used to examine different forms of digital control. The computer used is an ARCH 9000. During the past year, the instruments and computer systems of the plant have been modified and extended in various ways. It is only by re-designing the plant and processes that the full possibilities of computer control can be gauged. Short specialized training courses for chemical engineers from industry are being held at the laboratory so that detailed knowledge of the methods and techniques of process control may be diffused through the process industries. A second project to study computer control has now been started. This is a study of the control by computer of a fast chemical reaction (oxidation of hydrocarbons) in a transfer-line reactor, in which effective control to obtain conditions of maximum conversion can be done only by computer. The work so far has been mainly on computer control of large-scale and complex continuous processes. The laboratory has found, however, that there could also be improved control of a number of processes on a smaller scale. Research has been started, therefore, on control of batch distillation with an assembly of digital logic and analogue elements.

Several other investigations are described in the report. These include work on viscometers and flowmeters, and the development of an on-line particle size sensor and a new fluorine analyser based on neutron activation. The work on flow properties, mixing storage and bulk-handling of granular materials has led to designs for mass-flow hoppers. These have been successfully applied in a number of different industries.

It has been estimated recently that air pollution costs Britain £350 million annually, quite apart from any losses caused by its effect on health. The laboratory spends just over 20 per cent of its total expenditure on research in this field. In 1966, increased attention was paid to pollution arising from vehicular traffic and to the reactions of pollutants in the atmosphere.

Coexistence in Botany

Since the merger in September 1967 of the Departments of Botany and Agricultural Botany at the University College of North Wales, Bangor, a noteworthy effort has been made to break down the barrier between pure and applied plant sciences.

The School of Plant Biology—as it is now called—was instigated by Professor P. W. Richards with the aim of covering the widest possible range of botanical subjects and with the intention of ending the separation between pure and applied disciplines. According to Professor J. L. Harper, who is head of the new school, the union has brought in an academic staff of twenty-three, and a number of building alterations have made it possible to house almost all the academic staff in one main building. Teaching and some research activities are, however, still spread over a number of buildings.

The school now possesses two field stations. One of these is the 28 acre research station of the School of Agriculture at Pen-y-ffridd; it has research laboratories, and controlled environment facilities are provided. The other station—Treborth botanic garden—is approximately the same size and has laboratories, woodland and an experimental garden. The greater part of the work in systematics is concentrated at Treborth, and physiology and experimental ecology are developed at Pen-y-ffridd.

Within the school there are now two M.Sc. courses—one in pure and applied ecology and a second in crop protection. The latter with an intake of about ten graduates a year is particularly valuable, according to Professor Harper, for strengthening the links with agricultural industry.

Undergraduates are, however, the main interest in the school, which still offers degrees in agricultural botany and botany. Students are offered an extremely wide range of choices in the honours degree option, so that a student can specialize in an aspect of the plant sciences covering both pure and applied science disciplines. The merger has also produced a very large research group and there are at present thirty-four students working for postgraduate degrees—a healthy number for any university department. Part of the finance is provided by the Science Research Council, part by the Natural Environment Research Council, and the Ministry of Agriculture provides studentship awards. But in addition there are a number of students engaged in work supported by industry, and there are also students supported by the Copper Development Association, the Zinc Development Association and the International Copper and Zinc Research Association.

Parliament in Britain

Agriculture

Mrs Shirley Williams, Minister of State, Department of Education and Science, stated that in the United Kingdom in 1966, 431 students qualified for university first degrees in agricultural subjects and 194 for higher degrees or diplomas. In 1967, 482 qualified for diplomas, 1,799 for certificates following full-time courses and about 13,000 for certificates in individual subjects following part-time or block release courses in further education. (Written answer, January 25.)

Power

The Parliamentary Secretary to the Ministry of Power, Mr R. Freeson, stated that the inclusion of the allowance for the effect of devaluation changed the estimate of the cost of electricity from Dungeness *B* nuclear power station from 0·56*d.* to 0·57*d.*/kWh. The effect on the operating costs at Hunterston *A* station was likely to be marginal and at Hunterston *B* was unlikely to be significant, although depending largely on the final terms of individual contracts. The total consumption of fuel oil at power stations in the United Kingdom was estimated at 7·3 million tons in 1966 and 7·4 million tons in 1967. Mr R. Marsh, Minister of Power, stated that the Central Electricity Generating Board estimated the construction cost of Hinkley Point *B* at £71/kW and the base load generating cost, including capital charges, at 0·52*d.*/kWh. For Drax, the most advanced coal-fired thermal power station offered to the board, the corresponding figures are £52/kW and 0·60*d.*/kWh. He was advised by the board that with pithead coal prices of 3*d.*, 4*d.*, 5*d.* and 6*d.* per therm, the estimated base load generation cost per kWh of the most advanced coal-fired power station would be 0·55*d.*, 0·65*d.*, 0·74*d.* and 0·83*d.*, respectively. (Written answers, January 19, 22 and 23.)

What French Scientists Do

THE results of a detailed survey of the origins and the employment of research scientists in the French public service, including higher education, have now been published by the Délégation Générale à la Recherche Scientifique et Technique (*Les Chercheurs du Secteur Public en Sciences Exactes et Naturelles*). Because the public sector employs close on 60 per cent of the 50,000 or so research workers thought to be active in France at present, the census is likely to be a reasonably accurate picture of the management of scientific resources in France. The analysis now published is based on detailed replies collected during 1963–65 from 11,000 scientists working either in the French civil service or in higher education.

Physicists account for 31 per cent of the research workers covered by the survey. Biologists account for 29 per cent, chemists for 25 per cent, mathematicians for 8 per cent and geophysicists of various kinds for the remaining 7 per cent of this population of research workers. The survey did not cover people working in medicine and related disciplines or in the social sciences. One striking feature of the results is the comparatively high proportion of women at work in French laboratories, particularly in biology. For the public sector as a whole, roughly 20 per cent of the research workers are women; the proportion varies from 11 per cent in physics to 28 per cent in biology. The report points out that in the United States the proportion of women research workers in the public sector is only 9 per cent.

As elsewhere, the population of research workers in France is comparatively young. For the public sector as a whole, the median age of active research workers works out at 35 years. Chemists and physicists tend to be younger than biologists—a third of active chemists are in their twenties compared with a fifth of biologists. Although the report raises questions about the likelihood that a person's productivity and creativity may be determined by his age, the chances are that these small differences are consequences of the different rates of growth of different disciplines.

The French labour force includes a surprisingly high proportion of people from abroad. For the public sector as a whole, it seems that 4 per cent of active research workers are from countries outside France, roughly half of them from elsewhere in Europe. For the labour force as a whole, 45 per cent of research workers are to be found in higher education and roughly a quarter of them work directly for the CNRS. There are, however, sharp differences between the distribution of people with different kinds of qualifications. The Atomic Energy Commission (CEA), for example, employs more than a quarter of all physicists, nearly two thirds of mathematicians are to be found in universities.

Of those engaged in research in France, the majority seem to be trained at French universities. The Grandes Ecoles and the similar institutions in France provide a great many qualified people in science and technology, but many of these finish up in the private sector. The result is that 53 per cent of active research workers are trained in universities, just over a third in the Grandes Ecoles and 2 per cent at institutions outside France. One interesting feature of the statistics which have been collected is that 6 per cent of those active in research appear to have had their training at Ecoles Normales Supérieures, chiefly at a handful of particularly distinguished institutions of this type. Most of the women who make a career of research seem to have a university background. The same statistics show how comparatively small a proportion of French scientists include in their training a spell abroad.

The statistics also illustrate how French scientists tend to acquire higher degrees at a comparatively advanced age. Of those active in research, 27 per cent had qualified as doctors of science, but at a median age of 42 years. The more recently introduced doctorate based on a programme of study and research at a university immediately after baccalauréat seems to be held by roughly 6 per cent of the population of active researchers but, even there, half of the scientists concerned seem to have had to wait until they were 30 before qualifying. If anything, the age of qualifying seems to have increased over the past four decades.

In the public sector as a whole, teaching seems to account for a substantial proportion of the time and energy of qualified scientists. Altogether, 18 per cent of a person's time was spent on teaching, compared with 60 per cent on research. Mathematicians as a whole are the most diligent teachers (25 per cent of everybody's time), physicists get off most lightly, with an average of 12 per cent of time spent on teaching and 63 per cent on research. Understandably, the proportion of time spent teaching is greatest among those employed in universities—just over 33 per cent compared with 41 per cent for research. In the universities, it seems to be the rule that the most junior people do the most teaching. Assistants—the lowest grade—can expect to spend 38 per cent of their time teaching and 43 per cent of it on research. By contrast, professors spent 24 per cent of their time teaching and just as much on research. The report does not define accurately what it means by teaching, but in comparison with university people elsewhere, those in France probably carry the heaviest burdens.

The survey of French research workers has set out to discover how well equipped they are to deal with scientific literature from abroad. According to the information which has been collected, 7 per cent of active research workers confess to having no knowledge of a foreign language, but the Délégation Générale is quick to point out that the corresponding proportion in the United States is 27 per cent. In France, mathematicians seem to be the least well equipped with foreign languages—13 per cent of them know no foreign language compared with only 3 per cent of chemists in a similar state of ignorance. Most scientists in France, apparently, know at least two foreign languages and among the languages quoted English was at the top of the list (83 per cent) and German next (42 per cent). Of the scientists included in the survey, 14 per cent seem to have spent some period abroad on scientific business, with doctors of science among the most widely travelled. In the physical sciences the United States seems to be the favourite visiting place (with roughly half of those included in the survey). Among geophysicists and biologists, Africa claims pride of place.

NEWS AND VIEWS

Problems of European Integration

It is, of course, disappointing that the meeting in Geneva last week about the continued financing of the activities of the European Molecular Biology Organization should have made so little progress (see page 401) This is particularly ironical at a time when the foreign ministers of several European countries, meeting in Brussels, were congratulating themselves on their willingness to collaborate in all kinds of schemes for the integration of European institutions and interests

At the same time, it is only proper to acknowledge that the proposals which EMBO has put forward raise important issues of principle To be sure, it is hard to see why these have arisen so late in the day It is also hard to see why the example of CERN does not serve, to scientists as well as to administrators, as a proof that putting money into EMBO would not be the same as pouring it down the drain If there is to be further discussion about the proposals, it is to be hoped that it will be at once public and quick It is a great misfortune that the British Government, now apparently embarking on another process of enquiry into the rightness or wrongness of the scheme, should not have done the job more thoroughly at the beginning, perhaps a year or more ago

That said, it is important not to overlook the developments which have already taken place towards the integration of scientific institutions in Western Europe The way in which several national academies in Europe are collaborating—with funds provided by their governments—on the exchange of fellows and students from one laboratory to another is a splendid development with great promise for the future In this connexion, the part which has been played in the past by the North Atlantic Treaty Organization should not be overlooked The organization has been particularly influential in supporting summer schools for senior postgraduate students and the like at dozens of centres in Europe on an increasing scale One way and another, several of these summer schools have already grown into more or less permanent and regular institutions The only trouble, of course, is to know at this stage what is to happen to activities like these, financed through NATO, when the treaty is revised in 1969

If all these schemes for increasing the cohesion of the profession of science in Western Europe are put together, however, it must be acknowledged that they do not by any means match the real need for closer integration and real exchange between laboratories and universities Moreover, it is hard to see how the existing machinery could adequately administer the very much larger sums of money that would be needed if travel between one place and another in Western Europe were to become as free as it should be for scientists and technologists

In the past few years there has also been welcome progress towards the federation of learned societies in several disciplines Biochemical societies, for example, have created not merely a common institution but a journal of their own as well The physical societies are at present in the thick of negotiating a constitution for a federal organization, and will probably be able to launch this officially in the autumn It remains to be seen how far they will be able to go in the rationalization of journals, the development of international conferences and the administration of exchanges between laboratories, but at present everybody seems to be hoping for the best But even here, unfortunately, a few swallows do not make a summer It is unthinkable that the integration of European science should be allowed to proceed by the independent decisions of academies of different disciplines to link themselves together

This is why governments should be much more ready to take a hand than they apparently were last week at Geneva The long-term need is for the creation of institutions on a European basis which can help somehow to administer policies as well as charitable donations Professor Waddington made a strong case, in his contribution to the new OECD report on science policy in the United States, for a sharing of decision-making within Western Europe on new projects of research What he had in mind was the inclusion in the national research councils such as the Science Research Council of council members from other European countries The virtues of this development would be not merely the avoidance of overlap between research projects undertaken in different countries but the creation of means for the improvement of the proposals put forward by teams of scientists in separate countries This, as it happens, is a modest version of the kind of proposal which ought now to be considered seriously in Western Europe

Applying Holography

The progress that is being made in holography was reviewed by Professor D Gabor and nine others in a symposium held during January 17–18 at the National Physical Laboratory The meeting was organized by the Optical Group of the Institute of Physics and the Physical Society in conjunction with the Stress Analysis and Materials and Testing Groups The audience numbered more than 300 for each of the three sessions

In his opening paper Professor Gabor drew attention to some of the ways in which holography could be used to fabricate unusual types of optical element If recording materials of improved phase-recording quality could be found, then several uses could be foreseen for composite reflectors containing a very large number of stacked Lippman reflecting layers. He referred also to the increasing American interest in acoustic holography, which could be an attractive alternative to X-rays in medical diagnosis.

Of the papers that followed, two dealt with ultrasonics and with the problems of recording particle tracks in a bubble chamber, and three were concerned with fundamental optical aspects of holography. The paper by J. W. C. Gates (NPL) emphasized the advantages of pulsed laser holography and included a live demonstration in which a hologram was handed round the audience only minutes after it had been recorded on the platform.

The remaining papers dealt with measuring applications and made it clear that, although holography as a means of processing information has been studied for many years, its more recent role as a measuring tool is at least equally important.

The paper by A. E. Ennos (NPL) reviewed some of the ways in which hologram interferometry can be used to measure either minute changes in the shape of an engineering component or departures from a holographically recorded master shape. The highlight of this presentation was a short cine film which demonstrated the advantage of a new stroboscopic method, as opposed to the time averaged technique, for studying the vibrations of an acoustic resonator the resonances of which are sharp. J. M. Burch (NPL) described a new holographic version of the "moiré shadow" test which should make it possible to test a diffusely reflecting component such as a turbine blade with a reduced sensitivity of perhaps 400 fringes per centimetre of error. W. R. B. Bradford (EMI) illustrated the experimental precautions that are needed for holographic metrology, and showed results that should be of forensic as well as of engineering interest. It appears, for example, that if a floorboard is dented, Dr Bradford can determine which if any of the dents is less than an hour old.

The brief symposium was accompanied by several holographic demonstrations which were arranged by the NPL Division of Optical Metrology in an adjoining laboratory. A high degree of interest was shown throughout, and this augurs well for a full international conference on "Engineering Uses of Holography" which is to be held at the University of Strathclyde between September 17 and 20.

Extraction by Bacteria

from our Microbiology Correspondent

FOURTEEN years ago it was found that certain sulphur-oxidizing bacteria could accelerate the extraction of copper sulphide ores. Considerable use is now made of this leaching process which has rapidly attained importance as the necessity to exploit low-grade ores has arisen. This extraction process has been studied both in the laboratory and the field, and reports in the current number of *Biotechnology and Bioengineering* indicate the present state of our knowledge and the problems involved in optimizing the conditions for a successful commercial operation. Beck (*Biotech. Bioeng.*, IX, 487, 1967) has surveyed the practice of copper leaching in several mines in the western United States and, although the control of the process and the extent of the recovery varied greatly, essentially the same procedure was employed at all mines. Where convenient, the waste or low-grade ore (up to 20 million tons) is deposited in a ravine which forms a natural collection system and water is percolated through the ore dump. Pregnant waters rich in soluble copper and ferric iron feed a collection pond and then are piped to a precipitation plant where the copper is recovered and the iron is in part reduced to ferrous sulphate. Spent waters are subjected to further microbiological action in an oxidation pond in which iron is precipitated as hydrated ferric sulphate with a concomitant decrease in acidity. Finally, the spent waters are recycled through the ore dump and sulphuric acid added *en route* to maintain optimal *p*H conditions. Beck's survey proved a consistent association of *Thiobacillus ferrooxidans* with leaching waters and exposed ores and a massive development of this bacterium in the oxidation ponds. Stimulation of thiobacilli in this latter site provides a large inoculum in the percolate and removes much of the iron which might precipitate and cement in the upper layer of the dump and thereby impede percolation. As the water passes through the packed ore the viable thiobacilli decrease in number, an observation which could be taken as inconsistent with their proposed activity on sulphide ores. The massive dimensions of the leaching system, however, are likely to impose absorption and filtering out of bacteria and produce stratification and localized accumulations of organisms. In turn, localized heating, up to 80°, would have a two-fold effect—a decrease in the bacterial population, and a possible enhancement of chemical oxidation at the expense of microbiological activity.

The exact contributions which bacteria make to copper leaching are not known fully, but the efficacy of their activity is determined by a complex interaction of physical, chemical and biological factors. Ehrlich and Fox (*Biotech. Bioeng.*, IX, 471, 1967) ascribe three major functions to the thiobacilli: (1) direct oxidation of the sulphide ores, (2) regeneration of ferric iron which becomes involved in chemical oxidation of the ores, and (3) depressing the release of iron from the ores. Although it is possible only to assess the combined contributions made by thiobacilli at present, Ehrlich and Fox have studied parameters such as the nature of the gangue and mineralogy of the ore, particle size, the availability of oxygen and carbon dioxide, the *p*H, E_h and temperature, and the nutritional status of the system. This last point may affect the growth of heterotrophic micro-organisms, and either promote or inhibit the extraction process, by, for example, acid and sulphide production respectively. Unfortunately the scaling up of laboratory findings to field situations has had only limited success so far, and it is clear that more thorough evaluation of the *in situ* process along the lines of Beck's survey is a very necessary adjunct to simulated experiments in the laboratory.

The Wake of the Moon

from our Magnetosphere Correspondent

IN July 1967, the National Aeronautics and Space Administration in the United States succeeded in injecting the satellite Explorer 35 of the IMP series into orbit around the Moon. The instruments included two magnetometers and a plasma probe, and the preliminary results from one of the magnetometers (Colburn *et al.*, *Science*, **158**, 1967) tell an interesting story which suggests that when there has been time for the data from one of the magnetometers and the plasma probe to be put together, progress will be made with a basic plasma problem. This kind of data analysis necessarily requires a considerable time, however, and the data from each instrument are first studied separately. It may be noted that the Russian lunar orbiter Lunik also carried a magnetometer, but the measurements (Zhuzgov *et al.*, *Cosmic Investigations*, **4**, 880, 1966) showed a magnetic field strength an order of magnitude stronger than that found by Explorer 35. Since it is much easier to imagine causes of error leading to spuriously large fields than to spuriously low fields, it is most probable that the Lunik measurements are erroneous. It may also be noted that some check on the Explorer 35 measurements can be obtained by comparison with Explorer 33. This was launched a year earlier, was intended to orbit the Moon, but went into an Earth orbit with apogee farther than the Moon and is still operating. Furthermore, Explorer 35 is spin-stabilized along an axis nearly perpendicular to the ecliptic and any spacecraft field perpendicular to this axis is corrected for. The altitude of periselene is about one half of a lunar radius and that of aposelene about 4.5 lunar radii.

One remarkable negative discovery is the lack of a bow shock in front of the Moon. This would have appeared as a rather sudden increase in field strength and onset of turbulence as the satellite crossed the shock from upstream. The Earth's bow shock is clearly seen in the variance of the magnetic measurements on Imp 1 (Ness *et al.*, *J. Geophysical Res.*, **69**, 3531, 1964) in a distance quite small enough compared with the size of the Moon. The absence of a bow shock implies that the Moon does not appreciably disturb the flow of the Solar Wind. An early speculation (Gold, in *The Solar Wind*, Pergamon Press, 1966) suggested that the Solar Wind protons would be neutralized at the lunar surface but that an electromagnetic interaction would be found if the whole Moon had an electrical conductivity comparable to that of the Earth's crust. Briefly, in the Solar Wind the electric field is zero in the frame of the plasma and hence non-zero in the frame of the Moon, unless the magnetic field is parallel to the wind—an unusual situation. Such an electric field would drive a current through a conducting Moon in a direction which would increase the magnetic field on the upstream side; if this increase were appreciable, a shock should occur. It can be concluded that at least the outer layer of the Moon is a good insulator; by studying the time-variable fields near the Moon, electromagnetic sounding of the internal conductivity should be possible and hence some indication of internal temperature.

The other discovery by Explorer 35 is an enhancement of magnetic field strength downstream of the Moon which is plausibly interpreted as the result of a plasma void where the wind has been intercepted by the Moon. This is best understood hydromagnetically as an increase of magnetic pressure in the void caused by the lateral pressure of the plasma outside. The increase of the field propagates inward at the Alfvén speed in the void (assumed to be less than the speed of light) while the plasma closes in at about its thermal speed. The shape of the void should be mapped out as the aposelene of the lunar satellite swings round on the dark side. The structure of the plasma front moving into the void is a basic plasma problem of great interest, previously inaccessible to detailed measurement. The enhancement of the field in the void is not particularly reproducible, but this is probably accounted for by variations in the interplanetary field. Still less reproducible, but usually present, are large but short-lived decreases of field strength on either side of the void. Hydromagnetically this implies an increase in lateral pressure in the plasma which has just missed the Moon. This is plausible but not properly understood, and again suggests that the combination of the data from the plasma probe and the magnetometer will be fruitful.

Sequences and Structures

from our Molecular Biology Correspondent

ONE of the most far-reaching technical advances in the nucleic acid field in recent years has been the development by Sanger of procedures for separating and identifying radioactively labelled oligonucleotides. This culminated in a determination of the complete sequence of an RNA of 120 residues, containing no "unusual" bases, such as could be used as markers in the sequencing of transfer RNA species. The subject of this *tour de force* was the 5*S* RNA associated with the ribosomes of *E. coli*. Similar 5*S* species are present in other ribosomes, but their function remains obscure. The sequence of one of them, from mammalian ribosomes, has now been reported by Forget and Weissman (*Science*, **158**, 1695, 1967).

This RNA, isolated from KB carcinoma cell ribosomes, has 120 residues, and has other features in common with its bacterial counterpart; the 3′-terminus is uridine in both cases, but the KB cell RNA has guanylic acid, rather than uridylic acid, at the 5′-end. The sequence presents the possibility of fairly extensive base-pairing, and in particular complementary sequences of nine nucleotides occur at the two ends of the chain (residues 1 to 9 complementary with 118 to 110), so that these must be presumed to be united in a double-helical segment. In *E. coli* 5*S* RNA there are sets of ten complementary bases at the ends, but in both molecules isolated G-U pairs occur in these sequences. Other sets of complementary sequences, including one of nine residues, are found. As in the bacterial RNA, there is a run of five residues which is complementary to a four- or five-base sequence common to all transfer RNAs so far determined. This then reinforces speculation about a site for the attachment of *t*RNA to the ribosome.

The emergence of the primary structures of the various 5*S* and transfer RNAs has naturally provoked a fever of activity in a number of quarters. Most of all, it has stimulated attempts to work out the secondary, and even the tertiary, structure of these molecules solely by contemplation of the sequences. In favour of this agreeable pursuit, it must be said that the "cloverleaf" model for *t*RNA, which was deduced by comparing the several known sequences, remains an unchallenged working hypothesis, at least in respect of the pattern of base-pairing. The first serious attempt to establish experimentally how it relates to the overall appearance of the molecule comes now from Lake and Beeman (*J. Mol Biol*, **31**, 115, 1968) They have measured X-ray scattering at low angles from yeast transfer RNA in aqueous solution The scattering profile depends on the distribution of matter within the solute molecule, and it is possible to calculate the scattering for given models for the tertiary structure with considerable precision. Starting from a "cloverleaf" arrangement, containing four paired segments, Lake and Beeman are able to assert that the limbs are not splayed out, the *t*RNA is in fact a compact and disymmetric structure, and a good fit between experiment and theory can be achieved if the arms are packed tightly together The best fit is for a two up – two down configuration, but three up and one down is not excluded Granted the original "cloverleaf", therefore, this work must be seen as a considerable step towards a picture of the molecule

No such working model exists as yet for the 5*S* RNA An attempt at characterizing the secondary structure has been made by Boedtker and Kelling (*Biochem Biophys Res Commun*, **29**, 758, 1967) They have shown the existence of a considerable degree of base-pairing by demonstrating that the structure collapses when treated with formaldehyde The absence of any significant ionic strength dependence of the sedimentation coefficient indicates that there is in fact a rigid tertiary structure Measurements of ultraviolet hypochromicity in the native state, by comparison with that in the formaldehyde-treated material, have been used to obtain a limiting value for the extent of base-pairing For reasonable values of mean hypochromicity for paired and unpaired RNA chains, it is found that some 60 per cent or more of the bases are paired

Zonal Centrifugation

from a Correspondent

MORE than 200 people attended the British Biophysical Society meeting on zonal centrifugation on January 26, organized by Professor P Alexander at the Chester Beatty Research Institute Laboratories at Sutton Professor D E Hughes in his opening remarks described the development of the zonal centrifuge as a major breakthrough in the separation of biological material One of the major fields of study in contemporary biological science is the relationship between function and structure In order to study this it is necessary to obtain sub-cellular components in as pure a state as possible The old method of differential centrifugation produced very impure fractions, while the introduction of separation by density gradient centrifugation in swinging bucket rotors improved the quality of the fraction at the cost of greatly reduced yield With zonal centrifugation using rotors with capacities of up to 1,700 ml, it is possible to obtain fractions in quantities sufficient for almost any analytical procedure

Dr N Anderson of Oak Ridge who, with his co-workers, has been responsible for the development of the zonal centrifuge, started the proceedings with a major contribution entitled "Recent Advances in Zonal Centrifugation" This paper, lasting nearly an hour, was delivered with expertise and humour He described a number of interesting modifications in technique which can be applied to zonal centrifugation and which considerably extend its potential Among the successful applications so far is the production of highly purified viral vaccines using a flow-through rotor to process 1 to 2 litres per hour of culture medium One of the latest developments at Oak Ridge is a new generation of rotors, the "*K*" series, capable of handling four or five times the quantities of the present "*B*" series

The following papers were each concerned with the isolation of one or more components from disintegrates of biological material The overall impression was that the workers had achieved a degree of resolution and reproducibility not obtainable with any other method Dr J Hastings (Chester Beatty Research Institute) described the separation of different species of RNA using the *B* XV titanium rotor

Dr C. D Birnie and Dr Harvey (Imperial Cancer Research Fund) then showed some excellent separations of polysomes, ribosomes and ribosomal sub-units In the afternoon session, together with Miss S M Fox, these authors also described the use of the continuous flow rotor for the isolation of viruses

During the luncheon break, demonstrations of *A*, *B* XIV and *B* XV rotor systems were given by MSE of Crawley, England The afternoon session was opened by Dr R G Wallis (Microbiological Research Establishment), who described a simple containment device for use in conjunction with the zonal centrifuge for the isolation of non-pathogenic viruses

The complex problem of the isolation of cytomembrane and plasma membrane fragments from liver homogenates was dealt with by Dr E Reid, Mr R H Hinton and Mr J T R Fitzsimons (University of Surrey), and this was followed by a paper on "The Separation of Mitochondria and Lysosomes" by Mr G C Hartman and Mr R H Hinton.

Dr D Lloyd (University College of South Wales and Monmouthshire), using the *A* rotor, showed that the zonal centrifugation of a fraction rich in mitochondria resulted in the mitochondria separating into fractions having different metabolic properties but appearing as a broad band on the zonal profile Dr A P Mathias (University College, London) showed what degree of separation of nuclei could be achieved using the *A* rotor Using low speeds of the order of 600 r p m, he had separated diploid and tetraploid nuclei from rat liver

The final paper by P J G Avis, Dr C Dean, Dr A Driedger and Dr L Hamilton (Chester Beatty Research Institute) dealt with the separation of disintegrates of *Micrococcus radiodurans* using the *B* IV rotor A novel feature was the lysing of the bacterial cells within the rotor to avoid excessive shearing of the DNA during the introduction of the sample through the rotating seal

This was the first meeting to be held in this country devoted entirely to the technique of zonal centrifugation and drew one of the highest attendances for a meeting organized by the society

Lactose Repressor

from our Cell Biology Correspondent

THE isolation at Harvard University of the lactose operon and λ-phage repressors by Gilbert and Muller-Hill and Ptashne has made possible fundamental experiments to detect how genetic repressors act at the molecular level Last year, Ptashne (*Nature*, **214**, 232, 1967) showed that the λ-phage repressor binds specifically to the operator region of the λ-DNA molecule. This result supports the simplest model of repression—namely, that the repressor protein itself binds to DNA and that this binding somehow prevents transcription of the subsequent genes into *m*RNA In the current issue of *Proc US Nat Acad Sci*, (**58**, 2415; 1967), Gilbert and Muller-Hill report that the lactose repressor binds to the operator region of lactose operon DNA molecules and, furthermore, that the repressor molecules are released from the complex with DNA by inducers.

To do the experiments, Gilbert and Muller-Hill labelled *E coli* with radioactive sulphur and then isolated from the cells protein sedimenting at 7–8*S*, which includes the repressor. About 1 per cent of this labelled protein binds to DNA isolated from a defective λ-phage which carries the *E coli lac* operon in place of part of the λ-genome Moreover, a synthetic inducer of the lactose operon, isopropyl-1-thio-β-D-galactoside (IPTG), releases the bound protein from the *lac* DNA This proves, first, that the 1 per cent of 7–8*S* protein which binds to the DNA is indeed *lac* repressor—not just sticky protein—and second, that inducers work by releasing repressors from the repressor DNA complex On the other hand, o-nitrophenyl-β-D-fucoside (ONPF), a substance which binds to repressor but does not induce expression of the *lac* operon *in vivo*, has no effect in the *in vitro* binding of repressor to *lac* DNA, nor does ribonuclease

Gilbert and Muller-Hill have proved that the *lac* repressor binds only to the operator region of the *lac* operon by measuring the binding of repressors to the DNA of two constitutive mutants of the *lac* operon In the absence of inducer, one of these mutants produces *lac* operon enzymes at 20 per cent of the fully induced level, and the other mutant, a low level constitutive, produces only 1 per cent of the full level in the absence of inducers The basal level of the enzymes in wild type cells, in the absence of inducers, is only 0 1 per cent the full level The expression of the *lac* operon in the absence of inducers is explained as a failure of the repressor to bind to the operator site If so, the *in vitro* binding of repressor molecules to the DNA of the two constitutive mutants ought to be weaker by a factor of several hundred for the 20 per cent constitutive and at least ten times weaker for the 1 per cent constitutive than to wild type DNA In binding experiments Gilbert and Muller-Hill have also found, as expected, that the amount of repressor bound to the *lac* operon DNA of the constitutive mutants is less than the amount bound to wild type DNA The repressor, therefore, must bind to a unique sequence in the operator and, because no binding occurs between repressor and denatured DNA, the repressor must bind to double-stranded DNA Ptashne (1967) has shown that this is also the case for λ-phage repressor

On the basis of these isolation procedures, Gilbert and Muller-Hill estimate that each haploid *E coli* cell contains 10 to 20 repressor molecules Each molecule must recognize a stretch of at least 11–12 base pairs, which corresponds to at least one turn of the DNA helix, a stretch of 35 Å The *lac* repressor, with a molecular weight of 150,000, is a sufficiently large molecule to cover this amount of DNA

Now that the Harvard group has established beyond doubt that repressors act by binding to the operator, what comes next? If sufficient crystalline repressor can be isolated, it should be possible to determine by X-ray crystallography the molecular structure of the repressor and the allosteric changes that occur when repressor binds to DNA And, of course, there is still the problem of how, by binding to the operator DNA, the repressor prevents transcription

A New Ribonuclease

from our Cell Biology Correspondent

ZINDER'S group at the Rockefeller University has now reported the discovery and partial purification of a new ribonuclease—ribonuclease III—from *E coli* (Robertson, Webster and Zinder, *J Biol Chem.*, **243**, 82, 1968, and *Virology*, **32**, 718, 1967) Ribonuclease III is apparently specific for double-stranded RNA molecules Because of this unique property—all other known ribonucleases digest single-stranded RNA molecules—RNase III should prove extremely useful as an experimental tool The new enzyme, which occurs in *E coli* loosely attached to ribosomes, is an endonuclease, it attacks RNA chains internally rather than by clipping single bases from one or the other ends of an RNA chain

Robertson *et al* originally detected the enzyme when they found that about 2 5 per cent of the Qβ RNA added to an *S*30 extract of *E coli* to programme *in vitro* protein synthesis was degraded while the rest of the RNA remained intact Because about 2 5 per cent of the RNA isolated from Qβ phage by phenol extraction is known to be double-stranded, they inferred that the *S*30 extract contained an enzymatic activity——RNase III—which specifically digests double-stranded RNAs This they confirmed by digesting other double-stranded RNA molecules, including *R*17 replicative form RNA, reovirus RNA and synthetic double-stranded ribopolynucleotides, with *S*30 extract Tests with *f*2 RNA as messenger, however, showed that the presence of RNase III in the *S*30 extract does not have any gross effect on *in vitro* translation

Starting from these observations, it has now been discovered that the RNase III activity is associated with the *E coli* ribosomes and that it remains bound to the ribosomes at low salt concentrations, but that it is released by washing the ribosomes in buffered 0 2 M (or above) ammonium chloride Unlike RNase I, RNase III is not released from *E coli* cells by osmotic shock Zurdo and his colleagues have partially purified the enzyme, released from ribosomes, on 'Sephadex' columns and find that it has an absolute specificity for RNA molecules containing double helical regions, it has no effect on single-stranded RNA or on single-stranded or double-stranded DNA, and these polymers do not competitively inhibit the digestion of double-stranded RNA This property of RNase III, together with its absolute requirement for Mg^{++} and Mn^{++} and NH_4^+, K^+ and Na^+ cations, its *p*H optimum in the range of *p*H 7 4–*p*H 9 7, and its endonucleolytic activity—the enzyme cleaves internal phosphodiester bonds to yield 3′-phosphate and 5′-hydroxyl groups—clearly distinguish RNase III from any previously described RNase or DNase

RNase III can distinguish between double-stranded RNA and double-stranded DNA, and the secondary structure of double-stranded RNA is closer to the *A* helical form of DNA than the *B* helical form. This suggests that part of the specificity of RNase III resides in the ability to distinguish the *A* form polynucleotide helix. Preliminary experiments have shown that RNase III digests the RNA from a DNA/RNA hybrid. This result supports the suggestion that substrates for RNase III are *A* helices because the hybrid molecule has an *A* helix configuration.

Whatever the function of RNase III *in vivo*, the enzyme will certainly be useful to experimenters. It can obviously be used to analyse the role of double-stranded RNA in RNA phage replication, to produce limited digestion of predominantly single-stranded RNA molecules which have some helical content, and in hybridization experiments to selectively digest the RNA from a DNA/RNA hybrid.

Brain Amines and Drugs

from our Clinical Biochemistry Correspondent

In the past ten years, drugs which affect brain metabolism and human behaviour have been consumed in ever increasing quantities. A biochemical theory of affective disorders has been developed in which depression is the result of a deficiency in the concentrations of serotonin and norepinephrine in the brain, and mania is the converse state with too high a concentration of amines (Schildkraut and Kety, *Science*, **156**, 21; 1967). Much of the evidence comes from animal experiments, and all drugs are developed by experimenting on animals. The relation between biochemical and behavioural effects in different species is therefore of great interest. G. R. Pscheidt (*Comp. Biochem. and Physiol.*, **24**, 249; 1968) has now collected comparative data on the distribution of serotonin and norepinephrine in the brain and of the effects of reserpine, monoamine oxidase inhibitors and lysergic acid diethylamide (LSD).

The hypothalamus usually contains the highest concentration of serotonin and norepinephrine (Amphibia have epinephrine instead of norepinephrine). Birds and fishes are unusual in having relatively high amine concentrations in the telencephalon. The cortex, which is very low in amines, makes up only a very small proportion of the telencephalon in these species. In general, the parts of the brain which are older in an evolutionary sense contain the greatest concentration of free amines, and these are the parts concerned with emotional behaviour.

The monoamine oxidase inhibitors are a group of drugs which are thought to increase the concentration of amines in the brain by inhibiting their breakdown. They certainly do increase the concentration of serotonin in all the species studied, but in the cat, dog and frog the concentration of norepinephrine was not increased. Although large numbers of depressed patients have been treated with monoamine oxidase inhibitors, only one study has shown that they increase amine concentrations in human brain and that was concerned with the action of only one drug. Because animal experiments indicate that there is no correlation with species or drug in the dose of drug necessary to produce a 50 per cent increase of amine concentration, the true effect of these drugs on human brain amines is largely unknown.

Reserpine reduces the concentration of brain amines and produces sedation or tranquillization in all species studied except the frog. (Sedation occurs only if the frog has been pretreated with a monoamine oxidase inhibitor.) When reserpine and a monoamine oxidase inhibitor were given together, the amine concentration was usually intermediate between the concentrations achieved by either drug alone, but in the monkey and rabbit the combination actually gave higher concentrations of serotonin than the inhibitor alone.

Origin of the Diffuse Interstellar Bands

by

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Unidentified diffuse interstellar bands may be caused by electronic transitions of impurity ions in grains which are coupled to the lattice vibrations. The 4430 absorption band has a significantly asymmetric profile, being somewhat steeper on the red side than on the blue. This feature is a prediction of the solid state model proposed for the band.

Broad diffuse absorption features, such as the 4430 band, which occur in the spectra of distant stars have defied identification for more than 30 years. The interstellar origin of these bands seems to be beyond serious question and their association with interstellar dust is likely. Most recent attempts at a theoretical explanation of these bands have involved a search for transitions to closely spaced rotational and vibrational energy levels in molecules attached to grains. Herbig[1] has proposed that transitions from the metastable $c^3\Pi_u$ level of the H_2 molecule may account for the 4430 band, but this model can be criticized on account of the unfavourable conditions which exist for the population of the $c^3\Pi_u$ level[2]. More recently, Johnson[3] has proposed that transitions in

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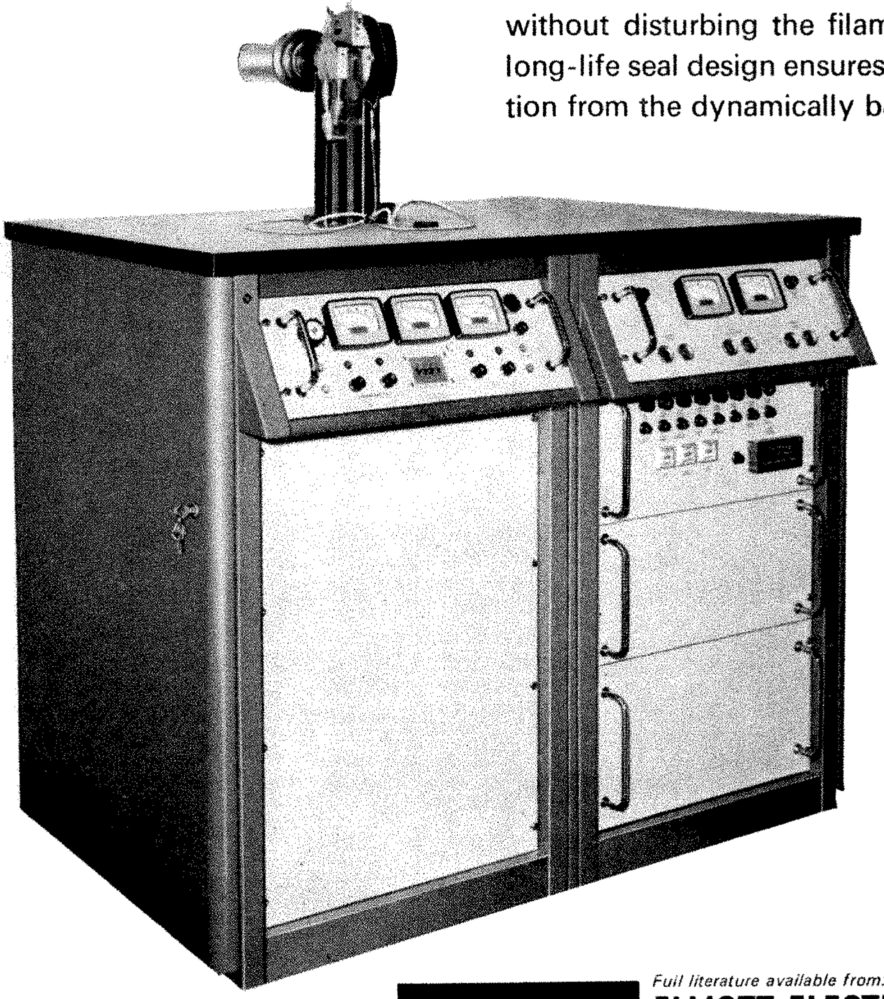

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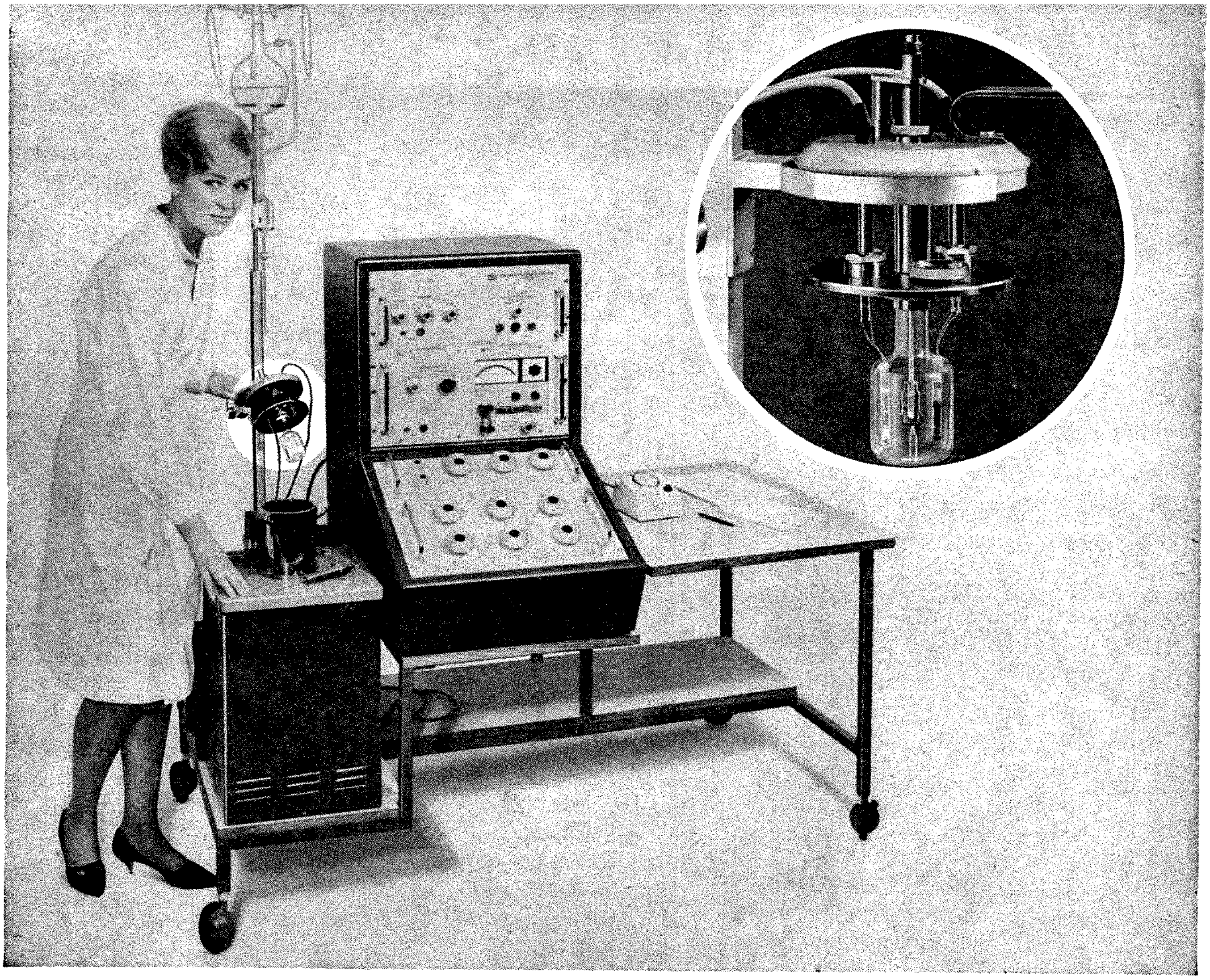

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complex molecular aggregates similar to chlorophyll could be responsible for the diffuse bands. Such a model does not seem to be even remotely plausible because of the lack of suitable conditions for the formation or the stability of such complex molecules in the interstellar medium.

A new line of investigation has recently been suggested[4]. It has long been known that the spectra of certain impure crystals exhibit broad absorption or emission features in the visible and near ultraviolet spectral regions[6-9]. Such bands may in general be attributed to electronic transitions in impurities which are coupled to the lattice vibrations. The widths of such bands may range from ~500–30 Å and the f-values may range from 1 to 10^{-2}. As already pointed out[4], some of these bands bear a striking resemblance to the 4430 band. Assuming that the 4430 band is caused by an electronic transition at an impurity site, we may calculate the concentration of impurities which is required. The absorption cross-section σ_0 near the band centre per impurity ion is given by

$$\sigma_0 = \frac{4\pi e^2}{\gamma mc} f \qquad (1)$$

where f is the oscillator strength and γ is the half-width of the band in frequency units. With $\gamma(=\Delta(c/\lambda))$ as appropriate for the 4430 band we have

$$\sigma_0 \approx 10^{-14} f \text{ cm}^2 \text{ per impurity} \qquad (2)$$

For a star at 1 kpc distance the observed absorption at the centre of the 4430 band is typically ~0.1 mag. In order to produce such an absorption we require a number density of impurities N given by

$$0.1 = \Delta m \cong 1.086\, \sigma_0 N \times (3\times 10^{21}) \qquad (3)$$

that is

$$N \cong 3 \times 10^{-9} f^{-1} \text{ cm}^{-3} \qquad (4)$$

For f in the range 1–10^{-2} and a total grain density $\sim 3\times 10^{-11}$ cm^{-3} we thus require ~10^2–10^4 impurity atoms per grain. Such an impurity concentration would seem entirely permissible in a grain consisting of ~10^9 atoms.

A particular class of defect band which has been studied in some detail is the so-called F-centre in alkali halides. In this case, we have an electron trapped at a negative ion vacancy. A small fraction of such vacancies is present in any crystal at thermal equilibrium, but their concentration could be enhanced by irradiating the crystal with X-rays or thermal neutrons. At such a vacancy an electron is in a potential well which may be approximated crudely by $\varphi = $ constant inside a spherical cavity representing the vacancy and $\varphi = -e^2/\varepsilon r$ outside it. The electron accordingly has hydrogenic energy levels with a $1s$ ground state and a $2p$ first excited level. By the usual selection rules the transition $1s$–$2p$ is strongly permitted with an f-value close to unity. The electronic transition gives rise to a broad band, for each of the electronic levels is split into many phonon states. Experimental results for a large number of alkali halides indicate that the central wavelength of this band depends almost entirely on the lattice spacing d, being approximately $\propto d^{1.8}$ (ref. 10). It varies from ~8000 Å in RbI to ~2500 Å in LiF. The width (at low temperatures) is typically in the range 300–500 Å, considerably broader than the generally recognized diffuse interstellar bands. It is, however, not impossible that such very broad diffuse features exist in stellar spectra, but do not show up above the noise level of the spectrograms. In this connexion it might be possible to attribute the sharp knee at ~4300 Å in the extinction curves of Nandy[11,12] to a weak, but very broad, F-centre at this wavelength superposed on a more gradual change of slope in the extinction curve given by the Mie scattering theory.

A more general class of impurity band is caused by an electronic transition in a foreign ion or radical occupying a lattice site of the crystal. In this case also it is an unfortunate circumstance that most of the available experimental data relate to impurities in alkali halide crystals. The physical processes involved, however, would apply to suitable impurity ions in any crystal lattice; but the central wavelength as well as the width of the band is determined by the particular impurity ion/host lattice combination. The purpose here is to recognize certain general structural features to be expected from these "solid-state bands" and attempt to identify such features with the known interstellar bands.

Wagner and Bron[8] have obtained absorption and emission bands in the blue region with widths ~100 Å for rare earth ions in alkali halide crystals. These bands arise from electronic transitions within the $4f$ configuration and are therefore parity forbidden in the free ion. Lattice vibrations, however, produce a non-zero electric field at the ion and the resulting Stark effect allows a mixing of states with odd and even parities. The net result is that the transition in the crystal becomes weakly permitted with an oscillator strength of ~10^{-2}. Similar effects may be expected to occur for impurity ions belonging to the iron group where electronic transitions may occur within a $3d$ configuration (personal communication from R. J. Elliot).

We urgently require experimental data for crystals of ice and/or graphite with sufficiently abundant impurity species such as Si, Mg, Fe, Ti. Until such data become available we can only hope to identify certain distinctive qualitative features to be expected on the basis of this model.

Let us now consider a very crude representation of the physical process involved. Suppose, initially, that the impurity ion in its ground electronic state is surrounded by host neighbours. Let q_0 be a co-ordinate parameter

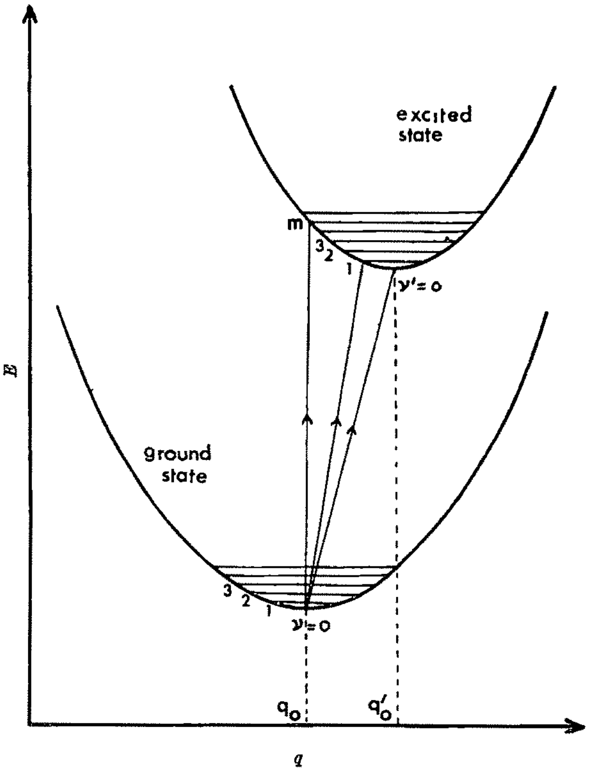

Fig. 1. Schematic energy level diagram showing the vibrational splitting of the electronic energy levels in the impurity ion.

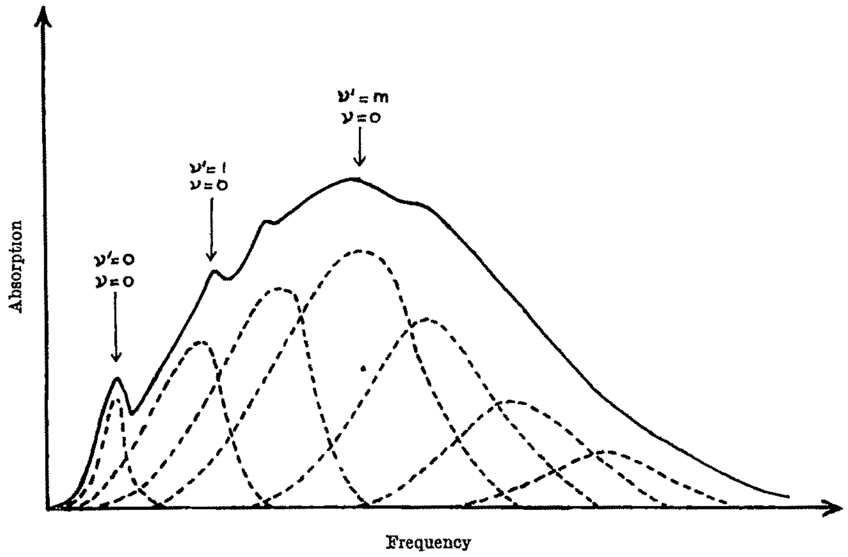

Fig 2 Schematic representation of the absorption profile to be expected due to transitions between phonon states associated with two electronic energy levels The red side of the band is in general steeper and may have more vibronic structure than the blue side (Compare with Fig 6 of ref 7)

which defines its mean equilibrium position relative to the nearest neighbours When the ion is in its upper electronic state the lattice will be locally distorted by the electric fields and q_0 will be increased to q'_0. In either situation the ion may be regarded as a harmonic oscillator vibrating about its mean position with equally spaced energy levels. The energy levels of the entire system may be represented schematically as in Fig 1. $\nu=0,1,2 \ldots$ and $\nu'=0,1,2$. denote the vibrational energy levels associated with the lower and upper electronic states, respectively $\nu'=m$ is the vibrational level of the excited electronic state which lies immediately above the $\nu=0$ level of the ground electronic state The "no phonon" transition from $\nu=0$ to $\nu'=0$ is usually very weak because it is forbidden by the Franck–Condon principle Transitions from $\nu=0$ to $\nu'=1$, 2 . become successively stronger until $\nu'=m$. This transition is strongest according to the Franck–Condon principle For $\nu'>m$ the successive transitions become progressively weaker Fig 2 shows schematically the additive effect of such transitions. The widths of the individual component bands are due to the dispersion of vibrational levels in the lower electronic state. For a more detailed explanation of the shape of these bands we refer the reader to M. H. L Pryce[7] Although varying degrees of asymmetry are found in the experimental solid state absorption bands, the general result which is borne out in Fig 2 is that the red sides of the bands are somewhat steeper and have more vibronic structure than the blue sides (see refs 7–9) The interesting possibility now arises that the predicted characteristics of the solid state band may be subject to an observational verification.

The determination of the precise shape of the 4430 band including the vibronic structures would require spectrograms at very high dispersion—somewhat higher than that available in the results of Seddon[13] Even with the available resolution, however, it may be possible to detect any marked asymmetry, if present, in the 4430 profiles Unfortunately, several stellar lines occur within the 4430 band and it will be necessary to make allowance for them. We have attempted to eliminate the stellar lines by differencing the 4430 intensities in pairs of stars of identical or very similar spectral type, one of which has weak 4430 and the other strong 4430 features Such a procedure would be expected to produce a cancellation of the stellar atomic lines and the residual profile may be

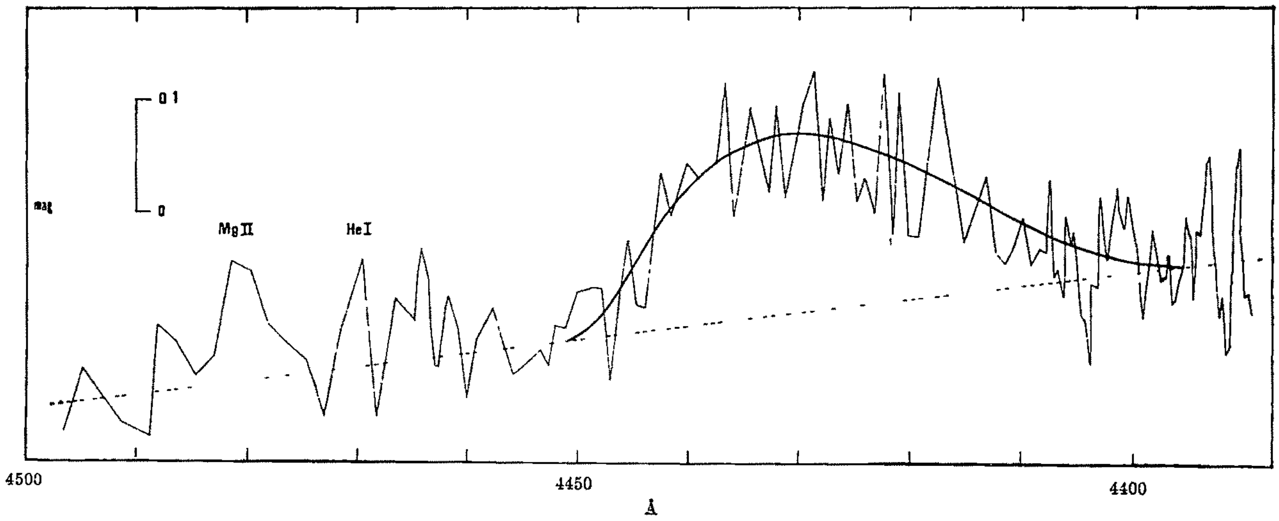

Fig 3 Mean profile of the 4430 band extracted from 3 pairs of stars Heavy line represents the best mean curve through the observational points, smoothing out fluctuations

Table 1 PAIRS OF STARS COMPARED

	Stars	Sp type	E_{B-V}	W_{4430}
(1)	*HD* 224055	*B3Ia*	0 91	2 11
	HD 198478	*B3Ia*	0 60	1 24
(2)	*HD* 224055	*B3Ia*	0 91	2 11
	HD 14134	*B3Ia*	0 67	1 24
(3)	*HD* 43384	*B3Iab*	0 65	1 54
	HD 42087	*B2 5Iab*	0 42	0 98

attributed to the features of the 4430 band including noise Three pairs of stars considered are listed in Table 1

The curve of the mean magnitude differences from the three pairs is plotted in Fig 3. It is interesting to note that the red side of the band is considerably steeper than the blue side, which is in good general agreement with our theoretical expectations (compare Fig 2) The spectral resolution in our data does not, however, permit us to detect vibronic structure which may also be expected on the red side of the band

The attempt by Herbig[14] to detect fine structure in the band occurring in the spectrum of *HD* 183143 was unsuccessful, this investigation may be worth repeating with a differencing procedure such as that employed here

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[1] Herbig, G H , *Astrophys J* , **137**, 200 (1963).

[2] Wilson, R , *Publ Roy Obs Edin* , **4**, 67 (1964)

[3] Johnson, F M , In *Interstellar Grains*, Proc Colloqu , Troy, N Y , August 1965 (edit by Greenberg, J M , and Roark, T P) (NASA *SP*-140) (Washington, NASA, 1967)

[4] Hoyle, F , and Wickramasinghe, N C , *Nature*, **214**, 969 (1967)

[5] Schulman, J H , and Compton, W D , *Color Centers in Solids* (Macmillan Co , New York, 1962)

[6] Pick, H , *Nuovo Cimento*, Ser X, **7**, suppl No 2, 498 (1958)

[7] Pryce, M H L , in *Phonons in Perfect Lattices and in Lattices with Point Imperfections* (edit by Stevenson, R W H) (Oliver and Boyd, 1966)

[8] Wagner, M , and Bron, W E , *Phys Rev* , **139**, A223 (1965)

[9] Dietz, R E , Thomas, D G , and Hopfield, J J , *Phys Rev Lett* , **8**, 391 (1962)

[10] Ivey, H F , *Phys Rev* , **72**, 341 (1947)

[11] Nandy, K , *Nature*, **208**, 274 (1965)

[12] Nandy, K , *Publ Roy Obs Edin* , **6**, 25 (1967)

[13] Seddon, H , *Nature*, **214**, 257 (1967)

[14] Herbig, G H , *Z für Astrophys* , **64**, 512 (1966)

Condensation of the Planets

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The early overluminous phase, during the condensation of the Sun can be a great help in understanding why the inner planets are largely made up of iron and magnesium silicate

It is now believed that condensing stars of solar mass are wholly convective, with structures of the kind first investigated by Homer Lane nearly a century ago The surface temperature is maintained at a value of 3,000°–3,500° K during much of the condensation process, eventually rising to ~4,000° K as the immediate pre-main sequence stages are reached These are characterized by a disappearance of complete convection To a moderate degree the star is then sub-luminous compared with the main-sequence state. During the earlier completely convective phases, on the other hand, the star is highly overluminous compared with the main-sequence state The latter is, of course, defined by the condition that nuclear reactions take over essentially the whole energy production, the contribution from gravitation becoming negligible.

In this article we shall examine the problem of the origin of the planets in accordance with the above picture for the early history of the Sun In particular we wish to examine the implication of the overluminous phase of the contracting Sun, especially as it affects the chemical composition of the planets

First, we notice there is clear-cut evidence that the original mass of the planetary material was at least 3,000 Earth masses, as compared with the present planets which total only about 450 Earth masses To see this we note the relative abundances for solar material shown in Table 1

These values are taken from a recent extensive review by Clayton, Fowler and Hoyle (unpublished results) of the abundances of the elements in stars These authors conclude that there is an uncertainty of about ±0 2 in the solar logarithmic abundances of many elements, although the uncertainty for Si may be worse than this because of uncertain *f*-values For our purpose these uncertainties are not, however, important

The densities of the planets Uranus and Neptune are too high (1 56 for Uranus, 2 27 for Neptune) for them to contain much H or He Their densities are appropriate to H_2O, NH_3, N_2, CO_2, after allowance is made for compression If we assume these planets to be largely composed of C, N, O we obtain a minimum estimate of ~3,000 Earth masses for the planetary material This follows because the relative abundances given in Table 1 require C, N, O to comprise about 1 per cent by mass of solar material, so that the ~30 Earth masses of Uranus + Neptune contain the total C, N, O belonging to ~3,000 Earth masses of material of solar composition It is implied here that we regard the planetary material as having originally had the same composition as solar material The estimate is a minimum because it supposes that the whole of the original C, N, O is now present in Uranus and Neptune

An estimate of ~3,000 Earth masses is supported by the Fe content of the inner planets About 30 per cent of the Earth itself is usually supposed to be present in an iron core With Venus possessing a similar structure, the total Fe content of the inner planets is about 0 5 Earth masses, which is the Fe content of some 2,000 Earth masses of solar material This again is to be interpreted as a minimum, for we have no knowledge that the inner planets contain the whole of the planetary iron We shall see at a later stage, however, that there are

Table 1 RELATIVE ABUNDANCE FOR SOLAR MATERIAL

Elements	Logarithm of abundance
H	12 00 (reference standard)
He	~11 0
C	8 5
N	8 06
O	8 80
Mg	7 36
Si	7 3 ?
Fe	6 8

theoretical reasons for believing that this might be the case. Furthermore, the coincidence of this iron estimate from the inner planets with the C, N, O estimate from Uranus and Neptune suggests that the original planetary material probably did constitute some 3,000 Earth masses. The solar mass is ~330,000 Earth masses and so we suggest that the ratio of the masses of the planetary material m to the Sun M was $m/M \simeq 10^{-2}$.

Even this minimum estimate shows that the condensing Sun must have become rotationally unstable at a radius R which much exceeded the present main-sequence radius. That is to say, the planetary material quitted the Sun during the wholly convective, highly luminous, phase of solar contraction. To see this we ask: what has happened to the excess mass, to the difference between $m \simeq 3{,}000$ Earth masses and the present-day 450 Earth masses? The natural answer is that H and He escaped in large quantities from the periphery of the solar system. The excess H and He belongs to the region of Uranus and Neptune and it was from such distances, $r \sim 3\text{–}5 \times 10^{14}$ cm, that these light gases escaped into interstellar space.

The total angular momentum A of the original planetary material must therefore have been

$$A \simeq mr\left(\frac{GM}{r}\right)^{\frac{1}{2}} \qquad (1)$$

the factor $(GM/r)^{\frac{1}{2}}$ being just the orbital velocity at distance r from the Sun. We shall see in a moment why the material may be expected to have been in Keplerian orbits, as (1) requires. First, however, we regard the angular momentum A as having been at one time present in the condensing Sun, and we ask at what radius R the Sun would have become rotationally unstable.

Rotational instability of a condensing gas cloud may be expected to follow the sequence of events described by Jeans[1], in which there is firstly a polar flattening into a spheroidal form which becomes more and more pronounced until the polar diameter is about half the equatorial diameter. At this stage the lenticular configuration develops a sharp edge, requiring material to break away from the main body and to be left behind in circular orbits as contraction proceeds. The condition for this "equatorial shedding" is

$$\frac{\Omega^2}{2\pi G\bar{\rho}} \simeq 0{\cdot}25 \qquad (2)$$

where Ω is the angular velocity and $\bar{\rho}$ is the mean density.

The coefficient on the right hand side of (2) is uncertain within a moderate range. Jeans worked out a number of cases in which the coefficient falls between a lower limit of ~0·19 for an incompressible fluid and ~0·36 for a compressible gas cloud of very strong central condensation. A value of 0·31 is given for a cloud with pressure $P \propto \rho^{2\cdot 2}$, ρ being the density. In our case we expect $P \propto \rho^{5/3}$, because of convection, and for the lower index the coefficient should be less than 0·31. A value ~0·25 therefore seems appropriate.

In (2) $\bar{\rho}$ is the mean density, given by $\bar{\rho} \simeq 3M/4\pi R^3$, so that (2) can be written in the form

$$\Omega^2 \simeq \frac{3}{8}\,\frac{GM}{R^3} \qquad (3)$$

The angular velocity Ω may be expected, because of magnetic viscosity, to be approximately uniform throughout the condensing solar cloud, so that Ω is related to R by

$$Mk^2\Omega = A \qquad (4)$$

where k^2 is the square of the radius of gyration about the polar axis. It is a little difficult to estimate k^2 because of the flattening. We note, however, that whereas k^2 is $2R^2/5$ for a uniform sphere, it is $R^2/2$ for a uniform disk, not much different. This suggests that k^2 will not be much different from the case of spherical symmetry, provided R is interpreted as the equatorial radius. With $P \propto \rho^{5/3}$ for a convective star, we can use the polytrope of index 1·5, for which $k^2 \simeq R^2/5$. Then using (1) for A, and eliminating Ω between (3) and (4), we obtain

$$\frac{R}{r} \simeq \frac{200}{3}\left(\frac{m}{M}\right)^2 \qquad (5)$$

Putting $r \simeq 4{\cdot}5 \times 10^{14}$ cm, close to the radius of Neptune's orbit, and $m/M \simeq 10^{-2}$, (5) gives

$$R \simeq 3 \times 10^{12}\ \text{cm} \qquad (6)$$

There are uncertainties in the numerical value to be used on the right hand side of (2), in k^2, and in the value of r, but all these uncertainties only affect R by factors that cannot be much different from unity. It seems unlikely that R could be less than 10^{12} cm, which is still more than ten times the present radius of the Sun. Indeed, the conclusion that the condensing Sun must have become rotationally unstable at a radius R much greater than present value holds *a fortiori*, because we have used a minimum estimate for m/M. If Jupiter and Saturn contain appreciable quantities of C, N, O, then m/M would have to be increased.

The next question concerns the manner in which the angular momentum A passes from the condensing Sun to the planetary material. Early attempts at a unified theory of the origin of Sun and planets, starting with Laplace, proceeded in terms of gas dynamics alone. It was first pointed out by Alfvén[2] that a magnetic coupling of the Sun and planetary material would tend to maintain uniform angular velocity and that this could lead to a transference of angular momentum in the required sense. One of us[3] showed that without a magnetic torque coupling of the kind suggested by Alfvén the condensing Sun would evolve into two closely separated stars of comparable mass, not into a star and planets. The requirement is for the angular momentum to become stored in a mass m that remains small compared with M. The solar condensation must not remain at the verge of instability, losing more and more material, as it shrinks. Instead the first loss of material must almost immediately take up the angular momentum, so that the Sun is then permitted to contract to the main-sequence without problems of rotational instability arising. This means that the angular momentum A must pass from M to m in a time no longer than that which is required for R to change by a moderate fraction of itself. This time interval is short, because the Sun is in its overluminous state and gravitational energy is being dissipated at a rapid rate. The loss rate is $\pi acR^2T_s^4$, where $T_s \simeq 3{,}000°$ K is the effective surface temperature. The gravitational energy is $\sim GM^2/R$, and the time required for R to shrink by an appreciable fraction of itself is

$$\sim \frac{GM^2}{R}\Big/\pi acR^2T_s^4 \approx 5{,}000\ \text{yr} \qquad (7)$$

when (6) is used for R. The angular momentum must be transferred from M to m in a time less than (7). Using the same method as was used formerly[4], it can readily be shown that a magnetic field with an intensity $\sim 10^2$ gauss would be needed to effect the transference in the required period. This is a considerably stronger field than that which was formerly calculated because the previous work did not take account of the overluminous phase. Nevertheless, a field of $\sim 10^2$ gauss is still not unreasonably high. When compressed by the continued shrinkage of the Sun to the main-sequence state the field strength would become $\sim 10^5$ gauss. It is an attractive possibility that the fields of up to 3,000 gauss still observed at the solar surface represent a decayed residue of this initial field.

The transference of angular momentum causes the planetary material to move outwards from the Sun. It must indeed move largely out to $r \simeq 5 \times 10^{14}$ cm in order to store the angular momentum. With increasing r the temperature of the material falls, approximately as

$$T \simeq T_s\left(\frac{r}{R}\right)^{\frac{1}{2}} \qquad (8)$$

and depending on their concentrations and saturation vapour pressures various substances condense out of the gas as T falls The density falls like

$$\rho \sim \frac{m}{r^3} \times [\text{Numerical factor of order unity}] \qquad (9)$$

the numerical factor depending on the degree to which the planetary material becomes flattened into a disk

Starting with material of solar composition we have carried out extensive computations of the thermodynamic properties of the planetary material, treating the system as a dissociating assembly of molecules, and including the formation of solids and liquids The density ρ was taken to fall as T^6, in accordance with the proportionalities suggested by (8) and (9), and the behaviour of the material was studied as a function of T Fig 1 shows the concentrations of a representative sample of molecules arising from such a calculation for the case $m \simeq 3{,}000$ Earth masses The calculations are much more sensitive to T than to ρ, so that the precise value chosen for the numerical factor in (9) was not very important The calculations were in fact performed for various values of the product $m \times$ [Numerical factor of order unity] About a hundred molecular species were included in the calculations, which led to details that go much beyond the scope of this article Here we are concerned only with the materials that condensed from the gas phase into the solid phase—no liquids were formed within the range of our calculations

The solids fell into two categories. refractory materials that condensed at temperatures above 1,000° K, and volatile substances condensing at low temperatures. The most important refractory materials were MgO, Fe and SiO_2 It is to be noted that the iron was given the opportunity to condense in a non-metallic form, for example, as the sulphide and oxides, but it was the metal phase that actually condensed The respective temperatures were ~1,600° K for MgO, ~1,500° K for Fe, and ~1,400° K for SiO_2 The temperature for silica was the lowest because at higher temperatures the silicon was overwhelmingly in the form SiO, which we took to have the rather low heat of vaporization of 65 Kcalories/mole. A significantly higher value, say, 100 Kcalories/mole, would have the effect of solid SiO appearing at ~1,600° K Unfortunately, there are no good data for the heat of vaporization of SiO, but we are informed that a low value is more likely to be correct than a high value

It is clearly significant that the inner planets are largely composed of Fe and of $MgOSiO_2$ in various mineral forms We also see why the inner planets contain an appreciable fraction of the total Fe and $MgOSiO_2$ contained in the whole of the planetary material Taking 1,500° K as a mean condensation temperature and setting $T_s \simeq 3{,}000°$ K in (8), we have $r \simeq 4R \simeq 1\ 2 \times 10^{13}$ cm, when (6) is used for R It is satisfactory that this value falls between the radii of the orbits of Venus and the Earth

The first volatile to condense was H_2O, at $T \simeq 150°$ K Again using (6) and (8), $r \simeq 400R \simeq 1\ 2 \times 10^{15}$ cm, comparable with but rather greater than the radii of the orbits of Uranus and Neptune Condensation at somewhat smaller values of r is indeed to be expected because (8) assumes that the solar radiation is not strongly absorbed within the planetary material Should the material become opaque, as it eventually does through absorption by water vapour in the infrared, the temperature must fall off more steeply with increasing r than is given by (8) This is because the material has a disk structure, so that a non-isotropic situation then occurs with the radiation being emitted normally to the disk, instead of continuing to propagate freely in directions parallel to the disk. The relevant strong water vapour band is centred at about 6μ When T falls so that the black-body curve has its peak at about 6μ, that is, when T falls to ~500° K, the opacity becomes large and T can be considered to fall quite steeply with r Using (8) again, but now with $T \simeq 500°$ K, leads to $r \simeq 36R \simeq 10^{14}$ cm, somewhat less than the radius of the orbit of Saturn An ambitious investigation, taking account of the geometry of the disk and of opacity effects, would be necessary to determine the details of the condensation process, but we see that the condensation of H_2O, and of the next most important volatile CO_2, extends to values $r \simeq 3 \times 10^{14}$ cm, although some condensation may occur at radii within the orbit of Saturn

This work differs from previous investigations in that we have taken account of the overluminous phase of the condensing Sun As far as the broad features of planetary condensation are concerned, this has many advantages First, if there were no high luminosity phase the volatiles would condense at too small values of r, near Jupiter rather than beyond Saturn Second, it is easier to understand the escape of H and He from the region of Uranus and Neptune, for $r = 3 \times 10^{14}$ cm, $R = 3 \times 10^{12}$ cm, $T_s = 3\ 900°$ K in (8) gives $T = 300°$ K Because of the shielding effect of water vapour at $r < 3 \times 10^{14}$ cm, this is an overestimate, but even allowing for the opacity due to water vapour we expect a temperature appreciably above the former value of 75° K or less[3] Third, the refractory materials condense in the region of Venus and the Earth, farther

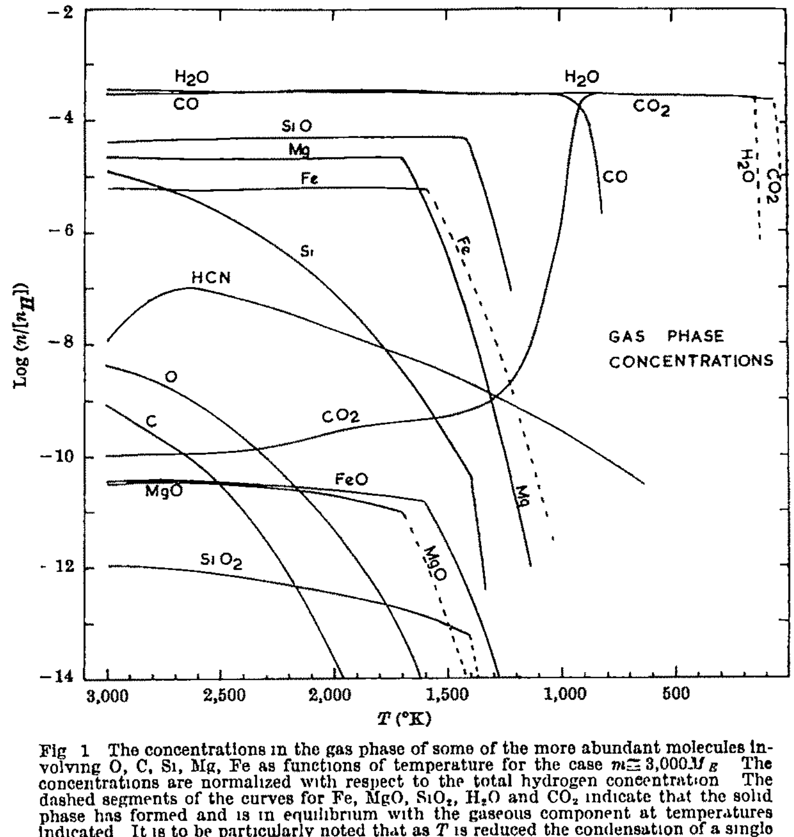

Fig 1 The concentrations in the gas phase of some of the more abundant molecules involving O, C, Si, Mg, Fe as functions of temperature for the case $m \simeq 3{,}000 M_E$ The concentrations are normalized with respect to the total hydrogen concentration The dashed segments of the curves for Fe, MgO, SiO_2, H_2O and CO_2 indicate that the solid phase has formed and is in equilibrium with the gaseous component at temperatures indicated It is to be particularly noted that as T is reduced the condensation of a single species involving a particular metal is accompanied by a steep drop in the concentrations of all species involving that metal As T falls, the metal (for example, Mg, whether as Mg metal or as MgO) is quickly drained out of the gaseous phase into the condensed phase

out than would be the case if the Sun had low luminosity. Fourth, at a temperature of 1,500° K metallic iron is sticky and likely to aggregate quickly into large lumps The MgO and SiO_2 could well have condensed as a fine smoke which continued to be carried along by the outward moving gases except when it became mixed in with the metal This could well explain the puzzling discrepancy between the ratio (Mg+Si)/Fe in the Earth and in the Sun In the Earth the mass ratio is $\sim 1/1$, whereas in the Sun it is $\sim 3/1$ The lumps of metal only succeeded in promoting the aggregation of about one-third of the $MgOSiO_2$ Fifth, it was previously supposed that the Earth aggregated as a cold body and this made it difficult to understand how the Fe in the Earth has managed to sink through to the centre With the Fe already in considerable lumps, and with the temperature greater than 1,000° K, this is now not so difficult Furthermore, the plastic flow of considerable lumps towards the centre would itself release energy that would raise the temperature considerably, as was first pointed out by Urey[4] It seems probable that the concept of an initially cold Earth will have to be revised, with a return to the earlier idea of a molten Earth[5], or at any rate to an Earth with large scale convection

An important question remains how did it come about that the Earth acquired volatiles, in particular H_2O, CO_2, N_2? Once the Earth had formed as a strongly gravitating body, it could acquire some volatiles by accretion from a solar wind The target area of the Earth for such a process, however, would be quite small It seems preferable therefore to argue that, although the main aggregation of the Earth may have occurred very quickly, say, in $\sim 10^4$ yr during the high temperature phase, a good deal of debris in the form of modest-sized planetesimals continued to be present for a time at least of the order of the time required for the Sun to reach the main-sequence, more than 10^7 yr This would include the overluminous phase that preceded the main-sequence state Further ejections of material, such as are observed in *T* Tauri stars, would lead to the condensation of volatiles at much smaller values of *r* These might well have become added first to the planetesimals, and later to the Earth and other inner planets as the planets continued to mop up the smaller bodies

It may also be worth noting in this connexion that Fe and $MgOSiO_3$ condense in an atmosphere containing high concentrations of H_2O, CO and N_2. It is possible that significant quantities of these volatiles become trapped as impurities within the refractory solids

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[1] Jeans, J H, *Astronomy and Cosmogony*, chap IX (Cambridge Univ Press, 1928)
[2] Alfvén, H, *On the Origin of the Solar System* (Oxford Univ Press, 1954)
[3] Hoyle, F, *Quart J Roy Astro Soc*, 1, 28 (1960)
[4] Urey, H, *The Origin of the Planets* (Oxford Univ Press, 1952)
[5] Jeffreys, H, *The Earth* (Cambridge Univ Press, 1952)

Prodromus of the Comparative Serology of Marsupialia

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Serological data, and the information about phylogeny which they supply, can be used as the basis of a revised classification of the Marsupialia

In spite of the great interest of marsupials to mammalogists, and a number of thorough and excellent taxonomic reviews which have appeared since marsupials were discovered[1–7], several problems of marsupial systematics remain unsolved This is largely because there is a poor understanding of the phylogeny of the group, which is in turn related to the paucity of Tertiary fossil material in Australia In this situation only the features of the living animals can provide clarification of marsupial affinities and relationships Accordingly, there has been in recent years an increase in interest in the systematic application of neontological characters to marsupials, often by workers who are not taxonomists in the classic sense Thus we now have a large body of data, taxonomically oriented, on marsupial reproductive anatomy and physiology[8–11], cytology[12–14] and sperm morphology and behaviour[15,16] Serology, however, has so far contributed little to marsupial systematics[17,18], and it was this omission, together with an awareness of the potential of serology in taxonomic work, which provided the stimulus for the present study Only three species of American marsupials have been included, but the coverage of the Australian radiation is comprehensive, and embraces about 1,800 individuals of eighty species, representing thirty-nine genera from all but one of the extant families listed by Simpson[19] A few results have already been reported (refs 20 and 21, and my unpublished work), but it will be some time before the details of this work can be published in full, and so it seems desirable to present a summary statement now A new classification of marsupials, incorporating the serological data, is also presented here, a detailed rationalization of this scheme will be given later

Standard serological techniques, including double-diffusion and immunoelectrophoresis in agar gels, nephelometry, starch-gel electrophoresis and the haemagglutination inhibition test, were applied to the study of the serum proteins of marsupials The greater part of the data summarized here came from the immunoelectrophoretic tests, which involved extensive use of preabsorbed antisera The results were scored and interpreted by a variety of techniques, including an original method of "logical analysis", and Goodall's probabilistic similarity index[22] was computed for certain sections of the data A feature of the study was the use of antisera prepared in marsupials this proved advantageous in studying families which contain numerous very closely related species (for example, Macropodidae)

As a group, the marsupials are more similar serologically to eutherian mammals than they are to monotremes (*Tachyglossus*), a point which accords well with contemporary concepts of the interrelationships of mammals[19] Fig 1 summarizes the serological affinities of forty-one marsupial genera to each other, generic limits are largely those adopted by Ride[23]. Fig 1 is neither a phylogeny—although it has phylogenetic implications—nor a classification The conventions of this phenogram are the usual ones, that is, the linkages represent groupings of increasingly similar taxa proceeding from left to right on the horizontal axis. The scale of the abscissa is roughly linear, but the ordinate has no dimension, and proximity of taxa in the vertical dimension does not necessarily indicate special affinity

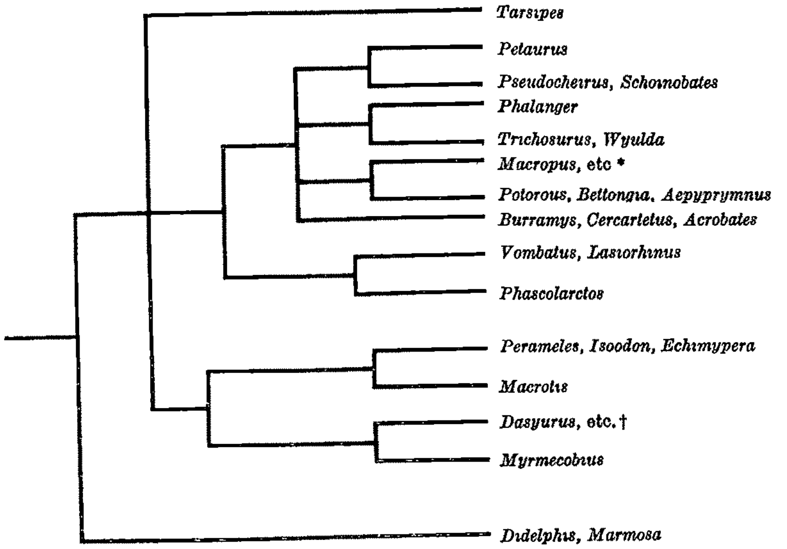
In many respects the groupings indicated by serology correspond to those of the generally accepted classification of marsupials[19] For this reason the details in which the serological clusterings differ from that arrangement are particularly interesting (1) The American marsupials, at least as represented by *Didelphis* and *Marmosa*, stand apart from all of the Australian genera, this result confirms the similar finding of Weymss[18] (2) The Australian marsupials comprise three groups *Tarsipes*, the remaining phalangeroids, and the peramelids plus dasyurids (including *Myrmecobius*) (3) *Phascolarctos* is most similar to the wombats (*Vombatus, Lasiorhinus*), and not to the "phascolarctine" possums (*Schoinobates, Pseudocheirus* and closely related genera) (4) The Phalangeridae, excluding *Phascolarctos* and *Tarsipes*, comprise at least three groups as distinct from each other as they are from the Macropodidae

The phenogram does not show in detail the affinities of the genera in diverse families such as Dasyuridae and Macropodidae Seven genera of dasyurids (apart from *Myrmecobius*, which represents a distinct line within Dasyuridae) have been examined *Dasyuroides, Sarcophilus* and *Dasyurus* (all species but *D maculatus* were tested) are very close, and *Sarcophilus* might be considered an aberrant member of *Dasyurus* *Sminthopsis* and *Antechinomys* form a second genus-group, and both groups are equally distinct from a third, consisting of *Phascogale* and *Antechinus* (including *Pseudantechinus*). The latter grouping is complicated by suggestions that *Antechinus* may represent more than one genus, and *A rosamundae* appears to be closer to *Dasyurus* than to *Phascogale-Antechinus*

The usual sub-familial arrangement of the Macropodidae is supported by serology, but Woodburne's recent suggestion[24] that *Dorcopsis* be included in the Potoroinae is not upheld; *Hypsiprymnodon* and *Caloprymnus* have not yet been examined Within Potororinae, *Bettongia* and *Aepyprymnus* form a group distinct from *Potorous* Species of all macropodine genera but *Dorcopsulus* and *Peradorcas* have been studied, and these experiments show that the strongest genus-group in Macropodinae is of *Thylogale* and *Petrogale*, with which *Setonix* and probably also *Lagostrophus, Dendrolagus* and *Dorcopsis* are associated The interrelations of the last three genera are still uncertain A second group of genera is represented by the kangaroos and wallabies of *Macropus* (sensu Calaby[25]), but on serological grounds this genus includes *Megaleia* and *Wallabia bicolor*. *Lagorchestes conspicillatus*, the single species of *Lagorchestes* available for study, is associated with *Macropus* *Onychogalea* appears to represent a third group of macropodines

The phylogenetic implications of data such as these are usually inferred by assuming that the similarities among organisms are proportional to their relationships, that is, that evolutionary change has been a regular function of time in each lineage If this is so, it is easy to establish the cladistic relationships of a group of living organisms from a consideration of their phenetic affinities. At the same time, the assumption of "time-dependent" evolution has certain logical consequences, the most obvious being that, with respect to the characters studied, no living organism is more primitive than any other Assuming time-dependency for biochemical or serological characters therefore precludes the possibility of discovering the relative primitiveness of members of the studied group, which is another, equally valid, aspect of phylogeny. Biological and theoretical considerations, as well as a large portion of the present and other published data, indicate that time-dependency cannot possibly hold true in detail for marsupial serum proteins; a further treatment of the problem is the subject of a paper now in preparation.

If any conclusions at all are to be drawn from the data of Fig 1, the assumption of time-dependency must be made, and its usefulness as a generalization for at least the broader aspects of marsupial relationships can only be assessed on the basis of the reasonableness of the resulting phylogeny Proceeding in this fashion, the results suggest that the Australian radiation is distinct from the American (at least, didelphid) radiation in this regard it would be most interesting to examine blood from caenolestids Within the Australian group, the dasyurids and peramelids must represent recent derivatives from the (presumably polyprotodont) stock, and thus neither family can be imputed to represent the ancestors of Phalangeroidea *Tarsipes*, which is so distinct from the rest of the Australian marsupials, presents a difficult and in many ways critical problem Recent unpublished work of Yadav at the University of Western Australia has shown that, in common with other Australian diprotodonts, *Tarsipes* possesses a superficial thymus gland, and this, together with its diprotodonty and syndactyly, would seem to remove all doubt that *Tarsipes* is a true phalangeroid Thus the only reasonable interpretation of the serological data is that this animal represents a lineage that arose from the Phalangeroidea as early as the basic polyprotodont–diprotodont division of Australian marsupials The remaining phalangeroids consist of two lines. koala–wombats and possums–kangaroos The latter comprises four equally related groups of genera, and in this sense "the macropodids are simply leaping phalangistids[26]"



* *Macropus*, etc , includes *Macropus, Megaleia, Wallabia, Lagorchestes, Lagostrophus, Setonix, Thylogale, Petrogale, Dendrolagus, Dorcopsis* and *Onychogalea*

† *Dasyurus*, etc , includes *Dasyurus, Dasyuroides Sarcophilus, Antechinus, Phascogale, Sminthopsis* and *Antechinomys*

Fig 1 Phenogram showing the serological affinities of forty-one marsupial genera The linkages represent groupings of increasingly similar taxa from left to right The scale of the abscissa is roughly linear, but the ordinate has no dimension

The serological data, and the data of phylogeny which they imply, can be considered together with all the other available information about marsupials as the basis for a revised classification of Marsupialia. The classification which follows purports to do this, but only a few comments in support of this arrangement can be made here It is likewise beyond the scope of this article to discuss nomenclature in detail, but as far as possible names have been chosen in accord with the provisions of the International Code of Zoological Nomenclature (second edition) A personal convention in this matter is that where the author did not use a family-group name at the indicated level, date and authorship are given in parentheses

Superorder Marsupialia Illiger, 1811
- Order Polyprotodonta Owen, 1866
  - Suborder Didelphimorphia Gill, 1872
    - Superfamily Didelphoidea (Gray, 1921)
  - Superfamily Borhyaenoidea (Ameghino, 1894)
  - Suborder Dasyuromorphia Gill, 1872
    - Superfamily Dasyuroidea (Goldfuss, 1820)
      - Family Dasyuridae (Goldfuss, 1820)
        - Subfamily Dasyurinae Goldfuss, 1820
        - Subfamily Myrmecobiinae Gill, 1872
      - Family Thylacinidae (Bensley, 1903)
  - Suborder Peramelemorphia *nov*
    - Superfamily Perameloidea (Waterhouse, 1838)
      - Family Peramelidae Waterhouse, 1838
        - Subfamily Peramelinae (Waterhouse, 1838)
        - Subfamily Thylacomyinae Bensley, 1903
- Polyprotodonta *incertae sedis*
  - Family Notoryctidae Ogilby, 1892
- Order Paucituberculata Ameghino, 1894
  - Superfamily Caenolestoidea (Trouessart, 1898)
- Order Diprotodonta Owen, 1866
  - Superfamily Vombatoidea (Iredale and Troughton, 1839)
    - Family Vombatidae Iredale and Troughton, 1839
    - Family Phascolarctidae Owen, 1839
  - Superfamily Phalangeroidea (Thomas, 1888)
    - Family Phalangeridae Thomas, 1888
      - Subfamily Phalangerinae Thomas, 1888
      - Subfamily Trichosurinae (Flynn, 1911)
    - Family Wynyardiidae Osgood, 1921
    - Family Petauridae (Gill, 1872)
      - Subfamily Petaurinae Gill, 1872
      - Subfamily Pseudocheirinae Winge, 1893
    - Family Burramyidae (Broom, 1898)
    - Family Thylacoleonidae Gill, 1872
    - Family Macropodidae Gray, 1821
      - Subfamily Macropodinae (Gray, 1821)
      - Subfamily Sthenurinae (Glauert, 1926)
      - Subfamily Potoroinae Trouessart, 1898
    - Family Diprotodontidae Gill, 1872
      - Subfamily Diprotodontinae (Gill, 1872)
      - Subfamily Nototheriinae (Lydekker, 1887)
      - Subfamily Palorchestinae Tate, 1948
      - Subfamily Zygomaturinae Stirton, Woodburne and Plane, 1967
  - Phalangeroidea *incertae sedis*
    - *Dactylopsila*, *Distoechurus*
  - Superfamily Tarsipedoidea (Gill, 1872)
    - Family Tarsipedidae Gill, 1872

I follow Ride[7] in considering the marsupials to represent several orders, for his reasons and also because this makes available a greatly expanded hierarchy for showing affinities and supposed relationships In such a multiordinal scheme, the Australian diprotodonts (Diprotodonta) and caenolestoids (Paucituberculata) represent two principal groups In spite of the separation of Australian and American marsupials on serological and other[16] grounds, I agree with Ride that it is useful to place the American polyprotodonts and the dasyuroids in a single order; however, unlike Ride, I believe that there are compelling reasons for including the peramelids in this order, and I would not separate them from it as he did To show the breadth of this third order (Polyprotodonta) as compared with the other two, I use suborders in it but not in Paucituberculata or Diprotodonta Gill's names are available for two of the suborders, and I propose the new name Peramelemorphia for the third

Because knowledge of fossil American marsupials is rapidly expanding, and the Didelphoidea have been revised recently[27], I do not offer opinions on the organization of the American marsupials below the level of superfamily. I have considerably reorganized Diprotodonta, however, as shown in the classification Here, *Tarsipes* is placed in a monotypic superfamily, somewhat compromising the serological results, and the koala and wombats are grouped as two families of another superfamily Phalangeroidea is restricted to the possums, kangaroos, and several extinct groups—inclusion of Diprotodontidae is somewhat tentative, however. The possums are reorganized into three families, the distinctness of which is supported by what is known of their cytology (refs 12 and 14 and unpublished work of M M Gunson, G B Sharman and J A. Thomson) and sperm morphology[15], this arrangement more adequately expresses what I believe to be the phylogenetic relationships of living phalangeroids Burramyidae may include *Distoechurus*, but *Dactylopsila* (including *Dactylonax*) is aberrant in so many respects that its affinities, like *Notoryctes*, can only be a matter of conjecture at present

This study formed part of a thesis submitted to the University of Western Australia, and was carried out while I held, successively, Fulbright student and US National Science Foundation graduate fellowships Funds were made available by the Research Grants Committee of the University of Western Australia, and a few experiments were carried out during my tenure as experimental officer with the CSIRO Division of Animal Health, McMaster Laboratories, Glebe, New South Wales, Australia

I thank the many people who helped me obtain or provided me with marsupial sera. I also thank Dr W. D L Ride for encouragement and criticism, and John Calaby for advice on nomenclature Mohan Yadav allowed me to quote from his unpublished researches on the superficial thymus

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[1] Waterhouse, G R , *A Natural History of the Mammalia*, 1, *Marsupiata* (Hippolyte Baillière, London, 1846)

[2] Gould, J , *The Mammals of Australia* (The Author, London, 1863)

[3] Thomas, O *Catalogue of the Marsupialia and Monotremata in the Collection of the British Museum (Natural History)* (The British Museum, London, 1888)

[4] Bensley, B A , *Trans Linn Soc Lond Zool* (2), 9, 83 (1903)

[5] Cabrera, A , *Genera Mammalium, Monotremata, Marsupialia* (Museo Nacional de Ciencias Naturales, Madrid, 1919)

[6] Simpson, G G , *Fossilium Catalogus*, 1 *Animalia*, Part 47 (W Junk, Berlin, 1930)

[7] Ride, W D L , *J Proc Roy Soc West Aust* , **47**, 97 (1964)

[8] Pearson, J , *Pap Proc Roy Soc Tasm* , *1945*, 13 (1946)

[9] Pearson, J , *Pap Proc Roy Soc Tasm* , *1949*, 211 (1950)

[10] Sharman, G B , in *Biogeography and Ecology in Australia* (edit by Keast, A , Crocker, A L , and Christian, C S) (W Junk, The Hague, 1959)

[11] Sharman, G B , in *Viewpoints in Biology*, **4** (edit by Carthy, J D , and Duddington, C L) (Butterworths, London, 1965)

[12] Sharman, G B , *Austral J Zool* , 9 38 (1961)

[13] Biggers, J D , Fritz, H I , Hare, W C D , and McFeely, R. A , *Science*, 148, 1602 (1965)

[14] Martin, P G , and Hayman, D L , *Chromosoma*, 20, 290 (1967)

[15] Hughes, L , *Austral J Zool* , 13, 533 (1965)

[16] Biggers, J D , and DeLamater, E D , *Nature*, 208, 402 (1965)

[17] Nuttall, G H F , *Blood Immunity and Blood Relationship* (University Press, Cambridge, 1904)

[18] Weymss, C T , *Zoologica*, 38, 173 (1953)

[19] Simpson, G G , *Bull Amer Mus Nat Hist* , 85, 1 (1945)

[20] Kirsch, J A W , *Austral J Sci* , 29, 430 (1967)

[21] Kirsch, J A W , and Poole, W E , *Nature*, 215, 1097 (1967).

[22] Goodall, D W , *Biometrics*, 22, 882 (1966)

[23] Ride, W D L , *A Guide to the Native Mammals of Australia* (Oxford University Press, Melbourne, in the press)

[24] Woodburne, M O , *Bur Min Resour , Austral Bull* , 87, 1 (1967)

[25] Calaby, J H *CSIRO Div Wildlife Res. Tech Pap* , No 10 1 (1966)

[26] Winge, H , *The Interrelationships of the Mammalian Genera*, 1 *Monotremata, Marsupialia, Insectivora, Chiroptera, Edentata* (C A Reitzels Forlag, Copenhagen, 1941)

[27] Clemens, W A , *Univ Calif Publs Geol Sci* , 62, 1 (1966)

Coral Community on Muddy Sand

by

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Heteropsammia michelinii, a free-living and free-moving scleractinian coral found in the Australian Great Barrier Reef, has a symbiotic association with a sipunculid worm, *Aspidosiphon corallicola*. The worm lives in a cavity on the under surface of the coral and it pulls the coral over the muddy surface of the sand.

SCLERACTINIAN corals are usually regarded as sessile organisms, providing the principal substance of coral reefs. We should like to record, however, some observations on a community of free-living and free-moving species of scleractinian corals which we found during October 1967 while we were participating in the Belgian De Moor Expedition to the Australian Great Barrier Reef. This community occurred at depths of around 12 m in the lee, that is north-west, of Lizard Island, at lat. 14° 39·5′ S. and long. 145° 26·5′ E. (Australian Survey Map SD 55.9, Grid Reference 334143). This granite mass is the highest of the continental islands within the northern half of the Great Barrier Reef. It is extensively vegetated, rises to a height of 382 m and provides the greatest shelter from the prevailing south-east trade winds. On the lee side, a gently sloping shelf 10–14 m deep extends to about 2 km from shore. The sediments of the outer part of the shelf consist of a relatively unsorted mixture of granite, sand, shell and *Halimeda* fragments, with a surface layer of detrital mud. The bottom has the "trampled" uneven appearance characteristic of sediments in which there is a large population of burrowing forms. Most of our collections and underwater observations were made about 1·5 km from the nearest reef.

There is a patchy flora of *Caulerpa*, *Avrainvillea*, *Udotea*, *Halimeda*, *Penicillus* and filamentous blue green algae. Benthic invertebrates include sponges, cerianthids, pennatulids, holothurians, crinoids, asteroids and some tunicates, more rarely *Pinna* sp. (*carnea* ?) and the white hammer oyster *Malleus albus*.

The coral population consists of *Cycloseris cyclolites* (Lamarck), *Diaseris distorta* (Michelin), *Heteropsammia michelinii* (M.E. and H.), and more rarely *Trachyphyllia geoffroyi* (Audouin). They are all free-living and except for *Trachyphyllia* have the corallum totally enclosed by living coenosarc. It is possible that *T. geoffroyi* is not typical of this environment, for it is found more frequently on clean shell and coral talus near the base of reefs where it begins life fixed to a hard substrate. The other species, *Cycloseris*, *Diaseris* and *Heteropsammia*, occur only on soft bottom, and they are never found on reefs.

It is paradoxical that all these forms by definition belong to the category of hermatypic (reef-building) corals because they contain zooxanthellae. In an ecological sense, however, they are ahermatypes (non-reef-builders) because their solitary free-living mode of life on the muddy bottom precludes participation in the process of reef-building. It is perhaps interesting that in the sub-order *Dendrophylliida*, *Heteropsammia* is one of only two genera known to contain zooxanthellae, the other being *Turbinaria*, an important Indo-Pacific reef-building coral.

Of the two fungiids, *Diaseris distorta* is the most numerous. It occurs in more or less dense patches some of which were estimated to contain more than a thousand individuals in a square metre. Most of the coralla are less than 1 cm across; they rarely reach to 2 cm, and are thin and flat. Several thousand *Diaseris* were inspected at Lizard Island; each showed traces of breakage along the septal radii, with subsequent regeneration from the

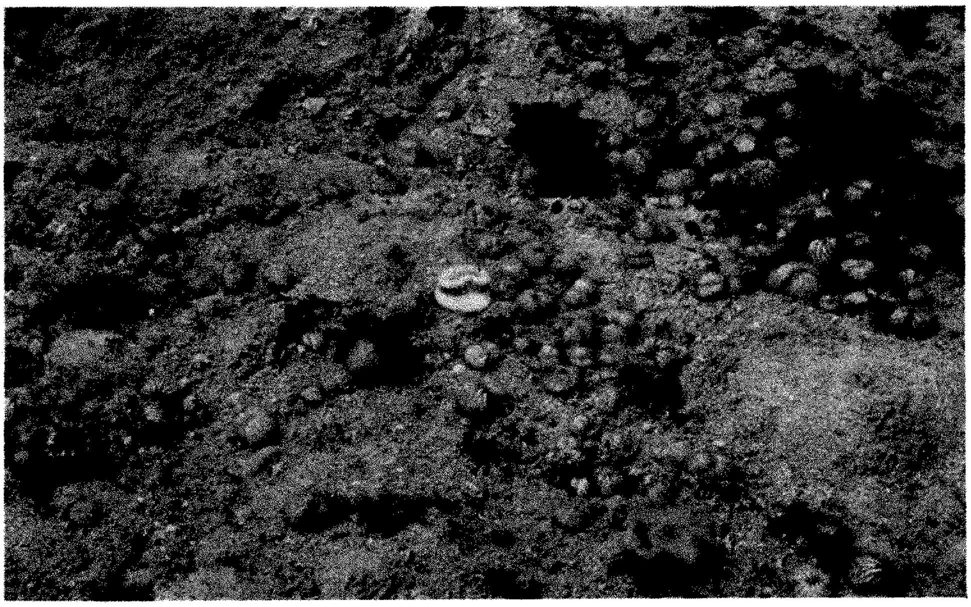

Fig. 1. Mixed community of *Heteropsammia michelinii*, *Diaseris distorta* and *Cycloseris cyclolites* at a depth of about 16 m on muddy silt bottom off Lizard Island. The most abundant coral in this field is *Diaseris*, small groups of which are congregated in hollows on the uneven bottom. Five *Heteropsammia* are visible in the foreground, and a single *Cycloseris* lies among the *Diaseris* on the right side of the photograph. All the corals here are fully expanded. The scale is given by the *Heteropsammia*, which are approximately 2 cm across.

stomodeal end. All the coralla were, as a result, asymmetric; indeed, most of the small individuals (less than 5 mm) were no more than wedge-shaped segments suggesting a very rapid rate of fragmentation. These corals, however, showed no signs of mechanical damage or injury as might be expected if the breakages were caused by external factors, such as browsing fish. It was also common to find specimens where the corallum was broken but the pieces were still held together by the tissues, which were intact and normal in appearance. The frequency of such skeletal breakage occurring without any obvious mechanical injury suggests that this may be a normal process of physiological segmentation, that is, asexual reproduction by transverse division of the anthocyathus instead of the anthocaulus, the latter being the usual method of increase among other monostomodeal fungiids[1]. This would explain the distribution of *Diaseris* in dense isolated patches each perhaps representing a clone, and also our failure to observe or collect any anthocauli of this species.

Cycloseris cyclolites is larger, dome-shaped and slightly oval. A typical individual has major and lesser diameters of 3·8 and 3·4 cm, respectively, and a height of 1·5 cm, the aboral surface being deeply concave. The coralla are fairly symmetrical and only a few showed healed scars caused by breakage and regeneration.

Both fungiids are usually expanded during the day. There are numerous small tentacles that retain particulate material which is carried to the stomodeum by local reversal of flagellar currents. When the corals are not feeding, the direction of these currents is towards the periphery, as in larger fungiids[2]. *Cycloseris* and *Diaseris* possess remarkable powers of movement. They are able to creep slowly over the bottom, right themselves if turned over and extricate themselves if buried by sediment. When corals of both species are mixed with their natural substrate and placed in a dish so that they are covered by mud, all the individuals gradually rise to the surface with their oral sides uppermost, that is, they uncover and, where necessary, turn over as originally described for larger fungiids by Marshall and Orr[3] and by Abe[4]. *Cycloseris* frees itself from mud by means of a combination of flagellar currents and tentacular movements. *Diaseris* in addition takes in water through the stomodeum; the resulting turgor causes the polyp to swell to more than double the size of the skeleton and so reduces its density that the animals literally "float" to the surface of the sediments. When the corals finally come to rest on top, they are surrounded by a well marked ridge of mud which has been carried off the oral surface by the combined action of the flagella and mucus. Compared with the larger fungiids, which usually occur in shallow turbulent zones on coarse sand or coral fragments, these smaller species are clearly very highly specialized for life on soft muddy substrates, in relatively calmer or deeper environments.

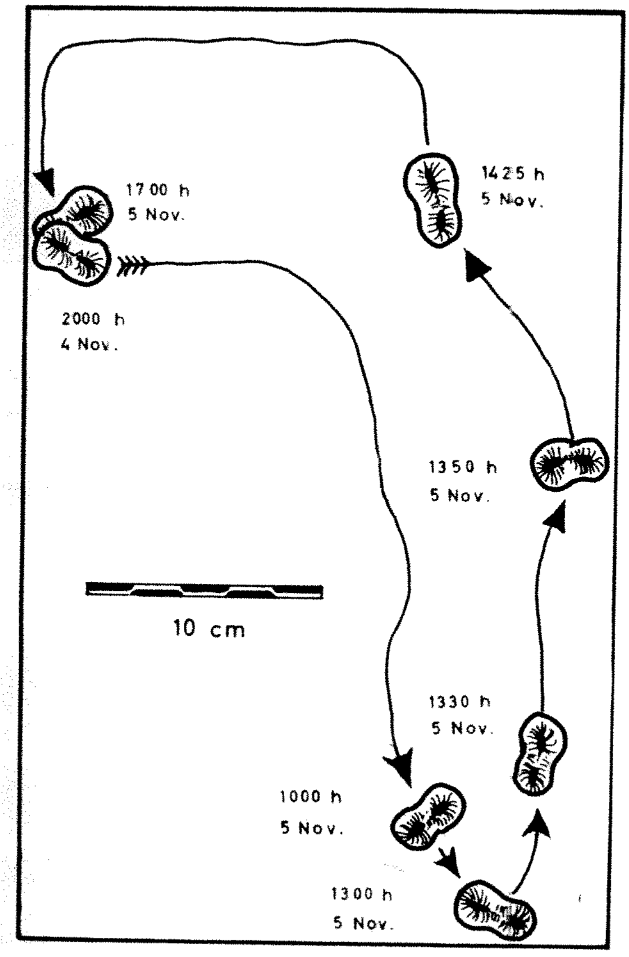

Fig. 2. Migration of a single *Heteropsammia michelinii* in a tank between 2000 h, November 4, and 1700 h, November 5, 1967. The path shown by the arrows is the trail left by the coral as it was being dragged along by its associated sipunculid worm *Aspidosiphon corallicola*.

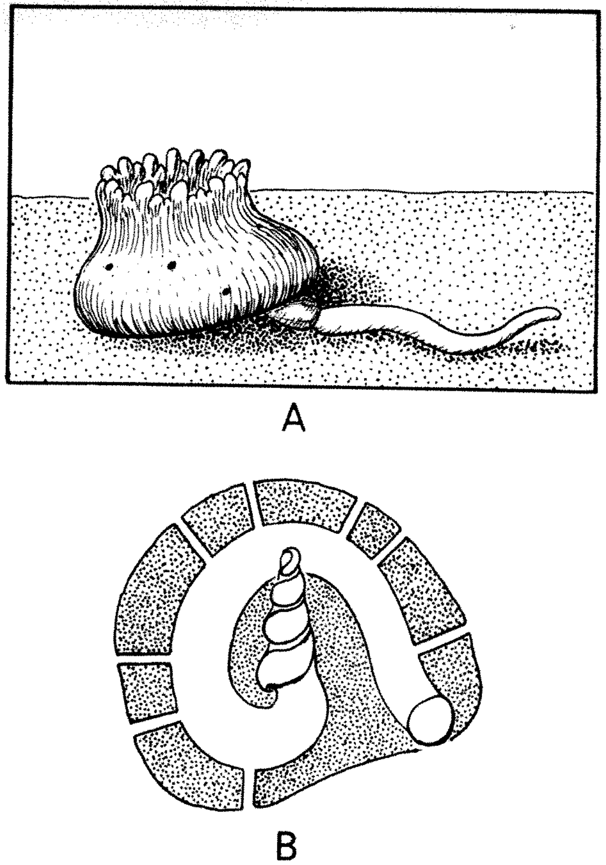

Fig. 3. *A*, *Heteropsammia michelinii* (in aquarium) with sipunculid reaching out from opening of cavity. Note openings of pores extending from this to exterior. (Redrawn after Bouvier[6].) *B*, Horizontal section through *H. michelinii* showing original gastropod shell and the spiral cavity extending from this occupied by the sipunculid, also pores, of unknown origin and function, extending to exterior. (Redrawn after Schindewolf[8].)

At Lizard Island, *Heteropsammia michelinii* occurs scattered among the *Cycloseris* and *Diaseris* (Fig. 1) and is of even greater interest. Completely free-living, each individual consists of a squat round base, with a column about 2 cm across and 1 to 2·3 cm high, crowned by one or two, rarely three, polyps. The upper calicinal surface is often wider than the base; such coralla tend to be unstable and they can topple over.

The most striking feature is the presence on the under surface of a rounded opening about 2 mm across which leads into a coiled cavity inhabited by a sipunculid worm, *Aspidosiphon corallicola* (Sluiter, as quoted in Vaughan and Wells[5]) There was little time for observations in life, but the worms were seen to emerge from the openings probing the sediments while retaining a very firm hold in the cavity They thus pull the corals over the surface of the muddy sand This movement is sporadic as shown by the track of a *Heteropsammia* on its natural substrate in an aquarium during a 21 h period (Fig 2), the wandering of the animal being clearly marked by the trail left in the sediment In their normal environments at Lizard Island many of the *Heteropsammia* were lying on their sides and a few were even upside down In one case the worm was seen to jerk the coral upright with one powerful movement The polyps are usually expanded, the tentacles reaching lengths of more than 10 mm Individuals leaning on their sides usually have their tentacles sweeping the bottom, and it is possible that they can collect in this way particular food of animal origin deposited on the sediment surface If this is so, then the activities of the commensal worm will be of great value as it continually moves the coral to the new feeding areas The sipunculid reaches down to feed in the subsurface sediments and does not compete for food with the coral

The only previous observations in life seem to be those communicated by Jousseaume to Bouvier[6] on which were based a figure, part of which is here reproduced (Fig 3*A*) The initial association is similar to that found in the sipunculid *Phascolion strombi*, which occupies empty shells of *Apporhais pes-pelecani, Dentalium entalis* and *Turritella communis* in European seas As first indicated by Milne Edwards and Haime[7], the further development of symbiotic relationship between sipunculid and coral involves settlement of planulae on similarly occupied gastropod shells As the coral comes to envelop the shell, the cavity of this is continued, still spirally wound but of much wider lumen (Fig 3*B*) This opens on the under side conferring a certain secondary bilaterality on the coral[8] The sipunculid cannot bore, but its active presence will modify the growth of the coral just as the external settlement of associates such as the bivalve *Pedum spondyloideum*[9] affects that of the "host" coral A series of minute passages of unknown function and certainly not formed by the worm open to the exterior (Fig 3*A* and *B*) In some cases (not personally observed) a calcareous secretion may cause slight constriction of the opening of the cavity. The sipunculid probably produces the secretion and it may assist it to retain firm contact while it explores the substrate The whole association, extended to cover a diversity of organisms connected with one or other of the symbionts, is briefly described by Schindewolf[8] It includes the small, byssally attached erycinacean bivalve, *Jousseaumiella heteropsammiae* Bourne (noted by Dr A Coomans), just within the opening of the cavity although it does also occur on the body of the sipunculid[10]

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[1] Wells, J W , *The Cnidaria and their Evolution*, 223 (Academic Press, 1966)
[2] Yonge, C M , *Sci Rep Great Barrier Reef Exped* , 1928–29, 1, 13 (1930)
[3] Marshall, S M , and Orr, A P , *Sci Rep Great Barrier Reef Exped* , 1928–29, 1, 93 (1931)
[4] Abe, N , *Palao Trop Biol Stud* , **2**, 105 (1940)
[5] Vaughan, T W , and Wells, J W , *Geol Soc Amer* , *Spec Pap* , 44 (1943)
[6] Bouvier, E -L , *Ann Sci Nat Zool* (7), **20**, 1 (1895)
[7] Milne Edwards, M , and Haime, J , *Ann Sci Nat Zool* (3), **10**, 65 (1848)
[8] Schindewolf, O H , *Akad Wiss u Lit Mainz, M -N Kl* , Jahrg 1958, 259 (1959)
[9] Yonge, C M , *Proc Malac Soc Lond* , **37**, 311 (1967)
[10] Bourne, G C , *Rep Pearl Oyster Fish Manaar, Roy Soc* , **5**, 243 (1906), *Proc Malac Soc Lond* , **7**, 260 (1907)

Mechanism of Olfactory Transduction

by

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A possible mechanism for olfactory transduction depends on the formation of a weak bound complex between an absorbed gas molecule and certain carotenoid pigments.

A THEORY of the olfactory transducer must satisfy several well established criteria The interaction energy between the odorous molecule and the receptor site must be a weak one—on the order of a few times kT—to allow rapid disappearance of the first signal when the surrounding atmosphere is changed and a second type of odour occurs Despite this weak interaction, it must produce a large effect because sensitivities of the order of a small number of odorous molecules per receptor cell have been reported at threshold. This large effect must be able to produce an increase in the conductance of a limiting resistance membrane, that is, it must increase the density of charge carriers, or the mobility of the charge carriers in the membrane, to depolarize the membrane (or a limited region of it) This last requirement, when coupled with the threshold sensitivity for smell, implies strongly that an amplification process is needed—the number of charge carriers traversing the membrane must be a large number times the number of adsorbed molecules present in that locality The theory must account for the nature of the different receptor types, and for the fact that a chemical will differentially excite these types, that is, it should allow us to organize the chemicals into different classes by the interaction mechanism It is also obvious that different chemicals have very different thresholds for detection—this, too, must be correlated through the proposed interaction mechanism And, as a final requirement of the theory, fatigue or adaptation, the diminishing of the signal strength with continuous or repeated exposure to high concentrations of certain odorous molecules, should be explicable

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Earlier theories of olfaction have been reviewed by Moulton and Beidler[1] We should like to present now the outline of a mechanism for olfactory transduction which seems to be consistent with all of the criteria we have mentioned It depends on the formation of a weak bound complex between an adsorbed gas molecule and certain carotenoid pigments Carotenoids, both free and bound to proteins, have been identified in the olfactory mucosa[2-6] This weak bound complex is not well understood, but it is most likely a donor-acceptor complex and we shall call it this Further investigations may modify this identification, but this modification will not alter the theory We have found that these weak bound complexes exert a large controlling effect on the conductivity of these pigments This is the fundamental observation

Carotenoids are semiconductors, both in the glass and crystalline states The electrical properties of β-carotene in particular have been extensively investigated[7] In an earlier investigation it was observed that the semiconductive properties of β-carotene were changed by the adsorption of oxygen molecules on the crystalline surfaces Effects of gas on the conductivity of other organic and inorganic semiconductors have been widely reported Gutmann and Lyons have reviewed this work recently[8] In this particular case, the surface conductivity of the β-carotene was increased by factors of up to 10^3, proportionally to the exponential of the partial pressure of oxygen in the surrounding atmosphere This effect was completely reversed by changing the atmosphere to one of pure nitrogen From the temperature and time dependence of the reversal, it was shown that the binding energy was 0 17 eV It was suggested then that a partial donor–acceptor complex had formed between the adsorbed oxygen and the carotene molecules on the surface of the crystallites This donor–acceptor complex caused a decrease in the activation energy for the thermal generation of charge carriers from 1 52 eV in a vacuum or inert atmosphere to 1 25 eV in the presence of oxygen This decrease in activation energy was sufficient to explain the increased conductivity

More recent investigations have shown that many other gases present in the atmosphere of the chamber can cause increases in current as great as 10^7 times the inert gas value[9,10] In Fig 1 we show, schematically, the apparatus used in these experiments These effects are all (in proper temperature conditions, that is, less than 50° C) reversible with about the same time course as found for oxygen, and suggest similar, if not identical, activation energies for the break-up of the weak bound complexes and subsequent desorption The gases which we have tested for this effect were chosen from three categories—no (or weak) odour, moderate odour and strong odour The results of these tests are shown in Table 1, where we have listed the gas and the increase in current caused by adsorption of this gas on crystallites of β-carotene The inert gases, as well as hydrogen and nitrogen, gave values no different from those obtained in a vacuum, and carbon dioxide, nitrous oxide, butanol and hexanol produced only small increases, less than a factor of 10 We shall discuss the esters later. The gases with moderate odours produced increases in current of a factor of 10^2–10^3, while gases with strong odours produced large increases in current of a factor of 10^5–10^7 With some gases, for example, ammonia and methanol, "puff" experiments can be done by injecting a 1 sec pulse of the gas into a stream of nitrogen and watching the current rise and then decay If β-carotene is only one of the pigments making up the different types of receptor, then it is possible for a gas with a strong odour to be found in the group producing small increases in current if this gas is not of the class to which β-carotene responds We believe this to be the case for the esters as described here If this theory is correct, however, we should not find a gas with no (or weak) odour in the category producing large increases in current This is shown by the results so far Oxygen is the only odourless gas in the group producing moderate increases in current and, indeed, a special case should be made for this gas as it is present in constant quantity in all normal air A detailed description of the experimental conditions is being published[9]

Table 1 CURRENT INCREMENTS IN A β-CAROTENE CELL WITH VARIOUS ADSORBED GASES (NOT CORRECTED FOR EQUAL VAPOUR PRESSURE)

Gas adsorbed	Approximate current increase ($\sigma_{\text{steady state}}/\sigma_{\text{initial}}$)
(1) Nitrogen	1
(2) Hydrogen	1
(3) Argon	1
(4) Helium	1
(5) Nitrous oxide	2
(6) Carbon dioxide	10
(7) Butanol	7
(8) Hexanol	5
(9) Heptanol	5
(10) Octanol	4
(11) Decanol	4
(12) Methyl acetate	10
(13) Ethyl acetate	3
(14) Butyl acetate	3
(15) Pentyl acetate	3
(16) Oxygen	10^2
(17) Isopropanol	10^2
(18) Sulphur dioxide	10^3
(19) Propanol	10^3–10^7 (?)
(20) Acetone	10^4
(21) Nitric oxide	10^4
(22) Ethanol	10^5
(23) Ammonia	10^5
(24) Hydrogen sulphide	10^5
(25) Methanol	10^6
(26) Nitrogen dioxide	10^6

In Fig 2, we have plotted the current rise on a log scale versus the time of flow for a series of alcohol vapours carried by nitrogen gas The rise and decay times are almost totally caused by the flushing time of the chamber containing the β-carotene cell The sizes of the responses are not corrected here for the different vapour pressures of the alcohols (all maintained at a temperature of 15° C) The results, nevertheless, show that the carotene cell responds very differently to the different alcohols With smaller chambers and higher flow rates, the speed of response is very fast In all the cases where large increases in current were found, it was observed that the semiconduction activation energy of the surface currents caused by gas adsorption was lowered enough to account completely for the increase in current

We have investigated this phenomenon in some detail and conclude that the same simple theoretical model proposed for the increase in current in crystalline proteins with hydration[11] is applicable A brief description of this

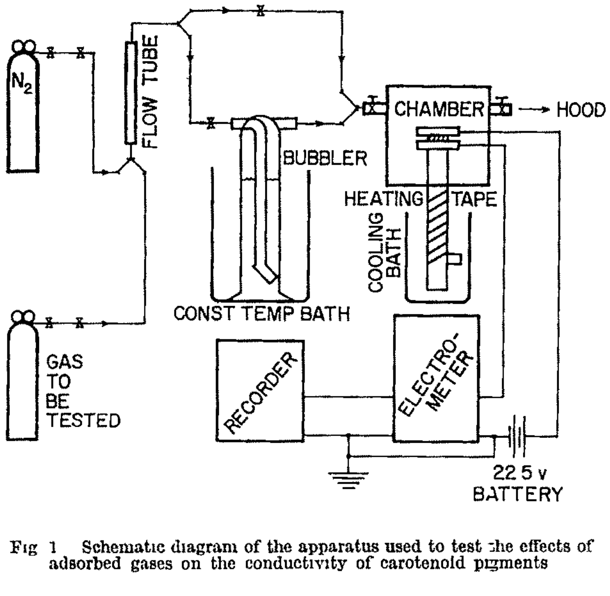

Fig 1 Schematic diagram of the apparatus used to test the effects of adsorbed gases on the conductivity of carotenoid pigments

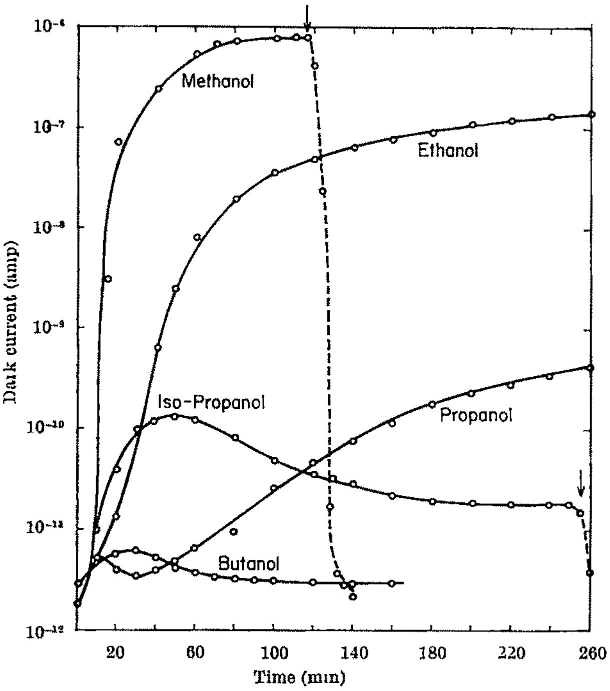

Fig. 2. Time course of the current rise and decay when nitrogen gas is bubbled through various alcohol liquids kept at 15° C and passed through the chamber. Arrows indicate when pure nitrogen alone is flushed through the chamber. Propanol shows a unique slow rise time.

model is essential. Experiments have shown that the conductivity, σ_A, of the carotene-gas complex increases with increasing gas adsorption, m (m = percentage by weight of adsorbed gas), at constant temperature and at low coverage according to the relation

$$\sigma_A = \sigma_v \exp[\alpha m] \qquad (1)$$

where σ_v is the conductivity in a vacuum or an inert atmosphere, and α is a constant for a given system. The temperature dependence of the conductivity of the carotene is that of a semiconductor, and so it is given by the relation

$$\sigma_v(T) = \sigma_0 \exp[-E_v/2kT] \qquad (2)$$

Combining equations (1) and (2) gives the empirical relation

$$\sigma_A(T,m) = \sigma_0 \exp[-E_v/2kT] \quad \exp[\alpha m] = \sigma_0 \exp[-(E_v - \gamma m)/2kT] = \sigma_0 \exp[-E_A/2kT] \qquad (3)$$

where $\gamma = 2kT\alpha$. The activation energy of the complexed crystals, E_A, is equal to $E_v - \gamma m$, and changes continuously with increasing gas adsorption. Measurements of the activation energies with different amounts of adsorbed gases have verified equation (3). We now calculate the energy necessary to separate a charge carrier from one molecule in the medium and place it on another a large distance away (that is, the coulomb interaction is neglected). This is given by

$$E_v = I_g - A_g - \frac{e^2}{R}\left(1 - \frac{1}{K}\right) \qquad (4)$$

where I_g is the gas state ionization energy of the molecule, A_g is the gas state electron affinity, K is the low frequency dielectric constant of the solid, e is the electronic charge, and R is the radius of the polarization cavity field. For the complex, if it is a weak binding type, we can approximately write

$$E_A = I_g - A_g - \frac{e^2}{R}\left(1 - \frac{1}{K'}\right) \qquad (5)$$

where K' is the new dielectric constant and $K' > K$. Eliminating $I_g - A_g$ from equations (4) and (5) results in the following relation

$$E_A = E_v - \frac{e^2}{R}\left(\frac{1}{K} - \frac{1}{K'}\right) \qquad (6)$$

The semiconduction relation is then

$$\sigma_A(T,K') = \sigma_0 \exp[E_v/2kT] \quad \exp[(e^2/2kTR)(1/K - 1/K')] \qquad (7)$$

where the second exponential term is the magnitude of the current increase as a result of the formation of the complex.

We have found for the two gases we have tested—oxygen and methanol—that the dielectric constant of β-carotene does increase when the complex is formed, and by almost exactly the correct amount required to predict the correct activation energies and conductivity increases using equations (6) and (7). Incidentally, this approach is free of any suppositions concerning injected charge carriers or on the nature (electronic versus ionic) or sign of the charge carriers.

It still remains a serious problem to understand why the formation of the weak bound complex changes the dielectric constant. A clue could be obtained from the theory of donor-acceptor interaction, which postulates a partially dative ground state (D^+A^-). This could lead to additional dipole fields. On this basis, we can only tentatively conclude that the weak bound complexes formed between the adsorbed gas and the β-carotene molecules are donor-acceptor complexes. In some other cases (such as the iodine-lipid and dinitrophenol-lipid complexes, see later) this conclusion is strengthened by spectral evidence of a donor-acceptor complex. We have not as yet found any charge transfer bands as evidence of the donor-acceptor complex of gases with β-carotene in the solid state (unpublished work of T. N. Misra and B. Rosenberg).

Differential Sensitivity and Receptor Types

Table 1 shows that the esters, methyl and ethyl acetate, produce less than a factor of 10 increase in the conductivity of the β-carotene cell. This is surprising, for the esters have a low threshold for detectability and, like other very odorous gases, it should have increased the current by a factor of about 10^6. We then measured the current increase in a cell of crystalline powder vitamin A alcohol exposed to these vapours. This chemical is a semiconductor with a low conductivity—on the order of that of β-carotene. The geometry of the conduction cell, as well as all other conditions, was maintained unchanged. The results are shown in Fig. 3. Here the current increase caused by adsorbing methyl acetate on β-carotene and vitamin *A* alcohol is compared. It is obvious that the vitamin *A* alcohol has the expected sensitivity to the esters, while the β-carotene does not.

This one case illustrates the possibility that the different carotenes and haplocarotenes function as the different receptor types, each with its own class of compounds which form the donor-acceptor complexes with it and therefore change the conductivity. Much more work remains to be done to test this crucial hypothesis of the theory and to sort out the respective classes of chemicals reacting with each receptor type. This particular example, however, is clear cut and significant, and serves to indicate the method for these tests. A more complete characterization of the pigments present in the olfactory organ is now necessary.

Fatigue Effects in Semiconduction

A second unexpected result of our experiments is the appearance of a fatigue effect in the conductivity process with certain gases. While the biological processes of fatigue or adaptation in sense organs are complicated by contributions occurring at each stage of the pathway between receptor and consciousness, there should still be fatigue or adaptation effects at the earliest stages. This

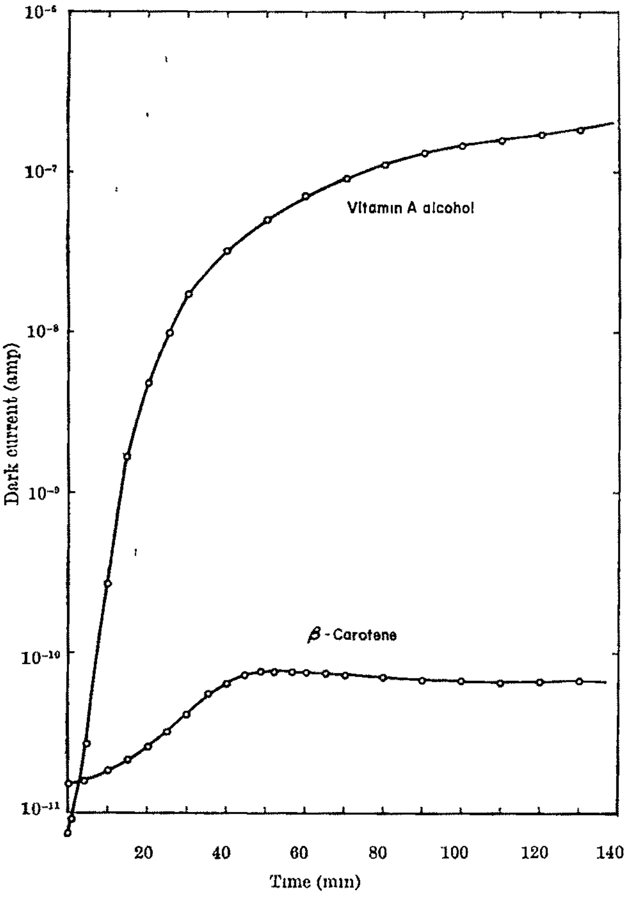

Fig 3 Comparison of the conductivity changes in β-carotene and vitamin A alcohol crystals (both all *trans*) when methyl acetate vapour carried by nitrogen gas is passed through the chamber

certainly holds in the case of the visual receptor, where a contribution to adaptation is known to occur in the receptor cell before the first synaptic connexion The anatomical simplicity of the olfactory system, in which only one synapse occurs in the pathway to the brain, makes a fatigue effect even more imperative at the receptor cell level

In Fig 2, the shape of the current versus time curve for isopropanol differs from the others, for with continuing exposure to the vapours at high concentration (partial pressure = 12 mm of mercury) the current increases to a maximum and then declines This effect is more clearly manifested with ammonia gas adsorbed on a β-carotene cell as shown in Fig 4 Here the current has risen by five orders of magnitude on first being exposed to the gas, and then decreases by three orders of magnitude before reaching a steady state When the gas is removed, by flushing the chamber with nitrogen only, the current returns to its original value This indicates that no marked chemical reaction has occurred between the ammonia gas and the β-carotene (at higher temperatures, however, there is such a permanent change in conductivity attributable to a chemical reaction) If ammonia gas is again passed into the chamber, the current rises again, but by only a factor of 10, indicating that the surface of the β-carotene has changed in some significant way In Fig 5, we show the effect of a number of such reversible flushes, each repetition of which causes a smaller change, until after five repetitions the current rise becomes reproducible and stable

After flushing with nitrogen gas for a few hours, the sensitivity to ammonia returns and the entire process can be repeated In essence, the surface of the β-carotene recovers its sensitivity There are at least two interpretations of this recovery process The first is that it is a result of the process of sublimation of the contaminated surface layers, thus exposing a virgin surface to the next ammonia gas exposure The second is the slow break-up of a more tightly bound complex and subsequent desorption of the ammonia—where the more tightly bound complex occupies the same sites as does the first type of complex but does not affect the conductivity While the sublimation process is attractive in explaining the recovery, it does not provide a basis for explaining the initial fatigue effect The second process can account for both effects Indeed, experimental evidence in favour of this second explanation has already been reported

We have already described the effect of oxygen adsorption on the conductivity of crystalline chlorophylls, *a* and *b*[12] It was shown that oxygen could exist in two states on the chlorophyll crystal surface In the first state, it increased the conductivity (donor–acceptor complex) and was easily desorbed In the second state, it did not affect the conductivity, and was more tightly bound to the surface (Ch^{+}–O_2^{-} ?) Irradiation by light converted the second form to the first form In the dark, the conductivity of crystalline chlorophylls was again higher, and the adsorption of gas was repeated a few times, with diminishing effects, until it almost disappeared A fresh exposure to oxygen would then start the same series of changes again

On the basis of these results, we suggest the following events as determining the fatigue effect and recovery The ammonia gas is adsorbed to a specific site on the crystallite, it forms a donor–acceptor complex with the β-carotene, there is an increase in the effective dielectric

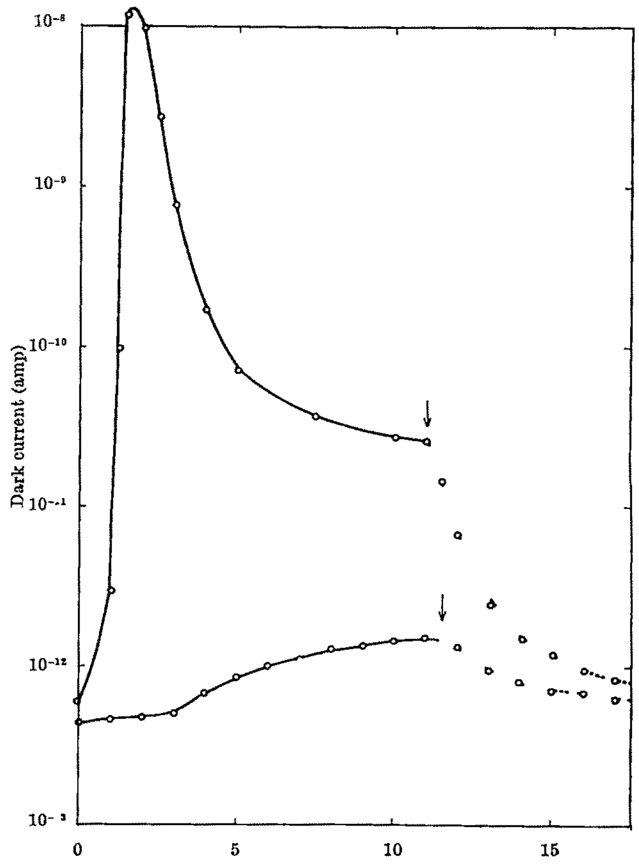

Fig 4 Fatigue effects in the conductivity of β-carotene when exposed to high concentrations of ammonia gas (NH_3 N_2, 1 1) The top curve is the first exposure and removal (arrows indicate the latter), the bottom curve is the second exposure

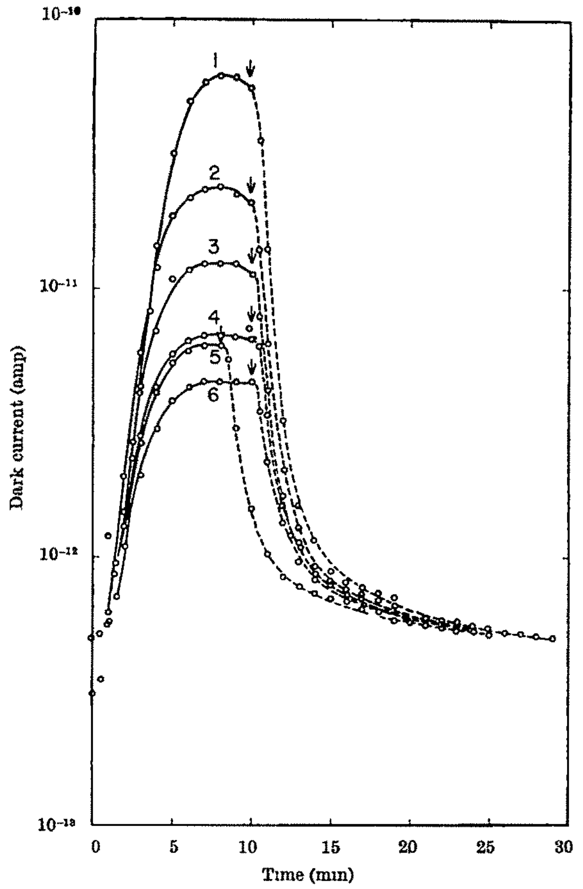

Fig 5 Fatigue effects in the conductivity of β-carotene after partial recovery of sensitivity, when exposed to ammonia as in Fig 4 Each repeat of the exposure and removal of the ammonia gas produces a smaller response until after six exposures the response stabilizes Repeats 7 to 10 (not shown here) are essentially the same as curve 6

constant, the activation energy for thermal charge generation decreases, and the conductivity increases This is state I The donor–acceptor complex converts in an equilibrium process to a more stable form (an ionic complex ?) on the same site which decreases the contribution to the effective dielectric constant, the conductivity decreases to a lower value, the binding energy of the complex is stronger and it therefore does not easily desorb This is state II (We thank L G Augenstein for recalling this two state hypothesis to our attention) When the chamber is flushed with nitrogen, the state I population decreases rapidly to zero and the conductivity returns to the usual nitrogen value The state II population slowly desorbs and/or slowly reverses to the state I type which desorbs, leading eventually to the total loss of the state II population The surface is returned to its initial condition Some more tests of this hypothesis are required and are under way at present in our laboratory The hypothesis is attractive in being able to explain the occurrence of fatigue and the subsequent recovery, and in a manner which agrees with the proposed theory We suggest that this may be at least a part of the fatigue effect in olfaction

Lipid Bilayers

So far, the experimental evidence submitted as the basis of this theory has been obtained on crystalline or glassy powders We do not suggest that such structures as these occur in the receptor membranes A more direct model system for a membrane is the bimolecular lipid films (lipid bilayers)[13] We have investigated such bilayers, using egg lecithin, synthetic lecithin and oxidized cholesterol The conductance of such bilayers increases by 10^5–10^6 when iodine (I_2, 10^{-5} molar) is added to the water electrodes[14] Spectroscopic evidence has been obtained which shows that charge transfer bands occur when iodine and these lipids interact in solution[15], and also that donor–acceptor complexes do form Solid state film experiments agree with these results and also show that the effective dielectric constant of the film increases when iodine vapour is adsorbed Again, these complexes are reversible

The same type of phenomena have therefore been found to occur in a system more like the biological situation In this case, no carotenoid was present in the bilayer, and only iodine and dinitrophenol have been tested so far Nevertheless, two important conclusions can be drawn from this work The first is that an unpigmented membrane may react to the presence of noxious chemicals by the proposed mechanism, and this may account for the response of the trigeminal nerve to higher concentrations of such chemicals The second conclusion is that bilayers incorporating various carotenoid pigments[16] form a simple and more significant model for the further testing of our theory Were it not for this possibility, we would hesitate to add yet another theory to the many already proposed to explain the phenomenon of olfactory transduction

On the basis of the experimental results described here, we propose the following as a possible mechanism for olfactory transduction (1) The pigments in the pigmented olfactory receptors constitute the recepter sites (2) These pigments exist to some degree in the limiting resistance membrane of the receptor cells (3) The odorous molecule when adsorbed on the receptor site forms a weak bound complex (a donor–acceptor complex) with the pigment molecule (4) The conductivity of the membrane increases because of an increase in the equilibrium number of charge carriers resulting from the donor–acceptor complex. (5) This increase in conductance is an amplifying process and leads to the depolarization of the membrane and signal generation (6) The donor–acceptor complexes are weakly coupled (binding energy less than 0 20 eV) and break up, leading to a desorption of the odorous molecule when the partial pressure of such molecules in the ambient atmosphere decreases (7) The nature of the donor–acceptor complex determines the amount of increase in conductance, and therefore the threshold sensitivity to the odorous molecule (8) The odorous molecule may form different strength donor–acceptor complexes with the different pigments of the various receptor types, and the resulting complex of signals defines the odour quality of that molecule (9) Such donor–acceptor complexes may form to a lesser degree between some molecules and the phospholipid constituents of an unpigmented membrane and produce similar, though weaker, signals through the same mechanism

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[1] Moulton, D G , and Beidler, L M , *Physiol Rev* 47, 1 (1967)
[2] Milas, N A , Postman, W M , and Heggie, R , *J Amer Chem Soc* , 61, 1929 (1939)
[3] Briggs, M H , and Duncan, R B , *Nature*, 191, 1310 (1961)
[4] Gerebtzoff, M A , and Shkapenko, G , *CR Assoc Anat* , 68, 511 (1952)
[5] Briggs, M H , and Duncan, R B , *Nature*, 195, 1313 (1962)
[6] Kurihara, K , *Biochim Biophys Acta*, 148, 328 (1967)
[7] Rosenberg, B , *J Chem Phys* , 34, 812 (1961)
[8] Gutmann, F , and Lyons, L E , *Organic Semiconductors*, 197–224 (New York, John Wiley and Sons, 1967)
[9] Misra, T N , Rosenberg, B , and Switzer, R , *J Chem Phys* (in the press)
[10] Cherry, R J , and Chapman, D , *Nature*, 215, 956 (1967)
[11] Rosenberg, B , *J Chem Phys* , 36, 816 (1962)
[12] Rosenberg, B , and Camiscoli, J F , *J Chem Phys* , 35, 982 (1961)
[13] Mueller, P , Rudin, D O , Tien, H T , and Wescott, W C , *Circulation*, 26, 1167 (1962)
[14] Rosenberg, B , and Jendrasiak, G L , *Chem Phys Lipids* (in the press)
[15] Bhowmik, B , Rosenberg, B , and Jendrasiak, G L , *Nature*, 215, 842 (1967)
[16] Leslie, R B , and Chapman, D , *Chem Phys Lipids*, 1, 143 (1967)

Origin of Nitrate Deposits

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New evidence from Antarctica supports the idea that nitrate deposits have been accumulated by precipitation from the atmosphere, in the absence of leaching and biological activity

DURING the summer of 1964–65 we surveyed soils in Antarctica at several localities along the western margin of the Ross Sea and Ross Ice Shelf We found that these soils showed many of the features of soils of arid climates, including the accumulation of water-soluble salts In particular, we found that included in these salts were nitrates of sodium and magnesium, together with traces of iodates Nitrate salts were found to be widespread in Antarctica in soils from various parent materials

The salt deposits that reveal most about the origin of the nitrates are those of the valley of the Shackleton Glacier, in the Queen Maud Range (Fig 1), in soils described earlier[1] These salt deposits can be divided into two groups, those of the Roberts Massif at the head of the Shackleton Glacier on the edge of the Polar Plateau, and those about 30 miles down the glacier near its junction with the McGregor Glacier

Roberts Massif is a large nunatak about 50 square miles in area which is encircled by two branches of the Shackleton Glacier and has an altitude of between 7,000 and 10,000 ft At some time in the past, the Polar Plateau was at least 1,000 ft higher than it is now, and covered the whole of the region As the level of the plateau has fallen, the ice retreated uncovering moraine-covered surfaces on which weathering has taken place and primitive soils have formed Each of these soils contains a horizon consisting predominantly of salts at a depth of 2–3 in below the surface The thickness of this horizon increases with the age of the soil, as indicated by weathering and topography, and so the accumulation of the salt is the result of a long-term process and is not some fossil feature The salts consisted of sulphates and nitrates of sodium, calcium and magnesium, together with traces of iodate, no chloride was detected and the ratio of chloride to nitrate was less than 1 15,000 (Table 1) The salt horizons contained minerals such as gypsum thenardite, epsomite, hexahydrite, bloedite, darapskite and soda-nitre

Table 1 WATER-SOLUBLE SALTS IN SOME ANTARCTIC SOILS

Depth (in)	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	SO_4^{2-}	NO_3^{-}	IO_3^{-}
				(mequiv /100 g soil)				
Upper part of Roberts Massif, at head of Shackleton Glacier, 85° 32′ S , 177° 20′ W								
0–2	3 4	0 5	6 7	<0 02*	<0 01*	5 7	0 2	0 1
2–3	6 0	25 1	35	<0 02*	<0 01*	38 0	40 0	0 01
3–7	7 6	116	200	0 07	<0 01	57 5	300	0 02
7–14	3 0	7 0	25 0	0 02	<0 01*	11 2	20 0	<0 01*
McGregor Glacier junction in middle part of Shackleton Glacier, 85° 11′ S , 174° 05′ W								
0–3	0 2	4 0	6 3	0 03	3 7	1 2	6 8	0 05
3–7	0 1	2 4	4 7	0 03	2 9	0 3	5 0	0 04
7–12	0 1	0 05	1 2	0 01	1 0	0 3	<0 1*	0 01
Coastal part of Darwin Glacier, 79° 51′ S , 159° 10′ E								
0–4	3 1	4 0	15 0	0 8	11 9	3 2	10 1	0 025
5–9	1 6	4 3	11 7	0 6	9 1	1 8	7 3	0 015
11–14	1 4	3 2	10 7	0 7	8 9	1 5	7 2	0 01
Saline ground water, results as mequiv /l								
14	49 0	254	400	16	231	69	440	4 8

* Not detected by the analytical method used

At the McGregor Glacier junction, where other extensive areas of bare ground occur, the proportion of chloride in the salts was greater, the ratio of chloride to nitrate approaching 1 1 in places (Table 1) In this area, salts do not occur as distinct horizons but are distributed throughout the soil—in places as nodules of salts, but commonly with no salt visible

These two regions differ markedly in climate, which accounts for the difference in composition, amount and distribution of the salts in the soils The Roberts Massif region on the edge of the Polar Plateau is constantly swept by a cold, dry katabatic wind from the south,

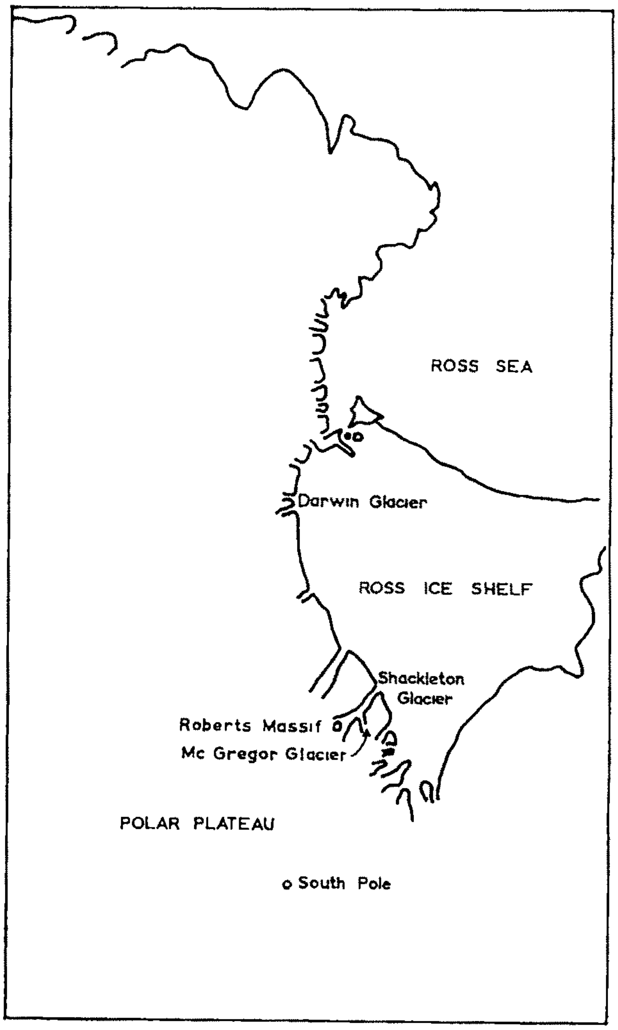

Fig. 1 Map of western margin of the Ross Ice Shelf showing localities of deposits on which nitrate studies were based

precipitation is very low and most of the snow that falls is blown from the Polar Plateau, temperatures are rarely, if ever, above freezing point and little if any melting of the snow takes place, instead the snow either sublimes or is blown away In this region any salts contained within the snow remain behind when it sublimes and may be transported a few inches into the soil by small amounts of moisture which may occasionally be present Consequently, the salts found in these soils should be related to the salt content of Polar Plateau snows

Further down the Shackleton Glacier at the junction with the McGregor Glacier the climate is warmer and precipitation is higher, the snowfalls occurring during periods when warm air masses move in across the Ross Ice Shelf bringing cloudy conditions to the coastal mountains We observed that, during the summer, cloudy conditions and light snow did not reach as far inland as the Roberts Massif, presumably being driven back by the increasing force of the katabatic wind from the south near the edge of the Polar Plateau Conditions in winter are unknown, but the precipitation pattern is expected to be similar

A third region even warmer and more moist than the other two can be defined near the coast and at a low altitude Although we could not examine the coastal region of the Shackleton Glacier Valley, it is thought to be very similar to the coastal portion of the Darwin Glacier Valley, where chlorides make up a greater proportion of the salts, but nitrates and iodates are still present (Table 1) Where temperatures are warm enough and drainage is impeded, salts may accumulate in highly saline ground water or as evaporite deposits around the edges of undrained basins[2]

Any differences in the nature of the salts contained in the soils of these regions should be related to the differences in origin of the precipitation—the Roberts Massif snows coming from the Polar Plateau, and those from areas nearer the coast being derived from local air masses gaining their moisture from the Ross Sea The principal difference is the virtual absence of chloride from salt deposits of the Roberts Massif region and its presence in soils closer to the coast

Wilson and House[3] have shown that snow falling at the South Pole contains small quantities of nitrogen in the form of nitrate Ammonia and organic nitrogen were not detected Small quantities of chloride and a smaller proportion of sulphate were also present, the molar ratio of chlorine to nitrogen being 2 1 The infall of nitrogen was reported to be 5×10^{-8} g/cm^2/yr They suggested that the nitrate was atmospheric nitrogen fixed by auroral processes, and that the chloride and sulphate are ultimately derived from the ocean

A considerable volume of work on the composition of atmospheric precipitation has been published and this has been reviewed by Eriksson[4] Both ammonia and nitrate–nitrogen have been detected in analyses of atmospheric precipitation together with compounds of chlorine, sulphur and iodine Most of these materials are thought to be derived from the sea Wilson[5] has shown that the upper, very thin layer of the ocean is enriched in nitrogenous biological materials which are transferred to the atmosphere during periods of strong winds He suggests that this is the source of the nitrogenous compounds found in the snows of temperate regions Near the oceans, most of the nitrogen is in the form of ammonia or protein compounds, but these are slowly oxidized, probably by ozone produced by the action of ultraviolet light in the upper atmosphere[6] Sulphides are similarly oxidized to sulphates and iodine compounds to iodates Lodge and Pate[7] have shown that the atmosphere over tropical oceans contains ammonia, but that the proportion of nitrogen oxides increases over land masses The proportion of chloride in atmospheric precipitation decreases with increasing distance from the sea, because of the removal of sodium chloride crystals which act as condensation nuclei and are thus precipitated from the atmosphere[8]

We believe that the compounds of nitrogen, sulphur and iodine in tropical and temperate regions are transported to the polar regions by the atmosphere in the course of circulation to maintain the permanent anticyclone induced by the altitude of the Antarctic continent and which is centred over the continent[9] During transport, they are oxidized to nitrates, sulphates and iodates The greater part of these compounds are removed with atmospheric precipitation but, as already explained, chlorides are removed preferentially

The remaining salts are precipitated with snow on the Polar Plateau in the quantities and proportions found by Wilson and House[3], and are transported by wind to areas of bare ground, such as the Roberts Massif, where they accumulate when the snow sublimes or melts and evaporates A further mechanism must be postulated, however, to account for the virtual absence of chloride from the soils of the Roberts Massif and its presence in snow at the South Pole We suggest that, because sodium chloride is more hydroscopic than other salts, it may dissolve and percolate deeper into the general mass of the ice, and that consequently it is progressively reduced in concentration during continual blowing around of drifting snow This can be supported by the fact that Wilson and House[3] report the presence of chloride in snow from a depth of 13 m but not from surface snow at the South Pole

The only other area in the world where nitrates and iodates are widely distributed is northern Chile Many hypotheses on the origin of the Chilean deposits are discussed by Goldschmidt[10], who suggests that, because Chilean nitrate contains approximately 0 2 per cent iodine as iodate, a solution to the problem of origin of these deposits might be found if the presence and state of oxidation of iodine compounds in salt deposits in other parts of the world were studied The proportion of iodate to nitrate in the Antarctic salts is similar to that in the Chilean nitrate deposits (Table 1), consequently, it seems likely that the nitrates and iodates are genetically related and have a similar origin in both the Chilean and Antarctic deposits

The possibility that the nitrates and iodates could have been derived by leaching from sedimentary rocks (as suggested by Johanneson and Gibson[11]) is disproved by their widespread distribution in soils formed from widely different rocks Igneous rocks have been found to contain small amounts of nitrogen[12], but the amount released at the slow rate of weathering prevailing in Antarctica seems to be insufficient to account for the quantities of nitrate present Furthermore, the iodine content of the salts is also left unexplained

A new general theory of the origin of principal nitrate deposits is proposed The atmosphere contains small quantities of nitrogenous material as well as compounds of iodine, sulphur and chlorine derived from the ocean surface in the manner suggested by Wilson[5], and these compounds, in varying proportions and states of oxidation, form part of the precipitation from the atmosphere throughout the world In most parts of the world, the compounds are intercepted by organisms or pass by leaching through the soil and are returned to the sea It is only in the almost complete absence of biological activity and leaching in such places as the Antarctic Continent and the deserts of northern Chile that nitrate salts can accumulate on a large scale

In both these areas, the deposits occur on old surfaces where the salts have been able to accumulate for long periods of time, the age of the Roberts Massif surface is considered to be at least several hundred thousand years[1], as are the surfaces on which nitrates are found in Chile[13]

Similar nitrate deposits might be expected in other parts of the world where conditions are sufficiently arid, however, in most of the world's desert areas they may be removed in drainage waters during the rate periods of torrential rainfall, and may be found in saline lakes and other drainage sinks. Nitrate deposits are in fact also known from the arid areas of California[14] where they seem to be similar to the Chilean deposits, but no other deposits of appreciable size are known except in Antarctica.

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[1] Claridge, G G C, and Campbell, I B, *NZ J Sci* (in the press)
[2] Campbell, I B, and Claridge, G G C, *NZ J Sci*, **10**, 563 (1967)
[3] Wilson, A. T, and House, D A, *J Geophys Res*, **70**, 5515 (1965)
[4] Eriksson, E, *Tellus*, **4**, 215, 280 (1952)
[5] Wilson, A T, *Nature*, **184**, 99 (1959)
[6] Ram, A, *Proc Acad Sci un Prov*, **4**, 147 (1934)
[7] Lodge, J P, and Pate, J B, *Science*, **153**, 408 (1966)
[8] Rossby, C G, and Egner, H, *Tellus*, **7**, 118 (1955)
[9] Weyant, W S, in *Antarctic Soils and Soil Forming Processes* (edit by Tedrow, J C F), 47 (Amer Geophys Union, 1966)
[10] Goldschmidt, V M, *Geochemistry* (Oxford University Press, 1954)
[11] Johanneson, J K, and Gibson, G W, *Nature*, **194**, 567 (1962)
[12] Wlotzka, F, *Geochim Cosmochim Acta*, **24**, 106 (1961)
[13] Vial, C D, and Wright, A C S, *Soils of the Arid Zones of Chile* (FAO Soils Bulletin No 1, Rome, 1965)
[14] Clarke, F W, *Bull US Geol Surv* 770 (1924)

Induction of the Primary Immune Response

by

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The cause of the induction of the primary immune response could be the depletion of a specific natural antibody

SERUM opsonins play a part in determining intravascular clearance rates of several types of particles, and it seems that recognition of the surface characteristics of the particle first occurs in the blood plasma of the host[1]. Antibodies, either natural or immune, act as opsonins to influence clearance rates by coating the surface of the particle, making it more liable to phagocytosis. Recognition comes about by the complexing of a specific opsonin with the particle at the moment of its contact with the blood, and this complex is removed from the circulation by the reticulo-endothelial cells, which results in a temporary depletion from the circulation of opsonins specific for that particle[2]. This sequence of events is similar to that which accompanies the intravenous injection of antigenic material, for example, bacterial, in non-immune as well as in immune animals.

The phagocytic cells of the reticulo-endothelial system or macrophages are usually found close to antibody producing cells and seem to be implicated in antibody production. A recent theory holds that the macrophage "processes" the ingested antigen by complexing RNA with the antigenic fragments and this complex is transferred to the precursors of antibody forming cells[3]. The minute amount of antigen which can be detected in antibody producing cells does not seem to supply the instruction necessary to support the high level of antibody synthesis that is usually found. Antigens may induce a primary response by indirectly priming cells genetically endowed with the ability to produce a specific antibody. These pre-formed antibodies would be represented in the body fluids by natural antibodies, that is, antibodies usually found in non-immunized animals. The macrophage, by ingesting the antigen–antibody complex[4], would cause a temporary depletion of the specific antibody in the extracellular fluid, an event which may be a signal to the cells producing that antibody. If the body fluids can recognize antigen—the specific natural antibody—and the macrophage removes it from the body fluids, the natural antibody producing cells bathed by the depleted fluid might recognize the absence of a specific natural antibody. Thus the exchange transfusion of blood previously depleted of natural antibodies against sheep red blood cells initiated a primary immune response in the rat equal to that obtained by the intravenous injection of sheep red blood cells.

Essentially the procedure was to adsorb the donor plasma with washed sheep red blood cells *in vitro*, recombine the adsorbed plasma with the donor blood cells and exchange with the recipient by transfusion.

Male rats of two strains were used, a Lewis inbred strain and a Sprague–Dawley (S–D) strain. Donor animals weighed between 250–300 g and recipients between 150–200 g. The citrate solution, 0.38 molar citric acid, 0.075 molar sodium citrate, 0.064 molar sucrose (ACS), was used in the proportion of 1.5 ml/8.5 ml of blood. ACS donor blood (35 ml) was centrifuged, 1,700*g*, for 40 min and 14 ml of the plasma was removed for adsorption. The remaining supernatant plasma was removed without disturbing the buffy coat and the donor red cell volume was adjusted to 10 ml by aspiration immediately before mixing the adsorbed plasma with the donor cells.

Sheep red blood cells (SRBC) in Alsever's solution were washed three times with 0.15 molar sodium chloride–0.01 molar phosphate buffer, *p*H 7.25 (PBS). The donor plasma was adsorbed by mixing with 1 ml of packed SRBC in plastic centrifuge tubes using siliconized pipettes and left at room temperature for 10 min followed by centrifugation, 300*g*, for 15 min at 10° C. The adsorbed plasma was filtered through a 'Millipore *HA*' membrane, pore size 0.45μ, using slight air pressure. After mixing the adsorbed plasma with the donor cells, the blood was passed through gauze and kept at room temperature for 30 min before transfusion.

The recipient was anaesthetized by intraperitoneal injection of 'Nembutal' and a No. 10 polyethylene catheter was inserted in the femoral vein through which 140 USP units of sodium heparin in 0.7 ml of saline was injected. The femoral artery was then cannulated and the outflow collected in a graduated receptacle. Donor blood was injected through the venous catheter at a rate corresponding to the outflow. The percentage exchange of blood was calculated from the formula of Trossman *et al.*[5] based on normal blood volumes of the rat[6].

Serum agglutinin titres against SRBC were measured on the fifth post-transfusion day. All sera were inactivated at 56° C for 20 min. Doubling dilutions of the antiserum with 2 ml final volumes were set up using 1 per cent normal rat serum in PBS as the diluent. The standard SRBC suspension was prepared by adding one volume of 1 per cent trypsin to nine volumes of an approximately 1 per cent suspension of washed SRBC and agitated for 10 min at 37° C. After a final washing the concentration of the SRBC was adjusted photometrically so that 1 ml of the SRBC suspension in 4 ml of water gave an optical density reading at 520 mμ of 0 30 A sample of the standardized SRBC suspension (0 1 ml) was added to the test system and agitated for 90 min at 37° C After centrifugation at 50g for 5 min the tubes were agitated and the highest serum dilution showing agglutination was recorded as the titre

My primary concern has been that solubilization of the surface antigens of the SRBC might have occurred during the adsorption process, for the 'Millipore' membrane would exclude SRBC stromata Three methods were used to test for soluble antigens in the adsorbed plasma ACS plasma, both SRBC-adsorbed and non-adsorbed, was converted to serum by adding 0 08 ml of a solution containing 0 075 moles of calcium chloride and 0 25 moles of magnesium chloride/ml of plasma The serum was heat-inactivated and used as the diluent for the titration of rat anti-SRBC serum Before the addition of the standard SRBC suspension, the tubes were incubated at 37° C for 60 min and followed by a further incubation for 90 min The agglutinin titre was not decreased by the adsorbed serum diluent Serum prepared from adsorbed plasma was diffused against rabbit anti-SRBC serum by the method of Preer[7] with no band formation Antigens were not detected in the adsorbed plasma by the passive cutaneous anaphylaxis reaction in the rat using rabbit anti-SRBC serum by the method of Ovary[8]

Natural antibodies in rat serum against SRBC can be demonstrated by the more sensitive haemolytic method, not by the agglutination method used here The antigenic effect of transfused SRBC-adsorbed blood was as potent as the intravenous injection of the optimum number of SRBC (Table 1) and the strength of the immune response was not influenced by the source of the donor rat blood Non-adsorbed blood, otherwise similarly prepared and exchanged, had no effect on the agglutinin titre The ineffectiveness of smaller numbers of SRBC to induce a primary immune response suggests that the degree of natural antibody depletion may influence the strength of the immune response Whether the stimulus for inducing the antibody response is the result of the contact of the donor cells with the adsorbed plasma *in vitro* or primarily a host response is not known, and for this reason the buffy coat was kept intact and the proportion of donor cells to plasma held constant. Exchange of volumes less than 50 per cent of the host's total blood volume gave significantly lower titres; however, in the studies reported here, between 75 per cent and 80 per cent of the host's blood was exchanged

Relatively large volumes of SRBC are required to adsorb adequately the natural antibody in rat plasma and multiple adsorptions using aliquots of the adsorbent seem to remove the antibody more effectively than one adsorption using the same total volume of adsorbent (Table 2)

The replacement of natural antibodies in SRBC-adsorbed plasma, before mixing with the donor cells, suppressed the immune response in the transfused recipient (Table 3) The euglobulin fraction containing natural antibodies was prepared from an equivalent volume (14 ml) of normal rat plasma by dialysis against 1/150 molar phosphate buffer, pH 5 8, for 48 h The addition of a similar fraction prepared from plasma previously depleted of the natural antibody against SRBC did not influence the degree of the immune response.

Table 1

No of rats	Donor strain	Recipient strain	Treatment of donor plasma	Reciprocal of titre log 2 (mean)
3	Lewis	Lewis	Non-adsorbed	< 1
3	S–D	Lewis	Non-adsorbed	< 1
3	S–D	S–D	Non-adsorbed	< 1
5	Lewis	Lewis	SRBC adsorbed	10 2
5	S–D	Lewis	SRBC adsorbed	10 8
5	S–D	S–D	SRBC adsorbed	10 4
6	2×10^8 SRBC*	Lewis	—	10 5
8	2×10^8 SRBC	S–D	—	10 0
5	1×10^6 SRBC	S–D	—	2 5

* The intravenous injection of SRBC and agglutinin titres on fifth day
S–D, Sprague–Dawley

Table 2

No of rats	Volume (ml) packed SRBC	No of adsorptions	Reciprocal of titre log 2 (mean)
3	4	1	3 3
5	1	2	6 0
5	1	3	8 2
15	1	4	10 4

Table 3

No of rats	Treatment of donor plasma	Addition to donor plasma	Reciprocal of titre log 2 (mean)
3	SRBC-adsorbed	Euglobulin from SRBC-adsorbed plasma	10 6
3	SRBC-adsorbed	Euglobulin from non-adsorbed plasma	2 3

Natural antibodies of surprising specificity occur in most animal species including man[9] Their origins have been attributed either to multiple environmental stimuli[10] or to the maturation of an immunological apparatus under genetic control[11] My thesis would imply that antibody forming cells are primarily under genetic control although dependent on the constant specific and non-specific challenges encountered in the host environment to express their full potential

There was further evidence of a genetic influence in the distinctive patterns of natural antibody concentrations in each animal species studied[9] Landy[12] has shown individual profiles of natural antibody titres within a species in which constant concentrations of specific natural antibodies are maintained for long periods It is suggested that the concept of homeostatically controlled concentrations of natural antibodies in the body fluids is crucial to an understanding of the sequence of events which follow the introduction of the antigen Temporary imbalance of the homeostatic mechanism resulting from the temporary depletion of the natural antibody induces the primary immune response while prolonged imbalance produces immunological tolerance

While an immune response is developing the circulating antibodies show gradually increasing avidity or affinity for the homologous antigen[13], but the variation in the degrees of avidity does not seem to serve any useful physiological function Antigens introduced into a mixture of the homologous 19S and 7S immune antibodies are preferentially complexed by the 7S antibodies with the likelihood that a similar class preference is exercised in the circulation[14] The relatively large quantities of antigen required for the *in vitro* adsorption of the homologous natural antibodies, despite their low titre in serum, may indicate a still lower degree of avidity The efficiency with which an antigen can preferentially deplete the body fluids of a particular class of antibody would be influenced by the degree of avidity of the antibody and thereby provide the induction stimulus to the cells synthesizing that class of antibody It is therefore proposed that the avidities of antibody are very important in determining the character of the immune response

If the cellular stimulus for the induction of the primary immune response is a demand for the replacement of a specific antibody, those cells phenotypically restricted to its production would, by the process of cell division and differentiation, provide antibodies of a similar specificity although not necessarily having the same physical characteristics In presenting a possible sequence of cellular events using the proposed stimulus for immunogenical induction, other significant findings[15–18] seem to be particularly pertinent

The small mobile lymphocyte seems to be the most likely source of natural antibodies and thus implicated in the first phase of the primary immune response[15] The feedback mechanisms by which these cells maintain homeostatic concentrations of natural antibodies in the blood and tissue fluids are unknown although circulating concentrations of a specific natural antibody were apparently independent of the number of cells producing that antibody[19] After contact of the antigen with the body fluids and the removal of the antigen–natural antibody complex by the macrophage, depletion of that specific natural antibody from the extracellular fluid may impose a sudden demand for replacement on those lymphocytes phenotypically restricted to that particular pattern of antibody synthesis Cytoplasmic budding or shedding of the lymphocytes may replace the extracellular antibody to concentrations greater than the homeostatically controlled one, providing the stimulus for replication and further differentiation of that cell clone

What are the factors which might influence the cell clone synthesizing the natural antibody to replicate and transform into a more efficient antibody producing cell ? Would the sudden cessation of demand for the antibody from a cell synthesizing it at increased rates result in a higher energy potential in that cell ? Perhaps the concept of a higher energy potential can be translated as the accumulation of those basic building blocks common to both DNA and RNA-protein synthesis Lack of cytoplasmic competition may permit, for example, the sharp rise in the pool size of amino-acids in the nucleus which precedes DNA synthesis[20] As a corollary, would the continuing increased demand for RNA-protein synthesis during antibody production result in the inhibition of lymphocytic replication and differentiation ?

The experimental evidence would implicate the blast cell as the successor to the small lymphocyte[15] Subject to the same homeostatic mechanisms as the small lymphocyte and stimulated by the antigen-induced depletion of its product, 19*S* immune antibody, the blast cell, unlike the lymphocyte, is not usually found in the circulation The attribute of "immunological memory" has been bestowed on the blast cell rather than the small lymphocyte, but the difference may be qualitative, for, if the strength of immunological memory of the antibody producing cell is determined by the avidity of its product and thus the efficiency with which the antigen can deplete the body fluids, the blast cell would have preference over the small lymphocyte Cessation of demand for the 19*S* immune antibodies may provide the stimulus for blast cell replication and transformation to the plasmocyte which is capable of synthesizing 7*S* antibodies of the highest avidity Circulating concentrations of 7*S* antibody do not seem to be under homeostatic control, for depletion of the antibody is not followed by increased synthesis nor does the mature plasma cell show mitotic activity

A potential role of natural antibodies in the induction of the primary immune response has been postulated by Jerne[21], Talmage[22], Boyden[23] and Landy[12] The mechanism by which natural antibodies exert their directive influence on antibody production has been attributed either to self-replication of antibodies[21] or to their cytophilic properties enabling the potential antibody forming cell to receive the antigenic stimulus[23]

Burnet[24] has pointed out that the mechanism by which the immune system recognizes foreign material is a crucial one in understanding antibody production If depletion of specific antibody from the body fluids is the common immunogenic stimulus, it is tempting to consider the following previously inexplicable findings with regard to antibody production the inhibition of antigen induced antibody production in infants with high circulating titres of maternal antibody, the failure to achieve an antibody response when antigen–antibody complexes are injected or the "blanketing effect" when the second antigen is given during the height of the primary immune response, the practice of coating particles with soluble antigens, thereby increasing the surface area of the antigen and permitting larger quantities of antibodies to be complexed

The enhancement of antibody production has been shown to be a non-specific effect[25] Endotoxin releases natural antibodies non-specifically without obvious injury to the antibody producing cells resulting in a five-fold increase in the bactericidal titre of the host serum[19]. The increased titres of circulating antibody were followed by the replication of the antibody producing cells With homeostatic concentrations of natural antibody in the extracellular fluid more than satisfied, could the cytoplasmic events in the cell now modulate activities in its nucleus ? The apparent synergism between the endotoxin-induced cellular depletion of antibody and the antigen-induced depletion of the homologous antibody from body fluids seem to give some understanding of the mechanisms by which adjuvants enhance antibody production

Perhaps the most intriguing relationships exist between immunological tolerance to foreign antigens and the apparent non-recognition of auto-antigens If excess antigen remains in contact with the body fluids for prolonged periods because of a relative deficiency in phagocytosis-promoting antibody, would the homologous antibody forming cells, in attempting to re-establish homeostasis, repress replication and transformation into cells capable of producing larger quantities of more cytotoxic antibodies ? Antibody producing cells in rats made tolerant to SRBC did not respond to repeated SRBC antigenic stimuli by replication although they were capable of producing the homologous antibody[26] There seems to be little reason to advance a different mechanism for the recognition and removal of auto-antigens resulting from the constant breakdown of the host's cellular elements In a situation where there is a continuous uniform release of either auto-antigens or foreign antigens from a successful cellular transplant at birth, replication of the homologous antibody forming cells may be inhibited by similar mechanisms The elucidation of those events occurring in the cytoplasm of the cell and their modulating effect on nuclear events may provide a vital link in understanding the mechanisms which control antibody production

While the hypothesis of specific antibody depletion as an inducer of the immune response seems to offer plausible explanations for some aspects of antibody production, it does, however, provide a ready means for its testing

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[1] Murray, I M , *J Exp Med* , **117**, 139 (1963)
[2] Murray, I M , *Amer J Physiol* , **204**, 655 (1963)
[3] Fishman, M , *J Exp Med* , **114**, 837 (1961)
[4] Garvey, J S , and Campbell, D H , *J Immunol* , **72**, 131 (1954)
[5] Trossman, C M , and Alzofon, F E , *Amer J Dis of Child* , **98**, 694 (1958)
[6] Belcher, E H , and Harriss, E B , *J Physiol* , **139**, 64 (1957)
[7] Preer, jun , J R , *J Immunol* , **77**, 52 (1956)
[8] Ovary, Z , *Intern Arch Allergy Appl Immunol* , **3**, 293 (1952)
[9] Gibson, H J , *J Hyg* , **30**, 337 (1930)
[10] Milgrom, F , and Swierzynska, Z , *Schweiz Z Allgem Pathol Bacteriol* , **19**, 189 (1956)
[11] Hirszfeld, L , *Ergebn Hyg Bakt* , **8**, 367 (1926)
[12] Landy, M , and Weidanz, W P , in *Bacterial Endotoxins* (edit by Landy, M , and Braun, W), 257 (Institute of Microbiology, Rutgers, The State University, New Brunswick, 1964)
[13] Eisen, H N , and Siskind, G W , *Biochemistry*, **3**, 996 (1964)
[14] Robbins, J B , Altemeier, W A , and Smith, R T , in *Phylogeny of Immunity* (edit by Smith, R T , Miescher, P A , and Good, R A), 76 (University of Florida Press, Gainesville, 1966)
[15] Gowans, J L , *Ann NY Acad Sci* , **99**, 432 (1962)
[16] McGregor, D D , and Gowans, J L , *J Exp Med* , **117**, 303 (1963)
[17] Nossal, G J V , *Adv Immunol* , **2**, 163 (1962)
[18] Nossal, G J V , and Makela, O , *J Exp Med* , **115**, 209 (1962)
[19] Michael, J G , *J Exp Med* , **123**, 205 (1966)
[20] Lieberman, I , Abrams, R , Hunt, N , and Ove, P , *J Biol Chem* , **238**, 3955 (1963)
[21] Jerne, N K , *Proc US Nat Acad Sci* , **41**, 849 (1955)
[22] Talmage, D W , *Ann Rev Med* , **8**, 239 (1957)
[23] Boyden, S V , in *Cell-bound Antibodies* (edit by Amos, B , and Koprowski, H), 7 (Wistar Institute Press, Philadelphia, 1963)
[24] Burnet, F M , *The Clonal Selection Theory of Acquired Immunity* (Cambridge University Press, London, 1959)
[25] Johnson, A G , in *Bacterial Endotoxins* (edit by Landy, M , and Braun, W), 252 (Institute of Microbiology, Rutgers, The State University, New Brunswick, 1964)
[26] Rowley, D A , and Fitch, F W , *J Exp Med* , **121**, 683 (1965)

LETTERS TO THE EDITOR

ASTRONOMY

Half Life of the Neutron and Cosmological Helium Production

I WISH to point out a cosmological consequence of the recent reduction in the experimental half life of the free neutron, if this is confirmed

Since the measurements of Sosnovsky *et al*[1], the best value for the neutron half life has been 11 7 min Recently, a new measurement by Christensen *et al*[2] has given the significantly lower value of 10 8 min Although there may be further measurements before this result is accepted, it had earlier been pointed out by Blin-Stoyle and Tint[3] that there were theoretical reasons for believing that the value of 11 7 min was too large

In the homogeneous isotropic Friedmann universe the amount of helium produced in the early stages is chiefly governed by the relative densities of protons and neutrons when nuclear reactions occur, essentially all of the neutrons and an equivalent number of protons combine to form deuterium and, subsequently, helium At very high temperatures the number densities of protons, n_p, and neutrons, n_n, are related by the formula

$$\frac{n_n}{n_p} = \exp\left(-2\ 54\ m_e c^2/kT\right) \qquad (1)$$

As the temperature drops, the reactions changing protons into neutrons and vice versa

$$\begin{aligned} n + e^+ &\rightleftarrows p + \bar{\nu} \\ p + e^- &\rightleftarrows n + \nu \end{aligned} \qquad (2)$$

are no longer rapid enough to maintain thermal equilibrium and the neutron number remains larger than that given by equation (1) The reduction of the neutron half life implies a corresponding increase in the cross-sections for reactions (2) so that the neutron number remains closer to that predicted by thermal equilibrium There are fewer neutrons when helium production occurs, so the amount of helium produced is reduced

A recent calculation of the helium production in the early stages of a Friedmann universe by Wagoner, Fowler and Hoyle[4] gives a helium production of between 25 per cent and 30 per cent by mass for plausible values of the present mean density of the universe, if it is assumed that the conventional interpretation of the 3° K black-body background radiation is correct This compares with abundances in the same range which have been observed in a variety of objects[5] It seems from an approximate calculation that, if the new value of the neutron half life is accepted, the numerical values of Wagoner *et al* should be reduced to about 23 per cent and 27 5 per cent Although this reduction is itself not very great and is probably within other errors of theory and observation, it is in the correct direction if the cosmological theory is to be correct It is well known that helium can be produced astrophysically but there is at present no belief that large scale destruction of helium has occurred in the universe. Thus all observed helium abundances should exceed that produced cosmologically The reduction in the predicted cosmological helium abundance might therefore prove significant in a detailed comparison between theory and observation

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[1] Sosnovsky, A N, Spivak, P E, Prokoviev, Yu A, Kutikov, I E, and Dobrinin, Yu P, *Nucl Phys*, 10, 395 (1959)
[2] Christensen, C J, Nielsen, A, Bahnsen, A, Brown, W K, and Rustad, B M, *Phys Lett*, 26B, 11 (1967)
[3] Blin-Stoyle, R J, and Tint, M, *Phys Rev*, 160, 803 (1967)
[4] Wagoner, R V, Fowler, W A, and Hoyle, F, *Astrophys J*, 148, 3 (1967)
[5] Tayler, R J, *Quart J Roy Astro Soc*, 8, 313 (1967)

Small Scale Distribution of Interstellar Absorption

A SURVEY programme to take Hα photographs of galactic emission nebulae has been in progress since October 1966 at the Tokyo Astronomical Observatory using the 50 cm Schmidt telescope at the Dodaira Station[1] A combination of Eastman Kodak 103*a-E* plate and Hoya colour glass *R*64 5 mm thick are being used The combination gives a pass-band about 300 Å wide centred at λ6563 Å, and provides Hα pictures of emission nebulae after subtraction of other nebular radiations contained within the passband Each plate is carefully calibrated by means of a tube sensitometer, the absolute brightness scale is obtained by measuring the brightness of selected star images

Seven galactic H II regions, *IC*1795, *IC*1805, *M*20, *M*8, *M*16, *M*17 and *NGC*7000 region, have been analysed so far The photographs are converted to isophotal contour maps using the iso-densitometer of the Tokyo Astronomical Observatory

Comparison of these contour maps of H II regions with those of microwave radio emission is of interest if the mechanisms of both types of radiation are known Both maps represent the emission measure, but the former includes interstellar absorption by solid grains. The comparison thus enables us to make a map of the interstellar absorption by assuming that both radiations are thermal

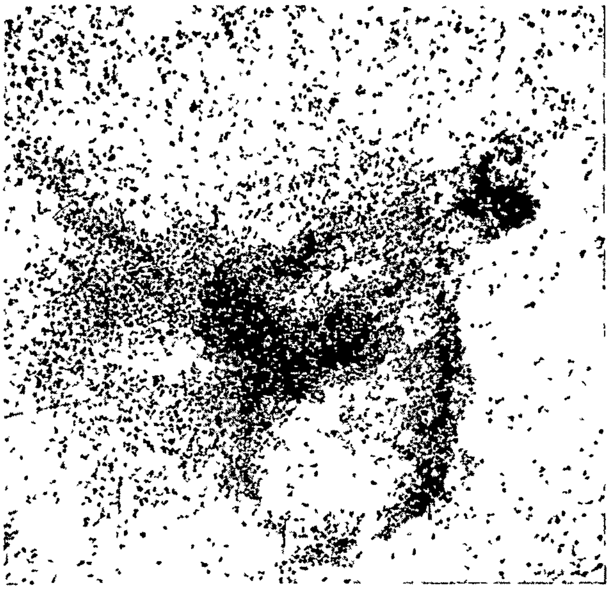

Fig 1 Hα photograph (Kodak 103*a-E* with Hoya *R*64 5 mm) of *IC*1795 (upper right of *IC*1805) and *IC*1805 taken with 50/65 cm F 2 0 Schmidt telescope of Dodaira Station, Tokyo Astronomical Observatory (Plate *SB*161 exposed for 60 min on October 7, 1966)

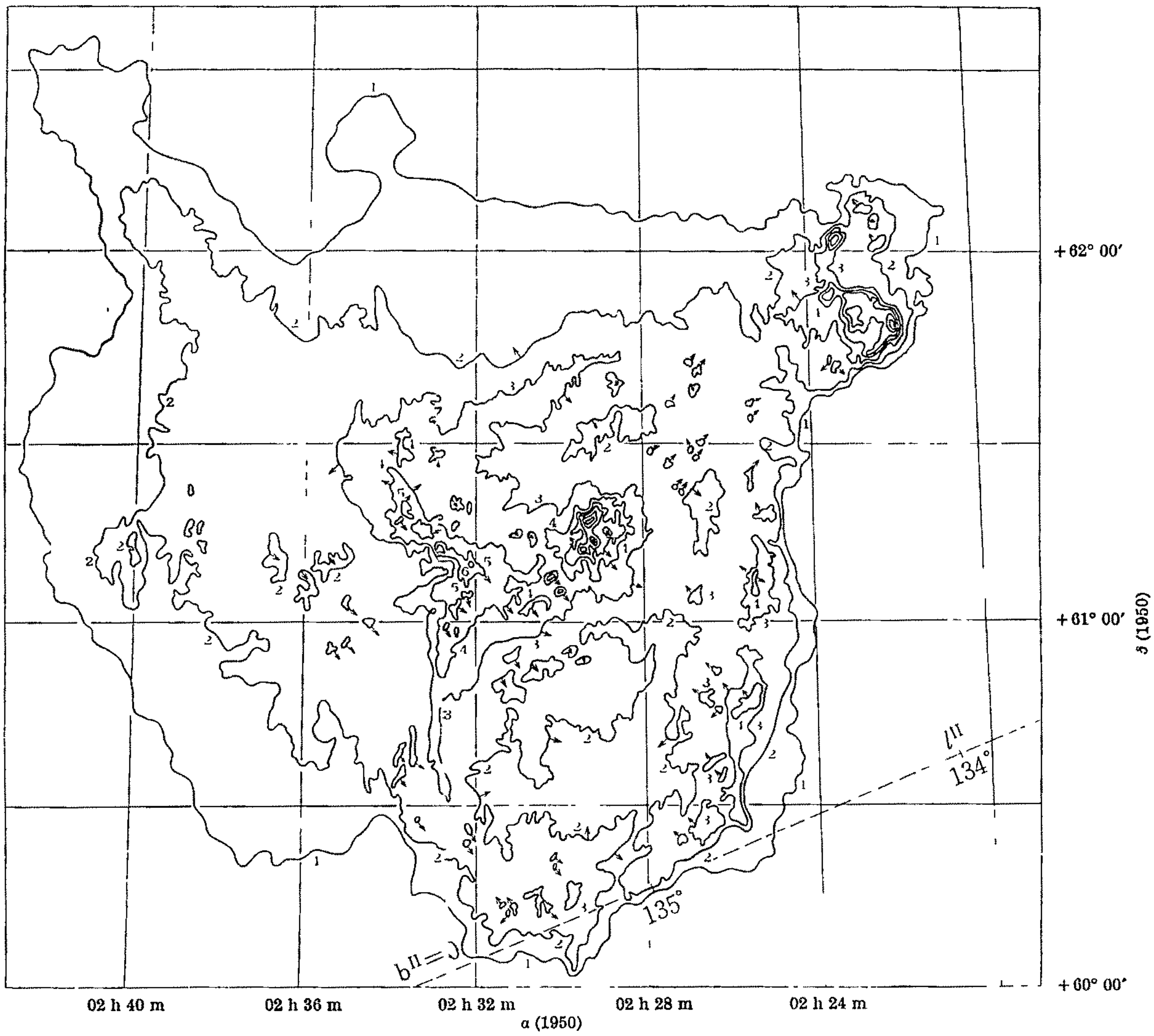

Fig 2 Hα contour map of *IC*1795 = *W*3 and *IC*1805 = *W*4 after Ishida and Ohashi[1]

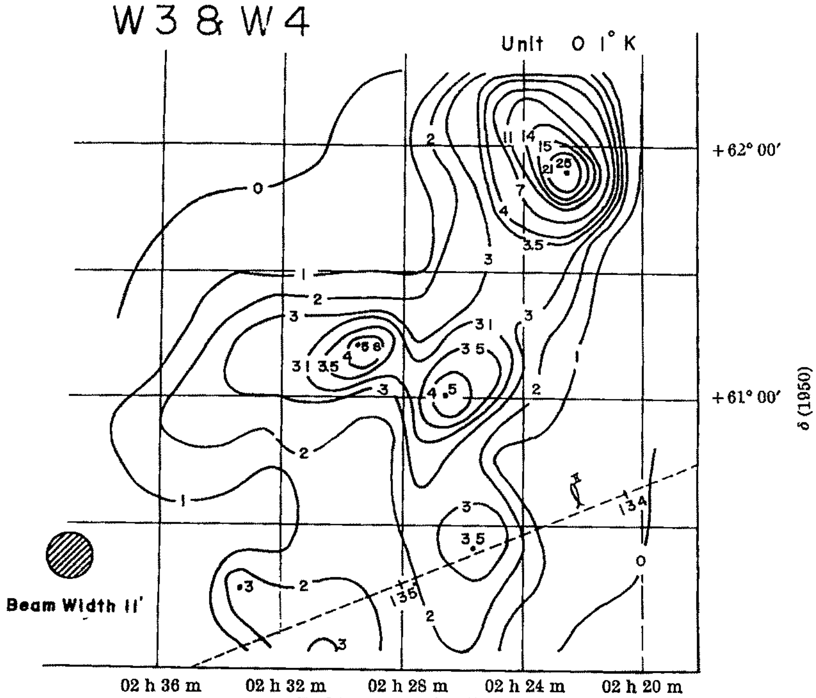

Fig 3 Microwave radio emission map of *IC*1795 = *W*3 and *IC*1805 = *W*4 at 4,170 MHz after Akabane *et al* [2]

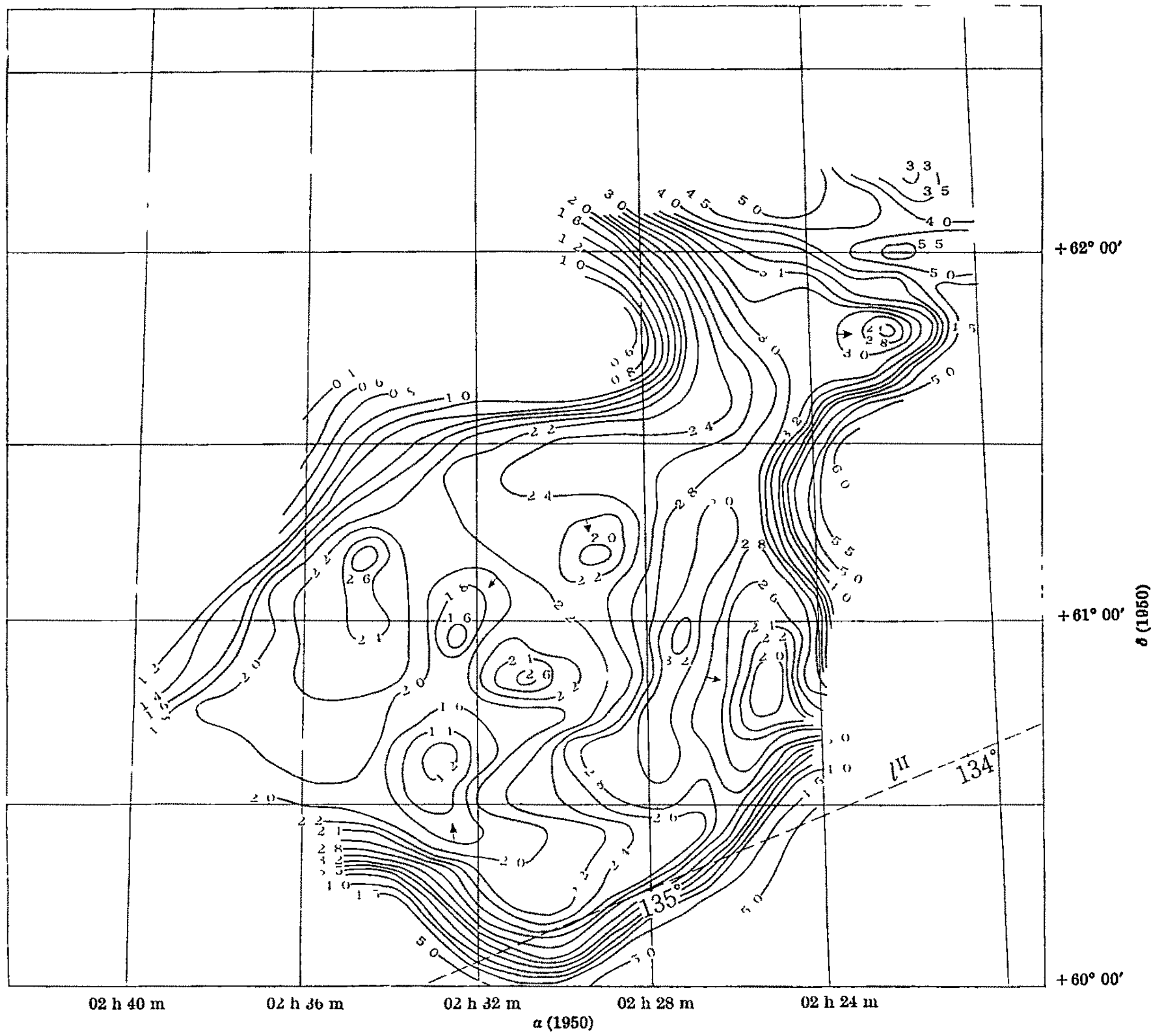

Fig 4 Interstellar absorption map in magnitude units of *IC*1795 = *W*3 and *IC*1805 = *W*4 at λ6563 Å

Figs 1–4 show a comparison for a complex region near the galactic equator (l^{II} is about 134 degrees) Fig 1 is a photograph in Hα light, Figs 2 and 3 are the contour maps in Hα and in microwave radio emission at 4,170 MHz (ref 2), and Fig 4 shows the surface distribution of interstellar absorption over the region The two emission nebulae in the field are *IC*1795 and *IC*1805 (*W*3 and *W*4, respectively, as radio sources)

Small scale structure of absorption is clearly seen in Fig 4 as well as the general tendency towards heavier absorption in lower galactic latitude[3] The absorption obtained is chiefly that within the H II region, but also includes a contribution from the space between the Earth and the H II region. In any case, this is an independent method of obtaining a map of interstellar absorption Interstellar absorption is currently obtained by stellar photometry The observational data, however, are not an absorption value but a colour excess, and the ratio of space absorption to colour excess is still not well known The technique presented here is one of the methods for deriving an absolute value of absorption and it will help to clarify the role of the interstellar dust grains in the H II regions

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[1] Ishida, K , and Ohashi, M , *Bull. Tokyo Astro Obs* , Ser 2, No 178, 245 (1967)

[2] Akabane, K , Miyazawa, K , Takahashi, T , Ishizawa, Y , and Nakajima, T , *Bull Tokyo Astro Obs* , Ser 2, No 177, 241 (1967)

[3] Ishida, K , and Kawajiri, N , *Publ Astro Soc Japan* (in the press)

Production of OH Molecules in Interstellar Shock Waves

MEASUREMENTS made recently at Berkeley have shown that there is interstellar OH absorption in most low latitude radio sources[1] Most indications are that the excitation, unlike that in the intensely emitting regions[2], is fairly normal, and that the state temperature is $T < 10°$ K If it is assumed that $T = 3°$ K (a minimum value, from the cosmic microwave background), the median value of OH/H is about 4×10^{-8} in sources in which both OH and H lines can be measured This ratio, which corresponds to OH/O $\sim 6 \times 10^{-5}$, is not far from that of CH and CH^{+}, seen optically An attempt by Bates and Spitzer to explain the abundance of CH by two body radiative association[3] failed by more than three orders of magnitude This led Stecher and Williams[4] to propose that chemical exchange reactions on grains of the form $GX + Y \rightarrow G + XY$ (where $X =$ H, $Y =$ O for OH) could operate in the heated regions behind interstellar shock fronts They calculated that $OH/H = 3\times10^{-9}$ after a shock passes. Photodissociation ($\tau = 10^4$ yr) between shock passages ($T = 10^7$ yr) reduces OH/H to an average value of 3×10^{-12}, which is four orders of magnitude too low According to Carroll and Salpeter[5], the chemical exchange reaction $O + H_2 \rightarrow OH + H$ produces comparable amounts of OH if $H_2/H \sim 10^{-4}$ According to Stecher and Williams[6], however, H_2/H is kept to the extremely low value of 10^{-7} by photodissociation of H_2

We have calculated the OH production caused by preassociation, as suggested by Solomon[7],

$$O + H \rightarrow OH, K_1 = 3\times10^{-16}\exp(-3,700/T)\ \mathrm{cm^3\ sec^{-1}} \quad (1)$$

where $K_1 = n(OH)/n(O)n(H)$ Obviously this reaction proceeds even if H_2 is absent, but, like the reactions involving H_2 and grains, it requires the high temperatures present behind a shock wave The primary destruction mechanism is[5]

$$OH + H \rightarrow H_2 + O, K_2 = 9\times10^{-12}\exp(-3,709/T) \quad (2)$$

Starting with no molecules, reaction (1) builds up OH behind the shock front, while reaction (2) converts some of these molecules to H_2 The inverse of reaction (2),

$$O + H_2 \rightarrow OH + H, K_3 = 2\times10^{-11}\exp(-4,630/T) \quad (3)$$

and the low-temperature reaction,

$$OH + O \rightarrow O_2 + H \rightarrow 2O + H, K_4 = 1\times10^{-10}\exp(-600/T) \quad (4)$$

were also included, we assumed that O_2 is photo-dissociated immediately The reaction $OH + H_2 \rightarrow H_2O + H$ was neglected because it becomes comparable with reaction (2) only when H_2/H approaches 10 per cent

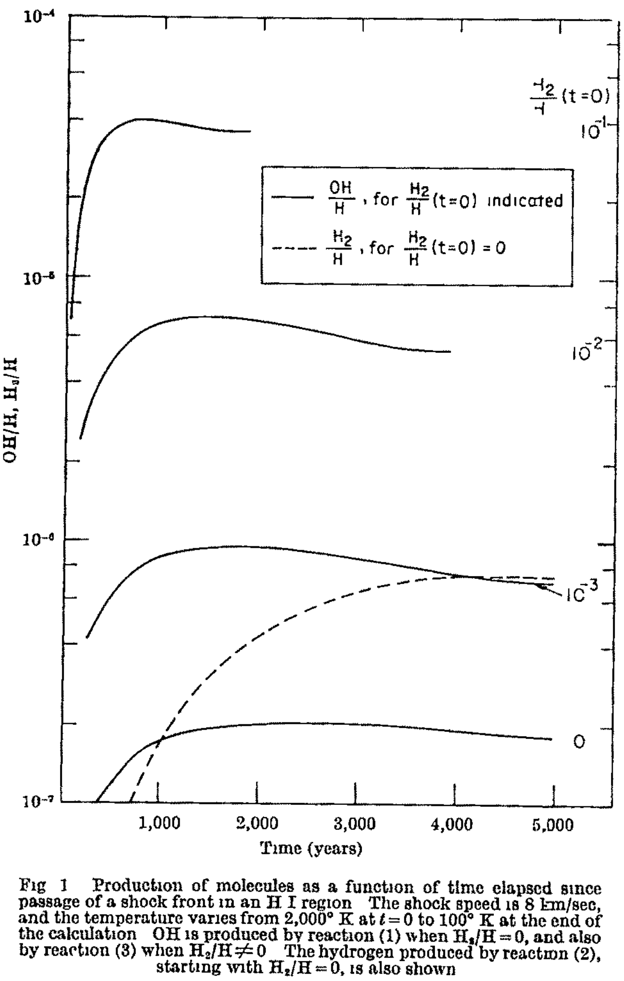

Fig 1 Production of molecules as a function of time elapsed since passage of a shock front in an H I region The shock speed is 8 km/sec, and the temperature varies from 2,000° K at $t = 0$ to 100° K at the end of the calculation OH is produced by reaction (1) when $H_2/H = 0$, and also by reaction (3) when $H_2/H \neq 0$ The hydrogen produced by reaction (2), starting with $H_2/H = 0$, is also shown

Both Stecher and Williams[4] and Carroll and Salpeter[5] used estimated postshock temperature density profiles We used a grid of profiles depending on mach number, initial H_2 abundance and preshock magnetic field strength calculated by Field, Rather, Aannestad and Orszag[8]. Their calculations, for preshock densities $n(H) = 10\ \mathrm{cm^{-3}}$ and $n(O) = 6\ 8\times10^{-3}\ \mathrm{cm^{-3}}$, show that the cooling time ($\sim 10^4$ yr) is reduced from previously accepted values because of frequent inelastic OH collisions with subsequent 63μ radiation. The short cooling time tends to limit molecule production, although it is somewhat compensated by increased densities far behind the shock

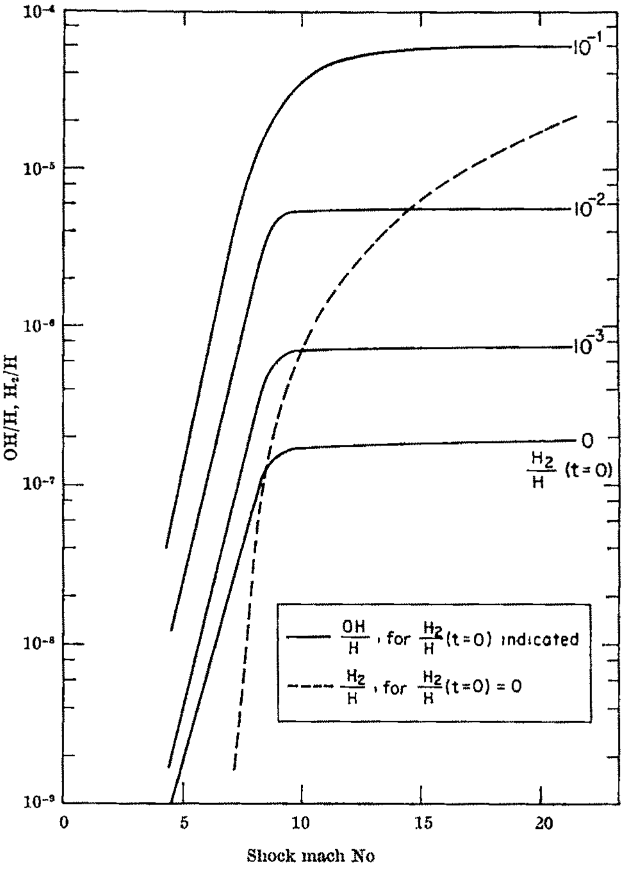

Fig 2 The final molecular abundances after shock passage as a function of mach number (= speed/0 8 km/sec) Significant contribution by reaction (3) requires $H_2/H > 10^{-3}$

Fig 1 shows the OH production for a mach 10 (8 km/sec) shock; the abscissa represents time elapsed since shock passage The calculations are terminated when the material behind the shock has returned to its preshock temperature, assumed to be 100° K For $H_2/H = 0$, equilibrium between reactions (1) and (2) is rapidly attained behind the shock, at $OH/H = 2\times10^{-7}$, independent of temperature Reaction (2) produces some H_2 as shown, but the amount is so small that reaction (3) and the reaction $OH + H_2 \rightarrow H_2O + H$ are safely neglected

Although at present it seems likely that the interstellar hydrogen abundance is very low[6], calculations were made for $H_2/H = 10^{-3}$, 10^{-2} and 10^{-1} to assess the importance of reaction (3) Photodissociation of H_2 was ignored

As expected, far more OH is produced by this exchange reaction. (The value for $H_2/H = 10^{-1}$ is an overestimate because of the neglect of $OH + H_2 \rightarrow H_2O + H$.) Our results agree roughly with those of Carroll and Salpeter, based on estimated cooling curves. The decrease at the end of the curves in Fig. 1 is produced by reaction (4), the only operative in the cooler regions far behind the shock. Fig. 2 demonstrates that reactions (1) and (3) require at least mach 10 shocks to be effective. Lower shock speeds do not result in sufficient temperatures, while higher speeds do not significantly increase molecule production further. In every case, the production of hydrogen is very small. A magnetic field changes the cooling times somewhat, and thus the molecular production. The minimum value achieved, $OH/H = 2 \times 10^{-7}$ when $H_2/H = 0$, is affected only in that larger fields require larger mach numbers for reactions (1) and (2) to come into equilibrium. The minimum effective mach number ranges from 10 for $B = 0$ to 17 for $B = 10^{-5}$ gauss.

If, with Stecher and Williams[6], we set $H_2/H = 0$, the relevant OH/H from reaction (1) is 2×10^{-7} for shocks of mach 10 or more. This is about two orders of magnitude greater than production by chemical exchange reactions on grains (except near hot stars where radiation pressure accelerates the grains through the gas, increasing the OH production[4]). The postshock value of OH/H is reduced by reaction (4) and by photodissociation, for which the combined lifetime is τ. At time t after shock passage, the ratio has dropped by the factor $e^{-t/\tau}$. When averaged for many shock passages (for which the mean interval is T), the reduction factor is τ/T. T is 6×10^6 yr (ref. 9) and τ (photodissociation) from the f-values of ref. 7 and the standard interstellar radiation field is 6,000 yr (ref. 10). Because this is smaller than that for reaction (4), the latter may be neglected. The reduction factor is therefore 10^{-3}, giving a mean value $OH/H = 2 \times 10^{-10}$, about 100 times the amount caused by grains[4]. It is still, however, 200 times smaller than the observed value, so that reaction (1) behind shocks cannot be solely responsible for the observed OH.

If hydrogen is present, Fig. 2 indicates that the factor of 100 can be recovered only if $H_2/H > 1$ per cent. This is much too high for the mean value of this quantity according to ref. 6. The possibility arises, however, that, in the immediate vicinity of shock fronts, production of hydrogen on grains is so rapid that a considerable abundance of hydrogen builds up. Although Stecher and Williams[6] estimate that H_2/H attains only the value 4×10^{-5} behind shocks, this depends on the details of the postshock cooling. We hope to check this in future calculations.

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[1] Goss, W. M., *Astrophys. J.*, Suppl., No. 137 (1968).
[2] Robinson, B. J., and McGee, R. X., *Ann. Rev. Astro. Astrophys.*, 5, 183 (1967).
[3] Bates, D. R., and Spitzer, jun., L., *Astrophys. J.*, 113, 441 (1951).
[4] Stecher, T. P., and Williams, D. A., *Astrophys. J.*, 146, 88 (1966).
[5] Carroll, T. O., and Salpeter, E. E., *Astrophys. J.*, 143, 609 (1966).
[6] Stecher, T. P., and Williams, D. A., *Astrophys. J.*, 149, L29 (1967).
[7] Solomon, P. M., *Nature*, 217, 334 (1968).
[8] Field, G. B., Rather, J. D. G., Aannestad, P. A., and Orszag, S. A., *Astrophys. J.*, 151 (in the press, 1968).
[9] Spitzer, jun., L., *Stars and Stellar Systems* (edit. by Kuiper, G., and Middlehurst, B.), 7, chap. 9 (University of Chicago Press) (in the press, 1968).
[10] Lambrecht, H., and Zimmermann, H., *Mitt. Univ. St. Jena*, No. 14 (1956).

PLANETARY SCIENCE

Fractionation of Iron in the Solar System

BANERJEE[1] has argued against the influence of ferromagnetism on the aggregation of particulate matter in a theory of Harris and myself[2] on chemical fractionation within the solar system.

His objection is based on one of the more unsatisfactory parts of magnetic theory—the prediction of spin structure. I quote a reference[3] he used (page 367). "Some calculations on small multidomain particles have been made but it is fair to say that to date such particles are not understood" (see also ref. 4, chapter 8). Calculations of the likely magnetization of any system are usually hampered by at least the following uncertainties: (*a*) proper enumeration and comparison of all the possible spin configurations, and (*b*) the occurrence of metastable spin configurations.

Assuming that the exact shape of particles formed in the conditions envisaged in the solar nebula is known, the difficulty of satisfying (*a*) is especially great near the Curie point. The simplified enumeration achieved by assuming a domain structure breaks down because of the extremely small crystalline anisotropy constants in the vicinity of the macroscopic Curie point. For the very slowly cooling particles in the completely thermalized environment postulated in the fractionation theory, metastable spin configurations become a real possibility for any particles much larger than the superparamagnetic size limit.

Thus I am surprised at the faith Banerjee has in his method of multidomain calculation and in the significance of his numerical results for the theory[2]. If the validity of his method is accepted, a trivial adjustment to the theory would remove the numerical discrepancy. It would be wrong, however, to conceal the ignorance that surrounds the magnetization of small particles and therefore the effectiveness of ferromagnetism in enhancing collision rates of ferromagnetic particles. In the conditions envisaged, the ratio of collision cross-section to optical cross-section of ferromagnetic particles probably rises from unity as the size exceeds the superparamagnetic limit, could reach very large values and probably declines towards unity for particles greater than 10^{-4}–10^{-3} cm. This size range probably includes a significant fraction of the particle size distribution of the primary condensate[2].

Fortunately, it is not necessary to take refuge in such ignorance. It was pointed out[2] that without long range ferromagnetic attraction, the binary collision rate of iron particles, if they averaged 10^{-4} cm in size, was of the order 1 per year. It would therefore still be possible for large aggregates to be built up. It seems probable that short range ferromagnetic interactions would create a thermally stable bond between any colliding iron particles of greater than superparamagnetic size. With no mechanism of comparable bonding efficiency between non-ferromagnetic particles a size difference of iron and non-metallic particle aggregates would occur. This is the essential step in the theory[2].

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[1] Banerjee, S. K., *Nature*, 216, 781 (1967).
[2] Harris, P. G., and Tozer, D. C., *Nature*, 215, 1449 (1967).
[3] Morrish, A. H., *The Physical Principles of Magnetism* (John Wiley and Sons, 1965).
[4] Brown, W. F., in *Magnetostatic Principles in Ferromagnetism* (North-Holland, 1962).

Lake Lappajarvi, Central Finland: a Possible Meteorite Impact Structure

LAKE LAPPAJARVI (lat 63° 10′ N, long 23° 40′ E) is situated about 60 km east of Vaasa about 200 km north-north-west of Helsinki (Fig 1) The depression in which the lake is located is largely filled by deposits from the last glaciation

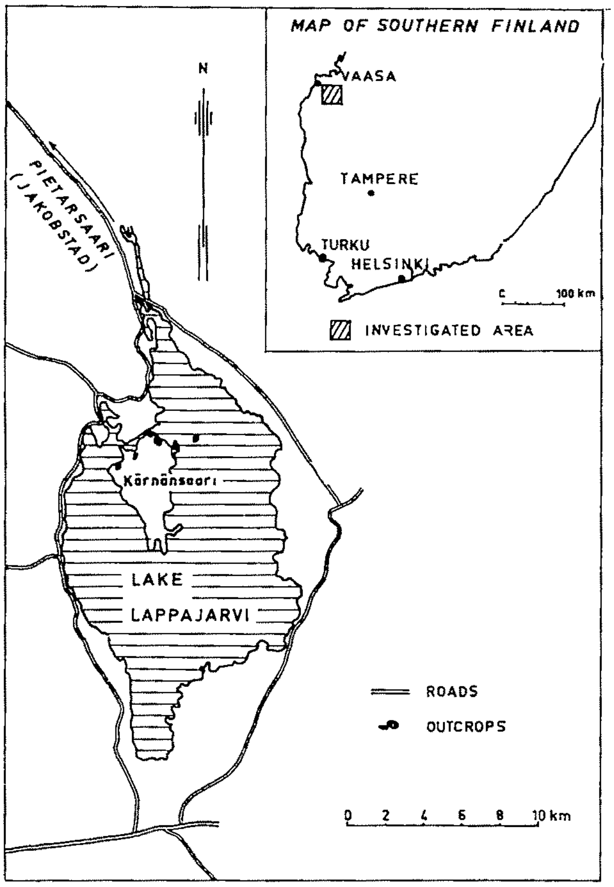

Fig 1 Map of Lake Lappajarvi and surroundings.

Holmberg described a peculiar rock on the island of Karna in Lake Lappajarvi as early as 1858 (ref. 1) The rock type was called karnaite Outcrops have been found on the islands of Karnansaari and Vartijasaari in the northern part of the lake and in boulders along the southern shore-line No karnaite in outcrops or as blocks is known along the northernmost shores[2] The bedrock around the lake is Archaeic and consists of gneisses and granites The boulders which are found to the south of the lake are thought to come from a neck of a volcano, the upper part of which has disappeared[3] Its age is probably post-Archaean but pre-Quaternary[4] The idea that the karnaite boulders emerge from Lake Lappajarvi has been further strengthened by studies of the distribution pattern of the boulders found south of the lake[5,6] The idea of a volcanic origin for the Lappajarvi structure is maintained in the most recent papers and books on the geology of Finland[7] There is, however, another possible interpretation of this structure The karnaite boulders are similar in appearance to corresponding samples from the Ries Basin in Germany and from Lake Mien in Sweden These structures have been interpreted as the result of the impact of meteorites[8,9]

Quartz in bedrock affected by shock waves from meteorite impacts will be deformed and give quartz deformation lamellae with definite characteristics[10] These lamellae follow certain crystallographic planes, for example, (0001), $(10\bar{1}3)$ and $(01\bar{1}3)$, $(10\bar{1}1)$ and $(01\bar{1}1)$, $(10\bar{1}2)$ and $(01\bar{1}2)$, the planes $(10\bar{1}3)$ and $(01\bar{1}3)$ being most common The lamellae are also closely spaced (5 to 30μ apart) and form parallel planar features which do not transgress grain boundaries These lamellae have been found in material from the Ries Basin and from Lake Mien[10] Such lamellae have also been reported from other circular structures such as the Holleford Crater in Canada, the Meteor Crater in Arizona and the Wabar Crater in Arabia[11,12]

Thin sections have been made from fragments of Archaean bedrocks in karnaite found as boulders along the southern shoreline of Lake Lappajarvi These have been studied under the microscope on a universal stage The quartz grains have the same appearance as those described from the Ries Basin, Lake Mien and other similar structures The following crystallographic planes of the lamellae have been measured (thirty-six measurements on sixteen grains) $(10\bar{1}3)$ or $(01\bar{1}3)$, $(10\bar{1}2)$ or $(01\bar{1}2)$, $(21\bar{3}1)$ or $(12\bar{3}1)$, and $(10\bar{1}1)$ or $(01\bar{1}1)$ The direction $(10\bar{1}3)$ or $(01\bar{1}3)$ is the most common, as von Engelhardt *et al* found in the Ries Basin and in Lake Mien[10] These measurements seem to support strongly the interpretation of the Lappajarvi structure as the result of meteorite impact The size of the structure is not exactly known but must be of the order 5–6 km

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[1] Holmberg, H J , *Finska Vetenskapssocieteten, Helsinki* (1858)
[2] Berghell, H , in Saksela, M , *CR Soc Geol Finland*, **22**, 20 (1949)
[3] Laitakari, A , *Suomen Geologinen Yleiskartta*, B 3, 39 (1942)
[4] Eskola, P , *Bull Comm Geol Finl* , **55**, 13 (1921)
[5] Molder, K , *CR Soc Geol Finland*, 21, 50 (1948)
[6] Saksela, M , *CR Soc Geol Finland*, **22**, 25 (1949)
[7] Simonen, A , in *Suomen Geologia* (edit by Rankama, K), 115 (Kirja Yhtymä, Helsinki, 1964)
[8] Shoemaker, E M and Chao, E C T , *J Geophys Res* , **66**, 3374 (1961)
[9] Svensson, N B , and Wickman F E , *Nature*, **205**, 1202 (1965)
[10] v Engelhardt, W , and Stoffler, D , *Naturwissenschaften*, **52**, 489 (1965)
[11] Bunch, T E , and Cohen, A J , *Science*, **142**, 379 (1963)
[12] Bunch, T E and Cohen, A J , *Geol Soc America Bull* , **75**, 1263 (1964)

Radiative Association : Possible Important Loss Process for Metallic Ions in the Ionosphere

THE maximum lifetime of a metallic ion, M^+, in the upper atmosphere is believed to be limited by radiative recombination, $M^+ + e \rightarrow M + h\nu$ The rate coefficient, γ_r, for this process is about (ref 1) 3×10^{-12} cm^3 sec^{-1} at 300° K

Radiative recombination should be contrasted with the loss of atomic ions through the radiative association processes

$$M^+ + O \rightarrow MO^+ + h\nu$$

$$M^+ + O_2 \rightarrow MO_2^+ + h\nu$$

Atomic nitrogen densities are too low at these altitudes for $M^+ + N \rightarrow MN^+ + h\nu$ to be important The ion XN_2^+, if not unstable, is much less likely to be formed than XO^+ or XO_2^+

Radiative association is quickly followed by dissociative recombination, with a rate coefficient of the order 3×10^{-7} cm^3 sec^{-1} Thus radiative association is the rate limiting step in the neutralization of metallic ions, $M^+ \rightarrow MX^+ (MX_2^+) \rightarrow$ neutrals

The loss rate for each metallic ion caused by radiative recombination, $\gamma_r[e]$, is compared in Table 1 with the radiative association loss rates $\gamma[O]$ and $\gamma[O_2]$ at altitudes of interest for the ions of sodium, calcium, magnesium, potassium, silicon, iron, aluminium and nickel The loss

Table 1 LOSS RATES FOR METALLIC IONS BY VARIOUS PROCESSES

Altitude	$3\times10^{-12}[e]$	$10^{-17}[O]$	$10^{-17}[O_2]$	$10^{-30}[O_2]^2$	$10^{-30}[O]\,[O_2]$
110 km	$4\ 5\times10^{-7}$ sec^{-1}	$2\ 0\times10^{-6}$ sec^{-1}	$3\ 5\times10^{-6}$ sec^{-1}	$1\ 2\times10^{-7}$ sec^{-1}	$7\ 0\times10^{-8}$ sec^{-1}
100 km	$1\ 5\times10^{-7}$ sec^{-1}	$5\ 0\times10^{-6}$ sec^{-1}	$2\ 0\times10^{-}$[illegible] sec^{-1}	$4\ 0\times10^{-6}$ sec^{-1}	$1\ 0\times10^{-6}$ sec^{-1}
90 km	$3\ 0\times10^{-8}$ sec^{-1}	$1\ 2\times10^{-6}$ sec^{-1}	$1\ 3\times10^{-4}$ sec^{-1}	$1\ 7\times10^{-4}$ sec^{-1}	$1\ 6\times10^{-6}$ sec^{-1}

rates $\beta[O_2]^2$ and $\beta[O]\,[O_2]^2$ are also indicated for the three body processes $M^+ + O_2 + O_2 \rightarrow MO_2^+ + O_2$ and $M^+ + O + O_2 \rightarrow MO^+ + O_2$, respectively The latter reaction may rather proceed as $M^+ + O + O_2 \rightarrow MO_2^+ + O$ The third body could be N_2

The value of 10^{-17} cm^3 sec^{-1} chosen for γ is not inappropriate Bates[2] calculated rates for radiative association of certain atomic species with rate coefficients roughly in the range $10^{-17\pm1}$ cm^3 sec^{-1} A rate coefficient of 10^{-30} cm^6 sec^{-1} is reasonable for a three body ion neutral attachment process Neutral number densities [O] and [O$_2$] are from CIRA 1965 while the [e] values are typical for daytime

Because sporadic *E* may attain an electron density of 10^6 cm^{-3}, radiative recombination may well be the dominant loss process leading to the neutralization of metallic ions near 110 km and above The altitude above which radiative recombination dominates radiative association will not be known, however, until the appropriate γ are accurately determined Somewhere below 100 km the three body recombination process must become more important than radiative association Values for γ and β are needed The crossover point occurs where $\gamma[O_2] = \beta[O_2]^2$, that is $[O_2] = \gamma/\beta$

The reaction $X^+ + O_2 \rightarrow OX^+ + O$ cannot remove metallic ions because the dissociation energies of FeO$^+$, MgO$^+$, seem to be less than for oxygen, 5 1 eV, making such reactions endothermic An exception may be $Si^+ + O_2 \rightarrow SiO^+ + O$ if the dissociation energy of SiO$^+$ is 5 66 eV (ref 3) All metallic ion reactions with nitrogen, $X^+ + N_2 \rightarrow XN^+ + N$, are presumably endothermic The dissociation energy of nitrogen is large, 9 76 eV

In conclusion, then, radiative association processes, hitherto believed unimportant in the ionosphere, may be an important mechanism in the neutralization of metallic ions near 100 km Three body reactions probably dominate near 90 km and below

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[1] Bates, D R , and Dalgarno, A , in *Atomic and Molecular Processes* (edit by D R Bates) (Academic Press, 1962)
[2] Bates, D R , *Mon Not Roy Astro Soc* ,111, 303 (1951)
[3] Wilkinson, P G , *Astrophys J* ,138, 778 (1963)

Calcian Dolomites and Dedolomitization

THE dedolomitization process, first named in 1847 (ref 1), has recently found increasing recognition, and has been reviewed briefly by Friedman and Sanders[2]

Three different mechanisms have been suggested to explain this process First, dedolomitization is a surface feature, and thus part of chemical weathering[3-5], second, reaction of dolomite with solutions containing sulphate results in the formation of calcite and soluble magnesium sulphate[1,6-8], and third, dedolomitization is a diagenetic process, related to physico-chemical changes in the overlying and interstitial water system[9,10] The evidence presented here not only strongly favours the diagenetic theory of dedolomitization, but points to an early stage of diagenesis and to the importance of particular crystallochemical features of the original dolomite crystals

During a study of eighty-five samples of sedimentary carbonate rock from Israel, ranging in age from Jurassic to Turonian dedolomitization was found to affect only calcian dolomites, that is, those containing at least 8 per cent calcium in excess of the ideal composition of dolomite (Ca Mg = 1 1 molar) (I collected and analysed forty-five samples of rocks from the Mahmal Formation (Jurassic) in Makhtesh Ramon, and E Sass chemically analysed forty samples of Cenomanian and Turonian age)

The fact that this state of calcium excess is original and antedates dedolomitization is shown by several samples in which the ratio of calcium to magnesium is not the same throughout the calcian dolomite crystal, but is zoned, parallel to its rhombohedral outline This variation in composition which was revealed by several independent observations was confirmed by electron probe analyses of two specially chosen crystals, and is analogous to the well known zoned distribution of iron in ferroan dolomites

X-ray powder patterns show that calcian dolomite crystals, which look perfectly homogeneous under polarized light, are aggregates of subparallel crystallites, less than 100 Å in diameter This crystal mosaic[12] results from the introduction of calcium ions into the sites of magnesium ions in the dolomite lattice Because calcium ions are larger than magnesium ions, lattice defects increase with growth and limit the size of the crystallites

The preferential dedolomitization of calcian dolomites or of zones or cores in dolomite crystals which are relatively rich in calcium can be explained by (*a*) the increase in free energy of calcian dolomite compared with "normal" dolomite, or (*b*) the ease of replacing calcium ions in the microcrystalline aggregate through the intercrystallite boundaries

Dedolomitization occurs either after the formation of a calcian dolomite crystal or at definite intervals during the growth of a dolomite crystal In the latter case, the replacing calcite will constitute rhombohedral cores or zones within the dolomite crystal In the Jurassic samples as many as three calcite zones are observed in individual crystals The early age of this dedolomitic calcite is shown by its frequent intimate association with zones of haematite within crystals of ferroan dolomite These zones are followed towards the outside by non-oxidized ferroan dolomite, and therefore represent interruptions in the process of crystal growth

Further evidence for the early diagenetic character of dedolomitization is provided by the presence of numerous dedolomitic intraclasts in different dolomites, which are not affected by this process

Calcian dolomites probably form in a geochemical environment intermediate between that corresponding to the formation calcite and "normal" dolomite respectively Dedolomitization occurred if and when the chemical system shifted towards the calcite stability field during diagenesis These conclusions agree well with the observation[5] that, in a given dolomitic limestone, increasing calcite content is associated with an increasing content of calcium ions in the dolomite crystals

A surface layer, rich in calcium (Ca Mg = 4 1) and about 100 Å thick, has been found on Recent dolomite crystals[11] This could represent one of the zones rich in calcium the development of which has just been described Because of the very high calcium content of ions in this surface layer, the considerably thicker calcian dolomite zones in older dolomite crystals have to be explained by the short range diffusion of calcium ions into the adjoining "normal" zones

I found no evidence in favour of the "sulphate hypothesis" during this investigation Sulphate is absent, or present only in traces Some rocks contain geothite, pseudomorphous after pyrite, but there was no dedolomitization in these rocks

Dedolomitization in the Jurassic rocks which I have studied is therefore an early diagenetic process There was no indication of surface weathering, such as an increase in the ratio of calcium to magnesium towards the exposed rock surfaces This does not exclude the possibility of dedolomitization being induced by surface weathering in special conditions, for example, along joints or associated with the formation of caliche.

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[1] Von Morlot, A , *Haidinger Naturwiss Abhandl* , 1, 305 (184")

[2] Friedman, G M , and Sanders, J E , in *Developments in Sedimentology*, 9A, 267 (edit bv Chilingar, G V , Bissell, H J , and Fairbridge, R W) (Elsevier, Amsterdam, 1967)

[3] Lang, H B , dissertation, Univ Tubingen (1958)

[4] Goldberg, M , *J Sedimentary Petrol* , 37, 3, 760 (1967)

[5] Schmidt, V , in *Dedolomitization and Limestone Diagenesis* Symp Soc Econ Paleontologists and Mineralogists Spec Pub No 13, 124 (1965)

[6] Tatarsky, V B , *Dokl Akad Nauk SSSR*, 69, 849 (1949)

[7] Evamy, B. D , *Sedimentology*, 2, 164 (1963)

[8] Shearman, D J , Khouri, J , and Taha, S , *Proc Geologists' Assoc Engl* , 72, 1 (1961)

[9] Sass, E , thesis, Hebrew Univ , Jerusalem (1966)

[10] Sass, E , and Oppenheim, M J , *Israel J Earth Sci* , 14, 91 (1966)

[11] Peterson, M N A , von der Borch, C C , and Bien, G S , *Amer J Sci* , 264, 4, 257 (1966)

[12] Smith, F. G , *Physical Geochemistry* (Addison-Wesley, Reading, 1963)

PHYSICS

Radio Pulses from Extensive Air Showers at 520 MHz

RADIO pulses at frequencies below 100 MHz have been detected in association with extensive air showers at the zenith by many workers[1–6], and subsequently at large zenith angles[7,8] Current theories do not predict a detectable flux of radio frequency power at frequencies greater than 100 MHz—the highly idealized model of Kahn and Lerche[9] imposes a limiting frequency, because of coherence conditions. of about 75 MHz, while Colgate[10] predicts a maximum emission at 1 MHz Tentative evidence supporting these predictions has been found in several experiments[1,2,5] Because of the nature of the theoretical models used and the possibility of other mechanisms contributing strongly in the ultra high frequency region of the spectrum, an investigation at a frequency greater than 100 MHz was undertaken

In this experiment, a master pulse (Fegan *et al* [11]), generated by a phased coincidence between two 44 MHz receivers (system beamwidth ± 35°), was used to interrogate the outputs of both a 520 MHz receiver and a particle detector The former consisted of a nineteen element Yagi antenna connected through a masthead tuned pre-amplifier and a line repeater to a radar scanning receiver (detector bandwidth $d\nu = 2$ MHz) All radio systems were orientated, at a zenith angle of about 80°, towards geomagnetic west, and were aligned using a hydrogen filled spark gap The particle detector, of 1 m^2 sensitive area, consisted of sixty liquid scintillation modules in a staggered vertical array, viewed by a 5 in photomultiplier tube; it was biased to operate at the single μ-meson level The system was operated only during night hours because of a high man-made interference level in the radio receivers by day

In the analysis of results obtained during [illegible] months running time, a coincidence ($\tau = 1$ μsec) between the master radio pulse and a particle in the detector was assumed to be caused by an extensive air shower and classified as an event. During 340 h of observation, seventy events were recorded It proved feasible to trace sixty-three out of seventy of the ultra high frequency video output waveforms associated with these events, each tracing being of 20 μsec duration. The other seven were not resolvable because of prohibitively high triggering rates A bandwidth-limited pulse, which occurred at the expected position of such tracings and which was greater than 1·5 times the average noise fluctuation of the trace, was taken to indicate ultra high frequency radio emission accompanying an extensive air shower Eleven such pulses were observed, varying in amplitude from 1 5 to 3 0 times, the average noise fluctuation with no other large pulse appearing at any point along the trace No pulses of comparable magnitude were found at any point on the other fifty-two tracings and at no time was the receiver saturated

To eliminate the possibility of (*a*) random coincidences between radio and particle systems giving rise to the seventy events, and (*b*) spurious pick-up within the display and trigger electronics giving rise to ultra high frequency pulses, two subsidiary experiments were carried out

(*a*) The observed μ-meson master pulse coincidence rate of about 0 2 h^{-1} was well above the calculated random rate of 0·01 h^{-1} To verify this the scintillation medium was screened off and both a cobalt-60 gamma source and a scintillation crystal were substituted in front of the photomultiplier tube The discriminator was slightly adjusted to give the same rate as previously, but all other parameters of the radio systems remained the same. No coincidence was observed in 32 h of observation, indicating that the observed events were unlikely to be random coincidences

(*b*) The 44 MHz receivers were disconnected and artificial pulses injected at random into (i) the trigger system and (ii) the display electronics If pick-up were absent no cumulative effect would be expected at any point of the corresponding ultra high frequency time base tracings Sixty ultra high frequency tracings obtained by (i) and one hundred and thirty obtained by (ii) have shown no such cumulative effect.

In a more detailed analysis of results, all traces were divided into ten channels and the amplitude of the largest pulse on each trace together with its position were noted Histograms of the integrated amplitudes were drawn—in (*A*) for the sixty-three events and in (*B*) and (*C*) for the different modes of random triggering. All three histograms were normalized at the same mean amplitude for channels 3 to 10 The expected position for genuine events lies between channels 1 and 2. The histograms are shown in Fig 1 Histogram (*A*) shows a significant positive effect in the expected position, the integrated amplitudes being 12 and 10 standard deviations above the mean in channels 1 and 2, respectively; whereas in (*B*) and (*C*) the slight positive effect is not statistically significant, being at most only 2 standard deviations above the mean

The sixty-three events which comprise histogram (*A*) denote an integral representation of the detected pulse amplitude. The amplitudes of these ultra high frequency events were converted to corresponding energies at the input by allowing for the detector law; and an integrated spectrum of the form $N(>E) = k(E)^{-\gamma}$ was drawn. An equivalent noise spectrum, based on the amplitude analysis of channels 9 and 10 of the same traces, was also drawn. The exponent for the pulse spectrum was 2 0±0 6, while that for the noise spectrum was 4 7±1 6. With the detector used and for the pulse amplitudes considered, $V_{\mathrm{out}} \propto E_{\mathrm{in}} \propto (V_{\mathrm{in}})^2$ For a coherent signal the expected value of γ is about 1 1, and for an incoherent mechanism 2 2, assuming that the shower energies are in the region, 10^{16}–10^{19} eV The observed value may therefore indicate that ultra high frequency emission is caused by incoherent radiation from extensive air showers.

In conclusion, it seems that, using a radio-particle coincidence, tentative evidence has been obtained for ultra high frequency radio emission from extensive air showers. This emission is consistent with an incoherent process Radio bremsstrahlung from delta ray electrons

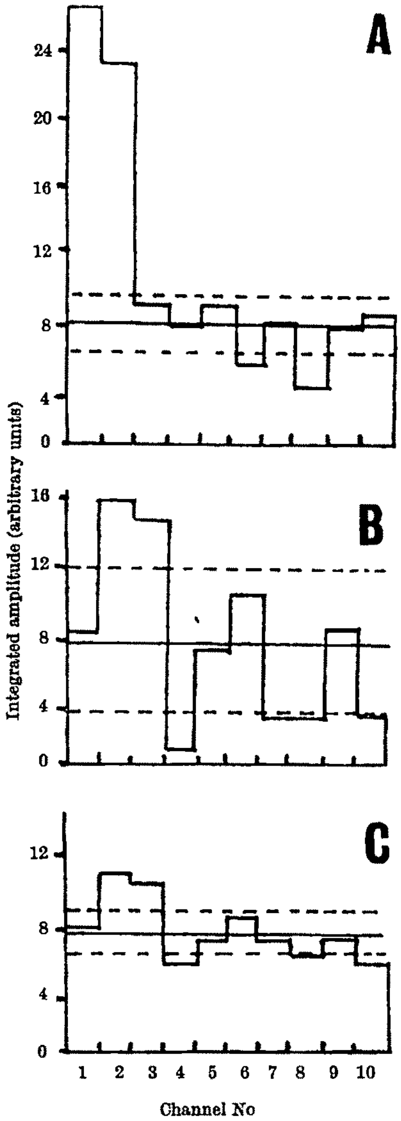

Fig 1 Histograms of the integrated amplitudes, *A* for the sixty-three events and *B* and *C* for the different modes of random triggering All three histograms are normalized at the same mean amplitude for channels 3 to 10 ———, Mean, - - -, standard deviation

has been postulated by Rosenthal and Filchenkov[12], and later re-examined by Charman and Jelley[13], who conclude that the intensity of radiation in the radio spectrum from this mechanism is insignificant compared with the geomagnetic effects Isotropic recombination radiation from atmospheric atoms and molecules, or the radiative Coulomb scattering of low energy shower electrons, are further possible mechanisms for ultra high frequency emission. The detailed quantum mechanical problems involved in calculating the power output for the latter process are considerable[14]

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[1] Jellev, J V, Charman, W N, Fruin, J H, Smith, F G, Porter, R A, Porter, N A, Weekes, T C, and McBreen, B, *Nuovo Cimento*, 46, 649 (1966)

[2] Porter, N A, Long, C D, McBreen, B, Murnaghan, D J B, and Weekes, T C, *Phys Lett*, 19, 415 (1965)

[3] Borzhkovskii, I A, Volovik, V D, Kobizskoy, V I, and Shmatko, E S, *Sov Phys J Exp Ther Phys Lett*, 3, 118 (1966)

[4] Allan, H R, and Jones, J K *Nature*, 212, 129 (1966)

[5] Barker, P R, Hazen, W E, and Hendel, A. Z, *Phys. Rev Lett*, 18, 51 (1967)

[6] Vernov, S N, Abrosimov, A E, Volovik, V D, Zalvubovskii, V I, and Khristiansen, G B, *Sov Phys J Exp Theor Phys Lett*, 5, 126 (1967)

[7] McBreen, B, O'Mongain, E P, Porter, N A, and Slevin, P J, *Phys Lett*, 23, 677 (1966)

[8] Charman, W N, Fruin, J H, Jellev, J V Porter, R A, and Smith, F. G, *Sov Phys J Exp Ther Phys*, 53, 88 (1967)

[9] Kahn, F D, and Lerche, I, *Proc Roy Soc*, A, 289, 206 (1965)

[10] Colgate, S A, *J Geophys Res*, 72, 19, 4896 (1967)

[11] Fegan, D J, McBreen, B, O'Mongain, E P, Porter, N A, and Slevin, P J, *Proc Tenth Int Conf Cosmic Rays Calgary*, 1967 (in the press)

[12] Rosenthal, J L, and Filchenkov, M L, *Izv Akad Nauk*, 30, 703 (1966)

[13] Charman, W N, and Jellev, J V, *Proc Tenth Intern Conf Cosmic Rays Calgary*, 1967 (in the press)

[14] Jauch, J M, and Rohrlich, F, in *Theory of Electrons and Photons*, 365 (Addison-Wesley, 1955)

THE SOLID STATE

Porous Layers at Glass Surfaces

PREVIOUS investigations have shown that porous modifications of the surfaces of glasses may be formed by the action of liquid water[1] and water vapour[2]. I have now studied the formation of porosity in the surface of grains of soda–lime–silica glass in various conditions.

Table 1 COMPOSITION OF SODA–LIME–SILICA GLASS

Component	Percent by weight
Silica	72 6
Calcium oxide	8 2
Sodium oxide	13 5
Potassium oxide	0 4
Magnesium oxide	3 9
Alumina	1 0
Sulphur trioxide	0·2
Total iron (expressed as ferric oxide)	0 15

The glass has the composition shown in Table 1 Structural properties of the grains were inferred from benzene physical adsorption isotherms measured by frontal and elution gas chromatography[1]. The monolayer capacity (N_m) was determined from the Brunauer–Emmett–Teller (BET) equation

$$\frac{P}{N(P_0-P)} = \frac{1}{N_m c} + \frac{(c-1)}{N_m c}\cdot\frac{P}{P_0} \qquad (1)$$

where P is the pressure of adsorbate in the gas phase in equilibrium with N moles of adsorbed adsorbate; N_m is the amount of adsorbate in the first adsorbed layer and may be readily related to the surface area of the adsorbent if the adsorbed molecules conform to a close packed configuration; P_0 is the saturation vapour pressure of the adsorbate, and the constant c is related to the average heat of formation of the monolayer, E_1, by the equation

$$c = \varphi \exp \frac{E_1 - E_L}{R\ T}$$

where φ is the ratio of the evaporation–condensation coefficients for the first and higher adsorbed layers and may often be approximated to unity[1], R is the gas constant, T is the absolute temperature at which the isotherm was measured, and E_L is the heat of condensation of the adsorbate The BET parameters for various treated grains are given in Table 2

Degassing treatments involved heating the grains for 3 h in a stream of dry nitrogen Isotherms measured after 3 h degassing and after a further 6 h degassing at the same temperature were in excellent agreement For the water treatment, 24 g of grains of approximately 160μ diameter (but containing an appreciable quantity of fine material) was heated in 100 ml of distilled water under reflux for 24 h After this time, the pH of the water was in the region 10 to 11 The alkali treatment involved heating the grains at 100° C in 10 per cent sodium

Table 2. SUMMARY OF BET PARAMETERS FOR BENZENE ADSORPTION ISOTHERMS AT 40° C

Treatment	Degassing temperature (°C)	$N_m \times 10^7$ mole g^{-1}	c
None	100	4.86	10.43
Water	50	18.30	8.16
	100	18.50	7.36
	150	17.70	8.63
	200	15.08	7.54
	245	13.71	9.23
	300	14.07	6.71
	350	13.94	7.84
	400	14.73	7.38
	450	13.90	5.80
	500	13.70	5.38
Rehydration	50	15.79	7.00
Sodium hydroxide	100	1,766.00	23.60
	400	1,577.00	14.41
Rehydration	100	2,348.00	11.83

hydroxide w/v. In both instances, the treated grains were finally washed in a stream of distilled water. Rehydration was effected by passing a stream of water over the grains for 24 h at 90° C.

The water treatment increased N_m and hence the surface area (because there is a surplus of hydroxyl adsorption sites for the adsorbate to conform to a close packed structure in the monolayer) by a factor of four. From the value of c, which is related to the heat of adsorption[1], it may be concluded that pores in the modified surface are greater than 35 Å in diameter[3]. The decrease in c value induced by the water treatment may be attributed to the different chemical compositions of the two surfaces, as a result of the preferential leaching of the weathering process. Alternatively, the ratio of free hydroxyl groups (which are the strongest sites for adsorption[4]) to adjacent hydroxyl groups which are cross hydrogen bonded may differ for surfaces produced by the two hydroxylation processes, namely the water treatment of the grains and the production of the grains by crushing glass in a humid atmosphere. Separate experiments have shown that the c value was 10 for freshly prepared vitreous silica grains. This would favour the latter mechanism, that is, the existence of more single hydroxyl groups on the freshly prepared grains than on the weathered grains.

Thermally degassing the grains at successively higher temperatures causes a general decrease in N_m and c. It is possible that at the higher degassing temperatures, steric effects control adsorption, because benzene is adsorbed specifically on surface hydroxyl groups. Thus the removal of these hydroxyl groups by heating the adsorbent would not only decrease the heat of adsorption, and hence c, but the adsorbed benzene molecules may not conform to a close packed configuration, for close packing, a surface hydroxyl concentration of greater than 2.4 hydroxyl groups/100 Å^2 is required, and at 500° C this concentration is within the range 1.6 to 2.9 (ref. 4). The increase in values of N_m and c after rehydration of the surface substantiates the existence of these steric effects. The reasons for N_m not returning to its original value may be explained in two ways. First, the degassing procedure causes an irreversible sintering process and consequent loss of surface area to occur within the porous layer. Second, the porous layer undergoes an irreversible shrinking process which would also cause a decrease in surface area. Because the degassing temperatures involved are rather low for sintering processes, it seems probable that the second mechanism has occurred, and this most likely over the temperature range up to 200° C.

Adsorption of benzene on glass surfaces which have been attacked by strong alkali shows high values for N_m and c. The high c value is indicative of the presence of micropores (that is, pores of less than 35 Å diameter) because the potential fields of the opposite walls of micropores overlap and are responsible for the high attractive force (and high heat of adsorption) for the adsorbate. Furthermore, it can be appreciated that because of the proximity of the pore walls, monolayer capacity has not the usual significance in microporous systems and therefore N_m cannot be readily related to the surface area, although the high value of N_m shows the extensive nature of the porous layer. The latter is confirmed by the results of the final rehydration where the low value of c indicates the absence of micropores, but the value of N_m is very large and the surface area is approximately 500 times its initial value. Thermal ageing of the microporous system shows, once again, a decrease in N_m and c. It was observed that if the microporous grains were immersed in water, the pH of the water rose at an appreciable rate. Rehydration therefore removes material which was responsible for the microporosity.

The following principal conclusions may be stated. When the soda–lime–silica glass is only lightly attacked by water or dilute alkali, a wide pore structure is formed which has thermal ageing properties similar to silica gel[5]. It is unlikely that the porous structure is globular, however, because the silica content of the glass is large enough for the wide pore structure, formed by a preferential leaching process, to be a continuous silica network. The microporous structure contained in the extensive wide pore structure (also observed by Liberti[6]) is probably formed from soluble or partly soluble reaction products which have not been removed from the deep porous layer.

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[1] Sewell, P. A., *Glass Tech.*, **8**, 108 (1967).

[2] Sewell, P. A., and Morgan, A. M., *Nature*, **215**, 325 (1967).

[3] Kiselev, A. V., *The Structure and Properties of Porous Materials*, Colston Papers (edit. by Everett, D. H., and Stone, F. S.), (Butterworths, London, 1958).

[4] Davydov, V. Ya., Kiselev, A. V., and Zhuravlev, L. T., *Trans. Farad. Soc.*, **60**, 2254 (1964).

[5] Makrides, A. C., *J. Phys. Chem.*, **63**, 1789 (1959).

[6] Liberti, A., *Sixth Intern. Symp. Gas Chromatography, 1966* (Institute of Petroleum, 1967).

Preparation of Fibre Reinforced Nickel–Gold Alloys using a Solid State Reaction

THE most obvious and frequently used method of making fibre reinforced alloys is to take fibres of a metal or alloy with a relatively high melting point and permit a matrix of metal or alloy with a lower melting point to solidify around them. A more sophisticated approach, which has received attention for a number of years, is to attempt to grow the fibres *in situ* within the matrix. This has been achieved by Hertzberg[1,2] and others using a technique in which eutectic alloys are permitted to undergo unidirectional solidification. This method is, however, rather limited in application because the alloy composition, and thus the volume fraction of fibres, is fixed. This communication gives a preliminary account of another technique that we have recently developed which does not suffer from this limitation.

This new method, which uses a solid state reaction to create the fibres, has been demonstrated using alloys in the gold–nickel system. The phase diagram for this system, which contains a simple miscibility gap, is shown in Fig. 1. In a series of experiments on the equiatomic alloy, the specimens were solution treated at 900° C, quenched at room temperature and then aged, usually for 1 h, at various temperatures within the miscibility gap. It was found that at temperatures above about 400° C the metastable solid solution which formed on quenching dissociated directly into the two equilibrium phases by a discontinuous solid state reaction which nucleated at grain boundaries. The reaction rate was found to go through a maximum at about 600° C, and it became infinitesimally small as the miscibility gap boundary was approached.

When the reaction products were examined by stereoscopic electron microscopy using a replica technique, it was found that at the lowest reaction temperatures the nickel rich phase was precipitated in the form of roughly spherical particles with diameters up to about 1000 Å within a matrix of the gold rich phase. As the reaction temperature increased the particles formed became larger, and then near 700° C the morphology changes to fibres a few tenths of a micron in diameter and many microns in length. In each grain several discrete fibre orientations were observed. Mechanical property studies showed that the small spherical particles produced a strong strengthening effect, but there seemed to be no significant effects attributable to the fibres. The volume fraction of fibres was, however, only a few per cent and so this was not considered surprising.

The occurrence of only a few per cent of the nickel rich phase, that is of fibres, in the equiatomic alloy after equilibration at 700° C is consistent with the phase diagram and results from the marked asymmetry in the miscibility gap. This may readily be seen by applying the lever rule to this case. The lever rule also predicts, of course, that the proportion of the nickel rich phase should increase with increasing nickel content. This suggested to us that higher volume fraction of fibres might be obtainable by using alloys containing more than 50 atomic per cent nickel. Accordingly, the experiments were repeated using alloys containing 50, 55, 60, 65 and 70 atomic per cent nickel. This time, however, hardness rather than mechanical property measurements was used to assess strengthening effects.

The results of this work showed that the morphology and kinetics of the dissociation reaction changed little with composition except that, as expected, the volume fraction of the nickel rich phase increased with increasing nickel content. In particular, the volume fraction of fibres increased and in the alloy having the highest nickel content exceeded 50 per cent. The fibres could be seen most convincingly in the stereoscopic pairs of electron micrographs, but quite convincing conventional electron micrographs were also obtained by careful manipulation of the shadowing technique. One of these, in this case for an alloy containing 60 atomic per cent nickel, is shown in Fig. 2. The hardness measurements showed peaks caused by fibres in every case except that of the equiatomic alloy, thus demonstrating that the fibres do cause reinforcement when there are enough of them. The results for the alloy containing 70 atomic per cent nickel are shown in Fig. 3. The peak at 450° C is caused by the small spherical particles and that at 700° C by the fibres.

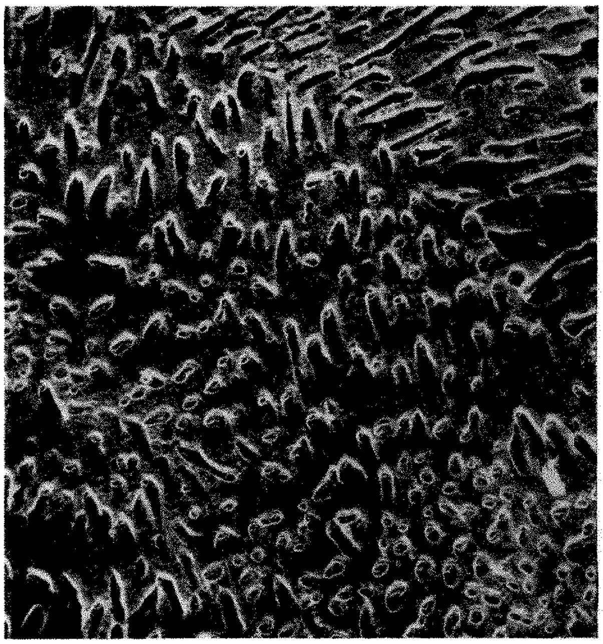

Fig. 2. Nickel rich fibres in an alloy containing 60 atomic per cent nickel and 40 atomic per cent gold (× 6,000).

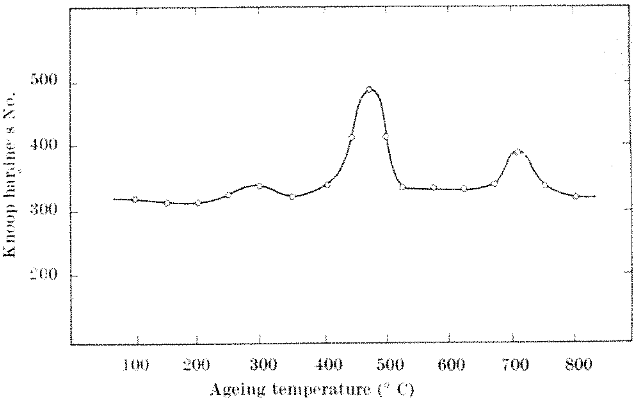

Fig. 3. An isochronal hardening curve for an alloy containing 70 atomic per cent nickel and 30 atomic per cent gold.

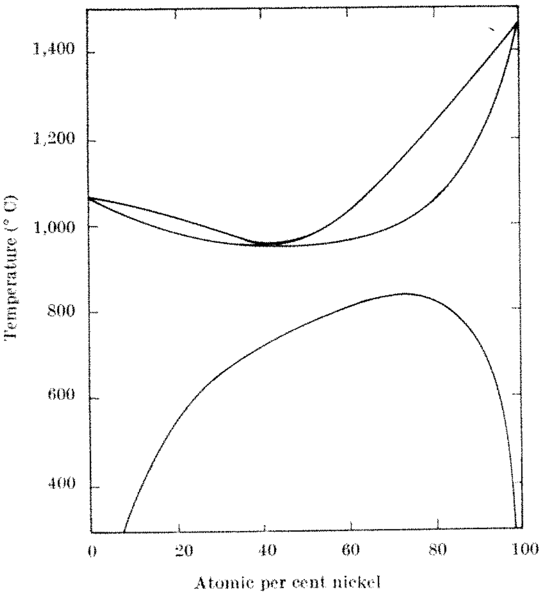

Fig. 1. The gold–nickel phase diagram.

Although these experiments show unequivocably that fibre composite nickel–gold alloys can be prepared, and that the fibres produce a strengthening effect, proper control of the fibre orientations to give directional strengthening has yet to be achieved. Experiments using directional heat treatments, and heat treatments in a magnetic field, are now in progress in our laboratory and may accomplish this. The applicability of the method to other alloy systems also remains to be explored. There are many other systems with miscibility gaps, but presumably the existence of a miscibility gap is not the only criterion to be satisfied before the method will work. It seems likely, for example, that the interfacial energy of the two equilibrium phases has to be highly anisotropic if fibres rather than spheres are to be formed.

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[1] Hertzberg, R. W., and Kraft, R. W., *Trans. Met. Soc. Amer. Inst. Met. Eng.*, **227**, 58 (1963).
[2] Hertzberg, R. W., *Trans. Amer. Soc. Metals*, **57**, 434 (1964).

Synthesis of Optical Quality Proustite and Pyrargyrite

The compounds silver orthothioarsenite, Ag_3AsS_3, and silver orthothioantimonite, Ag_3SbS_3, occur in nature as small imperfect crystals and have been given the mineral names proustite and pyrargyrite, respectively. Harker[1] determined their structures and assigned them to the space group $R3c$. They are non-centrosymmetric uniaxial crystals with a large refractive index, large negative birefringence ($n_0 - n_e > 0{\cdot}2$) and transmission over a wide spectral range (0·6–13 μm). They also exhibit semiconducting, photoconducting and piezoelectric properties.

It was thought that these materials, having such properties, might have useful applications in non-linear optics (K. F. Hulme and J. Warner, personal communication). Previous attempts to synthesize these compounds have been made by crystallization from aqueous solution[2], by melt growth[2-4] and by hydrothermal growth[5,6]. The latter resulted in transparent crystals of maximum dimension < 1 mm; other methods yielded opaque polycrystalline material. We have succeeded in growing large single crystals suitable for the proposed applications[7].

The compounds may be considered as binary mixtures of two sulphides and the appropriate phase diagrams are known[8,9]. Because the two compounds are so similar we shall describe only the growth of Ag_3AsS_3 (melting point 480° ± 3° C (M. M. Faktor, private communication). The growth of Ag_3SbS_3 follows a very similar pattern. In the preliminary experiments, we melted together stoichiometric proportions of high purity silver sulphide and arsenic trisulphide (British Drug Houses, Ltd., Poole, Dorset) in sealed quartz tubes and solidified the melts by the normal freeze method at rates from 5 to 200 mm/h. These experiments yielded opaque products. No improvement was obtained by varying the composition of the melt about the stoichiometric composition. We confirmed the low vapour pressure of volatile components over the melt, previously reported by Wernick[3,4]. Zone refining at various rates in the range 5 to 25 mm/h for up to thirty passes, taking care to avoid loss of volatile constituents, yielded translucent material of large grain size which contained numerous inclusions throughout, as shown in Fig. 1.

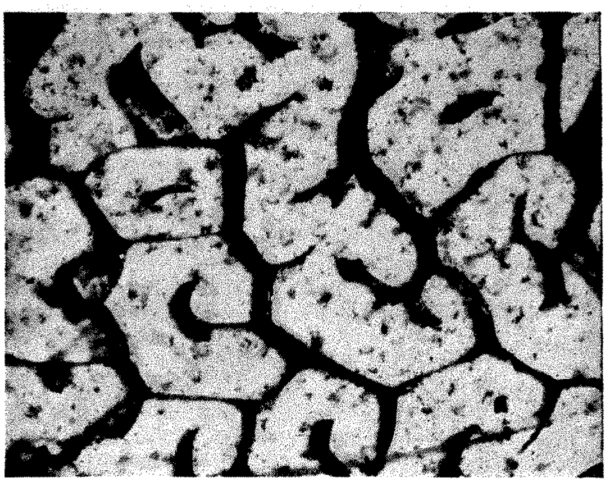

Fig. 1. Transmission micrograph of a slice of ingot showing characteristic cellular growth (× c. 57).

Careful microscopic examination of the ingots showed a cellular structure characteristic of constitutional supercooling[10]. We therefore decreased the growth rate and increased the imposed temperature gradient until at a growth rate of 8 mm/day and with a temperature gradient of 6 C°/mm at the liquid–solid interface we obtained large single crystals which were devoid of cellular structure and inclusions which result from the entrapment of melt[11] in the cell walls. Crystals of 12 mm diameter and 50 mm length have been grown in sealed quartz tubes by normal freezing of molten Ag_3AsS_3 in both vertical and horizontal apparatus. The melt can exhibit considerable supercooling; as much as 20 mm length has been observed to grow rapidly after nucleation. We found it convenient to rapidly freeze the first part of the melt and then to melt back to leave a 2–3 mm seed of polycrystalline material from which to propagate slow growth. Single crystals are readily obtained by promoting growth through a constriction in the quartz tube; in the case of vertical growth it was found that ten of the first twelve crystals grown were oriented within 30° of the optic axis.

The interesting optical parameters and the potential uses of this material have been described elsewhere[7]. Examination of the conoscopic figure in polished slices indicates that there is little strain in the crystals as grown. Although we have not yet found a suitable etch to reveal the dislocation content, the uniformity of extinction between crossed Nicols indicates a reasonable quality of single crystals.

The total impurity content of the material used was approximately 450 p.p.m. (excluding 250 p.p.m. of antimony). Of this 450 p.p.m., 200 p.p.m. was selenium, 100 p.p.m. was iron and other appreciable impurities were silicon, gold, lead and copper. The cellular structure obtained under the conditions quoted is unlikely to be caused by impurity segregation and indeed the zone-refined material showed no improvement. Segregation of the chief components of the melt is more likely the cause of the cell structure observed[12]. Treating the compound as a pseudo-binary compound of Ag_2S and As_2S_3, it was determined experimentally that with a growth speed of 8 mm/day and an imposed temperature gradient of 6 C°/mm the onset of cellular structure occurs when the concentration of arsenic trisulphide in the melt falls outside the approximate limits 22–28 mol per cent; at a growth speed of 4 mm/day and with the same temperature gradient these limits widen to approximately 18–32 mol per cent of arsenic trisulphide. Optical quality single crystals have been regrown in the same apparatus, however, at growth rates up to 112 mm/day without marked deterioration in crystal quality.

These results and observations show that the chief problem in growing optical quality single crystals is the occurrence of a cellular structure. As is well known[13,14], to avoid cellular growth the ratio of the growth rate to the temperature gradient in the melt must not exceed some critical value which is dependent on the degree of segregation at the solid–liquid interface. The origin of the segregation in these materials seems to be one of departure from stoichiometry rather than the presence of impurities.

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[1] Harker, D , *J Chem Phys* , 4, 381 (1936)
[2] Mellor, J W , in *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, 9, 293, 343 and 537 (Longmans, Green and Co , 1947)
[3] Wernick, J H , Gellor, S , and Benson, K E , *Anal Chem* , 30, 303 (1958)
[4] Wernick, J H , and Benson, K E , *J Phys Chem Solids*, 3, 157 (1957)
[5] Peacock, M A , *Univ Toronto Studies, Geol Ser* , No 51, 85 (1947)
[6] Chang, L L , *Amer Mineral* , 48, 429 (1963)
[7] Hulme, K F , Jones, O , Davies, P H , and Hobden, M V , *Appl Phys. Lett* ,10, 133 (1967)
[8] Jaeger, F M , and van Klooster, H S , *Z Anorg Chem* , 78, 245 (1912)
[9] Hansen, M , in *Constitution of Binary Alloys*, 4, 46 and 176 (McGraw-Hill, 1958)
[10] Rutter, J W , and Chalmers, B , *Canad J Phys* , 31, 15 (1953)
[11] Bardsley, W , Boulton, J S , and Hurle, D T J , *Solid State Electron* , 5, 395 (1962)
[12] Hurle, D T J , Jones, O , and Mullin, J B , *Solid State Electron* , 3, 317 (1961)
[13] Tiller, W A , Jackson, K A , Rutter, J W , and Chalmers, B , *Acta Met* , 1, 428 (1953)
[14] Mullins, W W , and Sekerka, R F , *J Appl Phys* , 35, 444 (1964)

MOLECULAR STRUCTURE

Experimental Observation of an Antisymmetric Raman Scattering Tensor

IT is well known that the scattering tensor for vibrational Raman scattering is symmetric[1] This is, however, not true for electronic Raman transitions (refs 1, 2 and unpublished results), and this has important implications in the selection rules for these transitions

We have recently demonstrated (ref 3, and results to be published) by *a priori* theoretical calculations the remarkable degree of antisymmetry of the scattering tensor for electronic Raman transitions of compounds containing trivalent lanthanides We report here some experimental observations of electronic Raman transitions where the antisymmetry of the scattering tensor is directly demonstrated.

In Fig 1, part of the 100° K Raman spectrum of yttrium aluminium garnet doped with about 5 per cent Eu^{3+} is shown The two relatively weak lines at 286 cm^{-1} and 307 cm^{-1} are not present in the Raman spectra of other yttrium aluminium garnet crystals and must be attributed to electronic Raman transitions taking place between the $^7F_0$ groundstate and the crystal field $^7F_1$ levels of the Eu^{3+} ion (The possibility of the observed lines being caused by local mode vibrations can be excluded because of the temperature dependence and the intensity of the transitions) The positions of the electronic levels as found from the Raman spectrum are in perfect agreement with the corresponding values derived from the optical fluorescence and absorption spectra[4]

Raman spectra of crystals of yttrium gallium garnet and yttrium vanadate doped with small amounts of trivalent europium have been reported previously[5,6]. Also in these other lattices electronic Raman transitions between levels of $^7F_0$ and $^7F_1$ have been found, so that the occurrence of electronic Raman transitions between these two levels must now be considered to be firmly established

According to Placzek[1] the components of the scattering tensor must transform as the matrix element $<\psi_r|\alpha_{\varrho\sigma}|\psi_k>$, where ψ_r and ψ are the wave functions for the levels between which the Raman transitions take place For the low lying electronic states of rare earth ions in crystals, the total angular momentum, $\mathcal{J}$, can be considered to be a good quantum number so that, neglecting the crystal field splitting of the excited state, the transformation properties of the scattering tensor will be determined by the symmetry operations in the total rotation group It is well known that it is possible to reduce the conventional cartesian tensor to a sum of three irreducible tensors which transform as states of total angular momentum 0, 1 and 2, respectively. The unitary transformation linking the cartesian basis set to the irreducible set has been given elsewhere[7] and it is found that the antisymmetric part of the scattering tensor is determined solely by that part of the irreducible tensor with angular momentum $\mathcal{J}=1$

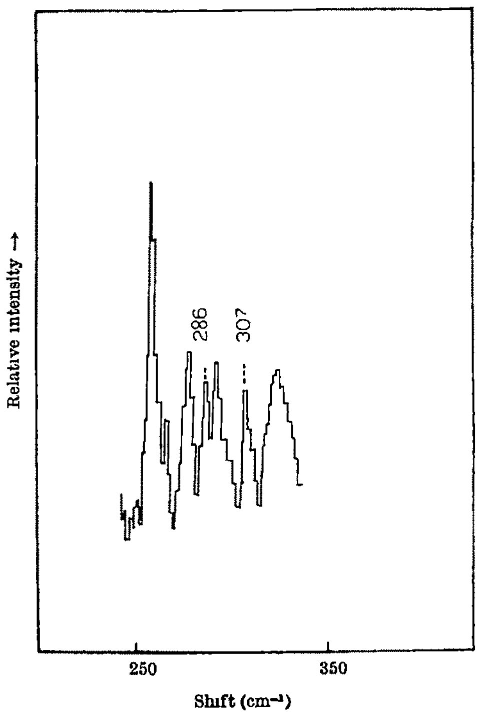

Fig 1 Part of the Raman spectrum of Eu^{3+} in yttrium aluminium garnet The spectrum was excited with the 6328 Å radiation of a helium–neon laser The time lapse between successive steps was 16 sec

For the trivalent europium ion the total angular momentum of the groundstate is equal to zero while the angular momentum of the first excited state is equal to one From the vector model of the addition of two angular momenta it is then found that only that part of the scattering tensor with quantum number $\mathcal{J}=1$ is non-vanishing for the electronic Raman transition $^7F_0 \rightarrow {}^7F_1$ That such a transition is readily observed is then an experimental proof of the theoretical prediction given previously that the scattering tensor for electronic Raman transitions of rare earth ions in crystals is highly asymmetric

This has important implications for the selection rules of electronic Raman transitions as compared with the conventional Raman effect For the latter the selection rules will be given completely by the transformation properties of products of the type $xy+yx$ and tabulations of these products can be found in many places (see, for example, ref. 8) For the electronic Raman effect, however, terms of the type $xy-yx$ also contribute and it should be remembered that the operators in the product do not commute so that this difference is usually a non-vanishing quantity. The transformation properties of this antisymmetric part can, of course, be handled by the powerful means of tensor operators, but there is an easier way out.

The antisymmetric part transforms under all rotations as a vector, but under inversion as an axial rather than a polar vector[9] The same is true for the transformation properties of rotation about cartesian axis, so that the transformation properties of the antisymmetric part of the scattering tensor can be found directly from the character tables given, for example in Wilson Decius and Cross[8]

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[1] Placzek, G in *Handbuch der Radiologie*, Bd VI, Teil II, 205 (Leipzig, 1934).
[2] Hougen, J T , and Singh, S , *Proc Roy Soc* , A, **277**, 193 (1964)
[3] Mortensen, O Sonnich, and Koningstein, J A , *Chem Phys Lett* , 1, 409 (1967)
[4] Koningstein J A , *Phys Rev* A, **136**, 717 (1964)
[5] Koningstein J A , *J Chem Phys* , **46**, 2811 (1967)
[6] Koningstein J A , and Mortensen, O S , *Phys Rev Lett* , **18**, 831 (1967)
[7] Mortensen, O S , and Koningstein, J A , *J Chem Phys* (in the press)
[8] Wilson, jun , E B , Decius, J C , and Cross, P C , in *Molecular Vibrations* (McGraw-Hill New York, 1955)
[9] Tinkham, M., in *Group Theory and Quantum Mechanics* (McGraw-Hill, New York, 1964)

Mossbauer Effect Study of the State of Iron in Spinach Ferredoxin

THE ferredoxins are an important group of enzymes which catalyse photochemical reactions in plants and photosynthetic bacteria They contain non-haem iron which is believed to be close to the active centre but which is in a chemical form which has not been unambiguously determined Spinach ferredoxin is one of the simpler of these compounds, has a molecular weight of about 12,000 and contains two iron atoms/molecule A Mossbauer study on this system has been made by Bearden and Moss[1] and on other non-haem iron proteins by several groups[2-4] In this communication, we describe Mossbauer effect measurements made both in the presence and absence of a magnetic field on spinach ferredoxin, and it is concluded that the iron atoms are both low-spin Fe^{2+} in the oxidized state, and there is one low-spin Fe^{2+} and one high-spin Fe^{2+} in the reduced state

The Mossbauer effect has become an extremely powerful technique for the study of the oxidation state of atoms because (1) it is non-destructive, in contrast to chemical methods, (2) it is a property of a specific nucleus (for example, iron-57), enabling investigations without knowing the complete X-ray structure of the molecule, and (3) the atoms can be identified, unlike in electron spin resonance or susceptibility measurements

The non-haem iron proteins give no electron spin resonance signal in the oxidized state, but on reduction they show an anisotropic resonance centred about $g = 1$ 94, which is quite unlike those found in any other compound of iron investigated For spinach ferredoxin, the values are $g_x = 1$ 89, $g_y = 1$ 94 and $g_z = 2$ 04 (ref 5) The structure and state of the iron atoms are not known, though it has been assumed from the similarity of the electron spin resonance signals that it is the same for all the compounds The electron spin resonance line was broadened when one of these proteins (*Azotobacter vinlandii*) was enriched with iron-57 (ref 6) and sulphur-33 (ref 7), which suggested that the resonance arises from iron atoms which have sulphur neighbours Bayer *et al* [8] have made ferredoxin which is free from iron (apoferredoxin) which shows no electron spin resonance signal, but on re-incorporation of the iron the $g = 1$ 94 signal reappears

Thus electron spin resonance measurements do not enable us to establish the valence state of the iron atoms and hence the Mossbauer effect may, in principle, be especially valuable here Because in highly covalent compounds the isomer shift is not very sensitive to the state of iron, we studied the magnetic hyperfine interactions which together with isomer shift measurements may distinguish between high and low spin states, as well as between ferrous and ferric assignments The spectra observed in strong magnetic fields at low temperatures are complicated for polycrystalline materials by the effects of magnetic anisotropy and quadrupole splittings, but nevertheless certain usual features may be enumerated[9] (1) High spin Fe^{3+} shows a sharp six line pattern corresponding to an effective magnetic field at the nucleus of 400 to 600 k gauss[10,11] because the ion is isotropic or almost isotropic (2) Low spin Fe^{3+} shows much broader lines because it has a large electric field gradient and anisotropic g-values ranging between 1 and 3, although usually a six line pattern may be distinguished which corresponds to an effective field of 200 to 300 k gauss (3) High spin Fe^{2+} may show a wide variety of possible Mossbauer spectra in magnetic fields because the quadrupole coupling and the g-values are sensitive to its ligand environment (4) Low spin Fe^{2+} shows[10] only a small broadening caused by the direct effect of the external magnetic field on the nucleus because this ion is non-magnetic and thus the internal field is zero

Thus observations of the effect of a magnetic field on a non-magnetic Mossbauer spectrum should clearly identify high spin Fe^{3+} or low spin Fe^{2+} The remaining configurations of single ions low spin Fe^{3+} and high spin Fe^{2+}, should be distinguishable by the large positive isomer shift (1 mm/sec or more) of the latter

Spinach ferredoxin was prepared from spinach bought in the market which was carefully selected, washed and then stored at − 20° C The purification procedure was a modification of the method of Hill and Bendall[12] We used Whatman *DE*22 anion exchangers, slightly different concentrations of acetate buffer, pH 5 5, and did not attempt to crystallize the protein The specimens were in frozen aqueous solution and the iron was not exchanged for enriched iron-57 so as to avoid any possible damage to the molecule, thus the intensity of the Mossbauer effect was weak but not impossibly weak The samples, 0 7 ml in volume, were estimated to contain 118 mg ferredoxin/ml , based on $E_{420} = 10$ 32 cm^{-1} $mmoles^{-1}$

The Mossbauer spectra were measured using the spinach ferredoxin as the absorber and cobalt-57 in chromium as a source of mono-energetic 14 4 keV gamma rays The source was driven on the vibrator designed by Cranshaw[13], and the spectra were recorded on a multichannel analyser

The spectra were measured for both oxidized and reduced ferredoxin Reduction was produced with sodium dithionite, and was monitored by observing the electron spin resonance signal both before and after the Mossbauer effect measurement The specimens were also checked for biological activity immediately after being prepared The Mossbauer spectra were measured at temperatures of 195° K, 77° K, 4 2° K and 1 5° K At the latter two temperatures magnetic fields of up to 30 k gauss could be applied Fields up to 5 k gauss were produced by an iron cored electromagnet Fields up to 30 k gauss were produced by a superconducting solenoid

After measurement the reduced samples were further checked for possible deterioration by allowing them to melt and re-oxidize in air, when they gave no electron spin resonance signal, but gave the Mossbauer spectrum of Fig 1 (*a*) and were still active On re-addition of dithionite the reduced spectra of Fig 1 (*b*) reappeared

The Mossbauer spectrum of oxidized spinach ferredoxin at 77° K is shown in Fig 1 (*a*) The spectrum at 4 2° K is similar, and agrees in general with those of Bearden and Moss[1], as does that of the reduced sample It consists of a pair of quadrupole split lines of almost equal intensity with separation of 0 6 mm/sec, and with an isomer shift of 0 2 mm/sec with respect to iron metal The spectrum

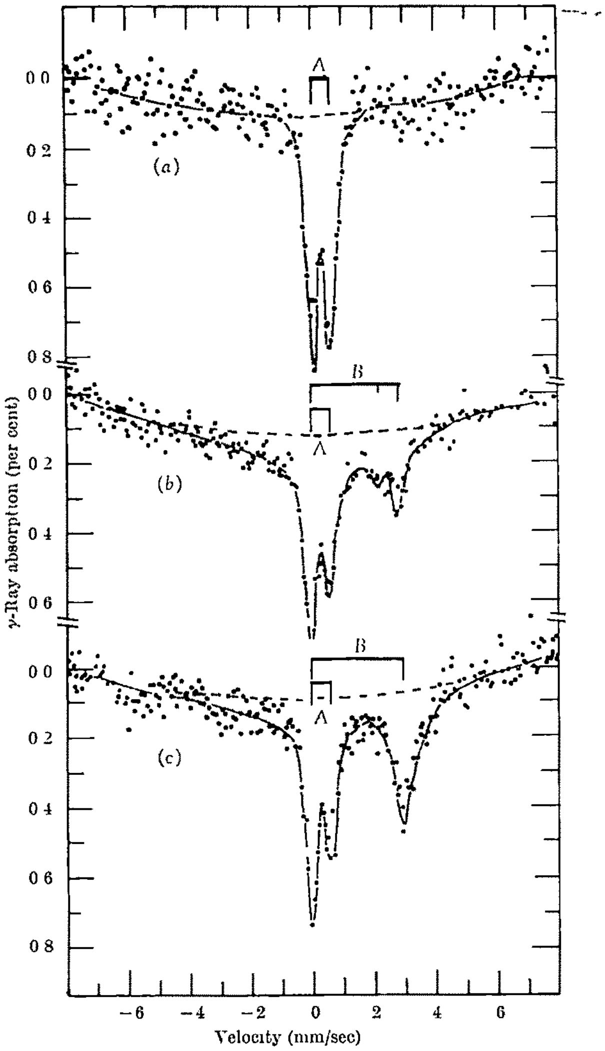

Fig. 1. Mossbauer spectra of spinach ferredoxin. (a) Oxidized, $T=77°$ K, (b) reduced, $T=77°$ K, (c) reduced, $T=4.2°$ K. The dashed line corresponds to the zero of absorption and is curved because of geometrical effects. Decomposition into the spectra A and B is described in the text.

varied only slightly between 195° K and 1.5° K. Further absorption was looked for at velocities up to ± 8 mm/sec but was not found. Application of an external magnetic field of 30 k gauss at temperatures between 1.5° and 4.2° K produced only a small broadening of the lines, corresponding to the iron being in a non-magnetic state. This suggests that the two iron atoms are both in a low spin Fe^{2+} state.

The spectrum at 77° K and at 4.2° K of reduced spinach ferredoxin is shown in Figs. 1 (b) and (c), respectively. It seems to consist of a pair of lines of unequal intensity at the same velocities (−0.1 and +0.5 mm/sec) as in the oxidized spectrum, and in addition a line or lines at positive velocities of between 2 and 3 mm/sec, which are temperature dependent. At 4.2° K there is a line at +2.9 mm/sec which appears to become less intense as the temperature increases. This seems to be caused by some of the absorption moving to lower velocities (about +2 mm/sec) and there is also a slight change in the line at +0.6 mm/sec which is sharper at 77° K than at 4.2° K.

The simplest interpretation of the spectrum at 4.2° K is that it consists of a superposition of a spectrum A, with a small quadrupole splitting (0.6 mm/sec) and isomer shift (+0.25 mm/sec), and a spectrum B, with a larger quadrupole splitting (about 3.0 mm/sec) and shift (about +1.5 mm/sec), in approximately equal intensities, although the spectrum with the larger splitting and shift seemed to be slightly more intense. The superposition is such that the low energy lines of each spectrum coincide so that the resulting line at −0.1 mm/sec of the composite spectrum is the deepest. It is natural to associate spectrum A with iron which has not been reduced because the lines are at velocities undistinguishable from those in the oxidized spectrum. The isomer shift and quadrupole splitting by themselves do not allow an assignment to be made for the state of these atoms. High spin Fe^{2+} can be ruled out, however, because it would have a large isomer shift and quadrupole splitting, which leaves either low spin Fe^{2+} or high or low spin Fe^{3+}. Spectrum B, if we are correct in analysing the spectrum in this way, clearly arises from high spin Fe^{2+} ions because of its large isomer shift and quadrupole splitting.

The spectra of the reduced specimen in external magnetic fields applied at 4.2° K are shown in Fig. 2. A comparison with the spectrum in zero field (Fig. 1 (c)) shows that, unlike the oxidized spectrum, an external magnetic field affects the spectrum, which shows that there are magnetic iron ions present, and is consistent with the observation of electron spin resonance in the reduced state. Spectrum A shows no internal magnetic field at the nucleus. Thus of the three possible alternatives (high spin Fe^{2+} having been already eliminated) it is identified as arising from low spin Fe^{2+} ions, as Fe^{3+} (high or low spin) would show large hyperfine fields. Spectrum B splits in the magnetic field more or less as one would expect for high spin Fe^{2+}. The spectra do not seem to vary greatly with temperature (compare spectra in 5 k gauss in Fig. 2 (a) and (b)), and a value of about 150 k gauss is deduced for the mean hyperfine field at these sites. The spectra in 30 k gauss were not as good as those in small or zero fields because the experimental geometry in the superconducting magnet was not so favourable. The principal point, however, that spectrum A is not drastically affected by an external field of 30 k gauss at 4.2° K is clear.

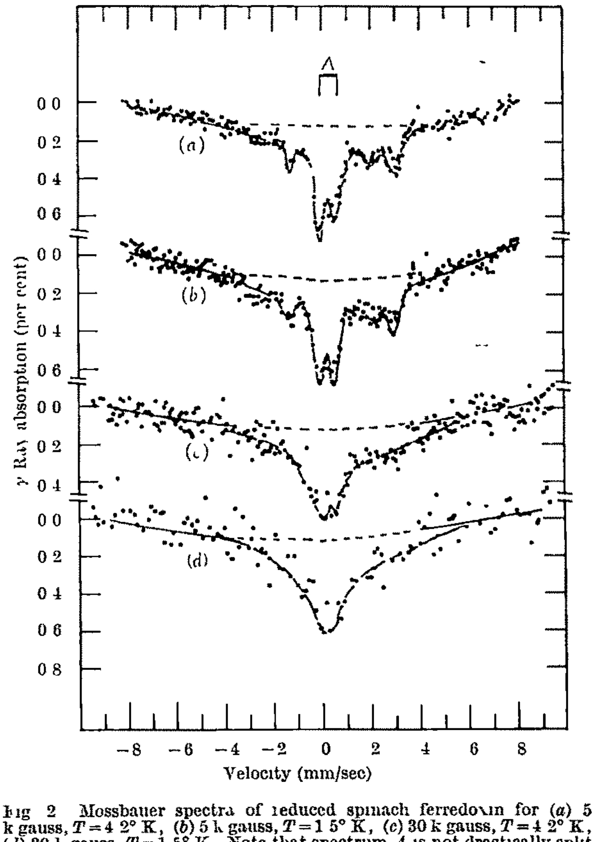

Fig. 2. Mossbauer spectra of reduced spinach ferredoxin for (a) 5 k gauss, $T=4.2°$ K, (b) 5 k gauss, $T=1.5°$ K, (c) 30 k gauss, $T=4.2°$ K, (d) 30 k gauss, $T=1.5°$ K. Note that spectrum A is not drastically split by magnetic fields.

It seems from the observed Mossbauer spectra that spinach ferredoxin in the oxidized state contains low spin Fe^{2+} ions, and that on reduction about one-half of the ions are converted into high spin Fe^{2+} ions That only a fraction of the iron atoms is affected on reduction was found previously from the intensity of the electron spin resonance signal, and it is consistent with the observation that spinach ferredoxin is a one electron donor[14]

If these assignments of the chemical states are accepted, there remain two important questions to be answered (1) how do the $g = 1\ 94$ electron spin resonance signals originate, and (2) why do only about half of the iron atoms change their state on treatment with sodium dithionite ?

Low spin Fe^{2+} has a non-magnetic ground state and so cannot give an electron spin resonance signal, while high spin Fe^{2-} usually does not give an electron spin resonance signal presumably because it is a non-Kramers ion and all degeneracies are removed by the Jahn–Teller effect Several people have proposed theories and models to explain the g-values in spinach ferredoxin (*a*) Brintzinger, Palmer and Sands[15] proposed a scheme showing that the resonance could arise from low spin Fe^{3+} tetrahedrally co-ordinated to four sulphur atoms (*b*) Gibson, Hall, Thornley and Whatley[5] proposed that in the oxidized state two high spin Fe^{3+} ions were antiferromagnetically exchange-coupled to give an $S = 0$ state, and in the reduced state one of these is converted to high spin Fe^{2+} so that the resulting Fe^{2+}–Fe^{3+} coupled system has $S = \frac{1}{2}$ and could have the observed g-values This theory has the added attraction of explaining why only half of the iron atoms may be reduced. (*c*) Blumberg and Peisach[16] proposed that the electron spin resonance arises from a free radical which is antiferromagnetically coupled to the excited ($S = 1$) state of low spin Fe^{2+}

The Mossbauer effect data on the reduced state do not seem to be consistent with model (*a*) involving low spin Fe^{3+} or (*b*) with coupled Fe^{3+}–Fe^{2+} states because the Fe^{3+} ions would give rise to a large hyperfine field when a magnetic field was applied The high isomer shift (1·5 mm/sec) of the high spin Fe^{2+} ion rules out tetrahedral co-ordination (as in (*a*)). There remains suggestion (*c*) that the iron is in a low spin Fe^{2+} state, which could fit in with the Mossbauer data of site A if the effect of the reducing agent is to produce a free radical with an unpaired electron spin, close to the iron

We would emphasize that these conclusions have been made on the assumption of simple models There is the possibility that the effective component of the hyperfine interaction tensor for site A is very small because of accidental cancellation. Such a situation occurs in ferrous chloride[17] and in the $FeCl_4^{2-}$ ion[18] Because of the small quadrupole splitting and the fact that the g-values are close to 2, however, both the dipolar and orbital hyperfine fields are small and it is unlikely that they could cancel the large negative core polarization field

It is possible that there are more than two iron sites The apparent decrease in intensity of the line at +2 9 mm/sec in the reduced spectrum at 77° K and the appearance of absorption near 2 mm/sec may result from a third iron state There also seems to be a continuous unresolved background in the spectra of the reduced samples which is not present in the oxidized form, which may be evidence for iron in a third state with relatively slow electronic relaxation rates, but which might be able to give the electron spin resonance signal

Although it therefore seems likely that spinach ferredoxin in the oxidized state contains two low spin Fe^{2+} in the molecule, and in the reduced state one low spin Fe^{2+} and one high spin Fe^{2+}, it is not possible to decide what is the origin of the $g = 1\ 94$ electron spin resonance signal until all the details of the Mossbauer spectra have been explained Studies with ferredoxin extracted from plants grown with enriched iron-57 are planned and should be valuable in understanding the details of the magnetic behaviour

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[1] Bearden, A J , and Moss, T H , *Proc Second Intern Conf Magnetic Resonance in Biological Systems*, Stockholm, June, 1966, 391 (Pergamon Press, 1967)

[2] Bearden, A J , Moss, T H , Bartsch, R G , and Cusanovitch, M A , in *Non-Heme Iron Proteins Role in Energy Conversion*, 87 (Antioch Press, 1965)

[3] Blomstrom, D C , Knight, jun , E , Phillips, W D , and Weiher, J F , *Proc US Nat Acad Sci* , **52**, 762 (1964)

[4] Johnson, C E , Bray, R C , and Knowles, P F , *Biochem J* , **103**, 10C (1967)

[5] Gibson, J F , Hall, D O , Thornley, J H M , and Whatley, F R , *Proc US Nat Acad Sci* , **56**, 987 (1966)

[6] Shethna, Y I , Wilson, P W , Hansen, R E , and Beinert, H , *Proc US Nat Acad Sci* , **52**, 1263 (1964)

[7] Hollocher, T , Solomon, F , and Ragland, T , *J Biol Chem* , **241**, 3452 (1966)

[8] Bayer, E , Josef, D , Krauss, P , Hagenmaier, H , Roder, A , and Trebst, A , *Biochim Biophys Acta*, **143**, 435 (1967)

[9] Johnson, C E , *Proc Second Int Conf Magnetic Resonance in Biological Systems*, Stockholm, June, 1966, 405 (Pergamon Press, 1967)

[10] Lang, L G , and Marshall, W , *Proc Phys Soc* , **87**, 3 (1965)

[11] Johnson, C E , *Phys Lett* , **21**, 491 (1966)

[12] Hill, R , and Bendall, F , *Nature*, **187**, 417 (1960)

[13] Cranshaw, T E , *Nucl Inst Meth* , **30**, 101 (1964)

[14] Whatley, F R , Tagawa, K , and Arnon, D I , *Proc US Nat Acad Sci* , **49** 266 (1963)

[15] Brintzinger, H , Palmer, G , and Sands, R H , *Proc US Nat Acad Sci* , **55**, 397 (1966)

[16] Blumberg, W E , and Peisach, J , in *Non-Heme Iron Proteins Role in Energy Conversion*, 101 (Antioch Press, 1965)

[17] Ono, K , Ibo, A , and Fujita, T , *J Phys Soc Japan*, **19**, 2119 (1964)

[18] Edwards, P R , Johnson, C E , and Williams, R J P , *J Chem Phys* , **47**, 2074 (1967)

CHEMISTRY

Visible Absorption Spectrum of Bromine Chloride

A VISIBLE and near infrared band system in emission, produced from the combination of atomic chlorine with atomic bromine, $Br + Cl + M = BrCl^* + M$, $BrCl^* = BrCl + h\nu$, has been reported recently[1] The transition involved was thought to be $^3\Pi_{0^+} \leftrightarrow {}^1\Sigma^+$ (ref. 1). Because no visible band absorption spectrum of bromine chloride has so far been published, no accurate vibrational or rotational constants for the states of this molecule are known The infrared absorption spectrum of bromine chloride[2,3] gave only an approximate value for ω_e'' of 442 5 cm^{-1}, because the ^{79,81}Br and ^{35,37}Cl isotopic splittings were not resolved. This result is in agreement with an estimate of $\omega_e'' = 440$ cm^{-1} by Herzberg[4], based on an early study of two vacuum ultraviolet band systems of bromine chloride[5]

In the present work, we have recorded the $^3\Pi_{0^+} \leftrightarrow {}^1\Sigma^+$ band system of bromine chloride in absorption using equilibrium mixtures of bromine, chlorine and bromine chloride

Although the equilibrium

$$Br_2(g) + Cl_2(g) \rightleftharpoons 2BrCl(g); \quad \Delta H^\circ = 0$$

lies to the right hand side at all temperatures used in this work (294° < T < 690° K), with $K_{eq} = 6\ 5$ at 300° K, a difficulty arises from the visible absorption bands of small quantities of bromine in the equilibrium mixtures. Because the banded spectrum of bromine chloride lies in the same wavelength region and is not well developed at room temperature, this probably accounts for the negative results of previous searches for the bromine chloride bands In the present study, absorption caused by molecular bromine was minimized by using a large excess of chlorine (initial ($P_{\mathrm{Cl_2}}/P_{\mathrm{Br_2}}$) between 20 and 60) at total pressures near 700 mm of mercury; typical partial pressures of bromine chloride were thus about 20 mm of mercury The gaseous mixtures were contained in a uniformly heated 200 cm 'Pyrex' tube with 7 cm diameter 'Pyrex' windows sealed on The continuum from a 55 W quartz iodine lamp was used with a system of plane mirrors and lenses to give a path length of 600 cm for absorption. All gas mixtures were allowed to equilibrate overnight

The spectra were photographed with a 10μ slit in the first order of a grating spectrograph of inverse linear dispersion 40 Å mm^{-1}, the resolving power (10,000) was limited by the resolving power of the photographic emulsion Wavelength calibration was by means of a neon discharge tube The spectral range studied was 5000 Å to 7200 Å, which was covered by 'Panatomic *X*' and *I-N* emulsions

The absorption spectra of various mixtures of bromine and chlorine were examined at different temperatures At 294° K, the $^3\Pi_{\mathrm{ou}+}$—$^1\Sigma_g^+$ band system of chlorine[6] at 700 mm of mercury extended to about 5400 Å ($v'' \leqslant 2$). The spectrum of a similar pressure of chlorine mixed with 12 mm of bromine contained several new, rather weak bands, degraded towards the red, in the region 5400 Å to 5800 Å Bromine $^3\Pi_{\mathrm{ou}+}$—$^1\Sigma_g^+$ bands[7,8] were very weak under these conditions.

On increasing the temperature (up to 690° K), the spectrum of chlorine (700 mm of mercury) developed progressions in v' from higher values of v'' ($v'' \leqslant 5$), so that at the highest temperature the chlorine band system extended to about 6100 Å With a similar increase in temperature, the new band system from the chlorine+bromine mixtures was relatively more intense, and extended even further towards the infrared. About twenty-five additional bands extending to 7000 Å were observed with medium intensity The same system was observed with greater intensity at 690° K using a mixture of 700 mm chlorine with 35 mm bromine

Measurements of the wave-numbers of the heads of the new bands resulted in an identification with the same bromine chloride band system observed in emission from the combination of bromine and chlorine atoms[1] With the same vibrational numbering, short v' progressions from $6 \geqslant v'' \geqslant 0$ of the $^1\Sigma^+$ state were observed in the absorption spectrum. The (v', 0) progression was not particularly intense, and was difficult to identify at higher temperatures on account of overlapping by chlorine ($^3\Pi_{\mathrm{ou}+}$—$^1\Sigma_g^+$) bands and by bromine chloride continuum Bromine chloride heads near 6000 Å were quite sharp, but some other bromine chloride heads were diffuse on account of the finite Br 79/81 isotopic splittings, which were not resolved with the dispersion of our spectrograph Cl 35/37 isotopic splittings were observed for a number of bands, however. Table 1 shows the wavelengths of the band heads of the system bromine chlorine ($^3\Pi_{0+}$—$^1\Sigma^+$) observed in absorption and emission[1]. The agreement is good in view of the limited precision of measurements in the emission spectrograms

The termination of the bromine chloride band system at the $v''=0$ progression, followed by continuous absorption to shorter wavelengths, and the relative intensities of the $v''=0$ and $v''>0$ progressions, strongly suggests that the vibrational numbering of the upper state given in the earlier work[1] is correct In the absorption spectrum of bromine chloride, transitions were observed to vibrational levels with $v'=8$ and 9, which lie at 18,172 ± 5 cm^{-1} and 18,288 ± 5 cm^{-1}, respectively, above $v''=0$ of the $^1\Sigma^+$ state Because the dissociation limit for Br($^2P_{\frac{3}{2}}$) + Cl($P_{\frac{3}{2}}$) lies[1] 18,007 ± 50 cm^{-1} above $v''=0$, these observations show that the excited state dissociates to Br($^2P_{\frac{3}{2}}$) + Cl($^2P_{\frac{1}{2}}$) for which the dissociation limit is 18,888 cm^{-1} (ref 1) The earlier identification of the upper state[1] as $^3\Pi_{0+}$ is therefore confirmed

Table 1 BAND HEADS OF BrCl($^3\Pi_{0+} \longleftrightarrow {}^1\Sigma^+$)

v'	v''	λ (Å) (Absorption, this work)	λ (Å) (Emission, ref 1)
8	0	5500	—
7	0	5543	—
(6 (10	0) 1)	(5586)	—
9	1	5604	—
8	1	5638	—
7	1	5680	5686
6	1	5732	5735
9	2	5744	—
8	2	5780	5784
7	2	5824	5829
6	2	5877	5880
9	3	5891	—
8	3	5930	5932
7	3	5976	5978
6	3	6031	6032
8	4	6085	—
5	3	6091	6091
7	4	6133	6133
4	3	6159	6159
6	4	6191	6190
5	4	6256	6254
7	5	6301	—
4	4	6329	6324
6	5	6360	6358
5	5	6429	6425
7	6	6471	—
4	5	6501	6499
6	6	6534	—
5	6	6608	6604
4	6	6685	6682
3	6	6771	6766

The band head analyses of the absorption and emission systems of bromine chloride ($^3\Pi_{0+}$—$^1\Sigma^+$) gave the same values for the vibrational constants, within the estimated limits of error. The mean values of all these data can be represented by the expressions

$$G''(v)(\mathrm{cm}^{-1}) = 443\ 5(v''+\tfrac{1}{2}) - 1\ 8(v''+\tfrac{1}{2})^2 \text{ for } 0 \leqslant v'' \leqslant 14$$

and

$$G'(v)(\mathrm{cm}^{-1}) = 16{,}705 + 239(v'+\tfrac{1}{2}) - 5\ 5(v'+\tfrac{1}{2})^2 - 0\ 08 (v'+\tfrac{1}{2})^3 \text{ for } 2 \leqslant v' \leqslant 9$$

The relatively large value for $\omega_e' y_e'$ is consistent with the expected maximum in the potential curve of the $^3\Pi_{0+}$ state of bromine chloride (compare the $^3\Pi_{0+}$ state of iodine chloride[9]) The observations of intense continuous absorption and unfavourable Franck–Condon transitions from $v''=0$ at 294° K also support the prediction of a potential maximum at an energy just above $v'=9$

The absorption spectrum of bromine chloride is currently under further study in this laboratory, using a Jarrell-Ash 3 4 metre Ebert spectrograph

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[1] Clyne, M A A, and Coxon, J A, *Proc Roy Soc*, A, 298, 424 (1967)

[2] Brooks, W V F, and Crawford, B, *J Chem Phys*, 23, 363 (1955)

[3] Mattraw, H C, Pachucki, C F, and Hawkins, N J, *J Chem Phys*, 22, 1117 (1954)

[4] Herzberg, G, *Molecular Spectra and Molecular Structure II, Spectra of Diatomic Molecules* (Van Nostrand, 1950)

[5] Cordes, H, and Sponer, H, *Z Phys*, 79, 170 (1932)

[6] Douglas, A E, Møller, C K, and Stoicheff, B P, *Canad J Phys*, 41, 1174 (1963)

[7] Darbyshire, O, *Proc Roy Soc*, A, 159, 93 (1937)

[8] Barrow, R F, and Horsley, J A, *Trans Farad Soc*, 63, 32 (1967)

[9] Hulthén, E, Jarlsater, N, and Koffman, L, *Ark Fys*, 18, 479 (1960)

Potassium Selective Microelectrodes with Precipitate in the Tip

THE thermodynamic analysis of physico-chemical processes in the living cell requires a knowledge of intracellular ionic activities. This problem has stimulated research into the design of special microelectrodes which are selectively sensitive to the different ions. This has been achieved by using either reversible, glass insulated, metal microelectrodes[1,2], or the so-called membrane electrodes[3]. The interphase potential of a metallic silver–silver chloride electrode, the most commonly used, is a function of the activity of chloride ions in the solution and its selectivity properties are determined by the presence of the silver chloride precipitate layer. In membrane electrode systems, selectivity depends on the ion exchange properties of the membrane material, and the transmembrane potential is a function of the ion activity ratio across the membrane for a particular ion. For the cations H^+, K^+ and Na^+, glass membrane electrodes[3] are usually used while silicone–rubber membranes packed with certain precipitates or ion-exchange substances are used for various anions[4].

In the course of experiments, it was noticed that, in certain conditions, even ordinary glass microelectrodes developed selectivity properties. The conditions in which this was observed were the following: a microelectrode in contact with the cell wall (cation-exchange phase) of *Nitella* cells[5,6] exhibited the characteristics of a K^+-electrode; the microelectrodes the tips of which were sealed with cellular materials maintained the potentials of the cell wall even after removal from the cell. This tip potential was a function of the sodium, a_{Na^+}, and potassium, a_{K^+}, ion activities, with a slight selectivity for K^+ rather than Na^+. After prolonged immersion in 3 molar potassium chloride solution (about a month) or after being held in air for a few hours, the microelectrode potential was seen to increase greatly because of a new phase forming in the tip, possibly a precipitate of potassium chloride from the saturated solution. Such microelectrodes were again selective for K^+ against Na^+ with a selectivity coefficient[7] (or permeability ratio) of 0.4.

These observations suggested that the usual microelectrodes might be converted into electrodes sensitive to selected ions by sealing their tips with an ion-exchange material which might be formed by precipitating a salt containing the particular ion. The formation of the precipitate inside the tip of the microelectrode leads to the distribution of ion activities between this newly created phase and the corresponding solutions contained inside and covering the outside surface of the microelectrode.

In the present work, the two reagents used to precipitate sparingly soluble potassium salts (K^+-precipitate) were nearly saturated solutions of sodium cobaltinitrite with a few drops of glacial acetic acid and dipicrylamine dissolved in 1 molar sodium hydroxide (according to IUPAC[8]). There are two principal steps in the manufacture of a K^+-sensitive microelectrode of the precipitate type. First, the tip of the microelectrode is filled with the potassium reagent, and, second, a precipitate of a potassium salt is formed inside the tip.

Glass microelectrodes with a tip diameter of about 1μ could be filled by microcapillary forces[9], the tip being inserted into the potassium reagent for 5–10 sec (or minutes if it was intended to fill the shank). Filling the microelectrode could also be accomplished quite readily by rotating (screwing) a fine glass capillary rod on the inside of the shank. To form the precipitate in the tip, the electrode was lowered into a 0.1 to 0.2 molar potassium chloride solution. This operation was viewed under a microscope. The microelectrode was then filled with 1 molar potassium chloride, using the standard ethanol boiling technique, to make contact with the precipitate from the inside. To obtain mechanically stable precipitates the temperature of the microelectrode, before boiling, should be kept at about 70° C for several hours.

A more convenient method of making the K^+-selective microelectrodes was to fill the shank with the K^+-precipitating reagent and then to make contact with this using a fine silver–silver chloride electrode. The actual precipitate was deposited as described. For experiments with plant cells it is not necessary to deposit the potassium precipitate before inserting the microelectrode into the cell because the tip fills with cell sap as soon as it is introduced into the vacuole (by turgor pressure).

The calibration of K^+ microelectrodes was carried out in pure potassium chloride and sodium chloride solutions in the range of 10^{-5} molar up to 1 molar. The method of making electrical measurements is described elsewhere[10]. It was found that the potential of the K^+ microelectrode (E_{K^+}) was a linear function of the logarithm of activity for the range 10^{-3} molar to 0.6 molar. The potential E_{K^+} could be expressed as $E_{K^+} = (RT/F)\ \ln\ (a_{K^+} + \alpha a_{Na^+})$. The best selectivity coefficient, α, achieved in the present work was 0.12. Because the activity of Na^+ in living cells is low the effect of these ions on the K^+ potential is quite small.

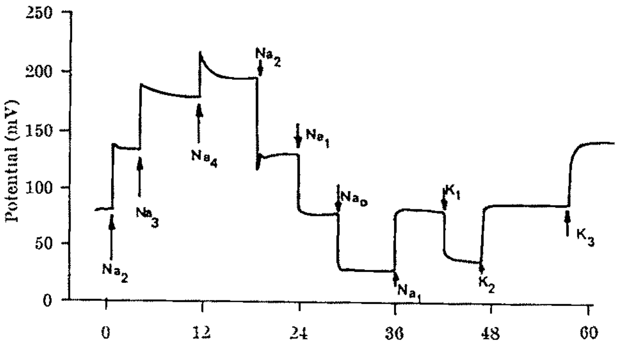

Fig 1. The potential of the K^+-selective microelectrode in potassium chloride and sodium chloride solutions of different concentrations (Na_2 refers to 10^{-2} molar sodium chloride, Na_1 to 10^{-1} molar sodium chloride, K_1 to 10^{-1} molar potassium chloride, etc.). The potential of the selective electrode is measured against a standard 3 molar potassium chloride filled microelectrode.

The sensitive area of these K^+ microelectrodes corresponds to the surface area of the open tip of ordinary microelectrodes. The advantage of this method of making cation sensitive microelectrodes is that no additional insulation of the shank is required. The resistance of the K^+ microelectrodes was found to be between 10^{10} and $3 \times 10^{10}\ \Omega$, a value which is considerably lower than the resistance of cation selective glass electrodes. The time response curves (Fig 1) suggest that there are two components of the electrode potential, a diffusion potential and a phase potential. The diffusion component of the total potential for Na^+ ions is much greater than that of K^+ ions, suggesting that the mobility of Na^+ ion is greater than that of K^+ ions. This is consistent with the idea that the K^+ ions are more strongly bound and thus potential determining. This is similar to the behaviour of K^+ glass selective microelectrodes[3].

Preliminary experiments have shown that the method of ion precipitation discussed can be extended to the manufacture of microelectrodes which are selectively sensitive to Ca^{++} and Na^+, and possibly to other ions.

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[1] Kerkut, G A, and Meech, R W, *Life Sci*, 5, 453 (1966)
[2] Coster, H G L, *Austral J Biol Sci*, 19, 545 (1966)
[3] Eisenman, G, and Conti, F T, *Gen Physiol*, 48 65 (1965)
[4] Pungar, E, Havas, T, and Toth, K, *Instrum Control Syst*, 38, 8, 105 (1965)
[5] Vorobiev, L N, and Kurella G A, *Biofizika*, 10, 788 (1965) (Russian)
[6] Nagai, R, and Kishimoto, U, *Plant Cell Physiol*, *Tokyo* 5 21 (1964)
[7] Lev, A A, *Biofizika* 9 686 (1964) (Russian)
[8] *Inter Union Pure Appl Chem*, Fifth Rep (1964)
[9] Vorobiev, L N, *Biofizika*, 20 358 (1965) (Russian)
[10] Vorobiev, L N, *Nature*, 216, 1325 (1967)

Discrimination of Chemiluminescent Mixtures: the Chemiluminescencegram

A METHOD has been developed for the continuous measurement of the intensity of light obtained in oxychemiluminescence experiments with changes in the *p*H of the solutions The plot obtained, which has been called a chemiluminescencegram, permits differentiation between the various compounds in a mixture capable of showing chemiluminescence In our experiments, luminol was chosen to test the efficiency of the method

Luminol was first shown to be a mixture of five components and not a pure compound as is usually accepted Indeed luminol samples ('Fluka' for analysis, 'Schuchardt' for analysis, and a sample prepared by ourselves[1]) were chromatographed on Whatman No 3 paper with 0 1 normal sodium hydroxide as eluent and the luminol sample was prepared as a 10^{-2} molar aqueous solution with the stoichiometric amount of alkali added to produce the mono-salt The chromatogram which was developed in 135 min showed five bands, all of which gave strong fluorescence when illuminated with ultraviolet light (2537 Å) Band 1, 13 5 cm from the start, showed a blue fluorescence, band 2, 19 cm from the start, had a greenish fluorescence, band 3, 23 cm away, had a weak blue fluorescence, and band 4, a double band at 28 cm showed a greenish fluorescence Chromatography of 3-nitrophthalhydrazide, prepared according to the method of Huntress *et al* [2], on the other hand, showed only two bands which when illuminated with ultraviolet light produced no luminescence at all The components appeared as two dark bands This result indicates that the possibility that one or more of the bands which appeared on the luminol chromatogram were caused by 3-nitrophthalhydrazide which had not been reduced in the preparation procedure could be excluded

The five components of the luminol mixture were then separated chromatographically, but, unfortunately, they were not in sufficient quantities for their structures to be established Enough material was obtained, however, for us to carry out some oxychemiluminescence experiments The five luminol components were oxidized, using the standard procedure with hydrogen peroxide and potassium ferricyanide in alkaline solution, with simultaneous recording of the intensity of the light evolved

Out of the five components only four showed chemiluminescence, namely, bands 1, 2, 3 and 4 while band 5 produced no light at all The light versus time curves obtained from these four components were not similar to each other in these experiments or in experiments in which potassium ferricyanide was replaced by haemin It should be noted that the spectral distributions of the light in these experiments were also different, and the same was true for the ultraviolet absorption spectra of these components

The presence of four fluorescent, as well as chemiluminescent components, in what was considered to be a pure compound suggests another explanation for the chemiluminescence and fluorescence spectral distributions in different solvents reported[3] by White and Bussey Indeed the occurrence of the two emission peaks in their spectra might be caused by the presence of two distinct molecular species instead of two different kinds of excited state of the same molecule

The chemiluminescent components of the luminol mixture were distinguished on the chemiluminescencegram produced as follows A solution was prepared consisting of 10 ml of 10^{-2} molar luminol (the molecular weight of 5-amino-2,3-dihydro-1,4-phthalazinedione), 10 ml of 30 per cent sodium chloride, 10 ml of 1 normal sodium hydroxide, 5 ml 3 per cent hydrogen peroxide, 0 1 ml 10^{-3} molar manganese chloride, and 10 ml of water Carbon dioxide (400 ml /min) was bubbled through the solution (4 5 ml) which was in a tube placed in front of a photomultiplier connected to a recorder The temperature in these experiments is critical and should be carefully maintained between 8° and 14° C The recording obtained by this procedure was called a chemiluminescencegram and it is shown in Fig 1 ('Schuchardt' for analysis) Fig 2 was obtained from a luminol sample which we

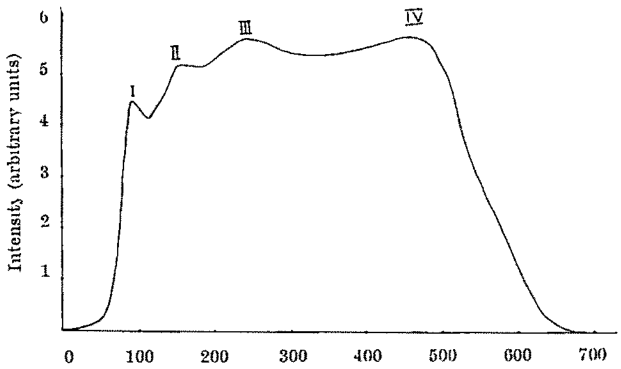

Fig 1 Chemiluminescencegram of luminol (commercial for analysis)

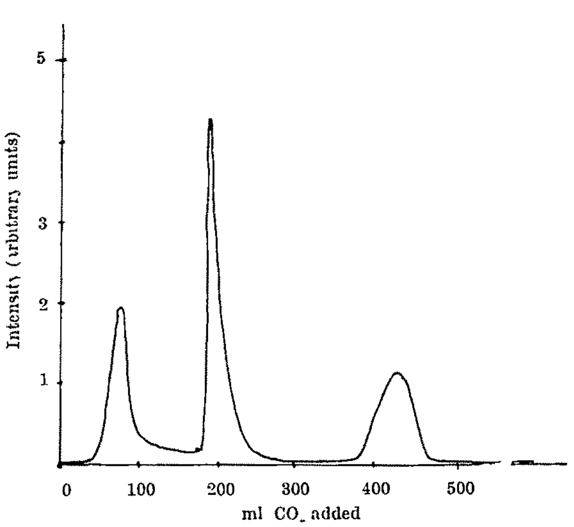

Fig 2 Chemiluminescencegram of luminol (sample prepared by ourselves)

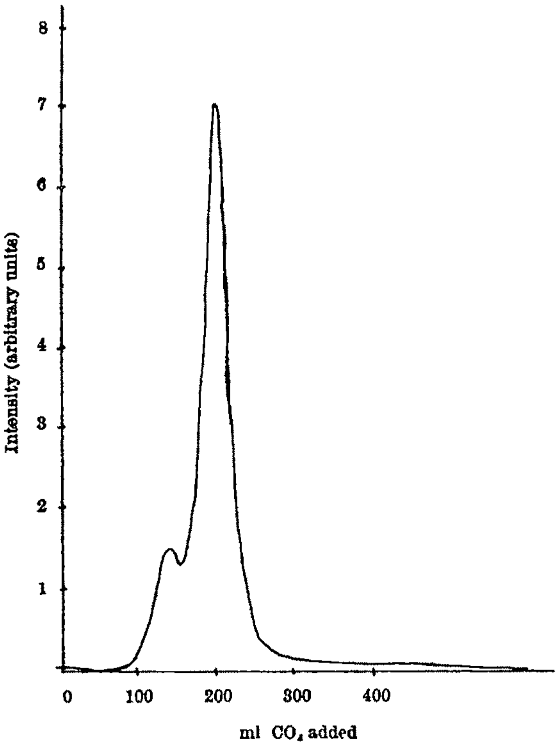

Fig. 3 Chemiluminescencegram of 3-nitrophthalhydrazide

prepared. The chromatographically separated components were then chemiluminescencegraphed and the peaks of the luminol chemiluminescencegram (Fig 1) were attributed No I to band 1, No. III to band 3, No. IV to band 2 and No II to band 4 of the chromatogram. A chemiluminescencegram of 3-nitrophthalhydrazide showed two peaks (Fig 3), indicating that both components of the mixture, established by chromatography, are chemiluminescent. Unsubstituted phthalhydrazide, however, showed the presence of several chemiluminescent components Finally, a similar method was used on a mixture of very pure rhodamine *B* and fluorescein and only two peaks were obtained.

This method, when carried out in strictly standardized conditions, not only offers a simple way of distinguishing the chemiluminescent components in a given mixture but also of identifying the known ones. Another characteristic of this process is that it offers a method of studying not only the light decay but also the light build-up, during chemiluminescence measurements Finally, it should be noted that the use of coloured reagents such as potassium ferricyanide and haemin is avoided, thus facilitating the study of the spectral distribution of chemiluminescence

The self-luminescence of luminol, which is a handicap in its use as a blood stain detector, has been overcome[4] by adding small amounts of 1-naphthol or 3-indazolinone-4-carboxylic acid to the luminol mixture used Adding 1-naphthol to a solution of luminol resulted in an almost complete disappearance of peak No I This indicates that the component corresponding to band 4 on the luminol chromatogram is responsible for the self-luminescence of luminol, and that luminol, free from this component, would be an adequate detector of blood stains without any addition being necessary, especially because the additives mentioned are known to cause an appreciable fall in the luminescence intensity

Preparative scale chromatography on a cellulose powder column resulted in a complete separation of band 1 but the other bands were only enriched. Fractional precipitation by the controlled neutralization of alkaline solutions of luminol seems more promising and we are working in this direction at present.

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[1] Redemann, C T, and Redemann, C E, *Org Synth Col*, 3, 69
[2] Huntress, E H, Stanley, L N, and Parker, A S, *J. Amer Chem. Soc*, 56, 241 (1964)
[3] White, E H, and Bussey, M M, *J. Amer Chem Soc.*, 86, 941 (1964)
[4] Uemura, K, *Sci Crime Detect*, 7, 6 (1954)

BIOCHEMISTRY

3-Methyl Histidine and Adult and Foetal Forms of Skeletal Muscle Myosin

RECENT independent investigations in this laboratory[1] and by Asatoor and Armstrong[2] have shown that 3-methyl histidine is a normal component of actin isolated from the skeletal muscle of a number of species In the case of rabbit actin, which has been most vigorously purified and intensively studied, approximately one histidine in nine is methylated and the 3-methyl histidine was found only in one of the peptide fractions isolated from tryptic digests of this protein[1].

On careful study of the acid hydrolysates of highly purified samples of the two other principal myofibrillar proteins, myosin and tropomyosin, using the Beckman system for amino-acid analysis of biological fluids, ninhydrin-positive material of identical elution rate to 3-methyl histidine could be detected only in myosin[1] Milligram quantities of this material were isolated from 15 g of myosin, and study of its properties and those of its dinitrophenyl and phenyl thiohydantoin derivatives confirmed that it was 3-methyl histidine This compound was present in myosin treated repeatedly with trichloroacetic acid to remove any non-covalently-bound peptide and amino-acid contaminants and in myosin purified by chromatography on DEAE cellulose[3] followed by fractionation with lithium chloride and ammonium sulphate The 3-methyl histidine was not a result of actin impurity in the myosin, for, although the former protein is present in very low amounts (probably < 1 per cent) in normal preparations, it could not be detected in the more highly purified myosin preparations when these were examined by the Ouchterlony procedure using antibody to rabbit actin raised in the fowl

Asatoor and Armstrong[2] also briefly mention the presence of small amounts of 3-methyl histidine in myosin but report that the amount decreased on purification. This has not been our experience 3-Methyl histidine was present only in the major components obtained on dissociation of the molecule by the alkali procedure of Gershman, Driezen and Stracher[4] and by succinylation[5] This evidence, and the fact that 3-methyl histidine is absent from light meromyosin fraction I but is present in heavy meromyosin and subfragment I (ref 1) in appreciably larger amounts relative to histidine than in myosin itself, strongly suggests that the residue is an integral part of the myosin molecule and not a result of traces of a protein impurity containing 3-methyl histidine

The amount of 3-methyl histidine estimated in rabbit skeletal muscle myosin varied from 1·44 to 1·88 moles per molecular weight of 500,000 Although there might be some variation in the 3-methyl histidine content of myosin from different rabbits, particularly in those of

Table 1 3-METHYL HISTIDINE CONTENT OF MYOSINS

Muscle	Animal	Type of preparation	Age of animal	No of preparations	No of determinations	3-Methyl histidine Residues/10^5 g	3-Methyl histidine Residues/mole
Longissimus dorsi	Rabbit	DEAE cellulose purified[3]	Adult	5	9	0 33 ± 0 033	1 63
		HAC*	Adult	3	3	0 35	
		Heavy component separated by succinylation[5]	Adult	1	2	0 34	
Heart	Rabbit	Heavy component separated by succinylation[5]	Adult	2	2	0 27	1 35
Mixed skeletal	Rabbit	DEAE cellulose purified[3]	28 Day foetus	7	11	Nil	Nil
Mixed skeletal	Rabbit	DEAE cellulose purified[3]	23 Day	1	2	0 23	1 15
		HAC*	23 Day	1	2	0 24	1 22
Breast	Fowl	DEAE cellulose purified[3]	Adult	1	2	0 32	1 59
		Heavy component separated by succinylation[5]	Adult	2	4	0 32	
Mixed thigh	Monkey	Standard preparation[6]	Adult	1	2	0 36	1 78

* HAC, heavy alkali component isolated as described by Gershman *et al* [4]

3-Methyl histidine values calculated on the basis of the estimated ratio of 3-methyl histidine to histidine assuming myosin contains 14 4 residues of histidine per 10^5 g and has a molecular weight of 500,000

different strains, it is considered that the observed scatter arose more from the difficulty in obtaining an accurate estimation of this amino-acid in the conditions employed rather than from animal variation. The fact that some variation occurred between duplicate assays on the same myosin sample confirmed this view. The high ratio of histidine to 3-methyl histidine in myosin made accurate evaluation of the true area of the 3-methyl histidine peak very difficult, particularly if the histidine peak trailed to any extent The average 3-methyl histidine content of myosin purified by DEAE cellulose chromatography, and using only estimates made from those chromatographic runs in which there was clear separation of the two peaks, was 1 63 residues per mole (Table 1) This value incorporates more data and is slightly lower than the previously reported figure[1] of 1·71 residues per molecule of 500,000

In contrast with the results with myosin isolated from skeletal muscle of adult rabbit, ninhydrin-positive material corresponding to 3-methyl histidine could not be detected in significant amounts in acid hydrolysates of myosin isolated from skeletal muscle of the 28 day old foetus (Table 1) In some preparations a very small amount of material which has not been identified was eluted just after the normal position for 3-methyl histidine This material was not consistently observed, and so it was not considered to be a significant component The results indicate that in the rabbit there is little if any significant methylation of the histidine of myosin before birth and provides further evidence of structural differences between the foetal and adult forms of myosin[6,7]. The situation regarding methylation of the histidines in myosin seems to be different from that with actin where the foetal and adult forms appear to be very similar if not identical in 3-methyl histidine content[1].

Investigations in this laboratory on the rabbit[8] and by Severin on the duck[9] show that the enzyme system responsible for the *N*-methylation of the imidazole ring, as judged by the rise in anserine content of the non-protein fraction of muscle, does not become appreciably active until some days after birth. The difference between stages of development of the muscle at which occur the methylation of the histidine residues of actin and myosin raises some interesting questions about the biosynthesis of these proteins, the methylation systems involved and the metabolism of the myofibril as a whole

In view of the hypothesis put forward elsewhere[10] that the myosin from red muscle represents a form intermediate between that associated with fast white muscle and that present in foetal muscle, it was interesting to compare the 3-methyl histidine content of myosin isolated from cardiac and skeletal (longissimus dorsi) muscles of the adult rabbit 3-Methyl histidine was clearly present in the cardiac protein, and although the data are too limited for a firm conclusion the average values suggest that the 3-methyl histidine content of cardiac myosin is lower than that of myosin isolated from white skeletal muscle

The significance of the methylation of the histidines in the two myofibrillar proteins is not yet apparent, although the process is specific for a particular residue in actin and is probably so in myosin, as far as can be judged from our preliminary studies It is interesting, however, that the other well-documented example of a protein containing a methylated amino-acid, in this case lysine, is flagellin[11] which also has a contractile function. Methylation of the histidine would be significant for the structure of the polypeptide chain because it confers a non-polar character on the imidazole ring and destroys its hydrogen bonding potential. Histidine has often been shown to have a role in the specific biological activity of proteins; and although it is striking that actin and myosin, with their unique properties for interaction, both contain 3-methyl histidine, neither the presence nor absence of this residue in myosin seems to be essential for its ability to combine with actin to form a viscous complex dissociated by ATP, or to act as an ATPase. Foetal myosin combines with adult actin apparently as effectively as adult myosin although no careful comparative studies have been carried out Foetal myosin does, however, hydrolyse ATP more slowly than the adult form of the enzyme[6] Possibly the differences in the reactivity of its lysine residues and thiol groups to succinic anhydride and 5-5′-dithio *bis* (2-nitrobenzoic acid), respectively, and the quantum yield of the ultraviolet fluorescence of the tryptophan residues[7] have more bearing on the differences in enzyme activity of the adult and foetal forms of myosin

Methylation of specific histidines of the myosin molecule is an interesting but unexplained aspect of the modification of the myosin molecule which occurs during the transition from the foetal to the adult form of a contractile protein and may reflect an aspect of specialization in muscle development It is also possible, as has been suggested elsewhere[6], that myosin exists in isoenzyme forms, some of which contain 3-methyl histidine, and the complement of which in a given myosin preparation will be characteristic of the type of muscle from which the protein is isolated

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[1] Johnson, P , Harris, C I , and Perry, S V , *Biochem J* , 103, 79P (1967), 105, 361 (1967)

[2] Asatoor, A M , and Armstrong, M D *Biochem Biophys Res Commun* 26, 168 (1967)

[3] Perry, S V , *Biochem J* , 74, 94 (1960)

[4] Gershman, L C , Driezen, P , and Stracher, A , *Proc US Nat Acad Sci* , 56, 966 (1966)

[5] Oppenheimer, H , Barany, K , Hamoir, G , and Fenton, J , *Arch Biochem Biophys* , 115, 233 (1966)

[6] Trayer, I P , and Perry, S V , *Biochem Z* , 345, 87 (1966)

[7] Trayer, I P , Perry, S V , and Teale, F W J , *Abstracts Seventh Intern Congr Biochem* , 943 (1967)

[8] Kendrick-Jones, J , thesis, Univ Birmingham (1965)

[9] Severin, S E , *Proc Plenary Sessions Sixth Intern Cong Biochem* , 45 (1964)

[10] Perry, S V , in *Muscle* (edit by Paul, W M , Daniel, E E , Kay, C M and Monckton, C), 29 (Pergamon Press, Oxford, 1964)

[11] Ambler, R P , and Rees, M W , *Nature*, 184 56 (1959)

Characterization and Rapid Labelling of Pituitary RNA

ALTHOUGH there is a great deal of evidence that hormones may directly influence the production of rapidly labelled RNA in many different tissues[1], some of it is equivocal[2]. Changes in rapidly labelled RNA of rat liver after the administration of hormones have been demonstrated by countercurrent distribution[3]. The interpretation of these changes is by no means clear, but cortisone increased the amount of rapidly labelled RNA in rat liver and decreased it in rabbit lymphocytes[4]. The pituitary is a source of many hormones, the secretion of which may be influenced by various factors, including the highly specific feed-back effects of the target gland hormones on trophic hormone synthesis. It may then be possible to examine the relationships between hormone action and rapidly labelled RNA production in this gland.

The RNA of young rat brain was easily labelled by direct injection of ^{3}H-orotic acid[5], but injection of radioactive orotic acid or uridine produced no labelling of the RNA from rat pituitaries. The RNA was successfully labelled by incubation of whole or halved glands in Eagle's medium[6] in the presence of ^{3}H-uridine.

Rats were killed by a blow on the head, were decapitated and the pituitaries were rapidly removed and the anterior lobes were placed in cold medium. Two glands are sufficient for each experiment, but between four and eight may be used. A sample of medium (1 ml.) (with streptomycin and penicillin) containing 500 μc. of ^{3}H-uridine, was used and the incubation at 37° C lasted 30 min for rapidly labelled RNA or up to 4 h for ribosomal RNA. The pituitaries were removed and broken down in 1 ml. of a cold mixture of 1 g of tri*iso*propylnaphthalene sulphonate, 6 g of 4-aminosalicylate, 1 g of NaCl, 100 ml. of water and 6 ml. of 2-butanol. The mixture was extracted with a mixture of phenol and cresol[7]. A second extraction was carried out in the presence of 0.5 molar NaCl and then the nucleic acids in the top phase were precipitated with two volumes of ethanol. Nucleic acids were dissolved in 0.1 molar sodium acetate and ribosomal and rapidly labelled RNA were precipitated with 4 molar NaCl. RNA was washed and purified as before and characterized by centrifugation in gradients of glycerol, sucrose, sulpholane with ethylurea or chloral[8].

The mean weight of the anterior pituitary gland from female hooded rats (4–6 months old) was 9.8 mg, whereas that from males of the same age was 7.0 mg. The content of RNA/mg of gland was the same in both sexes, 7.0–9.5 μg/mg and usually the final recovery measured by isotopic dilution of added rat liver RNA labelled with carbon-14 was about half this amount. The RNA of male pituitaries was more highly labelled in 30 min than that of females by ^{3}H-uridine and, using 500 μc./ml. of incubation medium, specific activities of 700–3,000 c.p.m./μg of RNA for males and 300–600 c.p.m./μg for females were obtained (32 per cent efficiency). Using 50 μc. of ^{3}H-uridine/ml. of incubation medium, the RNA from five male pituitaries had a mean activity of 3,700 counts/mg of pituitary while that from females had 505 counts/mg. When ^{3}H-guanosine (50 μc./ml. of incubation medium) was used for labelling, the RNA from male pituitaries had a mean of 5,130 counts/mg of pituitary, while the RNA from females had 2,120 counts/mg of pituitary.

Patterns of RNA from male and female pituitaries are shown in Fig. 1*a*–*d*.

The rates of sedimentation of the two ribosomal components are the same as those of ribosomal RNA from rat liver, judged by sedimentation of RNA in the presence of labelled (carbon-14) ribosomal RNA from rat liver and the radioactive pattern after incubating the gland with ^{14}C-uridine during 3.5 h followed by ^{3}H-uridine for 0.5 h, but even in these conditions a small proportion of carbon-14 label is found on the bottom of the gradient (Fig. 1*d*).

A large proportion of the rapidly labelled RNA is sedimented in the region between the 28*S* RNA and the base. This property is also characteristic of RNA from tumours[9], from young rat brain[5] and other tissues[10]. Still more radioactive RNA can be extracted from the base of the tube after the gradient solution has dripped out. This may be a separate fraction but accounts for a high proportion of the radioactive RNA. As postulated before[8], this material may result from the method of preparation using 4 molar NaCl to aggregate and precipitate ribosomal with rapidly labelled RNA. The nature of this rapidly labelled RNA with a high rate of sedimentation is at present being examined.

It is hoped to use this method to examine the effect of hormones and of target gland ablation on the RNA from the anterior pituitary and the hypothalamus.

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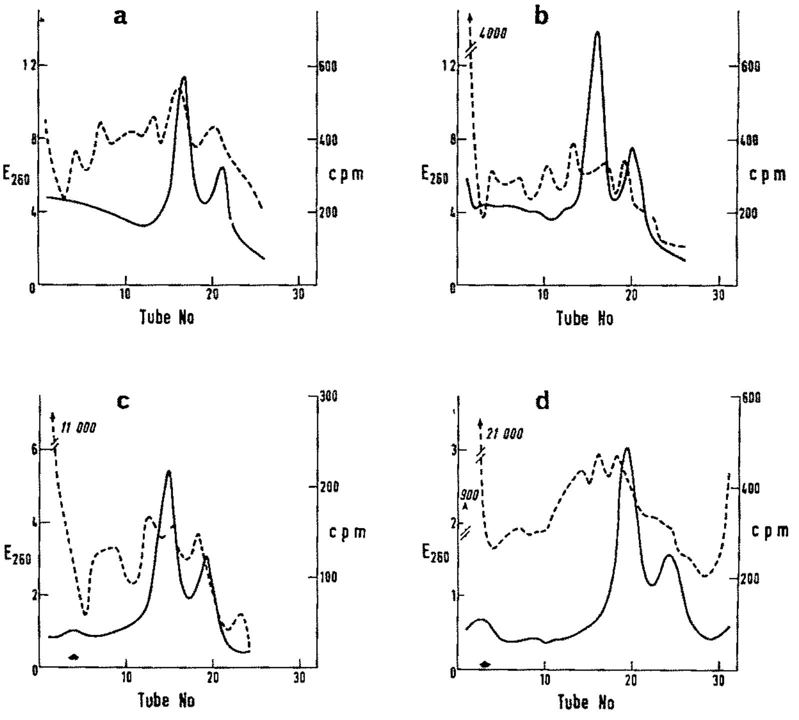

Fig. 1. Sedimentation profiles of RNA from the anterior pituitaries of male (*a* and *c*) and female (*b* and *d*) rats. RNA (*a*) from three glands and (*b*) from two glands in 0.1 molar sodium acetate buffer (*p*H 7.0) layered on a gradient of sulpholane (12.5–50 per cent) containing 20 per cent ethylurea, 0.1 molar NaCl, 0.01 molar *tris* (*p*H 9.0) and centrifuged at 40,000 r.p.m. for 5.5 h at 10° C in the 3 × 5 ml. rotor of MSE *TC*/50 centrifuge. RNA (*c*) from two and a half glands in 0.1 molar NaCl, 0.01 molar *tris* HCl, *p*H 7.4, layered on a gradient of glycerol (5–20 per cent) in 0.1 molar NaCl, 0.01 molar *tris* HCl, *p*H 7.4 and centrifuged at 50,000 r.p.m. for 100 min in the *SW* 50 rotor of the Spinco Model *L*. RNA (*d*) from one gland in 0.1 molar NaCl, 0.01 molar *tris* HCl (*p*H 7.4), layered on a gradient of glycerol (5–30 per cent) in 0.1 molar NaCl, 0.01 molar *tris* HCl, *p*H 7.4 and centrifuged at 40,000 r.p.m. for 190 min at 3° C in the 3 × 5 ml. rotor of the MSE TC/50 centrifuge. Incubation was for 30 min with 500 μc./ml. of ^{3}H-uridine for 3.5 h, (*d*) with 50 μc./ml. of ^{14}C-uridine. ———, Extinction at 260 mμ; - - - - - radioactivity of tritium, of carbon-14. The thick arrow indicates the penetration of the needle, the number at the side of the radioactivity patterns indicates the radioactivity extracted from the base of the tube plus that in the liquid which remained when the gradient had been fractionated (figure not available for *a*).

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[1] Karlson, P , *Mechanism of Hormone Action*, Nato Advanced Study Institute (Acad Press, New York, 1965)

[2] Korner, A , *Progress in Biophysics and Mol Biol* ,17, 61 (1967)

[3] Kidson, C , and Kirby, K S *Nature*,**203**, 599 (1964)

[4] Kidson, C , *Biochem Biophys Res Commun* ,**21**, 283 (1965)

[5] Nievel, J G , and Kirby, K S , *Biochem J* ,**101**, 7C (1966)

[6] Eagle H , *Science*,**130**, 432 (1959)

[7] Parish, J H , and Kirby, K S , *Biochim Biophys Acta*,**129**, 554 (1966)

[8] Hastings, J R B , Parish, J H , Kirby, K S , and Cook, E A , *Biochim Biophys Acta* (in the press)

[9] Wilkinson, B R , and Kirby, K S , *Biochem J* , **99**, 780 (1966)

[10] Scherrer, K , Marcaud, L , Zajdela S , London, I N , and Gros, S , *Proc US Nat Acad Sci* , **56**, 1571 (1966)

Radiation Stimulated Incorporation of Thymidine into the DNA of Human Lymphocytes

TRITIATED thymidine is incorporated into both strands of DNA in HeLa cells which have been exposed to ultraviolet and ionizing radiation[1,2] This stimulated incorporation can occur in concentrations of hydroxyurea[3] which inhibit normal DNA synthesis

We have found that human blood lymphocytes are also stimulated by radiation to incorporate thymidine into their DNA This process which occurs in about 90 per cent of irradiated lymphocytes is not inhibited by hydroxyurea and occurs in the absence of phytohaemagglutinin or other mitogens It can be readily demonstrated after exposure to as little as 25 ergs/mm^2 of ultraviolet light, a dose which inactivates less than 30 per cent of the lymphocytes

The whole blood of young healthy donors was passed through a column packed with nylon fibres[4] and one drop was collected every 8 sec Plasma gel in the ratio of 1 3 was added to the filtered whole blood and the mixture allowed to settle for about 20 min Of the white blood cells present in the upper layer, 98–99 per cent were lymphocytes The cells were washed once in phosphate buffered saline, *p*H 7 2 (PBS), and immediately irradiated in open Petri dishes by a low pressure ultraviolet lamp delivering the bulk of its radiation at 2537 Å with an intensity of approximately 5 ergs/mm^2/sec The thickness of the cell suspension was about 1 mm and the concentration of cells for all irradiations was adjusted to 10^6/ml

For autoradiography the cell suspensions, after irradiation in PBS, were centrifuged for 5 min at 1,200 r p m (200*g*) and the pellet was resuspended in NCTC 199 medium supplemented with 20 per cent calf serum (Microbiological Associates, Maryland) and containing 10 μc /ml (6 7 c /mmole) of tritiated thymidine (New England Nuclear) The cells were kept in suspension during a 2 h incubation at 37° C in an atmosphere of 95 per cent air and 5 per cent carbon dioxide and then washed once in PBS containing 100 μg/ml of thymidine to block any further uptake of isotope After a further wash the cells were hypotonically treated in 1 per cent sodium citrate solution for 20 min Carnoy fixer was added to the cell suspension and the mixture was centrifuged The pellet was resuspended in fixer and slides were prepared Kodak *NTB*2 nuclear track emulsion was used for the autoradiographs Duplicate slides were treated with 1 mg/ml of DNase for 1 h at room temperature, *p*H 7 2 After 3 weeks at 4° C the slides were developed, stained with haematoxylin, mounted in 'Permount' and examined

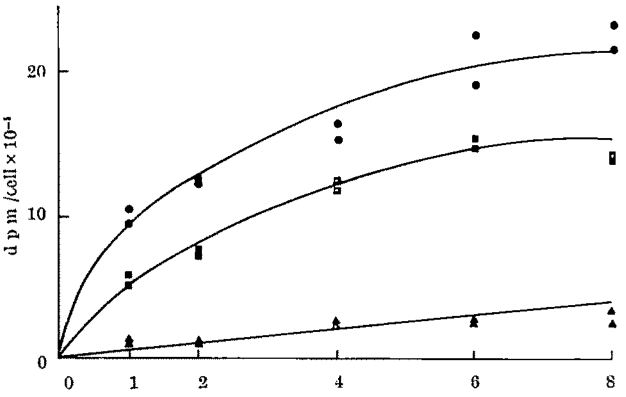

Fig 1 The kinetics of radiation-stimulated incorporation of thymidine into human lymphocytes The lymphocytes were exposed to ultraviolet radiation immediately after separation from whole blood, then incubated at 37° C in the presence of 10^{-3} molar hydroxyurea and 5 μc /ml of tritiated thymidine but without exposure to phytohaemagglutinin or other mitogens ●, 500 ergs/mm^2, ■, 25 ergs/mm^2, ▲, 0 ergs/mm^2

When liquid scintillation counting was used, 10^{-3} molar hydroxyurea was included in the incubation medium, to suppress the normal DNA synthesis occurring in a very small fraction of cells, in order to follow the stimulated incorporation Samples were removed from the culture bottles at various times, the number of cells per ml was determined and the samples were transferred to Millipore' filters Following washes with PBS, 5 per cent trichloroacetic acid and 95 per cent ethyl alcohol, the filters were completely dried at room temperature and placed cell side up in counting vials A sample of 10 ml of 'Premix *M*' (Packard, Illinois) in toluene was added to the vials and the activity counted in a Packard 'Tri-Carb' counter using a channel ratio method to determine efficiencies

Data for the survival were obtained by incubating the irradiated cells as before but using NCTC 109 medium Samples were removed from the cultures on the fifth day after irradiation and centrifuged The pellets were resuspended in PBS, the vital stain nigrosine (0 5 per cent, K and K Labs, Hollywood, California) added in a ratio of 1 4 and viable cells were counted Viable and dead cells were distinguished by their appearance under the phase contrast microscope viable cells showed clear cytoplasm with a soft-green nucleus, whereas dead cells showed black cytoplasm with a dark homogeneous nucleus

Table 1 shows that some 90 per cent of the lymphocytes incorporate tritiated thymidine into their DNA following doses of ultraviolet light of 25–400 ergs/mm^2 Less than 0 2 per cent of the cells incorporated thymidine in the absence of radiation This small fraction in normal DNA synthesis incorporates more than ten times as much thymidine as the stimulated cells in the 2 h incubation

Table 1 EFFECT OF ULTRAVIOLET LIGHT ON TRITIATED THYMIDINE INCORPORATION INTO HUMAN LYMPHOCYTES AND THEIR SURVIVAL

Ultraviolet dose (ergs/mm^2)	Percentage of labelled cells*	Fraction surviving after 5 days †
0	0 2	1 0‡
25	91	0 77
50	92	0 41
100	93	0 094
200	89	0 059
400	90	0 030

* Based on 500 cells at each dose The average number of grains per cell resulting from background was less than five, whereas the number in labelled cells varied between eleven and fifteen

† Each value is based on four haemocytometer readings

‡ Adjusted values based on a 60 per cent survival for the control culture

The incubation period was 2 h at a concentration of tritiated thymidine of 10 μc /ml (6 7 c /mmole) period and thus can be readily distinguished in autoradiographs from the radiation-stimulated cells.

The cells that had been stimulated by the radiation to incorporate thymidine could respond to phytohaemagglutinin when it was added to cultures of cells in fresh incubation medium by transforming and entering cell division.

The stimulated incorporation of thymidine after doses of ultraviolet radiation as determined by liquid scintillation counting is shown in Fig 1. Hydroxyurea, although it does not completely suppress the normal synthesis of DNA, as shown by the 0 ergs/mm^2 curve, has enabled us to follow the stimulated incorporation and also to correct the data obtained for the irradiated cells The incorporation of tritiated thymidine resulting from radiation alone occurs with no apparent delay following irradiation and is essentially complete after 6 h

Doses of ionizing radiation of between 1 and 16 krads also resulted in clearly demonstrable stimulated incorporation of thymidine The number of thymidine molecules incorporated per cell per rad following 1 krad in a 2 h incubation period is three—a number similar to the calculated number of single strand breaks per rad in the DNA of human lymphocytes

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[1] Rasmussen, R E , and Painter, R B , *J Cell Biol* ,29, 11 (1966)
[2] Cleaver, J E , *Abstract, Fifteenth Ann Meeting Radiat Res. Soc* (1967)
[3] Young, C W , *Cancer Res* ,27, 535 (1967)
[4] Walford, R L , *Ann NY Acad Sci* ,124, 563 (1965)

Peptide Inhibitors of the Renin–Angiotensin System

RENIN is said to split a leucyl–leucine bond in alpha 2 globulin substrate—angiotensinogen—to yield a decapeptide, angiotensin I. Angiotensin I is subsequently converted into an octapeptide, angiotensin II, by a converting enzyme contained in plasma Angiotensin II has a pressor activity We have studied the effects of synthetic peptides with a leucyl–leucine bond and their derivatives on the activity of renin in the formation of angiotensin The peptides were di-, tri-, tetra- and pentapeptides with leucyl–leucine bonds at the C-terminal end, in the middle position, at the N-terminal end or with one of the other structures as shown in Table 1

The experimental conditions were as follows Synthetic peptide (10 mg) was added to the reaction system containing 0 2 ml. of rabbit renin[1], 0 5 ml of plasma from a normal rabbit as substrate and 2·0 ml of 0 9 per cent sodium chloride solution The mixture was incubated at 37° C for 10 min and the reaction was then stopped by heating the mixture in a boiling water bath for 10 min Pressor activity of the supernatant was tested on the blood pressure of an anaesthetized, vagotomized rat treated with pentolinium An inhibitory effect of synthetic peptide was expressed as a ratio of the pressor activity of experimental and control samples

Table 1 SYNTHETIC PEPTIDES WITH LEU-LEU BOND EXAMINED

With Leu-Leu bond at C-terminus

No	
2	z Leu-Leu
3	Leu-Leu
4	Leu-Leu oMe
7	CHO Leu-Leu
51	CHO Leu-D-Leu
52	CHO D-Leu-Leu
53	CHO D-Leu-D-Leu
59	Acetyl Leu-Leu
60	Benzoyl Leu-Leu
101	z Leu-Leu NHNH$_2$
24	His-Leu-Leu
25	His-Leu-Leu oMe
141	z Pro-Phe-His-Leu-Leu

With Leu-Leu bond in middle position

No	
123	z His-Leu-Leu-Val
137	z Phe-His-Leu-Leu-Val

Without Leu-Leu bond

No	
15	Leu-Val
54	Acetyl Leu-Val
155	z Leu-Val
21	His-Leu
21	His-Leu
154	Leu-Val-Tyr oMe
244	Ileu-Leu-Val-Phe oMe
245	Val-Leu-Val-Phe oMe
265	Val-Leu-Val-Tyr oMe

With Leu-Leu bond at N-terminus

No	
113	z Leu-Leu-Val
117	Leu-Leu-Val oMe
178	Tos Leu-Leu-Val
184	CHO Leu-Leu-Val
186	Benzoyl Leu-Leu-Val
187	$CH_3(CH_2)_{10}$ CO Leu-Leu-Val
188	C_2H_5OCO Leu-Leu-Val
215	z Leu-Leu-Leu
217	z Leu-Leu-Gly
219	z Leu-Leu-His
220	z Leu-Leu-D-Val
*105	Leu-Leu-Val-Tyr oMe
221	Leu-Leu-Val-Gly oMe
*223	Leu-Leu-Val-Phe oMe
224	z Leu-Leu-Val-Gly
225	z Leu-Leu-Val-Phe
226	z Leu-Leu-Val-His
227	Leu-Leu-Val-His oMe
*242	Leu-Leu-Val-Phe oEt
243	D-Leu-Leu-Val-Phe oMe
261	D-Leu-Leu-Val-Tyr oMe
*263	Leu-Leu-Val-Tyr oEt

z, Benzyloxycarbonyl, D,D-form, oMe, methyl ester, oEt, ethyl ester
* Effective as inhibitor

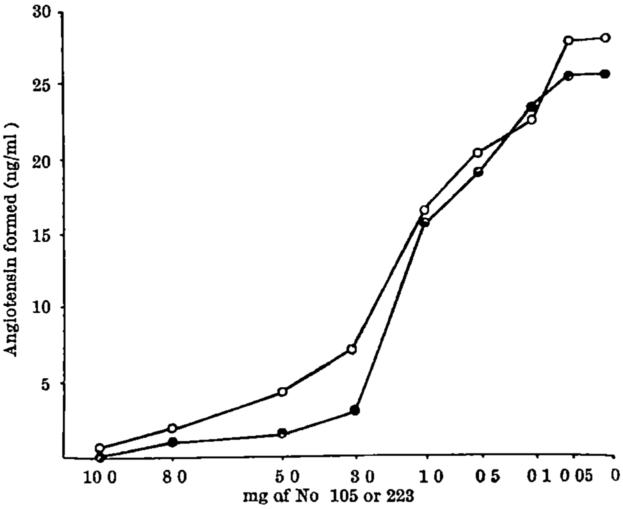

Fig 1 *In vitro* inhibition of the formation of angiotensin in the presence of varying amounts of synthetic peptides ○, No 105, Leu-Leu-Val-Tyr oMe, ●, No 223, Leu-Leu-Val-Phe oMe Rabbit plasma 0 3 ml., rabbit renin 0 05 ml , peptide in saline 2 75 ml ⟶Incubation *p*H 6 8, 37° C, 5 min

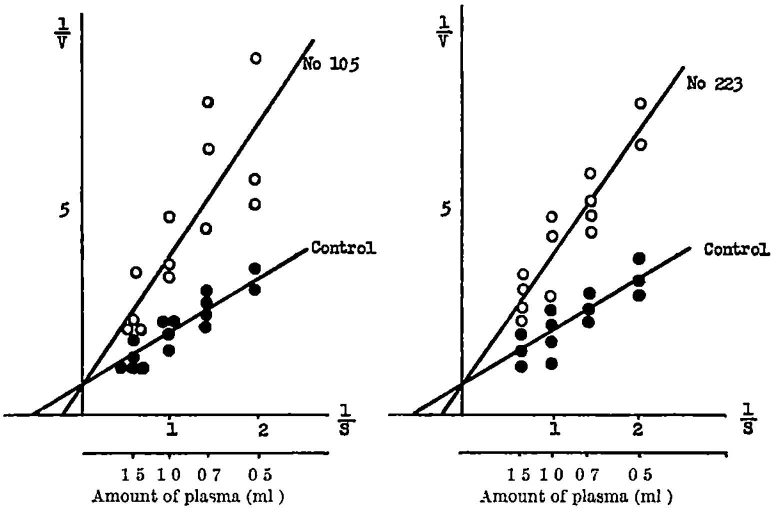

Fig 2 Inhibitory effects of synthetic peptides (No 105 (left) and 223 (right)) Lineweaver–Burk-type plots of the relationship between the reciprocals of substrate concentration and reaction velocity Control rabbit plasma 0 5, 0 7, 1 0, 1 5 ml , rabbit renin 0 05 ml and saline Experiment (with compound) rabbit plasma 0 5, 0 7, 1 0, 1 5 ml , rabbit renin 0 05 ml , compound (1 mg) in saline Total volume 3 1 ml , *p*H 6 8, 37° C, 5 min

Only the compounds Nos 105, 223, 263 and 242 among the synthetic peptides examined inhibited the formation of angiotensin in this *in vitro* system The ratios were 0 4–0 55 (average, 0 5) for No 105, 0–0 4 (average, 0 2) for No 223, 0 1–0 7 (average, 0 5) for No 263, and 0 4–0 7 (average, 0 5) for No 242 These four peptides were methyl or ethyl esters of Leu-Leu-Val-Tyr and Leu-Leu-Val-Phe, and they did not depress the blood pressure of the rat None of the other peptides inhibited the formation of angiotensin

Fig 1 shows the inhibitory effects of the varying amounts of synthetic peptides Nos 105 and 223 on the formation of angiotensin The pressor activity was expressed as angiotensin produced (ng) compared with synthetic α-angiotensin II amide (Ciba) About 25–30 ng of angiotensin was produced in 1 ml reaction mixture The formation of angiotensin decreased as the concentration of synthetic peptide was increased Fig 2 shows that the inhibitory effects of both Nos 105 and 223 were competitive according to the Lineweaver–Burk-type plot

It is not clear whether the peptides have an inhibitory effect on the renin alone or on both the renin and the converting enzyme The decrease of pressor response, however, indicates the inhibition of the enzyme hydrolysis of angiotensinogen, that is, it indicates the inhibition of renin activity, because the bioassay used demonstrates not only the amount of angiotensin II, but also the amount of angiotensin I formed in the reaction system So it is clear that the peptides might inhibit at least renin activity Furthermore, no decrease of the pressor response of synthetic α-angiotensin II amide in the rat was observed by adding the peptides, Nos 105 and 223, to the angiotensin injection This phenomenon suggests the peptides do not inhibit the rise of blood pressure caused by angiotensin

To summarize, methyl or ethyl esters of synthetic tetrapeptides, Leu-Leu-Val-Tyr and Leu-Leu-Val-Phe, acted as competitive inhibitors in the renin plasma reaction system used The chemical structures needed for the antagonistic activity are as follows (1) a leucyl–leucine bond at the N-terminal end in the tetrapeptide, (2) a leucyl–leucine bond with L-leucine (there was no inhibitory effect of the peptide with a leucyl–leucine bond with D-form instead of L), and (3) the presence of tyrosine or phenylalanine at the C-terminal end The last condition suggests that the phenyl group in the C-terminal amino-acid might be essential

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[1] Haas, E, Lamfrom, H, and Goldblatt, H, *Arch Biochem Biophys*, 48, 256 (1954)

Binding of Calcium and Strontium by Alginates

SODIUM alginates markedly inhibit the intestinal absorption of strontium in rats[1-4] and man[5,6] without an appreciable effect on calcium absorption so that they are of interest as possible therapeutic agents in cases of ingestion of strontium-90 Alginic acid is obtained from certain seaweeds and is a polymer of D-mannuronic acid and L-guluronic acid[7] joined by 1,4 linkages[8] It has been shown that guluronic-rich fractions of alginate are the most effective in reducing strontium absorption in rats *in vivo* and *in vitro*[4,9]

A measure of the calcium–strontium ion exchange reactions with various alginates has been obtained by calculating the selectivity coefficients for these ions[10]. In this study the formation constants of the polyuronic acids were found by the ion exchange resin procedure developed by Schubert[11,12], which assumes that the free metal ions in solution are neither hydrolysed nor polymerized[13], which is highly probable in the case of strontium and calcium The formation constant of an organo-metallic complex is given by

$$K_f = \frac{(K_d^0/K_d) - 1}{A^n}$$

where K_d^0 and K_d are the distribution coefficients of the metal between resin and solution in the absence and presence of the ligand respectively, A is the molar concentration of ligand and n is the number of moles of the ligand relative to the metal in the associated molecule[12] The distribution coefficient is defined as follows

$$K_d = \frac{\text{per cent metal in resin}}{\text{per cent metal in solution}} \times \frac{\text{vol solution}}{\text{wt resin}}$$

The data obtained by this method can be represented in several ways dependent on the manner in which A is expressed[14] In these experiments, concentration (A) is expressed in equivalents of uronic acid assuming an equivalent weight for sodium uronide of 198

The sodium uronides (0 01–0 001 equiv /l) were equilibrated with known weights of cation exchange resin saturated with sodium ('Dowex 50') in a solution containing 0 16 molar sodium ions and 10^{-7} molar calcium-45 or strontium-85 at 25° C and *p*H 7 2 Distribution coefficients were calculated from the radioactivity remaining in the supernatant The alginate derivatives were similar to those used previously for the study of inhibition of strontium uptake by an *in vitro* intestinal slice technique[4] and two to five different concentrations of uronide were used in each experiment L-Guluronic acid monomer was synthesized by a modification of the method of Fischer and Dorfel[7] and separated from other reduction products by chromatography on a column of 'Dowex-1'[15].

Table 1 FORMATION CONSTANTS OF THE CALCIUM AND STRONTIUM COMPLEXES OF A SERIES OF ALGINATE DERIVATIVES OF VARYING GULURONIC ACID CONTENTS AND DEGREES OF POLYMERIZATION (D P)

Percentage guluronic acid	D P	K_f Ca	K_f Sr	$\frac{K_f \text{ Sr}}{K_f \text{ Ca}}$
96	13	104	449	4 3
93	8	124	443	3 6
86	226	109	429	3 9
72	75	110	353	3 2
61	57	119	317	2 7
41	440	143	208	1 5
30	74	80	163	2 0

The results are presented in Table 1 together with guluronic acid content and number average degree of polymerization, determined as previously described[16] Monomeric L-guluronic acid has no detectable binding capacity for calcium or strontium ions in the experimental conditions By plotting log $(K_d^0/K_d - 1)$ against log A it was found that calcium and strontium ions formed a 1 1 complex with all the alginate fractions studied in the concentration ranges employed. The formation constants for strontium alginate are one and a half to four times greater than those for calcium alginate in these conditions Inhibition of intestinal cation transport by alginate should therefore have a greater effect on strontium than calcium absorption This series of formation constants seems to parallel the *in vitro* inhibition of strontium uptake by rat duodenal slices as shown by a similar series of alginate derivatives[4]

There is a good correlation between guluronic acid content and strontium–alginate formation constants which supports recent data[10] The calcium alginate formation constants do not appear to be related to guluronic acid content except that there is a marked increase from 30 per cent to 40 per cent guluronic acid The relative binding of strontium compared with calcium tends to increase with guluronic acid content As guluronic acid monomer has little or no specific cation binding capacity, this property of uronides is solely one of the polymer. The smallest unit exhibiting this binding is not known but must be between the degrees of polymerization of one and eight There is no evidence of the formation constants of the calcium and strontium polyuronide complexes depending on the degree of polymerization in this alginate series

No marked inhibitory effect of alginate on the intestinal absorption of other cations has been described Such investigations of the stability of an alginate–metal complex are useful in predicting the ligand's effectiveness in reducing the absorption of a given metal ion.

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[1] Paul, T M, Waldron-Edward, D, and Skoryna, S C, *Canad Med Assoc J*, **91**, 553 (1964)

[2] Skoryna, S C, Paul, T M, and Waldron-Edward, D, *Canad Med Assoc J* **93**, 404 (1965)

[3] Moore, W, and Elder, R L, *Nature*, **206**, 841 (1965)

[4] Patrick, G Carr, T E F, and Humphreys, E R, *Intern J Radiat Biol*, **12**, 427 (1967)

[5] Hesp, R, and Ramsbottom, B, *Nature*, **208**, 1341 (1965)

[6] Harrison, J, McNeill, K G, and Janiga, A, *Canad Med Assoc J*, **95**, 532 (1966)

[7] Fischer, F G, and Dorfel, H, *Z Physiol Chem*, **302**, 186 (1955)

[8] Hirst, E, and Rees, D A, *J Chem Soc*, 1182 (1965)

[9] Harrison, G E, Humphreys, E R, Sutton, A, and Shepherd, H, *Science*, **152**, 655 (1966)

[10] Haug, A, and Smidsrød, O, *Nature*, **215**, 757 (1967)

[11] Schubert J, *J Phys Chem*, **56**, 113 (1952)

[12] Schubert, J, in *Methods of Biochemical Analysis* (edit by Glick, D) **3**, 247 (1956)

[13] Feldman, I, Toribara, T Y, Havill, J R, and Neuman, W F, *J Amer. Chem Soc*, **77**, 878 (1955)

[14] Gosselin, R E, and Coghlan, E R, *Arch Biochem Biophys*, **45**, 301 (1953)

[15] Haug, A, *Norwegian Institute of Seaweed Research*, Report No 30, 26 (1964)

[16] Humphreys, E R, *Carbohydrate Res*, **4**, 507 (1967)

Influence of *p*H, Sodium and Calcium Ions on the d.c. Resistance of Black Egg Lecithin–Cholesterol Films

CONFORMATIONAL changes in coherent arrays of amphiphiles, such as phospholipids, are believed to be important in the function of biological membranes[1] Danielli and Davson[2] suggested that the bimolecular lipid leaflet comprised the basic structural unit in many natural membranes, but only recently has this fundamental structure become available for direct experimental investigation[3] These so-called black lipid films have d c resistances of 10^6–10^8 Ω cm^2 (refs. 4 and 5), but it is known that with certain preparations the resistance characteristics are markedly affected by the composition of the aqueous environment[5–7] This communication outlines some preliminary observations on the effects of *p*H, in the presence of sodium and calcium ions, on the d c resistance of egg lecithin–cholesterol films

The black membranes were prepared by smearing a solution of 10 mg of chromatographically pure egg lecithin and 10 mg of recrystallized cholesterol in 1 0 ml of *n*-decane across a 1 75 mm diameter hole bored in the wall of a 'Delrin' (Dupont de Nemours) vessel immersed in the appropriate aqueous phase Membrane resistances were determined by applying potentials up to 100 mV across silver–silver chloride electrodes situated in the inner and outer compartments and monitoring the current by a sensitive ammeter

In the first series of experiments, the aqueous phase comprised 0 1 normal sodium chloride to which various amounts of hydrochloric acid or sodium hydroxide were added to adjust the *p*H to values varying from 1 to 11 The exact value was measured before and after the determination of conductance using a standard *p*H meter Comparison experiments using solutions the *p*H of which was controlled by *tris* and phosphate buffers gave identical results In the second series of measurements, sodium chloride was replaced by 0 1 normal calcium chloride In a third series of experiments, the effect of varying concentrations of calcium chloride on membranes in 0 1 normal sodium chloride and known *p*H was investigated The rate of growth of black film varied with *p*H and the nature of the ion At both high and low *p*H in sodium chloride, film formation was rapid, but at *p*Hs greater than 9 in calcium chloride thinning was very slow and the film became rather rigid

In all cases d c resistance was found to be ohmic, at least up to applied potentials of 60 mV At higher potentials the current–voltage curve became convex towards the voltage axis, with the departure from linearity occurring at lower voltages at the extremes of *p*H The dielectric breakdown voltage was reduced to about 70 mV at high and low *p*H compared with about 150 mV at near neutrality Spot checks at 40 mV showed that the conductance increased in proportion to the growth of black film It was concluded from this that the conductance occurred through the bimolecular region

Curve *a* of Fig 1 shows the resistance obtained with films prepared in 0 1 normal sodium chloride at *p*Hs of between 0 and 9 All points are means of at least five determinations on two different phospholipid–cholesterol solutions Attempts to form films in solutions of higher *p*H were unsuccessful Replacement of sodium chloride by calcium chloride of the same normality completely altered the form of the resistance–*p*H curve (Fig 1, curve *b*) and black films formed at *p*Hs as high as 11 Addition of calcium chloride in the range 10^{-4} to 1 normal to an aqueous phase of 0 1 normal sodium chloride at various predetermined *p*Hs showed the membrane resistance to increase with the concentration of calcium chloride (Fig 2) It is particularly interesting to note that at *p*H 10 and 11 the presence of only 0 001 normal calcium

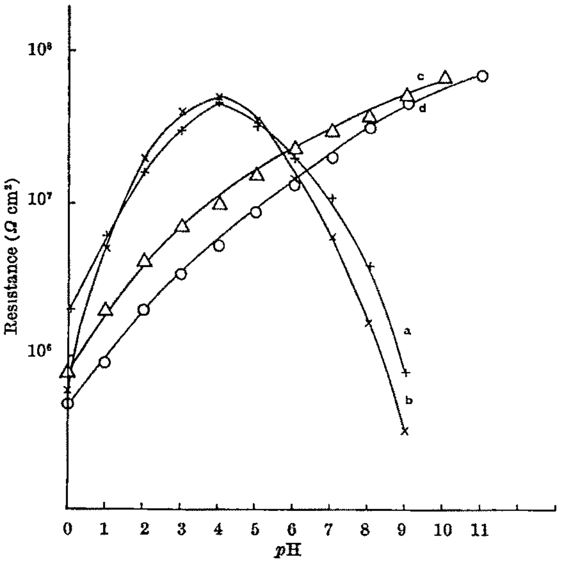

Fig 1 Resistance as a function of *p*H in 0 1 normal sodium chloride (×, +) and calcium chloride (△, ○) (Applied Science Egg Lecithin, General Biochemicals Egg Lecithin)

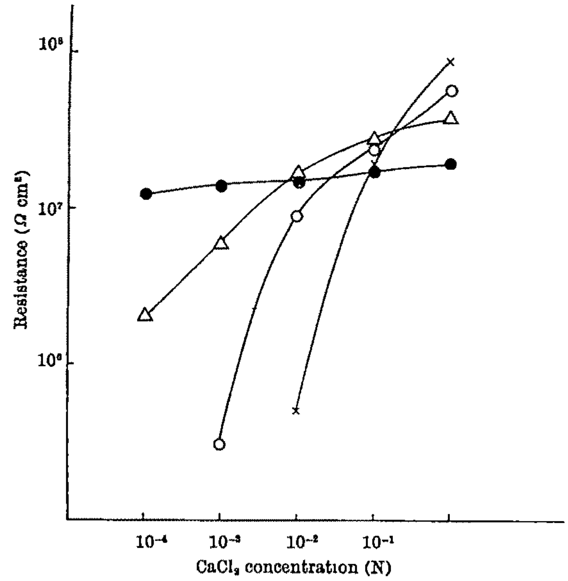

Fig 2 Resistance as a function of concentration of calcium chloride in 0 1 normal sodium chloride at various *p*Hs (Applied Science Egg Lecithin) ●, *p*H 6–4, △, *p*H 9, ○, *p*H 10, ×, *p*H 11

chloride was sufficient to enable films to be prepared whereas in the absence of calcium chloride complete black films could not be formed.

These results show that there is a striking difference in the resistance characteristics of lecithin–cholesterol films formed in the presence of sodium and calcium ions, and that when only sodium is present the resistance–*p*H curve passes through a maximum It is still only possible to speculate on the factors responsible for these observations Recent monolayer studies with egg lecithin have shown that exchange of calcium ions for sodium ions in the aqueous subphase produces considerable changes in both surface potential and pressure–area curves[8] These changes are attributed to the ability of calcium ions but not sodium ions to form bridge linkages between neighbouring phosphatidyl choline head groups A similar mechanism probably plays some part in the resistance changes observed with bilayers Ionization of the zwitterionic phosphatidyl choline head groups at the extremes of *p*H would tend to open the lattice and increase its permeability to ions, but at high *p*H, in the presence of calcium, this expansion is prevented by chelation

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[1] Kavanau, J L , *Structure and Function in Biological Membranes*, 1 and 2 (Holden-Day, Inc , 1965)

[2] Danielli, J F , and Davson, H A , *J Cell Comp Physiol* , 5, 495 (1935)

[3] Mueller, P , Rudin, D O , Tien, H T , and Wescott, W C , in *Recent Progress in Surface Science* (edit by Danielli, J F , Parkhurst, K G A , and Riddeford, A C), 1, 379 (Academic Press, 1964)

[4] Hanai, T , Haydon, D A , and Taylor, J , *J Theoret Biol* , 9, 433 (1965)

[5] Miyamoto, V K , and Thompson, T E , *J Colloid and Interface Sci* (in the press)

[6] Lauger, P , Lesslauer, W , Marti, E , and Richter, J , *Biochim Biophys Acta*, 135, 20 (1967)

[7] Tien, H T , and Diana, A L , *J Colloid and Interface Sci* , 24, 287 (1967)

[8] Shah, D O , and Schulman, J H , *J Lipid Res* , 8, 215, 227 (1967)

MICROBIOLOGY

Transducing Phage for *Pseudomonas putida*

The extensive biochemical knowledge of various *Pseudomonas* species[1] would be considerably enhanced if genetic analysis was possible in these organisms Conjugation and transduction have both been demonstrated for *P. aeruginosa*[2,3], but, despite apparently many other attempts, only recently has a transducing phage been characterized for *P putida*[4]

This communication describes the isolation of a transducing phage for *P putida* which seems to be different from the phage *pf*16 described by Gunsalus and his colleagues Ten strains of *P. putida* were provided by Professor A Rorsch (Rijswijk, The Netherlands) These were isolated from soil and are all characterized by the ability to use atropine as a sole carbon source The media and cultural techniques used in this work are the same as those described in publications from this laboratory on *P aeruginosa* phages[5] All cultures were grown at 30° C.

Preliminary work (unpublished results of B W Holloway and J Niblack) had shown that, in a limited range of strains, lysogeny could not be detected in *P putida*, and so we tried to isolate phages for *P putida* from sewage by the standard techniques One such phage was isolated; this has been called *PP*1 and has been characterized in the following ways

As isolated, *PP*1 gives indistinct hazy plaques when plated on *P putida PMBL-4Bl* A clear plaque variant (*PP*1*C*) occurs at a frequency of about 1/1,000 turbid plaques We have not been able to propagate the turbid plaque form to titres greater than 10^6/ml and all work has been carried out with the clear plaque variant. Plaques on *PMBL-4Bl* are 2–3 mm in diameter with a distinct edge and a slightly hazy interior because of the growth within each plaque of individual small bacterial colonies Such colonies have been isolated and are resistant to *PP*1*C* but are not lysogenic Phage adsorption is usually 80–90 per cent with no apparent dependence on the growth phase of the bacterium The burst size is about 90 with a latent period of 25–30 min The phage is comparatively resistant to ultraviolet irradiation, showing linear inactivation, with a 2 log reduction of infectious particles at a dose of 3,400 ergs/mm^2

Propagation of the phage has been best achieved by the layer agar technique For reasons which are not yet clear, the highest titres of phage achieved by this method have only been about 2×10^9/ ml and are usually lower than this In the preparation of transducing phage, the input of phage to the layers is critical and best transducing titres have been obtained where the plaques are just confluent after overnight incubation The phage has a limited host range, strain *PMBL-4Bl* being the only sensitive strain among fourteen strains of *P putida* so far tested In addition, *PP*1 did not plaque on any of eleven strains of *P fluorescens* tested Phage *pf*16[4] does not form plaques on any of these strains of *P putida*

*PP*1*C* is a general transducing phage This was demonstrated as follows an overnight broth culture of a recipient auxotroph was inoculated into fresh broth and grown until it was in exponential phase It was then centrifuged, resuspended in calcium (0 01 molar) saline and infected at a multiplicity less than 0 1 with phage grown on the prototrophic parent strain After 20 min adsorption at 30° C, the culture was centrifuged, resuspended in saline, and 0 2 ml aliquots were plated on to minimal medium

In these conditions, transduction occurs at a frequency of about 10^{-6}/plaque forming particle and 10^{-7}/recipient bacterium The low multiplicity of infection is essential for reproducible and workable levels of transduction for at higher multiplicities of infection the lytic action of *PP*1 evidently interferes with the recovery of transductants Transduction has been shown for a variety of auxotropic markers including the requirements for tryptophan, isoleucine plus valine, leucine and methionine Transduction does not occur when the recipient is made phage resistant (presumably a non-adsorbing mutant), or if the phage is propagated on the same auxotrophic strain used as the recipient The transduced colonies are still sensitive to the phage Transduction frequency increases by a factor of 3–5 when the phage is irradiated with ultraviolet light (2,500 ergs/mm^2) before adsorption

Phages active on *P putida* are much rarer than those plating on *P aeruginosa*, either as isolates from sewage or derived from lysogenic strains Transducing phages are usually not virulent and it is likely that phages such as *PP*1 or *pf*16 are not virulent or intemperate but are non-lysogenizing forms of temperate phages

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[1] Stanier, R Y , Pallerom, N J , and Doudoroff, M , *J Gen Microbiol* 43, 159.
[2] Holloway, B W , and Fargie, B , *J Bact* , 80, 362 (1960)
[3] Fargie, B , and Holloway, B W , *Genet Res* , 6, 284 (1965)
[4] Gunsalus, I C , Chakraborty, A M , Gunsalus, C F , and Crawford, I P , *Proc US Nat Acad Sci* (in the press)
[5] Holloway, B W , *Virology*, 25, 634 (1965)

IMMUNOLOGY

Neonatal Splenectomy : Absence of Runting in Mice

Kalpaktsoglou, Yunis and Good[1] recently reported that early splenectomy of mice can cause a runting and wasting syndrome similar to that associated with neonatal thymectomy The importance of this finding to our current concepts of the development of immunological competence was stressed, as were the clinical implications for a variety of haematological diseases and for resistance to extracellular pathogens Experiments performed several years ago in our laboratory (unpublished work) and others[2] had failed to detect such effects, so we felt it essential to re-investigate this question to determine whether the newer findings were generally applicable

We used mice of strains *C57BL6/JAu*, $C_3H/HeAu$, C_3H/Bi and *BALB/CAu* as well as the F_1 hybrid *BALB/C* × *C57BL/6*, which were splenectomized within a few hours of birth Mice were cooled on ice blocks, an incision was made in the left lateral body wall, the spleen was removed with curved forceps and the wound was closed with a single stitch Mice were kept warm for a few minutes after splenectomy before returning them to their mothers Unoperated mice served as controls in each litter Operative mortality was negligible once the procedure had been mastered Some animals were lost in the first few days following operation, because of post-operative neglect and cannibalism by the mother (Table 1)

Table 1 EFFECT OF NEONATAL SPLENECTOMY ON GROWTH AND SURVIVAL IN MICE

Strain	No of litters	Treatment	No	Survival at 1 week	Survival at 8 weeks
BALB/CAu	3	Operated	10	10	10
		Unoperated	9	9	9
C57BL6/JAu	6	Operated	19	11	10
		Unoperated	14	12	11
$C_3H/HeAu$	2	Operated	7	6	6
		Unoperated	7	7	7
C_3H/Bi	3	Operated	14	10	10
		Unoperated	10	8	8
$C \times C57BL (F_1)$	4	Operated	18	15	14
		Unoperated	14	14	13
Total	18	Operated	68	52	50
		Unoperated	54	50	48

Table 2 BODY WEIGHTS OF EXPERIMENTAL ANIMALS AT AUTOPSY (8 WEEKS)

	Females* No	Females* Weight	Males No	Males Weight
Operated	19	19 9 g	15	23 6 g
Unoperated	17	19 8 g	21	23 3 g

* Excludes pregnant females

Animals were examined at regular intervals before autopsy at 8 weeks old The results are presented in Table 1 Death after the immediate post-operative period was found to be minimal (4 per cent) in both the operated and control groups Runting was not observed (Table 2), the mean body weights of control and unoperated animals did not differ significantly, and there were no signs of wasting disease

These results are in sharp contrast to those of Kalpaktsoglou *et al* [1], who reported a mortality of 67 4 per cent which occurred primarily between the third and fourth week of life following a runting with an onset between the twelfth and sixteenth day Why these differences between experiments should exist is not clear It is, of course, known that runting may vary with laboratory conditions, for example, neonatal thymectomy fails to cause runting in germ-free animals[3,4] On the other hand, studies with mutant mice indicate that congenital absence of the spleen does not diminish antibody titres obtained in response to immunization with *Pasteurella pneumotropica*[5], and animals without spleens can survive and breed readily[6,7] Because the experiments of Kalpaktsoglou *et al* [1] offer no sham-operated control animals, one may wonder whether the observed runting and mortality might be caused by a non-specific effect of the operation, performed, after all, at a time when the immune system of the animal is not yet well established. Whatever the cause for the discrepancy, it is clear that it would be premature to generalize about the essential role of the spleen in murine post-natal survival

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[1] Kalpaktsoglou, P K , Yunis, E J , and Good, R A , *Nature*, 215, 633 (1967)
[2] Denys, jun , P , Leyton, R , and De Somer, P , *Life Sci* , 5, 1679 (1966)
[3] Wilson, R , Sjodin, K , and Bealmar, M , *Wistar Inst Symp* , *Monograph*, 2, 89 (1964)
[4] Miller, J F A P , *Wistar Inst Symp* , *Monograph*, 2, 104 (1964)
[5] Meier, H , and Hoag, W G , *Naturwissenschaften*, 14, 329 (1962)
[6] Searle, A G , *Genet Res* , 5, 171 (1964).
[7] Green, M C , *Develop Biol* , 15, 62 (1967)

Herpesvirus Infection of Human Mesodermal Tissue (Cornea) detected by Electron Microscopy

Herpesvirus hominis (herpes simplex virus) usually infects tissues of ectodermal origin, particularly skin, mucous membranes and the central nervous system Viral replication has been observed in mesodermal cells (principally leucocytes) in experimentally infected rabbits[1] and mice[2] and in tissue cultures of human leucocytes[3] and embryonic fibroblasts[4]. There has been little evidence, however, that infection of mesodermal cells plays a part in primary or recurrent human infections.

Herpetic infections of one commonly affected site in man, the cornea, frequently lead to pathological changes in the mesodermally derived stroma[5]. In corneal herpes, the virus can be easily isolated from the typical dendritic lesions in epithelium, but it is rarely recovered from eyes with chronic stromal herpetic keratitis[6]. For this reason it has been assumed that deep herpetic keratitis is a form of host response which does not depend on direct viral infection of the corneal stroma.

We have observed herpesvirus in the stroma of five corneas which were obtained at surgery from patients with chronic herpetic keratitis (Table 1). Two were the originally diseased tissue (*A* and *B*1); three others were corneal grafts which had been in place for 32 days (*B*2), 8 months (*C*), and 14 months (*D*), respectively. For electron microscopy, portions of all five corneas were fixed with cold 3 per cent glutaraldehyde in a 0·067 molar cacodylate buffer, and after 4 h–3 weeks they were post-fixed with 1 per cent osmic tetraoxide in a phosphate buffer. Specimens were embedded in 'Epon' and thin sections were cut for electron microscopy.

Particles resembling herpesvirus were found in the stromal layers of all five corneas. These particles consisted of polygonal, electron-dense shells (capsids) about 70 mμ in diameter (Fig. 1). Some of the capsids contained a mass of electron-dense material (nucleoid), others seemed to be empty. Still others, seen in the vicinity of intact cells or within cytoplasmic vacuoles, were surrounded by a membrane corresponding to the viral envelope (Fig. 1 inset). These naked and enveloped particles were identical with the herpesvirus particles which were observed by us in experimentally infected rabbit corneas which had been prepared in the same way[7].

Viral particles were seen in the corneal stroma in intimate association with intact cells (Fig. 1) or as aggregates mixed with unidentified debris. In four corneas, intact cells were found with capsids in their nuclei and with enveloped particles outside the cell membrane or within the cell vacuoles. In three specimens (*B*1, *B*2 and *C*), there were large masses of virus particles between the collagen lamellae of the cornea. Like the intact infected cells, these masses of particles occurred in the deepest layers of the cornea, distant from the surface epithelium. In one specimen (*B*2), virus particles were seen also within cytoplasmic vacuoles of the polymorphonuclear leucocytes, probably as a result of phagocytosis.

In four of the five corneal specimens which were examined (*A*, *B*1, *C* and *D*), many cells in the corneal stroma contained virus capsids within their nuclei (Fig. 1). Sequential studies of herpesvirus replication have shown that virus particles entering a cell are enclosed in a cytoplasmic vacuole[8], that the viral envelope is removed[9], and that naked particles can be found free in the cytoplasm but not in the nucleus during the early phase of infection[9]. As the growth cycle continues, newly formed virus capsids appear in the nuclei of infected cells shortly before infectivity increases[8]. In our specimens, the presence of virus particles in nuclei indicates that virus replication had indeed taken place within cells in the stroma of these corneas. The absence of viral particles in the nuclei of cells in specimen *B*2 could have been a result of treatment with 5′ iodo-2-deoxyuridine which is known to inhibit herpesvirus replication. The formation of enveloped particles outside the cell membrane and in cytoplasmic vacuoles of cells containing nuclear particles indicates that the final step in the formation of fully mature virus particles had taken place[8].

Table 1. HERPESVIRUS IN HUMAN CORNEAS

Specimen	Herpesvirus particles by electron microscopy	Herpesvirus isolation attempts	
		Corneal swab	Minced tissues
A	+	Neg.	N.D.
*B*1	+	Pos.†	Neg.
*B*2*	+	Neg.	Neg.
*C**	+	N.D.	N.D.
*D**	+	N.D.	N.D.

* Previously transplanted tissue.

† Eight months before the tissue specimen was obtained.

N.D., not done.

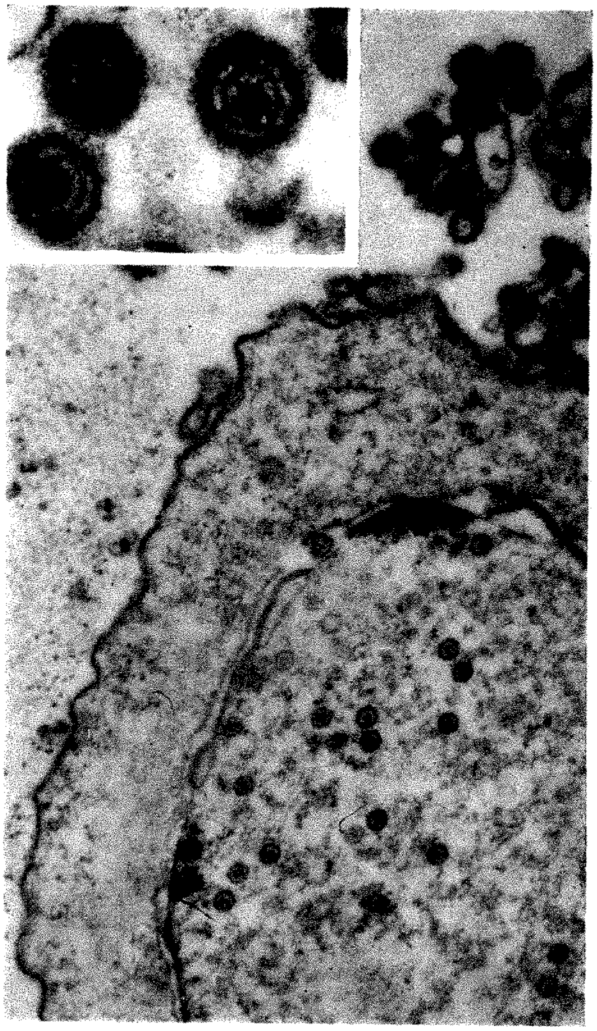

Fig. 1. Corneal stromal cell in specimen *B*1. There are numerous naked herpesvirus particles in nucleus (lower right), and particles with envelopes outside cell (top). Altered corneal collagen is at left of cell (×35,000). Inset: extracellular particles from same specimen with dense central nucleoid surrounded by capsid and outer envelope (×91,000).

Attempts to isolate herpesvirus before surgery were made by swabbing the cornea with a cotton tipped applicator which was then immersed in maintenance media with 2 per cent foetal calf serum (Table 1). Portions of two corneas (*B*1 and *B*2) were minced in the same media. Media surrounding the swab or the minced tissue were inoculated into Maben cells. A single isolate of herpesvirus was obtained from patient *B* during an episode of epithelial (dendritic) keratitis, 8 months before surgery. Swabs of cornea *A*, *B*1 and *B*2 immediately before surgery failed to yield virus, as did the minced tissue from specimens *B*1 and *B*2.

The failure to recover virus in cell cultures of corneal material from eyes not showing typical epithelial lesions confirms past experience[6]. The following are among possible explanations for this inability to isolate virus from corneas with herpes-virus particles.

(1) Only a few cells at a time might have been producing infective virus, and this process could have been limited to a small part of the cornea; this might have led to an accumulation of virus particles even though relatively little infective virus was present. The cornea, even in diseased states, is avascular and dense, and the removal of particulate matter such as virus particles could be a slow, inefficient process. Because herpesvirus is thermolabile[3], the particles seen in our specimens could have accumulated for many days and would have lost infectivity while retaining their morphological identity. (2) Only a small proportion of particles seen in section were structurally "complete". It has been suggested that "complete" particles are more infective than "naked" particles, but this has not been confirmed[9]. (3) These virus particles might belong to another member of the herpesvirus group, for example, varicella-zoster virus or cytomegalovirus. At some time in the course of their disease, however, all these patients had the typical dendritic corneal lesions commonly associated with herpes simplex, and they all exhibited clinical signs characteristic of chronic herpetic keratitis.

The exact identity of the cells which were infected with herpesvirus seen in the stroma of these corneas could not be established with certainty. Following experimental infection with herpesvirus, the stroma of rabbit corneas might contain regenerating keratocytes, fibroblasts, polymorphonuclear leucocytes, lymphocytes, plasma cells and macrophages (histiocytes)[10]. Cells which were infected with herpesvirus in the human corneas in this study had only one nucleus, little organized endoplasmic reticulum and few specialized organelles. They were clearly neither polymorphonuclear leucocytes nor lymphocytes. Probably derived from embryonic mesoderm[11], they could well have been fibroblasts or keratocytes.

The presence of herpesvirus in corneal stromal cells indicates that herpesvirus proliferates in mesodermal as well as ectodermal cells. Infection of connective tissue cells could also play a part in the pathogenesis of herpes infections at other sites, and could be concerned with the latency of the virus between recurrences of clinical activity.

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[1] Garabedian, G. A., and Syverton, J. T., *J. Infect. Dis.*, **96**, 5 (1955).
[2] Johnson, R. T., *J. Exp. Med.*, **120**, 359 (1964).
[3] Nahmias, A. J., Kibrick, S., and Rosan, R. C., *J. Immunol.*, **93**, 69 (1964).
[4] Smith, K. O., *J. Immunol.*, **91**, 582 (1963).
[5] Hogan, M. J., Kimura, S. J., and Thygeson, P., *Amer. J. Ophthalmol.*, **57**, 551 (1964).
[6] Hanna, L., Jawetz, E., and Coleman, V. R., *Amer. J. Ophthalmol.*, **43**, Part II, 126 (1957).
[7] Dawson, C. R., Togni, B., and Thygeson, P., *Nature*, **211**, 316 (1966).
[8] Holmes, I. H., and Watson, D. H., *Virology*, **21**, 112 (1963).
[9] Epstein, M. A., Hummeler, K., and Berkaloff, A., *J. Exp. Med.*, **119**, 291 (1964).
[10] Wolter, J. R., Shapiro, I., and Whitehouse, F., *Amer. J. Ophthalmol.*, **41**, 639 (1956).
[11] Mann, J., *Trans. Ophth. Soc. UK.* **51**, 63 (1931).

Failure to detect Interferon in the Urine of Persons with Virus Infections

WHEN rabbits or mice are inoculated intravenously with interferon or with an inducer of interferon formation, interferon appears in their urine[1-6]. Indeed, with rabbits, appreciably higher concentrations can be found at the same time in the urine than in the serum[2-4]. In contrast, we report here two studies in which interferon was not detected in urine from humans with viral infections.

One study which has been summarized elsewhere[7] involved children with mumps, measles or chicken-pox. The urine was collected from three groups of seven or nine children with each disease, from the first day when the rash appeared or the salivary gland swelled, for 4 days. A sample of urine which was collected from each child during each period of approximately 8 h was treated at *p*H 2 for 5 days at 4° C and was then returned to *p*H 7 and assayed for interferon. Serial dilutions were made in Eagle's medium from an initial one in four dilution, and these were added to groups of three tube cultures of human embryo lung cells. After incubation overnight at 37° C, the tubes were drained, rinsed twice with fresh medium and challenged with one hundred infecting doses of HGP rhinovirus. After 3 days at 33° C, the cytopathic effect (CPE) was scored on the conventional 1–4 scale, and the 50 per cent CPE-inhibition endpoint was determined graphically. A laboratory reference human interferon preparation was included in all assays as a control. No interferon was detected in any of the urine samples. Some of the urine sample from two children within each of the groups was then concentrated seven to nine times by pressure dialysis in 'Visking' tubing, but still no interferon was found on assay. Because of the theoretical possibility that the acidification or pressure dialysis treatments had caused losses of interferon, a number of urine samples were assayed without such treatments, but again with negative results. We also found that small amounts of human leucocyte interferon which were added to normal urine were stable at *p*H 2 and on pressure dialysis.

Because blood samples were not collected, it is not known whether interferon was present in the blood of the children during the period of urine collection. Petralli, Merigan and Wilbur[8], however, vaccinated children with live measles virus and found interferon in their blood 7–11 days later. In our investigation, urine was collected from three children with measles from the onset of prodromal symptoms, that is, about 10 days after infection, until 4 days after the appearance of the rash. By analogy, it seems likely that interferon was present in the blood during at least part of this time, but none was detected even in concentrated urine samples.

Merigan[9] found very small amounts of interferon in concentrated urine samples from children with mumps.

Wheelock and Sibley[10] found interferon in the blood of subjects vaccinated with yellow fever virus. We therefore subcutaneously vaccinated six volunteers with the 17*D* strain of yellow fever virus (10^4 mouse 50 per cent lethal doses). Serum and urine samples which were collected on days 0, 4, 5, 6, 7, 8 and 36 were assayed for virus, interferon and antibody. All volunteers showed a transient viraemia between the fourth and seventh day after vaccination, and all had a rise in antibody titre on day 36. Interferon was found in the sera of three volunteers at the time of the peak of viraemia or a day after. The maximum levels were 4, 10 and 1 units of interferon per ml., but no interferon was found in any of the urine samples. During the course of this work, Dr A. Quercioli of the University of Siena informed us that he also was unable to detect interferon in the urine of volunteers who were vaccinated with yellow fever virus.

From collaborative studies with workers in six different laboratories currently studying human interferon, we believe that our assay for human interferon is at least of average sensitivity. Our results, together with those of Merigan and Quercioli, suggest therefore that subjects infected with mumps, measles, chicken-pox and 17*D* yellow fever viruses do not excrete interferon in their urine in amounts which are readily detectable by present methods. In contrast, Soloviev[11] reported that interferon was present in urine from patients who were infected with influenza *A*2 virus in concentrations higher than those in the serum.

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[1] Oh, J. O., *Proc. Soc. Exp. Biol. and Med.*, 123, 493 (1966).
[2] De Somer, P., Le Clerc, E., Schonne, E., and Billau, A., *PAH$_3$/WHO Conference on Viral and Rickettsial Vaccines, Washington DC* (in the press).
[3] Ho, M., and Postic, B., *Nature*, 214, 1230 (1966).
[4] Bocci, V., Russi, M., and Rita, G., *Experientia*, 23, 309 (1967).
[5] Gresser, I., Fontaine, D., Coppey, J., Falcoff, R., and Falcoff, E., *Proc. Soc. Exp. Biol. and Med.*, 124, 91 (1967).
[6] Subrahmanyan, T. P., and Mims, C. A., *Brit. J. Exp. Pathol.*, 47, 168 (1967).
[7] Finter, N. B., *Ciba Foundation Symposium on Interferons* (Churchill, London, 1967) (in the press).
[8] Petralli, J. K., Merigan, T. C., and Wilbur, J. R., *New Engl. J. Med.*, 273, 198 (1965).
[9] Merigan, T. C., *Ciba Foundation Symposium on Interferons* (Churchill, London, 1967) (in the press).
[10] Wheelock, E. F., and Sibley, W. A., *New Engl. J. Med.*, 273, 194 (1965).
[11] Soloviev, V. D., *International Symposium on Interferons, Siena, Italy* (Academic Press, 1967) (in the press).

CYTOLOGY

New Simple Method of Micrurgy on Living Cells

A SIMPLE method has been developed whereby micrurgy on living cells such as nuclear transplantation and cytoplasmic injection in amoebae can be performed with greater ease and rapidity. The present method differs from those used currently[1-4] in that the need for a micro-hook to hold cells and the use of mineral oil (liquid paraffin) to prevent the evaporation of culture medium are eliminated. This is done by placing the cells on an agar surface. When the excess medium surrounding the cells is withdrawn, they remain firmly attached during operations. The agar gel apparently replaces the medium that evaporates, and there is no damage to the cells during the period required for micrurgy. Thus, for example, nuclei are transplanted between amoebae in open drops with a micro-probe alone.

This principle can be applied to both the original hanging drop method[1-3] and the modified slide method[4]. For preparation, 0.6 per cent agar in the cell culture medium is boiled, filtered and poured while hot onto clean glass slides or coverslips (agar with different purities and at different concentrations was tried, but ordinary bacteriological, non-nutrient agar obtained from Fisher Scientific Co. was found to be most suitable). As soon as the agar cools and gelates, cells can be placed on it and are ready for operations.

No hooks are used, and so it is necessary to place cells in desired positions beforehand. For example, the donor and recipient cells for nuclear transplantation are placed side by side before withdrawing the excess medium. After this, the cells cannot move and different ones can be easily distinguished by their relative positions during and after operations.

With a good objective that has a relatively long working distance, of which there are several makes available, the slide method is preferred to the coverslip method. In the slide method, no inversion of the operation stage is necessary and there is more freedom of movement both with the micro-tool and the slide. For example, using a circular rotating microscope stage, the direction of the arrangement of cells being operated can be easily changed up to 180 degrees. With the slide method, the micro-probe is bent downwards and applied to the cells from above[4].

The advantages of this new method are obvious and manifold: first, there is no hook to make and use, thus eliminating the most troublesome portion of the micrurgical work. As a result, operations can be performed with much greater ease and rapidity than with other methods. For example, as many as 200 amoebae can be easily enucleated in an hour and thirty nuclear transplantations can be carried out during the same period in optimal conditions (compare ref. 3).

While the agar-coated slides were designed primarily for micrurgy on amoebae, they were also found useful in immobilizing other motile cells such as *Tetrahymena* and *Paramecium* for microscopic examination and manipulation of these cells.

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[1] Comandon, J., and de Fonbrune, P., *CR Soc. Biol.*, 131, 744 (1939).
[2] Lorch, I. J., and Danielli, J. F., *Quart. J. Microsc. Sci.*, 94, 461 (1953).
[3] Goldstein, L., in *Methods in Cell Physiology* (edit. by Prescott, D. M.), 1, 97 (Academic Press, New York, 1964).
[4] Jeon, K. W., *Exp. Cell Res.*, 46, 609 (1967).

PATHOLOGY

Sebaceous Gland Activity in Breast Cancer

A NUMBER of observations suggest that human breast cancer develops under hormonal influence. Several such observations include (*a*) breast cancer is predominantly a disease of women, (*b*) the administration of oestrogenic materials produces breast tumours in experimental animals[1], (*c*) the course of breast cancer is influenced by ablative endocrine procedures or by the administration of exogenous hormones in both humans and experimental animals[2-4], and (*d*) the length of the menstruating years and the number of pregnancies affect the frequency of the disease in any given population[5,6].

The physiological role of oestrogens in breast cancer has not been clearly elucidated, although this has been examined in some detail. Measurements of the urinary excretion of oestrogen metabolites in women with breast cancer as compared with age-matched control populations of healthy and ill females have revealed no uniformly significant differences[7,8].

Studies of total urinary 17-ketosteroids, as well as androsterone and aetiocholanolone, have not demonstrated any significant abnormal pattern in women with breast cancer[9-11]. Bulbrook *et al.* have demonstrated that the clinical response to ablative surgery was predictable from the concentrations of urinary aetiocholanolone and 17-

hydroxycorticoids, arranged as a statistical phenomenon and termed the "discriminant" The higher the urinary values of aetiocholanolone in relation to hydroxycorticosteroid levels, the better the response rate to the ablative endocrine surgery The "discriminant" may also predict the clinical course of a woman with early breast cancer

Study of certain tissue and organs known to be influenced by hormonal factors may reveal biological information relevant to the problem of breast cancer The sebaceous gland is such an organ

The development and activity of the sebaceous glands of the skin are primarily under the direct influence of androgen[12] In prepuberal subjects, administration of as little as 5 mg of methyltestosterone daily will stimulate the sebaceous glands[12] This change can be observed in histological sections as well as in an increase in sebum production, a quantitative measurement which is an accurate indicator of sebaceous gland function[2] Marked development of the sebaceous gland occurs in both sexes at puberty, although throughout the adult years significantly higher concentrations of sebum are noted in men than in women In the female, sebaceous gland activity declines significantly after menopause In men such a fall in sebum production does not occur, rather there is a very gradual decline[13]. Bilateral orchiectomy in the elderly male, however, results in a marked fall in sebaceous gland secretion[4]

The administration of oestrogen will result in a reduction in sebaceous gland activity This effect, even from very large doses of oestrogen, is prevented by the concomitant administration of disproportionately small amounts of androgen, indicating that there is no target-site antagonism between oestrogen and androgen[12] Progesterone, in physiological amounts, does not stimulate the sebaceous gland[15] The role of corticosteroids has not been clearly defined, but there is evidence to suggest that they are needed in a permissive role to allow for sebaceous gland response to androgenic stimulation[16] The exogenous administration of suppressive doses of glucocorticoid may cause a reduction of sebum secretion in women[17]

Sebum production was measured by a gravimetric technique[18] in twenty-two postmenopausal women with advanced breast cancer, aged 50 yr or older, who had never received exogenous hormone therapy All these women had undergone radical mastectomy some years before sebum testing Twenty women had undergone spontaneous menopause, although three patients had surgical oophorectomy 1–3 yr after established menopause, and two women were still menstruating at the time of surgical oophorectomy

As controls, thirty-one healthy premenopausal women in the fifth decade (ages 40 to 49 yr), thirty-five postmenopausal healthy females (ages 50 to 86 yr), and twenty-six postmenopausal women with advancing cancer in other sites were studied The latter group included seven reproductive (three ovarian, two endometrial, one cervical, one pelvic), two urinary, six gastro-intestinal, four head and neck, four lung, two unknown primary, and one lymphoma Two of the patients in this latter group had been treated with bilateral oophorectomy for gynaecological cancer, but both were long past spontaneous menopause at the time of surgery No hormonal therapy had been administered in any of these patients One woman had received abdominal irradiation 2 yr before sebum testing

Table 1. MEAN SEBUM CONCENTRATIONS IN WOMEN

Group	Sebum production (mg/10 cm²/3 h ± 1 standard error)	P values
(*a*) Premenopausal, normal	1 87 ± 0 20	
(*b*) Postmenopausal, normal	1 02 ± 0 10	
(*c*) Cancer, non-breast	0 97 ± 0 15	
(*d*) Breast cancer	2 11 ± 0 28	
(*a*) versus (*b*) and/or (*c*)		< 0 01
(*d*) versus (*b*) and/or (*c*)		< 0 01
(*a*) versus (*d*)		Not significant
(*b*) versus (*c*)		Not significant

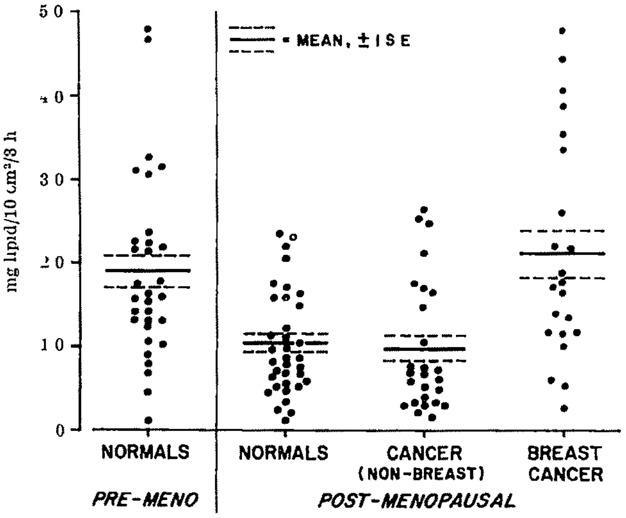

Fig 1 Sebum production in individual women studied

The mean ages of the three postmenopausal groups were postmenopausal healthy females, 67 0 yr, breast cancer patients, 62 0 yr, and cancer, non-breast patients, 65 0 yr

The sebum data (Fig 1 and Table 1) are expressed as mg of surface lipid/3 h period/10 cm² area of forehead skin Each subject was tested two or three times, 1–2 days apart, and the results averaged The mean level (mg/10 cm²/3 h) and one standard error of the mean in the groups is shown in Table 1 Statistical comparison of the postmenopausal healthy group versus the cancer, non-breast group (also postmenopausal), reveals no significant differences The value for the postmenopausal breast cancer group is significantly higher than the other two postmenopausal groups ($P < 0 01$) and is statistically similar to the premenopausal control group

Only three breast cancer patients had sebum values below the mean of the healthy postmenopausal control group Two of these three women had a prior oophorectomy (age 52 and 53 yr) Fifteen of the twenty-two breast cancer patients had values greater than two standard errors above the mean of the postmenopausal controls

It would seem that the sebaceous gland is stimulated by endogenous androgen, with testosterone, Δ^4-androstenedione and dehydroepiandrosterone as the likely agents in this regard These androgens have been shown to stimulate sebaceous gland activity when administered exogenously[16] The likely source of this stimulating material is the ovary or the adrenal gland or both

Seven women, from both postmenopausal cancer groups, had undergone surgical oophorectomy months to years before testing Resulting sebum values were 0 14, 0 51, 0 59, 1 16, 1 64, 2 18 and 3 35 mg That some of these women showed high levels of sebum production might indicate that the stimulating material is not uniquely ovarian in origin The mammalian adrenal is known to produce Δ^4-androstenedione and dehydroepiandrosterone, and is probably capable of synthesizing testosterone[19,20] A specific end organ sensitivity, however, cannot be excluded

Urinary androgen metabolites were not measured in these women, but reports in the literature indicate that excessive amounts of aetiocholanolone and androsterone have not been found in patients with breast cancer[21] The highly specific sebaceous gland target action of testosterone or Δ^4-androstenedione, however, may require only a small quantity of this material, which may not be reflected in either the urinary 17-ketosteroid level or the specific urinary 11-desoxy-17-oxysteroids Decreased biological activity of oestrogen by itself would not be expected to uncover a weak androgen effect on the sebaceous glands, inasmuch as studies have shown that there is no direct target-site antagonism between androgens and oestrogens[12]

The data suggest the possibility that postmenopausal women with advancing breast cancer are, in general, in an androgen-stimulated internal environment, and as a group are quite different in this regard from women with other advancing cancers and from healthy postmenopausal women, although virilizing effects were not observed in these patients (hirsutism, voice changes, acne lesions) Whether this androgen activity is secondary to advancing breast cancer or can be found with early non-metastatic cancer is now being investigated, as is the study of plasma androgen concentrations in relation to sebum production

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[1] Lacassagne, A , *CR Acad Sci* ,**195**, 630 (1932)

[2] McDonald, I , *Surg Gynecol Obstet* ,**115**, 215 (1962)

[3] Haddow A , Watkinson, J M , and Paterson, E , *Brit Med J* , ii, 393 (1944)

[4] Huggins, C , Briziarelli, G , and Sutton, jun , H , *J Exp Med* , **100**, 25 (1959)

[5] Logan, W P D , *Lancet*, ii, 1199 (1953)

[6] Lilienfeld, A M., *Cancer Res* ,**23**, 1503 (1963)

Crowley, L G , Demetriou, J A , Kotin, P , Donovan, A J , and Kushinsky, S , *Cancer Res* ,**25**, 371 (1965)

[8] Brown, J B , in *Endocrine Aspects of Breast Cancer*, 197 (E and S Livingstone, Ltd , Edinburgh, 1958)

[9] Marmorston, J , Crowley, L G , Myers, S M , Stern, E , and Hopkins, C E , *Amer J Obstet Gynecol* , **92**, 447 (1965)

[10] Schweppe, J S , Jungman, R A , and Lewin, I , *Cancer*, **20**, 155 (1967)

[11] Bulbrook, R D , Hayward, J L , Spicer, C C , and Thomas, B S , *Lancet*, ii 1235 (1962)

[12] Strauss, J S , and Pochi, P E , *Rec Prog Hormone Res* ,**19**, 385 (1963)

[13] Pochi, P E , and Strauss, J S , in *Advances in Biology of Skin* (edit by Montagna, W), **6**, 121 (Pergamon Press, NY, 1965)

[14] Pochi, P E , and Strauss, J S , *Amer Med Assoc Arch Derm* , **88**, 729 (1963)

[15] Strauss, J S , and Kligman, A M , *J Invest Derm* , **36**, 309 (1961)

[16] Pochi, P E , Strauss, J S , and Mescon, H , *J Invest Derm* , **41**, 391 (1963)

[17] Pochi, P E , and Strauss, J S , *J Invest Derm* (in the press)

[18] Strauss, J S , and Pochi, P E , *J Invest Derm* , **36**, 293 (1961)

[19] Lipsett, M B , and Korenman, S G , *J Amer Med Assoc* ,**190**, 147 (1964)

[20] Segre, E J , Klaiber, E L , Lobotsky, J , and Lloyd, C W , *Ann Rev Med* , **15**, 315 (1964)

[21] Grattarola, R , *J Endocrinol* , **38**, 77 (1967)

PHYSIOLOGY

Effect of Oxygen Concentration on the Development of Two-cell Mouse Embryos

UNTIL recently, studies on early mammalian embryos were relatively infrequent because of difficulties in obtaining material and in maintaining the viability of the embryos New methods for routine collection and culture[1] have partially overcome these obstacles and several specific biochemical requirements for growing mouse embryos *in vitro* have been determined[2–6]. Using these methods, we have determined the oxygen concentration required by mouse embryos developing *in vitro*

Two-cell mouse embryos were cultured for three days *in vitro* in Brinster's medium for ovum culture[4], with the following concentrations of oxygen in the gas phase 19 9 per cent, 5 0 per cent, 2 5 per cent, 1 7 per cent, 1 5 per cent, 1 0 per cent, 0 5 per cent and 0 per cent In each experiment, one control group of embryos was gassed with 95 0 per cent air plus 5 0 per cent carbon dioxide, and balance nitrogen All experiments were maintained at atmospheric pressure To assure equilibration of the culture medium with a known oxygen tension (pO_2), a continuous flow of gas was used throughout the experiment The various proportions of gases were fixed by a system of pressure reduction valves and flowmeters, using air from a central compressor, carbon dioxide from a cylinder and a mixture of 95 0 per cent nitrogen plus 5 0 per cent carbon dioxide from a cylinder[7] Flow rates were set according to calibration charts provided by the Matheson Company In one experiment, the composition of the mixture, as prepared with the flowmeters, was checked by the method of Scholander, which is accurate to ±0 015 volume per cent[8] The calculated oxygen concentration was 0 97 per cent, and the analysis indicated the actual concentration was 1 15 per cent Because this mixture was one of the lower values of oxygen concentration used in the culture experiments and the required flow rates were in the least accurate range of the flowmeters, other higher concentration oxygen mixtures were presumed to be at least as accurate as this one

For each experiment, two-cell embryos from several mice were pooled and then distributed in groups of fifteen in several drops of culture medium Three days later, the number of morphologically normal blastocysts in each drop was recorded The fractional response was converted to an angular value[9] for the purpose of statistical analysis (see ref 10) Table 1 shows the mean angular responses and standard errors, as well as the mean percentage responses, for data which were pooled from several experiments at each oxygen concentration from 1 7 per cent to 0 per cent To analyse for the significance of differences in response, an analysis of variance was done for data from each concentration from 1 7 per cent to 0 5 per cent oxygen

Two-cell embryos develop into normal blastocysts in concentrations of 1 0 per cent oxygen and greater There was no significant treatment effect at 1 7 per cent, 1 5 per cent or 1 0 per cent oxygen At 0 5 per cent oxygen there was a significant decrease in the number of blastocysts developing from two-cell embryos ($P<0\ 001$) In an anaerobic atmosphere, 0 per cent oxygen, there was no development This indicates that equilibration of the gas with the culture medium occurred in a few hours, because not more than one cleavage occurred after the embryos were placed in the anaerobic incubator

Variation in developmental response of two-cell embryos to cultivation *in vitro* is caused by a number of factors, one of the most important being small variations in the age of the embryos at the beginning of culture Such variation accounts for the difference in response of the

Table 1

Oxygen concentration (volume per cent)	Mean angular response of normal development	Percentage response
1 7	42 93 ± 3 22	46
19 9	47 45 ± 2 68	54
1 5	45 03 ± 1 95	50
19 9	50 64 ± 2 29	60
1 0	31 32 ± 2 18	27
19 9	28 96 ± 3 32	24
0 5	17 98 ± 2 04*	10
19 9	31 88 ± 2 95	28
0 0	7 4 ± 0*	0
19 9	46 67 ± 2 40	53

The first four figures are mean responses from twelve drops of culture medium, fifteen embryos per drop The remaining are means of eighteen drops All means are ± standard error

* $P<0\ 001$

controls among the different experiments This does not affect the validity of the results, because equal numbers of control embryos and experimental embryos were taken from a common pool in each experiment

The fact that embryos develop a pO_2 of approximately 7 mm of mercury in 1 0 per cent oxygen, is not inconsistent with an aerobic metabolism Chance, Schoener and Schindler[11] have demonstrated by *in vivo* measurements on the rat brain that the pO_2 of extracellular blood is much less than 4 mm of mercury, when the electroencephalogram response and breathing stop At this pO_2, the nicotinamide adenine dinucleotide in the rat brain is reduced 80–90 per cent. This seems to be the level of reduction of pyridine nucleotide above which cellular function ceases The intracellular pO_2 at this degree of nucleotide reduction as measured in yeast is less than 0 1 mm of mercury

These results indicate that the mouse embryo is capable of normal development *in vitro* at very low oxygen tensions Whether this capacity is ever utilized *in vivo* is not known, because oxygen tensions of 40–75 mm of mercury have been found in the Fallopian tube of the rabbit[12]

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[1] Brinster, R L , *Exp Cell Res* , 32, 205 (1963)
[2] Brinster, R L , *J Exp Zool* ,158, 49 (1965)
[3] Brinster, R L , *J Exp Zool* ,158, 69 (1965)
[4] Brinster, R L , *J Reprod Fertil* ,10, 227 (1963)
[5] Brinster, R L in *Preimplantation Stages of Pregnancy* (edit by Wolstenholme, G E W , and O'Connor, M), 60 (J and A Churchill, London, 1965)
[6] Brinster, R L , and Thomson, J L , *Exp Cell Res* , 42, 308 (1966)
[7] Brinster, R L , in *Eighth Biennial Symp Animal Reprod* (edit by Nalbandov, A V) (in the press)
[8] Scholander, P F , *J Biol Chem* ,167, 235 (1947)
[9] Fisher, R A., and Yates, F , in *Statistical Tables for Biological, Agricultural and Medical Research*, 70 (Hafner Publishing Co , New York, 1957)
[10] Biggers, J D , and Brinster, R L , *J Exp Zool* ,158, 39 (1965)
[11] Chance, B , Schoener, B , and Schindler, F , in *Oxygen in the Animal Organism* (I U B Symp Ser) (edit by Dickens, F , and Neil, E), 367 (Macmillan Co , New York, 1964)
[12] Mastroianni, jun , L , and Jones, R , *J Reprod Fertil* , 9 99 (1965)

Stimulus-induced Release of Substances from Olfactory Bulb using the Push–Pull Cannula

SEVERAL methods have been used to study neurohumoral release within the mammalian brain These include the use of collecting cups affixed to the cortical surface[1–5], ventricular cannulae for the perfusion of cerebrospinal fluid pathways[6–8] and the push–pull cannula[9] With the last device. perfusion fluid is passed through the inner tube of a concentric dual-barrelled cannula and is recovered by siphoning through the outer barrel Because it makes possible perfusion of small regions of cerebral tissue, even in the depths of brain, the push–pull cannula has been widely used to investigate the spontaneous and stimulus-induced release of acetylcholine[2,10–17]

Certain nerve terminals in the olfactory bulb contain substantial quantities of norepinephrine and serotonin[18,19] The microelectrophoretic administration of either of these monoamines to some bulb neurones alters their firing patterns[20] These findings, together with the well documented effects of drugs which deplete or antagonize monoamines in the olfactory bulb[21,22], suggest that norepinephrine or serotonin may serve as neuromediators in this structure and prompted the present study Using the push–pull cannula we found that olfactory stimulation caused a marked increase in the efflux of radioactively labelled norepinephrine and serotonin from the rat olfactory bulb Stimulation also increased the efflux of labelled inulin and other inert substances which are unlikely to have transmitter function Evidence for the release of potential neurotransmitters using the push–pull cannula in brain, which does not include controls for non-specific release, should therefore be interpreted with caution.

Adult Osborn–Mendel rats were lightly anaesthetized with 15 per cent urethane (approximately 1 ml /100 g body weight, injected intraperitoneally) Then either ^{3}H-7-D,L-norepinephrine (10 μc , specific activity 8 8 c / mmole) or ^{3}H-serotonin (25 μc , specific activity 2 1 c / mmole) was injected into the cisterna magna When norepinephrine or serotonin is administered intracisternally, its regional and subcellular distribution seems to follow that of the endogenous amine[23–26] In these experiments, assay of the olfactory bulbs of four rats killed 60–90 min after intracisternal injection of ^{3}H-norepinephrine confirmed the substantial accumulation (4–13 mμc /mg) of the exogenous amine and of its metabolites

Under stereotactic control, the push–pull cannula (fashioned from a 15 gauge needle and Clay–Adams *PE*-20 polyethylene tubing) was inserted 1 mm beneath the surface of the bulb, and perfusion with Elliot's *B* irrigating solution (Baxter Laboratories) at 37° C was begun at a rate of 0 2 ml /min The perfusate was collected during consecutive 1 min intervals and assayed for total radioactivity by liquid scintillation spectrometry Tritium levels followed a multiphasic exponential decay curve which tended to plateau after approximately 30 min of perfusion Throughout the experiment, a gentle stream of air was delivered at a constant rate into both nostrils by means of plastic catheters A switching device allowed the air current to be bubbled through either pure water or a dilute aqueous solution of such odorous substances as pyridine, isoamyl alcohol, *n*-heptane or almond extract

When the tritiated amine levels were steady (40–200 min after the intracisternal injection of either ^{3}H-norepinephrine or ^{3}H-serotonin), the animal was exposed to an odour for 1 min This stimulus consistently resulted in a three to ten-fold rise in the tritium efflux above baseline levels (Fig 1*A*) Such odour-induced alterations in tritium efflux could be repeated up to four or five times after a single intracisternal injection The increased efflux was detectable immediately on stimulation, was usually maximal during the second 1 min interval following cessation of stimulation and persisted for 3–5 min thereafter An exposure of at least 15 sec to an odorant was necessary to produce detectable release

Several tests of the specificity of these findings were then carried out (1) There was no change in the efflux of labelled monoamine during exposure to loud noises or on electrical stimulation (1–5 V, 3 msec, 20 Hz) of the contralateral forepaw for 1 min It thus seemed unlikely that the odour-induced alterations in tritium efflux resulted from a general arousal reaction (2) The changes induced by odour were not reduced in animals previously subjected to bilateral superior cervical ganglionectomy, thus indicating that release was not dependent on the sympathetic innervation of the olfactory bulb (3) In order to examine the possible influence of local alterations in the integrity of the blood–brain barrier or in the cerebral blood flow, the standard intracisternal dose of ^{3}H-norepinephrine (10 μc) was given intravenously In these circumstances, no spontaneous or odour-induced efflux of tritium from the olfactory bulb was detectable

As a further test of the specificity of the stimulus-induced release of potential neurotransmitters found in these experiments, several metabolically inert, ^{14}C-labelled substances such as inulin (1 μc , 3 8 mc /g), urea (1 μc , 5 1 mc /mmole), or α-aminoisobutyric acid (1 μc , 0 32 mc /mmole) were injected with the ^{3}H-amines On odorous stimulation, a substantial rise in the efflux of the ^{14}C-labelled compound invariably accompanied the augmented ^{3}H-amine efflux (Fig 1*B* and *C*) Tests with increasingly mild odorant exposures revealed no dif-

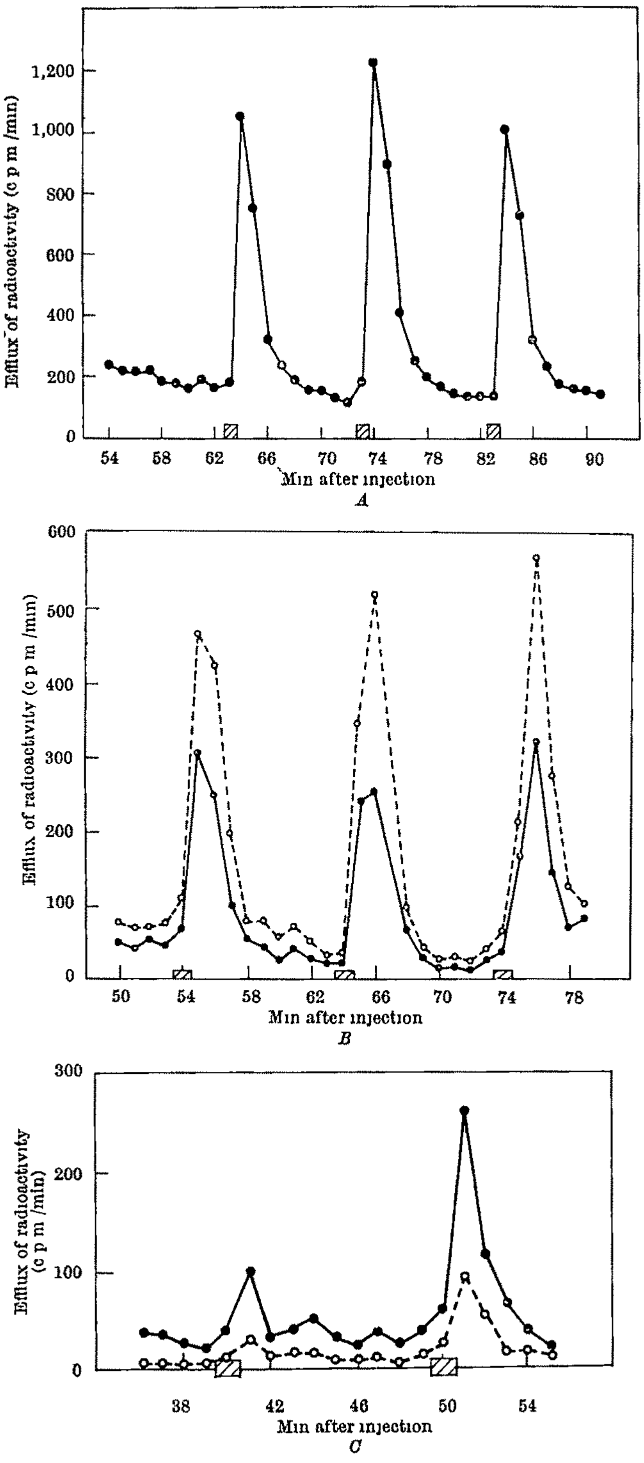

Fig 1 Odour-induced changes in the efflux of radioactivity from the rat olfactory bulb following intracisternal injection of (*A*) 10 μc ^{3}H-7-norepinephrine, (*B*) ^{3}H-norepinephrine (●) plus 1 μc ^{14}C-urea (○), or (*C*) ^{3}H-norepinephrine (●) plus 1 μc of ^{14}C-inulin (○) Nostrils were exposed to isoamyl alcohol vapour for 1 min at the times indicated In *A* and *B*, rats were anaesthetized with urethane, in *C*, immobilized with *d*-tubocurarine and artificially ventilated

ference in threshold between norepinephrine and inulin A wide anterior craniotomy was performed in several animals in an attempt to reduce possible stimulus-related changes in intracranial pressure This procedure failed to modify the odour-induced response of either of the compounds The odorant-increased efflux of both inulin and the amines was not disassociated in artificially ventilated, unanaesthetized rats immobilized with *d*-tubocurarine (Fig 1*C*). These animals were prepared under general anaesthesia and subsequently received local anaesthesia to all pressure points and wound margins It did not seem that the concurrent efflux of these substances could be attributed either to the anaesthesia or to the respiratory changes which occasionally accompanied odorant stimulation

Inulin injected into cerebrospinal fluid remains largely, if not exclusively, in the "extracellular" space[27], so the odour-induced alterations in its efflux found here suggest that local changes within this compartment may attend neural stimulation During perfusion with the push–pull cannula, a steep concentration gradient may be created in the artificial extracellular space surrounding the cannula tip Relatively small variations in hydrostatic pressure or in the transport or diffusion rates of extracellular components might produce substantial changes in the concentration of these substances in the push–pull cannula perfusate Although there is convincing evidence that labelled norepinephrine injected into the cerebrospinal fluid is rapidly taken up into intraneuronal stores[23], it is impossible to decide whether the stimulation-induced alterations in norepinephrine efflux reflect physiological release from such intracellular stores or merely extracellular shifts of residual exogenous amine

While these considerations may be less important when the push–pull cannula is used to study the release of endogenous substances, the inevitability of perfusing traumatized tissue in which the normal intra–extracellular barriers may be compromised seriously complicates the interpretation of results Improved controls are necessary before data derived from the push–pull cannula can be confidently cited as evidence of release of potential cerebral neurotransmitters

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[1] MacIntosh, F C , and Oborin, P E , *Abstr Intern Physiol Cong* , 580 (1953)
[2] Mitchell, J F , *J Physiol* , **165**, 98 (1963)
[3] Phillis, J W , and Chong, G C , *Nature*, **207**, 1253 (1965)
[4] Celesia, G G , and Jasper, H H , *Neurol* , **16**, 1053 (1966)
[5] Collier, B and Mitchell, J F , *J Physiol* , **188**, 83 (1967)
[6] Beleslin, D , Carmichael, A E , and Feldberg, W , *J Physiol* , **173**, 368 (1964)
[7] Feldberg, W , and Myers R D , *J Physiol* , **184**, 837 (1966)
[8] Portig, P J , and Vogt, M , *J Physiol* **186**, 131P (1967)
[9] Gaddum, J H , *J Physiol* , **155**, 1P (1961)
[10] Gaddum, J H , in *Pharmacological Analysis of Central Nervous Action* (edit by Patton, W D M), **8**, 1 (Pergamon Press, Oxford, 1962)
[11] Mitchell, J F , and Szerb, J C , *Abstr Twenty-second Intern Physiol Cong* , 819 (1962)
[12] Szerb, J C , *Nature*, **197**, 1016 (1963)
[13] Delgado, J M R , and Rubinstein, L , *Arch Intern Pharmacodyn* , **150**, 530 (1964)
[14] McLennan, H , *J Physiol* , **174** 152 (1965)
[15] McLennan, H , *Experientia*, **21** 725 (1965)
[16] Mitchell, J F , in *Mechanisms of Release of Biogenic Amines* (edit by Euler, U S von, and Rosell, S), 425 (Pergamon Press, Oxford, 1966)
[17] Metz, B , *J Physiol* , **186**, 321 (1966)
[18] Garattini, S , and Valzelli, L , *Serotonin* (Elsevier, Amsterdam, 1965)
[19] Dahlstrom, A , Fuxe, K , Olson, L , and Ungerstedt, U , *Life Sci* , **4**, 2071 (1965)
[20] Baumgarten, R von, Bloom, F E , Oliver, A P , and Salmoiraghi, G C , *Arch Ges Physiol* , **277**, 125 (1963)
[21] Bloom, F E , Costa E , and Salmoiraghi, G C , *J Pharmacol Exp Therap* , **146**, 16 (1964)
[22] Salmoiraghi G C , Bloom, F E , and Costa, E , *Amer J Physiol* , **207**, 1417 (1964)
[23] Glowinski, J and Baldessarini, R J *Pharmacol Rev* , **18**, 1201 (1966)
[24] Aghajanian, G K , and Bloom, F E , *J Pharmacol Exp Therap* , **156**, 23 (1967)
[25] Palaic, D , Page, I H , and Khairallah, P A , *J Neurochem* , **14**, 63 (1967)
[26] Fuxe, K , and Ungerstedt, U , *J Pharm Pharmacol* , **19**, 337 (1967)
[27] Woodward, D L , Reed, D J , and Dixon, M W , *Amer J Physiol* , **212**, 367 (1967)

Effect of Secretin on Human Rectal Mucosa *in vivo*

It is now more than 60 years since secretin was discovered and since its significance with respect to pancreatic and biliary secretion was recognized[1]. Later investigations have led to the conclusion that pancreatic juice[2] and bile[3] can both be considered to be a mixture of two fluid components which are probably secreted by different cells and under independent physiological control, that is, they are made up of a fluid which is rich in electrolytes and a fraction containing a high concentration of the macromolecular secretory products of the exocrine cells. The principal effect of secretin seems to be to stimulate formation of the "electrolyte fraction", but its precise mode of action is still unknown.

Despite widespread investigations, there is no conclusive evidence that secretin has any physiological effects on tissues other than the exocrine pancreas and the liver. In particular, detailed studies have failed to show any effect on Brunner's glands[4] or on the transfer of fluid and electrolytes across the rat ileum[5] and frog skin[6]. Recent observations which were made in this laboratory, however, suggest that secretin may have some action on human rectal mucosa—an epithelium across which considerable movement of water and electrolytes is known to occur.

During tests of pancreatic function on children with cystic fibrosis (CF) and on healthy controls[7] rectal suction biopsies were taken, both at the start of the test and 15 min after injection of secretin (2 IU/kg body weight in 5 ml of isotonic saline). A multipurpose biopsy tube was used and the specimens, which were obtained 5–6 cm from the anal orifice, were fixed in Bouin's solution. Paraffin sections (6µ) of the fixed tissue were stained with haematoxylin and eosin and with stains specific for mucosubstances (for example, colloidal iron-PAS and toluidine blue).

After secretin had been injected the surface epithelium of the rectal mucosa showed distinct changes which were not seen in the specimens at zero time: the nuclei seemed to be displaced towards the luminal surface while the area at the base of the cells, between the nuclei and the subepithelial connective tissue, took on a "fuzzy" appearance (Fig. 1). These changes were seen in every post-secretin specimen, but the entire surface layer was not always equally affected. Furthermore, there were no well defined effects on the goblet cell mucus which could be shown histochemically. Injection of 5 ml of isotonic saline alone produced no visible changes in the appearance of the mucosa. Similar results were observed in both CF and control tissue, but the CF response to secretin seemed to be less marked; it must be stressed, however, that this is a qualitative observation which was made on a small number of cases only (that is, three CF and three controls).

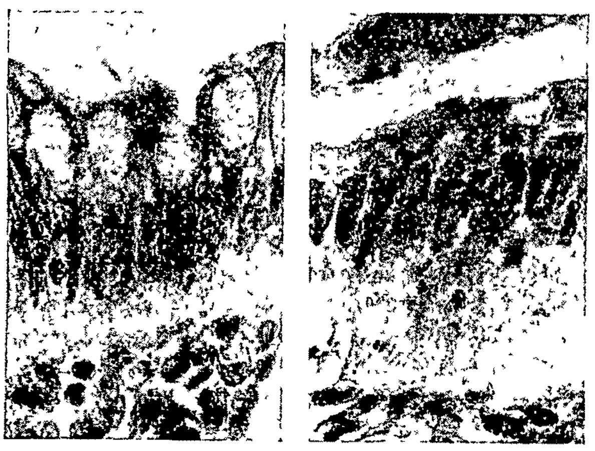
A *B*

Fig. 1. *A*, Surface epithelium of human rectal mucosa before injection of secretin (× 1,120). *B*, Rectal mucosa from the same subject 15 min after injection of secretin (× 1,120).

The precise interpretation of these observations is not clear, but we suggest that the appearance indicates changes at the base of the epithelial cells, possibly in the extracellular region—a site which may well be important in controlling the rate of movement of fluid from the connective tissue proper into the cells. Polyelectrolyte mucoid material has been shown external to the plasma membrane of many cell types[8], and could envelope virtually all cells (the "extraneous mucoid coat")[9]. Interaction of a polar polypeptide such as secretin with this extracellular layer could well cause structural alterations, resulting in the modification of its "porosity" towards fluid and electrolytes. Thus the changes which we have observed could indicate an effect on fluid distribution at the base of the epithelial cells which are actively involved in fluid transfer.

The effect of secretin both on rectal mucosa and on the exocrine pancreas is now being studied in greater detail in rats and kittens, with special emphasis on the mechanism by which fluid movement in these tissues is controlled.

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[1] Bayliss, W. M., and Starling, E. H., *J. Physiol.*, **28**, 325 (1902).
[2] Grossman, M. I., and Ivy, A. C., *Proc. Soc. Exp. Biol. and Med.*, **63**, 62 (1946).
[3] Wheeler, H. O., Ramos, O. L., and Whitlock, R. T., *Circulation*, **20**, 988 (1960).
[4] Cooke, A. R., and Grossman, M. I., *Gastroenterology*, **51**, 506 (1966).
[5] Hubel, K. A., and Colbert, B., *Experientia*, **23**, 337 (1967).
[6] Friedman, R. T., LaPrade, N. S., Alyawar, R. M., and Huf, E. G., *Amer. J. Physiol.*, **212**, 962 (1967).
[7] Hadorn, B., Johansen, P. G., and Anderson, C. M., *Canad. Med. J.* (in the press), *Austral. Paediat. J.* (in the press).
[8] Pease, D. C., *J. Ultrastruct. Res.*, **15**, 555 (1966).
[9] Bennett, H. S., *J. Histochem. Cytochem.*, **11**, 14 (1963).

Calcium Spikes of Nerve Cell Membrane: Role of Calcium in the Production of Action Potentials

This communication reports the observation that the membrane of bullfrog sympathetic ganglion cells is capable of producing action potentials (calcium spikes) in an isotonic solution of calcium chloride in response to cathodal currents which are applied through an intracellular recording microelectrode[1].

In record 1 of Fig. 1, the action potential (sodium spike) which was produced in Ringer's solution is shown. The calcium spike which was obtained after a ganglion was perfused with an isotonic solution of calcium chloride (83 mmolar) containing 2 mmolar potassium bicarbonate for more than 3 h is shown in record 2. The resting membrane potential and the effective membrane resistance in this solution (mean values for seven cells being approximately 100 mV and 200 MΩ, respectively) were much larger than those in Ringer solution (mean values for ten cells being approximately 65 mV and 40 MΩ, respectively). The threshold for initiation of calcium spikes was considerably higher than that for sodium spikes, the threshold membrane potentials ranged from −5 to −10 mV (among seven cells) in the isotonic

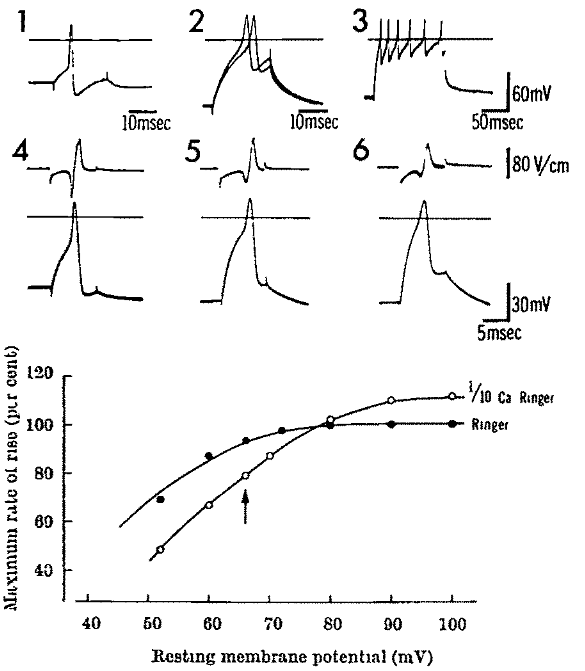

Fig 1 Record 1 shows the action potential of a ganglion cell in Ringer solution, and records 2 and 3 show the action potentials of two other cells of the same preparation in an isotonic solution of calcium chloride Records 4, 5 and 6 were obtained in succession from a cell of a different preparation during perfusion with a solution containing 57 mmolar sodium chloride, 1 8 mmolar calcium chloride, 2 ml mmolar potassium bicarbonate and 114 mmolar sucrose, 5 min after the perfusate was changed to a solution containing 57 mmolar sodium chloride, 41 5 mmolar calcium chloride and 2 mmolar potassium bicarbonate, and 8 min after addition of tetrodotoxin (5×10^{-6} g/c c), respectively An electrically differentiated record of each potential wave lies immediately above them The graph shows the maximum rate of rise of the spike action potentials of a cell induced at varying membrane potential levels during perfusion with Ringer solution and with a low calcium (0 18 mmolar) Ringer solution was plotted against the membrane potential level The membrane potential level was varied by applying anodal or cathodal currents through the recording microelectrode Arrow indicates membrane potential level in the absence of extrinsic currents The largest rate of rise in Ringer solution is taken to be 100 per cent

solution of calcium chloride and from −40 to −45 mV (among ten cells) in Ringer solution The overshoot of the calcium spike was larger than that of the sodium spike, the peak potentials of the sodium and calcium spikes ranged from 25 to 30 mV and 40 to 50 mV respectively The mean values of the maximum rates of rise of the calcium spikes (seven cells) were about 25 per cent of those of the sodium spikes (ten cells) Because the IS-component of the calcium spikes could not be isolated[1], it was assumed that the calcium spikes had originated from the soma membrane Repetitive responses (calcium spikes) were readily produced if a strong cathodal current was applied (record 3, Fig 1)

The value of the peak potential of the calcium spike was almost linearly proportional to the calcium concentration in the external solution (the calcium concentration was altered by replacing calcium chloride with equimolar amounts of sucrose), the increment for a ten-fold increase in the calcium concentration being about 30 mV This suggests that the inward movement of calcium across the membrane is responsible for the production of the calcium spike No action potentials were produced with calcium at a concentration less than 5 mmolar The calcium spike was more resistant to tetrodotoxin than was the sodium spike The sodium spike disappeared within 5 min in the presence of 5×10^{-7} g/ml of tetrodotoxin, whereas the calcium spike was depressed but not abolished in the presence of 5×10^{-6} g/ml of tetrodotoxin Procaine (5×10^{-3} g/ml), which blocked the sodium spike within a few minutes, markedly prolonged the falling phase and slightly depressed the rising phase of the calcium spike The characteristics of the calcium spikes resembled those of the anthropod muscle[2] and of the molluscous giant nerve cells[3-5]

In Ringer solution containing 1 8 mmolar calcium, the calcium current may be very small compared with the sodium current during the action potential The calcium current, however, seems to make a considerable contribution to the inward current during the action potential produced in a solution of sodium containing a relatively large amount of calcium Furthermore, the calcium in a solution of sodium is able to depress the sodium current[6] These actions of calcium can be seen in records 4, 5 and 6 of Fig 1 Record 4 was obtained in a solution containing 57 mmolar sodium and 1 8 mmolar calcium (isotonicity was maintained with 114 mmolar sucrose) When the calcium concentration was increased to 41·5 mmolar, the level of the threshold membrane potential was markedly raised and the maximum rate of rise of the action potential was decreased conspicuously, while the peak level of the action potential was increased, as seen in record 5 When tetrodotoxin (5×10^{-6} g/ml) was added to the perfusate, the threshold membrane potential level was raised further and the maximum rate of rise of the action potential was rapidly decreased to a certain extent within a few minutes without appreciably changing the peak level, and then remained there, this would be the calcium spike (record 6)

Another effect of calcium which was observed with the present preparation was that it removed inactivation of the sodium carrying system[6] This is graphically illustrated in Fig 1, in which the maximum rate of rise of the sodium spike produced at a relatively low resting potential level is decreased in Ringer solution containing 0 18 mmolar calcium

In conclusion, calcium is capable of (1) acting as a charge carrier, (2) removing sodium inactivation, and (3) depressing the sodium current during the production of the action potential of sympathetic ganglion cells

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[1] Nishi, S , and Koketsu, K , *J Cell Comp Physiol* **55**, 15 (1960)
[2] Hagiwara, S , *Ann NY Acad Sci* , **137**, 1015 (1966)
[3] Gerasimov, V D , Kostyuk, P G , and Aisku, V A , *Bull Exp Biol Med* **58**, 3 (1964)
[4] Meves, H , *Pflug Arch* , **289**, Part 2, 10 (1966)
[5] Chamberlain, S G , and Kerkut, G A , *Nature*, **216**, 89 (1967)
[6] Frankenhaeuser, B , and Hodgkin, A L , *J Physiol* , **137**, 218 (1957)

Effects of Vasopressin, Theophylline and Cyclic Adenosine Monophosphate on Short-circuit Current across Isolated Rabbit Ileal Mucosa

VASOPRESSIN causes rapid, equal increases in short-circuit current (Isc) and rate of transport of sodium when added to the serosal surface of frog skin or toad bladder[1,2]. Several investigators have presented convincing evidence that this alteration in the active transport of sodium is primarily, if not entirely, a consequence of increased passive permeability of the mucosal surface to sodium, as well as to other small hydrophilic solutes[3-5] Adenosine-3′ 5′-monophosphate (cyclic AMP) and theophylline have been shown to mimic the effects of vasopressin on Isc and osmotic water flow across toad bladder[6] It has been suggested that cyclic AMP is the more direct mediator of the permeability change and that vasopressin and theophylline exert their effects by increasing the concentration of cyclic AMP in the epithelial cells[7].

Theophylline and cyclic AMP have been observed to increase acid secretion but not Isc across frog gastric mucosa[8] Vasopressin has been observed to cause a marked but transient increase in Isc across stripped guinea-pig caecum[9] and to cause a small but more prolonged increase in Isc and osmotic water flow across toad colon[10] It would therefore seem that these compounds could alter ion transport in several epithelial membranes, including mammalian intestinal mucosa, but that the ion transport systems which are affected might not be the same in every instance

We have observed an increase in Isc when either vasopressin, theophylline or cyclic AMP was added to the serosal surface rabbit ileal mucosa *in vitro* Observations on the Isc alterations caused by these compounds in sodium chloride, choline chloride and sodium sulphate containing Ringer solutions are presented in this communication Changes in Isc were determined rather than changes in electrical potential alone, because the former directly measures net ion transport across the tissue while the latter depends on passive ion permeability as well, which can be greatly altered by changes in ionic composition of the bathing medium.

Segments of distal ileum from New Zealand white male rabbits were stripped of their outer mesothelium and two principal muscle layers, and were clamped as flat sheets between two identical half chambers Mucosal and serosal surfaces of the tissues were perfused at 37° C from separate reservoirs containing oxygenated solutions which were identical except for the addition of D-glucose (15 mmolar) to the serosal side and D-mannitol (15 mmolar) to the mucosal side Unless otherwise stated, the perfusates had the following ionic composition in mmoles/l sodium, 141, potassium, 10, calcium, 1 25, magnesium, 1 1; chloride, 127; bicarbonate 25, dihydrogen phosphate, 0 3; hydrogen phosphate, 1 6 In some experiments choline was substituted for sodium and sulphate was substituted for chloride (sodium, 185, sulphate, 83) or for both chloride and bicarbonate (sodium, 193, sulphate, 100) Perfusates were continuously gassed with 95 per cent oxygen, 5 per cent carbon dioxide if they contained bicarbonate and with 100 per cent oxygen if they did not Apparatus and methods which were used for the determination of Isc were essentially those previously described[11] In many experiments a continuous recording of Isc was obtained by using an automatic voltage clamp

Compounds were not tested until the Isc was stable and at least 20 min had elapsed after the beginning of perfusion At the end of each experiment the viability of the tissue was established by eliciting a substantial increment in Isc on the addition of D-glucose (15 mmolar) to the mucosal perfusate (choline was replaced by sodium before adding glucose)

Vasopressin ('Pitressin', Parke Davis) caused a large but transient (2–5 min) increase in Isc within 30 sec of addition to the serosal perfusate at concentrations as low as 5 mU/ml A maximal response seemed to be attained at a concentration of 25–75 mU/ml The short duration of the effect was manifested at concentrations as high as 2 U/ml Vasopressin (400 mU/ml) was ineffective when added to the mucosal perfusate alone The effects of synthetic arginine and lysine vasopressin (Sandoz Pharmaceuticals) on Isc were indistinguishable from that of 'Pitressin'

The increment in Isc induced by vasopressin disappeared despite the persistence of active hormone in the perfusate This was shown when the original perfusate was added to another piece of mucosa eliciting another large increase in Isc Furthermore, the addition of a second increment of vasopressin equal to the first failed to produce another response whether it was added immediately after the initial effect had disappeared or 1 h later A substantial second response was elicited, however, by first washing the serosal surface with vasopressin-free solution (three changes of solution) and then adding the amount of vasopressin which was originally used A response was produced in this way as early as 10 min after the first change of solution A moderate second response was also elicited when a modest initial concentration of vasopressin (7 to 20 mU/ml) was followed by a twenty-fold increment in concentration.

Table 1 INCREMENT IN ISC (μAMP/CM^2)

Compound	Ringer solution Sodium, chloride, bicarbonate	Choline, chloride bicarbonate	Sodium, sulphate, bicarbonate	Sodium, sulphate
Vasopressin (50–400 mμ/ml)	110 ± 9 (11)	0 (5)	11 ± 3 (5)	0 (8)
Theophylline (5–10 mmolar)	123 ± 14 (10)	22 ± 2 (9)	34 ± 10 (4)	0 (8)
Cyclic AMP (3–7 5 mmolar)	97 ± 11 (9)	30 ± 6 (4)	21 ± 1 (3)	0 (8)
D-Glucose (10–15 mmolar)	87 ± 5 (5)	0 (2)	44 ± 5 (5)	46 ± 11 (5)

Arithmetic mean values ± 1 S E M are given No of experiments in parentheses

While the present observations do not permit formulation of a precise mechanism for the transient character of the Isc response to vasopressin, they are consistent with the following two general mechanisms, one of which might be relevant (1) Vasopressin might be rapidly inactivated by intracellular enzymes and both active and inactive forms of vasopressin might compete for specific hormone receptor sites A second vasopressin response might only occur therefore if a critical ratio of active to inactive intracellular hormone concentrations were exceeded (2) The metabolic events set into motion by vasopressin could rapidly deplete the mucosal cell of a compound which is essential for the Isc response and the continued presence of the hormone or one of its metabolites could inhibit resynthesis of this compound

Cyclic AMP (1 to 7 5 mmolar) and theophylline (1 to 10 mmolar) caused rapid and sustained increases in Isc when added to the serosal perfusate (Table 1). This effect was almost completely inhibited by 10^{-4} molar ouabain which was added 15 min earlier The earlier addition of vasopressin did not diminish the subsequent response to theophylline or cyclic AMP. Maximal amounts of the latter compounds, however, completely prevented a subsequent vasopressin response

To determine which ions are involved in these increases in Isc, the effects of vasopressin, cyclic AMP, theophylline and glucose on Isc across mucosae perfused in choline, sulphate–bicarbonate and pure sulphate Ringer solutions were compared (Table 1) The effects of mucosal glucose are included here in order to show that sodium transport can be stimulated in the absence of any chloride or bicarbonate in the perfusate Although responses were considerably smaller in choline and solutions containing sulphate, definite sustained increases in Isc were caused by cyclic AMP and theophylline in the absence of sodium and in the absence of chloride, but not in the absence of both chloride and bicarbonate Further experiments with theophylline in bicarbonate-free sodium chloride Ringer solution showed that a substantial Isc response can also be obtained in the absence of bicarbonate provided that chloride is present

These results suggest that both chloride and bicarbonate are actively secreted by rabbit ileum in response to cyclic AMP and theophylline. Whether or not vasopressin also elicits an anion secretory response cannot be surmised as the hormone did not significantly alter Isc in solution of either choline or sulphate.

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[1] Ussing, H H , and Zerahn, K , *Acta Physiol Scand* , **23**, 110 (1951)
[2] Leaf, A , and Dempsey, E , *J Biol Chem* , **235**, 2160 (1960)
[3] Curran, P F , Herrera, F C , and Flanigan, W J , *J Gen Physiol* , **26**, 1011 (1963)
[4] Leaf, A , and Hays, R M , *J Gen Physiol* , **45**, 921 (1962)
[5] Civan, M M , and Frazier, H S , *J Clin Invest* , **46**, 1945 (1967)
[6] Orloff, J , and Handler, J S , *J Clin Invest* , **41**, 702 (1962)
[7] Handler, J S , Butcher, R W , Sutherland, E W , and Orloff, J , *J Biol Chem* , **240**, 4524 (1965)
[8] Harris, J B , and Alonso, D , *Fed Proc* , **24**, 1368 (1965)
[9] Ussing, H H , *Metabolic Aspects of Transport Across Cell Membranes* (edit by Murphy, O R), 49 (University of Wisconsin Press, 1957)
[10] Cofre, G , and Crabbe, J , *J Physiol* , **188**, 177 (1967)
[11] Schultz, S G , and Zalusky, R *J Gen Physiol* , **47**, 567 (1964)

Teratogenic Effects of Hypoglycin-A

HYPOGLYCIN-A[1] has been shown to be a non-proteinogenic amino-acid of the structure L-α-amino-β-methylenecyclopropanepropionic acid[2] It is the toxic element of the fruit of *Blighia sapida* (called "ackee" in Jamaica) which is a common Jamaican dietary item Hypoglycin is considered to be responsible for the "vomiting sickness", an acute condition, frequently observed in Jamaica, which is characterized by persistent vomiting and a coma[3,4] In experimental animals, hypoglycin-A causes a delayed hypoglycaemia, vomiting[1,5] and fatty degeneration of the liver[6]

The teratogenicity of hypoglycin-A was tested on pregnant albino rats weighing between 170 and 180 g The first day of pregnancy was considered to be the morning when sperm was found in the vaginal smear Fifteen pregnant rats were then given daily intraperitoneal injections of an aqueous solution of hypoglycin-A (70 per cent of the pure substance, 30 mg/kg body weight), from the first to the fifth day of gestation The animals were kept in individual cages at room temperature (22°–25° C) and were fed a standard 'Purina' rat chow and were provided with unlimited water Foetuses which had been recovered from pregnancies terminated on the nineteenth and twentieth days of gestation were weighed and examined for the presence of external malformations, foetal resorption sites were counted and random foetuses from each litter were fixed in Bouin's solution Free-hand sections were made and examined for visceral malformations[7]. Thirteen pregnant animals, which were not treated, served as controls

Table 1 TERATOGENIC EFFECTS OF HYPOGLYCIN-A ON THE RAT EMBRYO

Pregnant rats	Total implantations	Resorptions	Malformed foetuses	Normal foetuses
Treated with hypoglycin-A	125	7	(110) 18 E 8 E 84 E+S	8
Control	105	1	(6)	98

E, Exencephalocoele, S, syndactyly

The results are shown in Table 1 The incidence of foetal malformations, resportion of foetuses and retarded foetal development (decrease in size and weight) in the groups of animals which were treated was significantly higher than in the control groups The abnormalities observed were exencephalocoele and syndactyly The numbers in the litters were not affected

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[1] Hassall, C H , Reyle, K , and Feng, P , *Nature*, **173**, 356 (1954)
[2] von Holt, C , and Leppla, W , *Angew Chem* , **70**, 25 (1958)
[3] Scott, H H , *Ann Trop Med Parasitol* , **10**, 1 (1917)
[4] Jeliffe, D B , and Stuart, K L , *Brit Med J*, 1, 75 (1954).
[5] Chen, K K , Anderson, R C , McKowen, M C , and Harris, P N , *J Pharmacol Exper Therap* , **121**, 272 (1957)
[6] von Holt, L , and von Holt, C , *Biochem Z* , **331**, 422 (1959)
Wilson, J G , *Teratology, Principles and Techniques* (edit by Wilson, J G , and Warkany, J), 262 (Univ of Chicago Press, Chicago, Illinois, 1965)

Effect of Dietary Carbohydrate on Hepatic Lipogenesis in the Rat

IT has been suggested that dietary sucrose is implicated in the aetiology of ischaemic heart disease[1] If this were so, dietary sucrose would produce different metabolic effects from other dietary carbohydrates. In man and experimental animals, substitution of sucrose for starch in the diet can result in diminished growth, increased concentrations of serum cholesterol and alterations in liver lipids[2–4] Elevated concentrations of plasma triglyceride result in man from eating sucrose[5–6] or fructose[7] as compared with glucose, while the substitution of fructose or sucrose for glucose or starch in the diets of rats causes an increased liver lipid content[8] These results suggest that the effects of sucrose are caused by the fructose content of the sugar

The purpose of the experiments reported here was to study the influence of dietary sucrose, fructose, glucose and rich starch on hepatic lipogenesis and the activities of some enzymes thought to be associated with lipogenesis

Male Wistar rats were given the experimental diets *ad libitum* for 6 days and were 45–49 days old when killed The standard diet used contained 62 8 per cent soluble carbohydrate, 23 per cent protein 4 4 per cent fat, 3 8 per cent fibre and 6 0 per cent ash One group of animals was maintained on the standard diet, but their drinking water was replaced by 20 per cent fructose The rest of the diets consisted of 68 per cent of the stated carbohydrate, 24 per cent casein, 3 9 per cent mineral salts mixture[10], 0 48 per cent corn oil. 0 48 per cent cod liver oil, 2 9 per cent gelatine and 0 58 per cent yeast extract Enzyme activities and acetate incorporation into lipid were estimated by methods described by Taylor, Bailey and Bartley[9]

Table 1 shows that provision of dietary carbohydrate in the form of fructose rather than glucose results in higher activities of hepatic pyruvate kinase, malic enzyme and ATP citrate lyase This confirms the results of Freedland, Cunliffe and Zinkl[11] and Kornacker and Lowenstein[12] In animals given the diet containing sucrose enzyme activities were intermediate between those of the fructose and glucose fed animals When the dietary carbohydrate was given as diet 86, enzyme activities were similar to those of the animals fed on glucose In diet 86, the carbohydrate is predominantly barley and wheat starch It is interesting that much greater activities of enzyme are found if rice starch is used as the source of carbohydrate The difference in pyruvate kinase activities between rats fed sucrose and diet 86 supports the suggestion of Bartley *et al* [10] and Yudkin and Krauss[13] that the high rat liver pyruvate kinase activities reported by Krebs and Eggleston[14] when they fed animals high

Table 1 CHANGES IN LIVER WEIGHT AND ACTIVITY OF SOME RAT LIVER ENZYMES AND OF [1-^{14}C] ACETATE INCORPORATION INTO RAT LIVER LIPIDS WITH CHANGE IN DIET

Diet	Pyruvate kinase activity (μmoles substrate utilized/min/g wet weight of liver at 25° C)	Malic enzyme activity (μmoles substrate utilized/min/g wet weight of liver at 25° C)	ATP–citrate lyase activity (μmoles substrate utilized/min/g wet weight of liver at 25° C)	Acetate incorporated into lipid (μmoles/90 min/g of liver slice)	Liver weight (percentage body weight)
Diet 86	31.0 ± 1.1 (5)	6.5 ± 0.69 (6)	1.11 ± 0.12 (6)	1.06 ± 0.29 (11)	4.45 ± 0.17 (10)
Fructose	85.0 ± 10.9 (5)	13.9 ± 1.70 (6)	3.51 ± 0.83 (6)	0.58 ± 0.18 (12)	5.25 ± 0.17 (10)
Glucose	38.0 ± 4.4 (5)	7.5 ± 0.80 (6)	1.69 ± 0.51 (6)	1.00 ± 0.23 (12)	4.85 ± 0.20 (10)
Sucrose	66.5 ± 12.2 (5)	9.6 ± 0.86 (6)	2.99 ± 0.48 (6)	0.62 ± 0.19 (11)	4.70 ± 0.19 (10)
Rice starch	50.0 ± 3.3 (5)	9.2 ± 2.27 (6)	1.96 ± 0.35 (6)	0.40 ± 0.08 (12)	4.35 ± 0.21 (10)
Diet 86 + 20 per cent fructose	90.0 ± 5.9 (5)	NE	NE	NE	NE

The results are given as means ± *S.E.M.* with the number of observations in parentheses. NE, not estimated. The rats were given the experimental diets for 6 days.

carbohydrate diets were, at least in part, caused by the nature of the carbohydrate (sucrose). Table 1 also shows that hepatic pyruvate kinase activities can be raised if drinking water is replaced by 20 per cent fructose.

Although the activities of hepatic enzymes thought to be associated with lipogenesis are found to be higher in animals receiving dietary fructose or sucrose than in those fed diet 86 or glucose, acetate incorporation into lipid of liver slices was lower. A similar result was obtained for the animals fed on starch. Zakim *et al.*[15], also using liver slices, were unable to detect a difference in acetate incorporation between animals fed fructose, glucose and sucrose, but there was greater incorporation of acetate into lipid by homogenates of liver from rats which had been fed a sucrose rich diet than from those given a starch rich diet[16].

Although our results suggest that the activities of some liver enzymes depend on the nature of the dietary carbohydrate, it is possible that the effects were caused by ingestion of different quantities of the various carbohydrates. Table 2 shows the amount of carbohydrate eaten and the weight gained by the groups of rats. The increased enzyme activities produced by fructose and sucrose cannot be explained by greater intake of these two carbohydrates. If isocaloric diets had been given, the difference between the fructose and sucrose results and the others would probably have been greater. There seems to be a difference in enzyme activity produced by dietary rice starch compared with dietary wheat and barley starch (diet 86). Provision of fructose in the diet not only led to a low carbohydrate intake compared with the other diets but also resulted in the least weight gain (Table 2).

Table 2 AMOUNT OF CARBOHYDRATE EATEN BY, AND WEIGHT GAIN OF, RATS GIVEN VARIOUS DIETS

Diet	Carbohydrate eaten (g/day/rat)	Weight gain (g/day/rat)
Diet 86	11.1	2.9
Fructose	6.5	0.8
Glucose	9.3	2.0
Sucrose	8.0	2.0
Rice starch	11.7	1.9
Diet 86 + 20 per cent fructose	12.1 (7.1 from diet 86 and 5.0 from 20 per cent fructose)	1.9

The animals were given the experimental diets for 6 days. The results are the means for twenty-eight rats except for the diet 86 + 20 per cent fructose when only ten animals were used.

Changes in the activities of enzymes in response to changes (of quantity or kind) of dietary carbohydrate are presumably secondary to changes in the concentrations of specific metabolites which induce the enzymes. Three responses can be distinguished if the diets differ in quantity of carbohydrate[14], nature of the monosaccharide or the polymeric state of the carbohydrate. Two factors are important in considering how the diets affect amounts of inducer: first, the rate at which the sugar is presented to the liver, which is governed by the rate of digestion and absorption, and second, the manner in which different monosaccharides are metabolized by the liver.

In the absence of evidence that glucose and fructose are absorbed at grossly different rates, differences in their metabolism must explain nutritional dissimilarities. When fructose or sucrose are fed to rats a considerable amount of fructose is thought to reach the liver[17]. Fructose which is metabolized by the liver to dihydroxy acetone phosphate and glyceraldehyde and then through the glycolytic sequence to pyruvate, is utilized by liver slices much more rapidly than glucose[18]. By-passing the rate limiting phosphofructokinase step of glycolysis (which is the point of feedback control by ATP and citrate[19]) is likely to result in more rapid conversion of fructose to pyruvate than of glucose to pyruvate. In man, feeding fructose leads to higher concentrations of blood pyruvate than does feeding glucose[20,21]. The increase in pyruvate kinase produced by fructose diets is probably a result of accumulation of a metabolite intermediate between fructose and pyruvate, and because animals fed on starch have a higher content of hepatic pyruvate kinase than starved animals[14] it is probable that the inducer is on that part of the glycolytic pathway common to fructose and glucose metabolism. There is a species difference in control of pyruvate kinase by carbohydrate. Mouse liver pyruvate kinase activities show little alteration with change in diet (unpublished results).

An increase in pyruvate formation with fructose or sucrose diets would cause increased formation of acetyl-Co*A*. The excess acetyl-Co*A* may be utilized for fatty acid synthesis. The increase in the activities of malic and citrate cleavage enzymes, thought to be associated with lipogenesis, is in accord with this view, so it was surprising that the rise in these two enzymes was not accompanied by an increased incorporation of ^{14}C acetate into the lipid of liver slices. It is possible that the postulated increase in the acetate pool would cause dilution of the labelled acetate so that its incorporation would not give an adequate index of lipogenesis.

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[1] Yudkin, J., *Lancet*, i, 1335 (1963).
[2] Macdonald, I., *Proc. Nutrit. Soc.*, **23**, 119 (1964).
[3] Antar, M. A., and Ohlson, M. A., *J. Nutrit.*, **85**, 329 (1965).
[4] Al-Hagdy, S., Miller, D. S., Qureshi, R. U., and Yudkin, J., *Nature* **209**, 81 (1966).
[5] Macdonald, I., and Braithwaite, D. M., *Clin. Sci.*, **27**, 23 (1964).
[6] Macdonald, I., *Amer. J. Clin. Nutrit.*, **16**, 458 (1965).
[7] Kaufmann, N. A., Poznanski, R., Blondheim, S. H., and Stein, Y., *Israel J. Med. Sci.*, **2**, 715 (1966).
[8] Allen, R. J. L., and Leahy, J. S., *Brit. J. Nutrit.*, **20**, 339 (1966).
[9] Taylor, C. B., Bailey, E., and Bartley, W., *Biochem. J.*, **105**, 717 (1967).
[10] Bartley, W., Dean, B., Taylor, C. B., and Bailey, E., *Biochem. J.*, **103**, 550 (1967).
[11] Freedland, R. A., Cunliffe, T. L., and Zinkl, J. G., *J. Biol. Chem.* **241**, 5448 (1966).
[12] Kornacker, M. A., and Lowenstein, J. M., *Biochem. J.*, **95**, 832 (1965).
[13] Yudkin, J., and Krauss, R., *Nature*, **215**, 75 (1967).
[14] Krebs, H. A. and Eggleston, L. V., *Biochem. J.*, **94**, 3C (1965).
[15] Zakim, D., Pardini, R., Herman, R. H., and Sauberlich, H., *Biochim. Biophys. Acta*, **137**, 179 (1967).
[16] Cohen, A. M., and Teitelbaum, A., *Israel J. Med. Sci.*, **2**, 727 (1966).
[17] Kiyasu, J. Y., and Chaikoff, I. L., *J. Biol. Chem.*, **224**, 935 (1957).
[18] Spiro, R. G., and Hastings, A. B., *J. Biol. Chem.*, **230**, 751 (1958).
[19] Passonneau, J. V., and Lowry, O. H., *Advances in Enzyme Regulation* (edit. by Weber, G.), **2**, 265 (Pergamon Press, London, 1964).
[20] Dodds, C., Miller, A. L., and Rose, C. F. M., *Lancet*, ii, 178 (1960).
[21] Leahy, J. S., Noel, P. R. E., Street, A. E., and Worden, A. N., *Lancet*, ii, 1363 (1961).

Tetrapod Characteristics of the Pituitary Gland of *Calamoichthys calabaricus*

Calamoichthys and the better known *Polypterus* comprise the most primitive group of living ray-finned fishes, and are regarded as palaeoniscid relics[1-4]. Some of the earliest reports on the morphology of the fish pituitary gland concern this group[5-7]. In re-evaluating the morphology of the pars distalis in primitive actinopterygian fishes, a portal vascular system between the inferior surface of the infundibulum and the sinusoids of the pituitary gland was discovered in *Calamoichthys*.

India ink injected through the heart of the anaesthetized animal revealed the portal system to good advantage (Fig. 1). Two small arteries ramify within the meninges and converge along the inferior surface of the infundibulum. They then directly enter the pars distalis and constitute the sole demonstrable vascular supply to the entire pituitary gland. The morphology of the primary capillary bed of the portal vessels is similar to that of tailed amphibians[8]. Electronmicroscopy of the median eminence reveals a morphology indistinguishable from that of various amphibians[9,10]. Axons containing elementary neurosecretory granules measuring 1000–1500 Å and synaptic vesicles measuring 500 Å abut against a loose pericapillary space which is 4000 Å wide (Fig. 2). Occasional fenestrae are seen in the capillary endothelium. There seems to be no specialization of the pars distalis into a terminal zone or tuberal region as in the lungfish *Protopterus*[11,12] or in tetrapods.

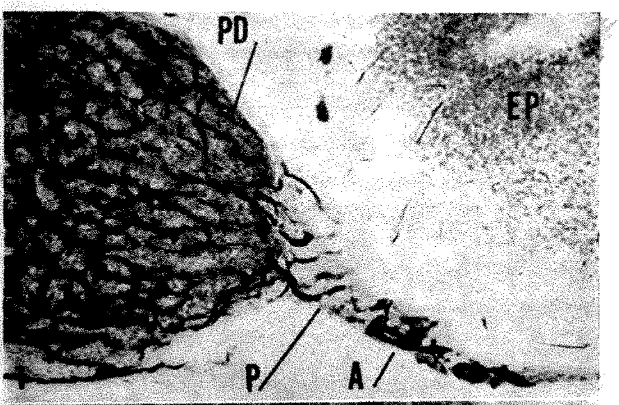

Fig. 1. Parasagittal 50μ thick nitrocellulose section of median eminence. India ink was injected. A, Artery of primary capillary bed; EP, ependyma of third ventricle; P, portal vessels; PD, pars distalis.

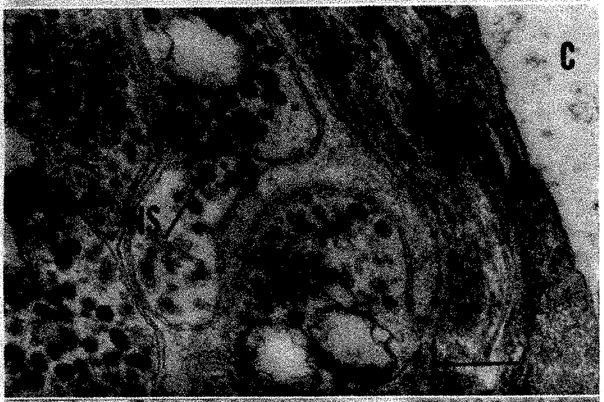

Fig. 2. Electronmicrograph of median eminence. Line at lower right indicates 0·5μ. C, Capillary lumen; E, endothelium; PC, pericapillary space; NS, neurosecretory granule.

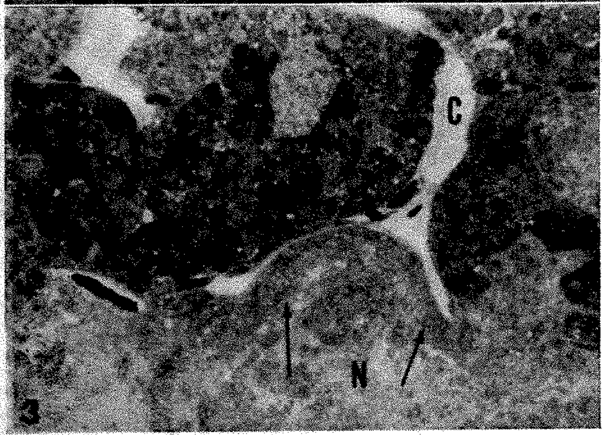

Fig. 3. Araldite 0·5μ thick section of pars intermedia, PI, and neurohypophysis, N. Arrows indicate concentration of neurosecretory material about capillary lumina.

In addition to a median eminence and hypothalamo–hypophysial portal system similar to that of amphibians, there are specialized pericapillary neurohaemal contacts within the neurohypophysis. These are similar to the neurohaemal contacts of the pars nervosa of the amphibian pituitary gland (Fig. 3). Electronmicroscopy of these areas reveals elementary neurosecretory granules measuring 1500–2000 Å and of lesser electron density than those of the median eminence.

Typical piscine features of the pituitary gland of *Calamoichthys* are the interdigitation of pars intermedia and neurohypophysis, and the presence of a saccus vasculosus with Kroenchenzellen.

Calamoichthys apparently retains the primitive hypothalamo–hypophysial portal system, a feature which it shares in a modified form with the elasmobranchs, chimaeras, lungfish and tetrapods. The loss of the portal system in ganoid and teleost fishes may be a secondary feature, associated with the evolutionary trend toward direct neurosecretory and secretomotor innervation of the pituitary gland in the latter group[13,14].

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[1] Goodrich, E. S., *Palaeobiology*, **1**, 87 (1928).
[2] Kerr, J. G., *J. S. Budgett Mem. Vol.* (Cambridge University Press, 1907).
[3] Berg, L. S., *Trav. Inst. Zool. Acad. Sci. USSR*, **5** (Ann Arbor, Michigan, 1947).
[4] Romer, A. S., *Vertebrate Paleontology*, third ed. (University of Chicago Press, 1966).
[5] Waldschmidt, J., *Anat. Anz.*, **2**, 308 (1887).
[6] Bickford, E. E., *Anat. Anz.*, **10**, 465 (1895).
[7] Gérard, P., and Cordier, R., *Ann. Soc. Roy. Zool. Belg.*, **67**, 87 (1937).
[8] Green, J. D., *Anat. Rec.*, **99**, 21 (1947).
[9] Oota, Y., and Kobayashi, H., *Zeit. f. Zellfor.*, **60**, 667 (1963).
[10] Smoller, C. G., *Gen. Comp. Endocrinol.*, **7**, 44 (1966).
[11] Wingstrand, K. G., *Vidensk. Medd. Dansk Naturhist. For.*, **118**, 193 (1956).
[12] Kerr, T., and van Oordt, P. G. W. J., *Gen. Comp. Endocrinol.*, **7**, 549 (1966).
[13] Follenius, E., *Arch. D'Anat. Microsc.*, **54**, 195 (1965).
[14] Vollrath, L., *Zeit. f. Zellfor.*, **78**, 234 (1967).

Biological Action of Blood Serum of Schizophrenic Patients in relation to Stress

THE presence in the blood of toxic substances, the action of which on the brain is presumed to cause disturbances in mental function, is frequently suggested as a factor in the pathogenesis of schizophrenia[1-4]. The sources and the chemical nature of such substances are still unknown. Many publications claim, however, that the body fluids, such as blood and urine, of schizophrenic patients show a wide spectrum of pathological activity on various test objects and the psychiatric literature usually refers to this as "toxic action". The body fluids of schizophrenic patients seem to change the functions not only of various processes in isolated biological systems (for example, isolated cells, tissue cultures and biochemical processes), but also the function of whole organisms.

It is not yet known, however, whether such reactions are specific or non-specific (for example, stress reactions). The non-specific reactions of the body fluids of schizophrenic patients have not been sufficiently investigated, although this question is of primary importance, especially in the study of responses in animals where the reaction to any injurious effect (for example, to the action of stressors) commonly includes many different patterns of adaptation-stress-reaction.

This communication presents the results of some investigations of stress reactions in animals to which blood serum from schizophrenic patients had been administered.

The experiments were carried out with rabbits and rats. The serum was administered intravenously to rabbits in a dose of 3 ml./kg body weight and a dose of 10 ml./kg body weight to rats. Sera from sixty-four patients with periodic schizophrenia, from fifty-six patients with nuclear schizophrenia and from fifty-seven normal individuals were used. 11-Hydroxy-corticosteroids were estimated before administration of the serum and at 2·5 h intervals afterwards. Blood sugar and lymphocyte counts were also taken before injection and afterwards at intervals of 1, 2 and 2·5 h. After the animals had been killed, internal organs were examined and the concentrations of adrenaline and noradrenaline in the hypothalamic region of the brain were estimated. The concentrations of corticosteroids in the blood and catecholamines in the brain were determined by fluorimetric methods[5–7].

After the administration of serum from the normal controls in the rabbits, corticosteroids showed a rise in concentration after 1 h (at 2·5 h the concentration was 5·9 ± 1·0 mg/100 ml. $P < 0{\cdot}05$ compared with normal—3·8 ± 0·7 mg) and there was a fall in the lymphocyte count (after 2·5 h—55 per cent from the baseline, $P < 0{\cdot}001$). There was also a temporary hypoglycaemia (13 per cent below baseline). In the internal organs there was no severe alteration, and only a hyperaemia was observed. In the hypothalamus there was an increase in the adrenaline content of brain tissue; 0·32 mg/g compared with the normal 0·07 mg/g, $P < 0{\cdot}05$. These results are presented in Fig. 1: we suggest that they show the activation of the function of the hypothalamus–pituitary hypophysis–adrenal cortex system.

The structure, the extent and the degree of the reaction of the animals to the injection of serum from schizophrenic patients were all different and, moreover, some of the differences in reaction seemed to be related to the pattern of development of the disease (that is, whether it was periodic or nuclear).

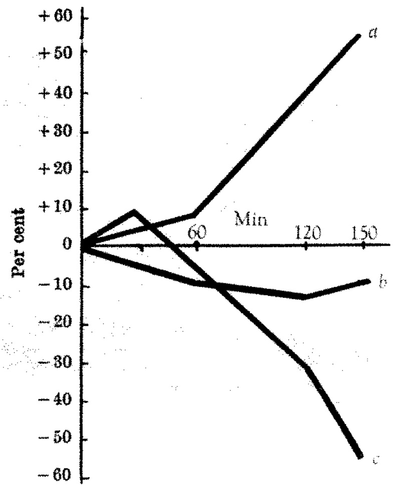

Fig. 1. Blood corticosteroids (*a*), sugar (*b*) and lymphocytes (*c*) after the administration of the serum of normal persons to rabbits.

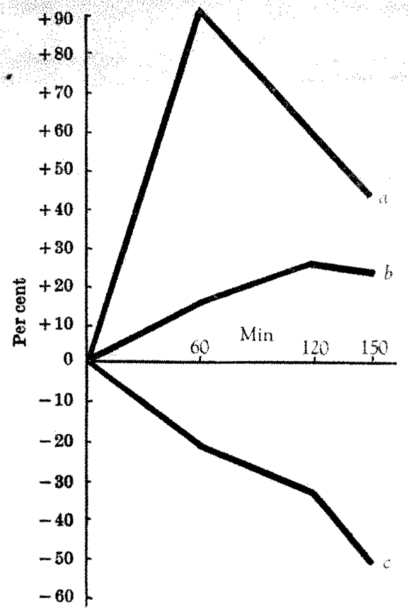

Fig. 2. Blood corticosteroids (*a*), sugar (*b*) and lymphocytes (*c*) after the administration of the serum of patients with periodic schizophrenia.

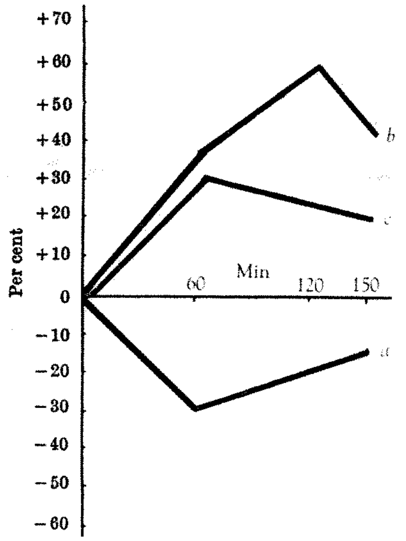

Fig. 3. Blood corticosteroids (*a*), sugar (*b*) and lymphocytes (*c*) after the administration of the serum of patients with nuclear schizophrenia to rabbits.

After the administration of serum from patients with periodic schizophrenia, a pattern of activation of the hypothalamic–hypophysis–adrenal cortex system was found similar to that after the injection of normal serum. There was a statistically significant increase in the concentration of corticosteroids after 1 h (1 h, 6·9 ± 1·1 mg/100 ml.; 2·5 h, 5·1 mg/100 ml., compared with a baseline of 3·6 ± 0·8 mg/100 ml.) and a fall in lymphocytes (after 2·5 h to 50 per cent of the baseline, $P < 0{\cdot}001$). In this group, however, a rise of 15–20 per cent in blood sugar was observed as well as the lymphopenia and this was assumed to be a corticoid hyperglycaemia because of the statistical correlation between the two reactions (Fig. 2). In this group there was also a statistical augmentation in the hypothalamus not only of adrenaline but also of non-adrenaline (non-adrenaline was 1·07 mg compared with 0·43 mg in normal animals). On examination of the internal organs a hyperaemia associated with very small haemorrhages along the intestinal tract was observed, especially in the diverticuli

After administration of serum from patients with nuclear schizophrenia (that is, schizophrenia with a gradual and malignant progress) the reaction pattern in the rabbits was different again The most important difference was the absence of any augmentation of the concentration of corticosteroids as well as the absence of a lymphopenic reaction (Fig 3) In these cases, typical stress reactions were not obtained, and in the hypothalamic region there was no increase in catecholamines In some cases, however, there were marked changes in the internal organs, consisting of massive haemorrhages and sometimes small ulcers especially along the intestinal tract Furthermore, sometimes in this group, there was a marked stable hyperglycaemia (up to 100 per cent of the normal) Similar results were found in the experiments on rats

These features of animal stress reaction to the administration of serum from patients with different forms of the disease could have great importance not only for the interpretation of the biological ("toxic") action of the serum of schizophrenic patients, but also in providing clues concerning the pathogenesis of this disease and providing an understanding of some of the clinical–somatic symptoms

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[1] Kety, S S , *Science*, 129, 1528, 1590 (1959).
[2] McGeer, E G , and McGeer, P L , *J Ment Sci* , 105, 2 (1959).
[3] *Serological Fraction in Schizophrenia* (edit by Heath, R. G.) (New York, 1963)
[4] Vartanian, M E , *J Chem Assoc USSR*, 4, 362 (1964)
[5] Bertler, A , *et al* , *Acta Physiol Scand* , 44, 3 (1958)
[6] Guillemin, R , *et al* , *J Lab Clin Med* , 53, 830 (1959).
[7] Zenker, N , and Bernstein, D , *J Biol Chem* , 4, 695 (1958)

Relationship between Total Body and Muscle Respiratory Rates in Infants with Malnutrition

Montgomery[1] and Monckeberg[2] have observed altered levels of oxygen consumption by malnourished infants before and during therapy of nutritional deficits There are indirect indications of changes in tissue oxygen metabolism in malnourished children Glucose tolerance tests reveal delayed clearance of intravenous glucose loads[3–4] It has been found that the malnourished infant has a reduced ability to utilize acetate, glucose and pyruvate when these were administered as ^{14}C isotopes[5].

There have been several enzyme studies of liver and muscle tissue from malnourished children[6–14] As previously noted[12], the study of isolated enzymes *in vitro* may have little bearing on the altered physiology of malnutrition *in vivo*. There is greater physiological significance in work on *in vitro* activities of enzyme chains or coupled reactions which are dependent on structural integrity[10] We have compared the oxygen uptake of skeletal muscle with the total metabolic rate of infants recovering from malnutrition

The infants varied in age from 8 to 30 months The clinical characteristics of infantile malnutrition in Jamaica have already been described[1,15].

The fasting infant was lightly sedated with chloral hydrate or paraldehyde Oxygen consumption was determined in an open system while the subject slept A constant flow of room air was maintained for 30 minutes through the system A portion of the effluent air was analysed for oxygen with an oxygen cell and for carbon dioxide by a manometric technique For comparison with tissue respiratory rates, the basal oxygen consumption is expressed as QO_2, ml O_2/kg tissue/h

Table 1 EFFECT OF MALNUTRITION ON OXYGEN CONSUMPTION

	Total body (mequiv /kg)	No of observations	QO_2 µl /mg wet wt/h Body	QO_2 µl /mg wet wt/h Muscle	QO_2 µl /mg protein/h Muscle
A	Less than 30	2	0 29	0 32	3 73
B	30 to 40	10	0 43 ± 0 03	0 35 ± 0 04	8 12 ± 1 88
C	Greater than 40	12*	0 59 ± 0 03	0 59 ± 0 08	7 43 ± 1 28
	P value (difference between groups *B* and *C*)		0 02	0 001	0 05

* Total body potassium was determined on only six of these subjects The others had completely recovered from malnutrition and are assumed to be in the normal range of total body potassium In this analysis the average of duplicate QO_2 determinations was utilized Means and standard error are given

Muscle samples weighing 10–20 mg were obtained by needle biopsy of the quadriceps muscle[12] within 2 h of the measurement of basal oxygen consumption. After weighing, the biopsy sample was homogenized by hand in 0 05 ml of 0·25 molar sucrose The glass homogenizer was kept in a brass block on ice The homogenate was centrifuged for 5 min at approximately 300*g* at 5° C and the supernatant was used for measurement of oxygen consumption Protein content of the homogenate was determined by the method of Lowry *et al* [17].

Tissue respiration was measured in duplicate by the cartesian diver method[10] The reaction mixture consisted of equal parts of potassium phosphate buffer (*p*H 7 4), magnesium chloride (0 2 molar) and sodium succinate (0·3 molar) After a 5 min equilibration period, three manometric readings were obtained at 10 min intervals and averaged to determine the rate of respiration Results were calculated as ml of O_2/kg wet weight/h (QO_2/wet weight) and µl. of O_2/mg protein/h (QO_2/protein).

Whole body potassium was measured in a 4 π whole body counter described by Garrow[16]. The subjects were counted to an accuracy of 6 per cent and the results corrected for self absorption

The total body potassium content can be used as one index of the degree of malnutrition These values were used to group the subjects in Table 1. A significant difference was observed between groups *B* and *C*, the two groups large enough for statistical evaluation Both the muscle and total body QO_2/wet weight were reduced in the subjects with reduced total body potassium No reduction in muscle QO_2/protein content of homogenate supernatant was seen

There is a correlation between basal oxygen consumption of the body and *in vitro* QO_2/wet weight With twenty-three duplicate samples the correlation coefficient was 0 525 The relation is described by $Y = 23 + 0\ 84X$, where 23 is the Y intercept and 0 84 is the computed slope, the points are plotted in Fig 1

A significant decrease in muscle respiration occurs in malnourished children It is proportional to the change in basal metabolic rate The fluctuations in oxygen consumption of the malnourished infants[1–2] are therefore related to alterations within tissues as well as altered respiring tissue mass

Succinate dehydrogenase is localized in mitochondria[20] The observed alterations in tissue respiration may be linked to alterations in muscle mitochondrial content Montgomery[19] demonstrated that a reduction in muscle cytoplasm occurs in severe malnutrition It is reasonable to assume that the number of mitochondria is also reduced The results could be explained by depletion of mitochondria because the oxygen consumption per mg protein (that is, per mitochondrion) is unchanged by malnutrition We cannot, however, rule out the possibility of the inhibition of mitochondrial respiration

In healthy adults, muscle oxygen consumption accounts for only 25 per cent of basal energy expenditure[21] In malnourished children, muscle mass/kg body weight is reduced to half compared with that of the adult[15] Muscle metabolism must account for only a small fraction of basal metabolism of the malnourished child

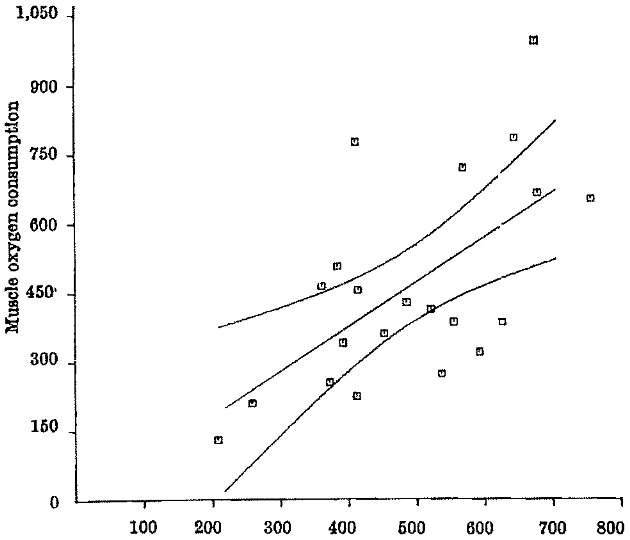

Fig 1 The relationship between *in vitro* muscle and *in vivo* total body oxygen consumption in infants recovering from malnutrition The points are means of duplicate observations on muscle homogenate The calculated slope and 94 per cent confidence limits are indicated on the graph

Because of the demonstrated relationship to total oxygen consumption, the alterations in muscle respiration must reflect alterations in other tissues

Waterlow[10] studied liver homogenate respiration with the same techniques in a similar group of malnourished children No significant alterations in succinate oxidation, based on fat-free dry weight, were observed Data from chemical analyses of the liver from malnourished children[22] reveal smaller changes in the K to N ratio than those seen in muscle[23] Sparing of the liver may be at the expense of muscle depletion We suggested that muscle has a buffering capacity against depletion of liver potassium and possibly other vital viscera[23]. This biological "law" has been proposed by Levinson and Watkin[24] and Waterlow and Stephen[25] for nitrogen homeostasis It may be true for tissue respiration as well as for electrolyte metabolism

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[1] Montgomery, R D , *J Clin Invest* , 41, 1653 (1962)

[2] Monckeberg, F , Beas, F , Horwitz, I , Dabancens, A , and Gonzalez, M , *Pediatrics*, 33, 554 (1964)

[3] Slone, D , Taits, L S , and Gikhirst, G S , *Brit Med J* , i, 32 (1961)

[4] Bowie, M D *S A Med J* ,1, 44 (1964)

[5] Gillman, J , Gillman, T , Scrugg, J , Savage, N , Gilbert, G , Trout, G , and Levy, P , *S Afr J Med Sci* ,26, 31 (1961)

[6] Waterlow, J C , *W I Med J* ,1, 44 (1958)

[7] McLean, A E M , *Clin Sci* , 30, 192 (1966)

[8] Burch H B , Arroyave, G , Schwartz, R , Padilla, A , Behar, M , Viteri, F., and Scrimshaw, N S , *J Clin Invest* , 34, 1579 (1957)

[9] Fletche, K , *Amer J Clin Nutrit* ,19, 170 (1966)

[10] Waterlow, J C , *Proc Roy Soc* , B, 155, 96 (1961)

[11] Mukherjee, K L , and Sarkar, N K , *Brit J Nutrit* ,12, 1 (1958)

[12] Waterlow J C , and Stephen, J M L , *Proc World Conf Gastroenterology*, 691 (1958)

[13] Metcoff, J , Frenk, S , Yoshida, T , Torres-Pinedo, R , Kaiser, E , and Hansen, J D L , *Medicine*, 45, 365 (1966)

[14] Cat, I , Campello, A P , Voss, D O , Graga, H , and Bacila, M , *Lancet*, ii, 416 (1963)

[15] Waterlow, J C , Cravioto, J , and Stephen, J M L , *Adv Protein Chem* ,15, 131 (1960)

[16] Nichols, B L , Hazlewood, C F , and Barnes, D J (submitted for publication)

[17] Lowry, O H , Rosebrough, N J , Farr, A L , and Randall, R J , *J Biol. Chem* ,193, 265 (1951)

[18] Garrow, J S , *W I Med J* ,14, 73 (1965)

[19] Montgomery, R D , *J Clin Pathol* ,15, 511 (1962)

[20] Mahler, H R , and Cordes, E H , *Biological Chemistry*, 398 (Harper and Row, New York, 1966)

[21] Mottram, R F , *J Physiol* ,128, 268 (1955)

[22] Garrow, J S , Fletcher, K , and Halliday, D , *J Clin Invest* , 44, 417 (1965)

[23] Nichols, B L , Alleyne, G A O , Hazlewood, C F , and Waterlow, J C (submitted for publication)

[24] Levinson, S M , and Watkin, D M , *Fed Proc* ,18, 1155 (1958)

[25] Waterlow, J C , and Stephen, J M L , *Brit J Nutrit* ,20, 461 (1966)

BIOLOGY

Interoceanic Colonization of a Marine Goby through the Panama Canal

THE Panama Canal (Fig 1) represents a potential pathway for the interoceanic dispersal of marine fishes The fresh waters of Lakes Gatun and Miraflores have, however, effectively maintained the isolation of all but a few, of the marine shore fishes which are found on both coasts of the isthmus, those species which are able to cross this barrier are not known to breed on the opposite coast[1]

The neotropical genus *Lophogobius* is represented in Panama by two species, *Lophogobius cristulatus* (Ginsburg) from the Pacific coast of Panama and Costa Rica and *Lophogobius cyprinoides* (Pallas) which is widespread throughout the Caribbean We report here a breeding population of *L cyprinoides* in the Pacific, and examine some aspects of this successful colonization through the canal

The Miraflores Third Lock area (Fig 2), in which this population was found, is unique in many ways The Third Locks project was begun by the Panama Canal Company in 1941 in an effort to provide an auxiliary set of locks for the canal. When the project was abandoned in 1943, a large excavation 4,400 ft long, 300–500 ft wide and 60–85 ft deep was left adjacent to the Pacific entrance of the Canal The excavation was filled with sea water from the nearby canal entrance and with fresh water from run-offs At present the exchange of water between this lagoon and Panama Bay is maintained only at high tides by means of several underground culverts As a result of this arrangement the water in the Third Lock is brackish, being about 12‰ at the surface and 18‰ at 50 ft This is in contrast to the figure of approximately 30‰ in adjacent areas of Panama Bay The Third Lock experiences only a few inches rise and fall of water level as compared with the Pacific tidal oscillation which averages 16 ft This relatively low tidal amplitude is analogous to that found on the Atlantic coast where the average tidal range is less than 1 ft The steep basalt sides of the Third Lock are covered with oyster shells which provide the major habitat for *Lophogobius* The presence of adherent oyster shells in a non-tidal situation is unusual on the Pacific coast; in most cases, their periodic exposure during low tides renders them unsuitable cover for fishes.

Lophogobius cyprinoides seems to be the dominant species in the Third Lock Other goby and eleotrid genera, centropomids and lutjanids are sometimes found along the occasional shallow mangrove areas, but only the latter group seem to be present in significant numbers

Numerous individuals of *Lophogobius* from the Third Lock and Atlantic localities were examined to determine whether any morphological differences existed between the two populations The evidence indicated that the specimens from both localities constituted one homogeneous population

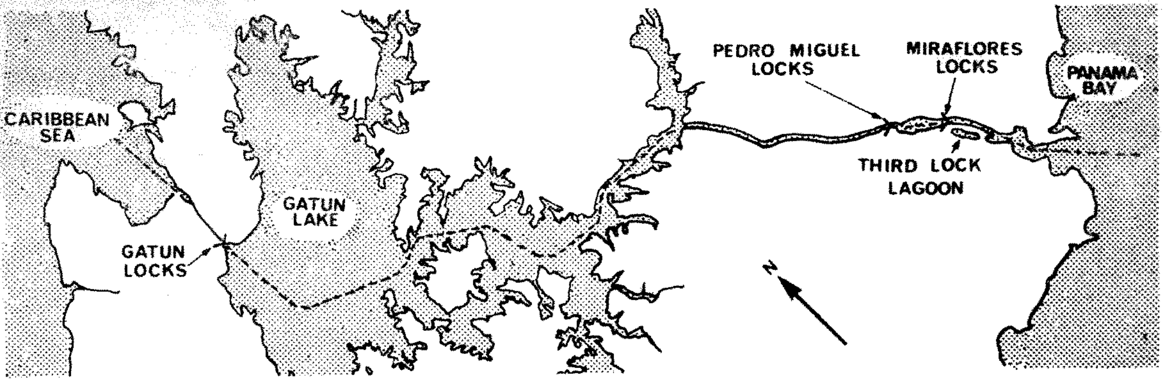

Fig. 1. The Panama Canal. The distance between the Atlantic and Pacific termini is approximately 50 miles.

Breeding tests were designed to detect the presence of any reproductive isolating mechanisms which could have evolved in these two groups. Control pairs of *L. cyprinoides* from both localities as well as pairs of the Pacific species *L. cristulatus* were established in 10 and 20 gallon aquaria which were continuously supplied with running sea water. Pairs of the six possible combinations between these three populations were also isolated. Fishes were fed minced shrimp and clams daily and each pair was provided with a triangular clay tile nest site. The results of these breeding experiments are summarized in Table 1. All the control groups, particularly those of *L. cyprinoides*, bred freely in the laboratory. All the crosses between individuals of *L. cyprinoides* from the Atlantic and Pacific localities produced frequent spawnings. No spawnings were obtained from the hybrid pairs of *L. cyprinoides* × *L. cristulatus*. This is not surprising because *L. cristulatus* is morphologically quite distinct from *L. cyprinoides*. It does indicate, however, that species of *Lophogobius* are unlikely to spawn indiscriminately when confronted with a no-choice mating situation. The larvae of both control (Atlantic × Atlantic) and experimental (Atlantic × Third Lock) spawnings of *L. cyprinoides* have now been raised past metamorphosis with no apparent differences in viability. It should be possible to determine the fertility of the F_1 generation in order to investigate further the presence of possible incipient genetic differences between the parental and colonizing population of *L. cyprinoides*.

Table 1. RESULTS OF BREEDING EXPERIMENTS WITH PANAMANIAN SPECIES OF *Lophogobius*

Species crossed	No. of pairs examined	Total days paired	No. of spawnings observed
L. cyprinoides × *L. cyprinoides* Atlantic Atlantic	3	167	12
L. cyprinoides × *L. cyprinoides* Third Lock Third Lock	2	90	10
L. cristulatus × *L. cristulatus* Pacific Pacific	7	371	5
L. cyprinoides × *L. cyprinoides* Atlantic Third Lock	5	286	22
L. cristulatus × *L. cyprinoides* Pacific Atlantic	3	311	0
L. cristulatus × *L. cyprinoides* Pacific Third Lock	3	160	0

This is the first record of a species of fish passing through the Panama Canal and successfully colonizing the opposite coast. The fauna of this region is not well known, however, and therefore it is quite possible that a careful survey would reveal other similar cases of successful amphi-American migrations. The success of *L. cyprinoides* in colonizing the Miraflores Third Lock and its failure to colonize other areas along the Pacific coast may be the result of a number of factors. When it was completed, the Third Lock represented an unoccupied habitat with a relatively large area capable of supporting demersal fishes. A euryhaline species such as *L. cyprinoides* was pre-adapted to colonize successfully a brackish water area[2,3]. At the same time the reduced salinity of this area undoubtedly prevented many of the stenohaline species which inhabit adjacent areas of Panama Bay from invading the Third Lock. The lack of competition from other bottom dwelling species, particularly Blennidae and Gobiidae, could account for the dense population of *L. cyprinoides*. In spite of the abundance of *L. cyprinoides* in the Third Lock, the species does not seem to have dispersed further into adjacent Pacific coastal areas. The factor limiting their distribution could be the lack of a suitable unoccupied niche rather than either the inability of the species to adapt to the Pacific environment or the fact that the numbers of individuals are insufficient to constitute a propagule. No indications of any morphological changes or presence of reproductive isolating

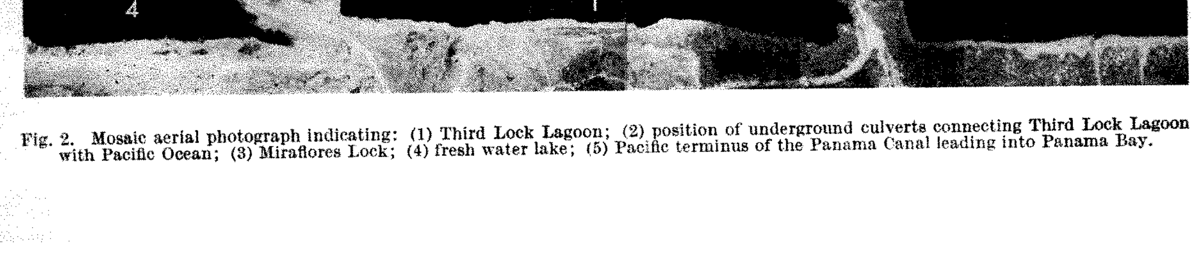

Fig. 2. Mosaic aerial photograph indicating: (1) Third Lock Lagoon; (2) position of underground culverts connecting Third Lock Lagoon with Pacific Ocean; (3) Miraflores Lock; (4) fresh water lake; (5) Pacific terminus of the Panama Canal leading into Panama Bay.

mechanisms were found, and at present the colonizing population seems to be identical to the parental stock. This is not surprising because the founding population could have been large and only a short isolation period has elapsed. Indeed, it is quite probable that gene flow is still maintained through the canal.

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[1] Hildebrand, S. F., *Zoologica*, **24**, 15 (1938).
[2] Breder, C. M., *Amer. Mus. Nov.*, **551**, 1 (1932).
[3] Hollister, G., *Bull. NY Zool. Soc.*, **37**, 183 (1934).

Differences in the Quality of Birds nesting in the Centre and on the Edges of a Colony

IN many bird colonies there is a surplus of adult birds in or about the colony which fail to breed[1,2]. In several species, the failure to breed can be attributed to the inability to obtain a suitable nest-site within the colony limits, and this results in competition for the limited number of available sites. Experiments on the kittiwake *Rissa tridactyla* have shown that some of these non-breeding adults will breed if suitable unoccupied ledges are made available in the centre of the colony, but similar sites remain unused on the periphery (Coulson, in preparation). This and other observations indicate that the competition among males, which are responsible for nest-site selection, is more intense in the colony centre and investigations were made to determine whether the birds nesting centrally in the colony differed in quality from those breeding near the edge.

The kittiwake colony at North Shields, Northumberland has been studied in detail during the past 15 years and all the birds have been individually marked for recognition[4]. For purposes of analysis, the colony has been divided into two sections, the centre and the edge, according to the position and density of the nests. The adult mortality rates and breeding biology of these two groups have been compared.

Mortality rates have been estimated from the disappearance of marked birds. The breeding birds show a very strong site and colony tenacity[3], and there is no evidence of birds moving from colony to colony. The annual mortality rates of male and female kittiwakes breeding in the centre and on the edge of the colony are shown in Table 1. The mortality rate among males nesting on the edge is about 60 per cent higher than in those nesting in the centre, but the difference between the two groups of females is not significant. This differential mortality could result from differences in the conditions in the two areas, or it could be caused by a selection of birds at the time of recruitment into the two areas. There is no indication that "stress" is greater at the edge of the colony; there is considerable evidence suggesting the opposite. The weight of males at the time of first breeding differs by a small but significant amount between the two areas (Table 2), but there is no such difference in the females, nor is there a significant difference between the mean wing lengths of the males. Those males recruited to the edge appear to be of a similar structural size to those which enter the centre of the colony, but they differ slightly in the quantity (and perhaps quality) of certain body tissues.

Table 1. MEAN ANNUAL MORTALITY RATES OF MALE AND FEMALE KITTIWAKES NESTING AT THE EDGE AND IN THE CENTRE OF THE COLONY

	Centre			Edge		
	No. of bird-years at risk	No. dying	Percentage mortality	No. of bird-years at risk	No. dying	Percentage mortality
Males	404	47	11·6	307	57	18·6
			($\chi^2 = 6·17$, 1 d.f. $P < 0·02$)			
Females	412	34	8·3	332	38	11·4
			($\chi^2 = 2·15$, 1 d.f. Not significant)			

Table 2. WEIGHTS AND WING LENGTHS OF MALE AND FEMALE KITTIWAKES BREEDING IN THE CENTRE AND ON THE EDGE OF A COLONY

	Centre			Edge			
	No.	Mean weight (g)	*S.E.*	No.	Mean weight (g)	*S.E.*	Difference (centre edge)
Males							
All breeding birds	108	393·5	±2·1	105	385·9	±2·0	+7·6*
Survived more than 5 yr	36	397	±4·1	28	391	±4·9	+6·0
Died within 5 yr	53	388	±3·4	51	383	±2·9	+5·0
Mean wing length	99	308·8	±0·65	101	309·7	±0·64	−0·9
Females							
All breeding birds	83	347·9	±2·9	84	350·9	±3·3	−3·0
Survived more than 5 yr	27	358	±5·2	21	360	±7·0	−2·0
Died within 5 yr	37	345	±3·5	43	344	±4·4	−1·0
Mean wing length	93	299·8	±0·88	94	301·8	±0·74	−2·0

* $P < 0·01$.

All weights and measurements were taken during the first year of breeding.

Table 2 shows that both the male and female kittiwakes which survive 5 years or more from the time of first breeding are significantly heavier at the time of recruitment than their neighbours which died within this period ($P < 0·01$ in each sex). It seems that subsequent survival is partly related to the body weight at the time of recruitment. It is possible to account for much of the observed differential mortality between males breeding on the edge and in the middle of the colony because of the higher proportion of low weight males recruited at the edge of the colony. The intense competition for nest sites in the centre results in very high selection for vigorous males with less severe selection towards the edge of the colony.

Other aspects of the breeding biology of the kittiwakes in the two areas show significant differences. There are a larger mean clutch size, higher hatching success and more young fledged per pair in the centre of the colony (Table 3). There is also a large difference in the proportion of birds which change their mate from one breeding season to the next. It has been shown that breeding success is lower among kittiwakes which are forming the particular pair for the first time and that age also influences the fecundity[4]. More investigations are required to establish the role of these factors in producing the differences in the breeding biology shown in Table 3. The differences in the reproductive rate, however, between birds breeding in the centre and on the edge of the colony seem to be the direct or indirect result of variations in the quality of the males recruited in different parts of the colony. None of the differences were the result of predation.

These results have important implications in ecological studies on colonial birds and in understanding colonial organization. It is important to study representative samples of birds when measuring the mortality rates of colonial birds and also when estimating the mean reproductive success of the species. These findings also suggest that the adult birds which fail to obtain a breeding site may be individuals of even poorer quality than those which succeed in acquiring an edge site. If this is the case, it would seem probable that their survival rate may be much lower than any group of breeding birds and the loss to the population of this breeding potential may not

Table 3. COMPARISON OF THE BREEDING BIOLOGY OF KITTIWAKES BREEDING ON THE EDGE AND IN THE CENTRE OF THE COLONY

	Centre	Edge
No. of nests	433	323
Clutch size	2·18 ± 0·024	2·03 ± 0·028*
Hatching success	76 ± 1·4 per cent	70 ± 1·8 per cent*
Breeding success	68 per cent	65 per cent
Young fledged/pair	1·48	1·32*
Per cent change mate/year	29 per cent	54 per cent*
Mean date of first egg	May 16	May 20*

* $P < 0·01$.

be as great as would appear from proportions of adult non-breeding birds.

I thank Smith's Dock Co., Ltd. (North Shields), for giving me access to the colony of kittiwakes. This work was supported by grants from the Nature Conservancy and the Science Research Council.

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Received December 27, 1967.

[1] Wynne-Edwards, V. C., *Animal Dispersion in Relation to Social Behaviour* (Oliver and Boyd, 1962).

[2] Coulson, J. C., and White, E., *Ibis*, **98**, 63 (1956).

[3] Coulson, J. C., and White, E., *Ibis*, **100**, 40 (1958).

[4] Coulson, J. C., *J. Anim. Ecol.*, **35**, 269 (1966).

Chemical Protection against X-irradiation by a New Reducing Agent 1,4-Dithiothreitol in Marsupial Leucocytes in Culture

THE sulphydryl compounds[1] are among the most effective and useful groups of chemicals with a protective action against the damaging effects of X-rays in living organisms. In 1964, dithiothreitol (DTT) was put forward by Cleland[2] as a new protective agent for sulphydryl groups against oxidation. Because DTT is a solid with high solubility in water with a very low vapour pressure and little tendency to be oxidized by air, it should be superior in these respects to the thiols already in use, some of which are sparingly soluble or have an objectionable smell[2]. No data have been reported on DTT as a protective agent against irradiation damage, for which it would seem to be particularly well suited. We have irradiated leucocyte cultures of the potoroo *Potorous tridactylus* in the presence and absence of DTT to determine the protective action of DTT against induced chromosome breakage.

The low chromosome numbers (2n = 13 in male and 12 in female) and the distinct morphology of the potoroo chromosomes enable an accurate estimation of damage at metaphase to be made. Translocations involving two or more chromosomes can be recognized without ambiguity.

Leucocyte cultures were prepared from oxygenated blood obtained from an anaesthetized potoroo by heart puncture using an intraperitoneal injection of 60 mg/ml. of 'Nembutal' (1 ml./4 lb. body weight). Samples of 5 ml., half of which received 0·5 ml. of DTT (0·1 mg of DTT/ml. of whole blood) 1 h before irradiation, were irradiated with 200 r. at 100 r./min (half value layer 0·5 mm copper; 235 kV peak). Treated and control cultures were kept at room temperature. One hour later, all blood samples were mixed with a few drops of phytohaemagglutinin (PHA, Wellcome) and after a few minutes the samples were centrifuged for 5 min at 100*g*. The supernatant plasma with suspended leucocytes was removed, and the leucocytes precipitated by centrifuging for 7 min at 100*g*. The supernatant was discarded and the cells were washed twice in complete medium to remove traces of DTT and autologous serum. A sample (0·5 ml.) of complete medium was added to each sample of washed leucocytes from 5 ml. of blood; the cells were dispersed and were placed in culture flasks with 8 ml. of complete medium (Parker's 199, 12 per cent foetal calf serum and 2 per cent PHA). DTT solution was made by dissolving 50 mg of dithiothreitol (Cleland's reagent, Calbiochem) in 50 ml. of Hanks balanced salt solution (BSS) sterilized by filtration. Cultures were incubated for 72 h at 37° C with 'Colcemid' (Ciba) added for the last 2 h of the culture period to give a final concentration of 0·004 per cent. Leishman microscopic preparations were made after hypotonic treatment and fixation (1 glacial acetic : 3 ethanol).

Table 1. CHROMOSOME DAMAGE IN LEUCOCYTE CULTURES FROM X-IRRADIATED FULLY OXYGENATED BLOOD OF *Potorous* WITH AND WITHOUT DITHIOTHREITOL

	X-rays	DTT	Chromatid deletions	Chromosome deletions	Two-hit events, chromosome	Cells scored
DDT treatment	+	+	5	66	5	100
	+	+	2	63	7	100
	+	+	2	62	10	100
Mean	+	+	3	64 ± 1·01	7 ± 1·4	300
DTT control	+	−	10	79	18	100
	+	−	8	70	26	100
Mean	+	−	9	75 ± 4·5	22 ± 4·0	200
Spontaneous aberration	−	−	2	12	0	100
Mean*	−	−	7 ± 1·6	12 ± 1·4	0	400

* Mean for parallel controls done at other times but in similar conditions. X-ray dose was 200 r. at a rate of 100 r./min; DTT dose was 0·1 mg/ml. whole blood.

All cultures gave good mitotic indices and chromosomal damage was noted as follows: chromatid and iso-chromatid deletions were taken as one-hit events, and chromosomal interchanges, such as conjugation of an acentric pair with another chromosome, rings and dicentrics, were noted as two-hit events.

All the damage can be expected to be chromosomal because all the cells were irradiated in G_1. Spontaneous aberration, however, will produce chromatid deletions and some iso-chromatid deletions. Some iso-chromatid deletions are indistinguishable from chromosome deletion produced by irradiation. In Table 1 the level of spontaneous breakage is shown in concurrent control cultures and in parallel controls made at different times. It is clear that there is no significance in the chromatid deletions produced by spontaneous breakage. Significant differences, however, occur in the number of aberrations of the chromosome type. The presence of DTT significantly decreases chromosome aberrations in irradiated cultures; the chromosome deletions are reduced by 17 per cent and the two-hit events by as much as 68 per cent. If the damage is calculated as the number of hits per cell, then the presence of DTT during irradiation reduces the total damage by a half (Table 2).

Table 2. PROTECTION AGAINST X-RAY INDUCED CHROMOSOME BREAKAGE BY DITHIOTHREITOL

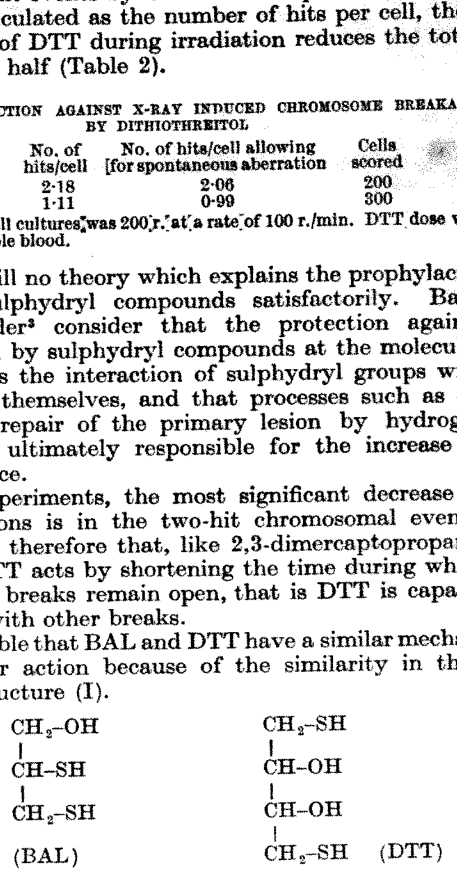
DTT present	No. of hits/cell	No. of hits/cell allowing [for spontaneous aberration	Cells scored
−	2·18	2·06	200
+	1·11	0·99	300

X-ray dose to all cultures was 200 r. at a rate of 100 r./min. DTT dose was 0·1 mg/ml. of whole blood.

There is still no theory which explains the prophylactic action of sulphydryl compounds satisfactorily. Bacq and Alexander[3] consider that the protection against X-irradiation by sulphydryl compounds at the molecular level involves the interaction of sulphydryl groups with the radicals themselves, and that processes such as instantaneous repair of the primary lesion by hydrogen transfer are ultimately responsible for the increase in radioresistance.

In our experiments, the most significant decrease in the aberrations is in the two-hit chromosomal events. It is possible therefore that, like 2,3-dimercaptopropanol (BAL)[4,5], DTT acts by shortening the time during which chromosome breaks remain open, that is DTT is capable of reacting with other breaks.

It is probable that BAL and DTT have a similar mechanism for their action because of the similarity in their chemical structure (I).



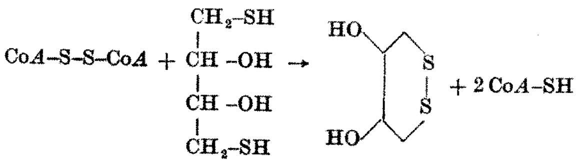
$$\begin{array}{c} CH_2\text{–}OH \\ | \\ CH\text{–}SH \\ | \\ CH_2\text{–}SH \\ \\ \text{(BAL)} \end{array} \qquad \begin{array}{c} CH_2\text{–}SH \\ | \\ CH\text{–}OH \\ | \\ CH\text{–}OH \\ | \\ CH_2\text{–}SH \end{array} \quad \text{(DTT)} \quad \text{(I)}$$

Sulphydryl compounds may act by enhancing the process of repair[3,6]; for example, they convert a coenzyme *A* molecule (CoA-SH) in an inactivated state to a disulphide through a free radical oxidation[2].

$$\mathrm{Co}A\text{–S–S–Co}A + R\mathrm{SH} \rightarrow \mathrm{Co}A\text{–SH} + R\text{–S–S–Co}A \qquad (1)$$

$$R\text{–S–S–Co}A + R\mathrm{SH} \rightarrow R\text{–S–S–}R + \mathrm{Co}A\text{–SH} \qquad (2)$$

The molecule of DTT has two sulphydryl groups, and so it is twice as effective as the monothiols, and it should be more effective in the repair mechanism mentioned in equations (1) and (2), because the disulphide formed is a sterically favoured cyclic one (equation (3)).


CoA–S–S–CoA + CH₂–SH / CH –OH / CH –OH / CH₂–SH → HO, HO, S, S + 2 CoA–SH
(3)

This increases its effectiveness, giving it an even lower redox potential than that of BAL; DTT is capable of maintaining monothiols in the reduced state and reducing dithiols quantitatively[2].

If we are correct in assuming that BAL protects the processes of active oxidative metabolism necessary for the healing of broken chromosomes, then our results would imply that DTT had a similar action on rejoining. The simplest hypothesis is that DTT acts as a reducing agent by almost eliminating the oxygen effect, because it does not reduce the total damage by more than 50 per cent. Whatever the mechanism, dithiothreitol is an effective protector against ionizing radiation in living cells and it decreases all types of chromosomal breakage significantly. It would be valuable to make a direct comparison with BAL in the same cell system and also to determine (by establishing the optimum concentration and period of treatment) whether a 50 per cent reduction in damage is the limit of effectiveness.

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[1] Doherty, D. G., in *Radiation Protection and Recovery* (edit. by Hollaender, A.), 45 (Pergamon Press, New York, 1960).
[2] Cleland, M. W., *Biochemistry*, **3**, 480 (1964).
[3] Bacq, Z. M., and Alexander, P., *Nature*, **203**, 162 (1964).
[4] Wolff, S., *Nature*, **173**, 501 (1954).
[5] Wolff, S., *Genetics*, **39**, 356 (1954).
[6] Wolff, S., and Luippold, H. E., *Proc. US Nat. Acad. Sci.*, **40**, 187 (1954).

Method for deriving a Survival Curve of Young Calves in Wild Ungulates

In animal demography attention is often drawn to the lack of data on very young animals[1,2], for the relatively delicate remains of juvenile corpses are easily destroyed or consumed by predators. The Uganda defassa waterbuck *Kobus defassa ungandae* Neumann, in the Queen Elizabeth Park, western Uganda, is continuously breeding and monotocous. In such an animal an approximate survival curve for the first 6 to 7 months of life can be constructed from the lactation status of the parent. Lactation state is determined by gross examination of the sliced mammary gland in slaughtered animals.

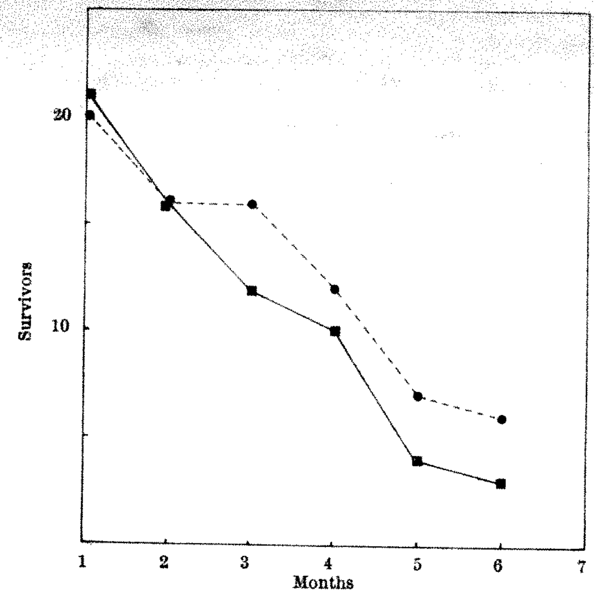


Fig. 1. Survival curves for the first six months of life in waterbuck calves derived from the numbers of lactating (■—■) and non-lactating (● - - - ●) females.

Females more than 3 years old are usually lactating up until the sixth month of pregnancy, or they have lost their calves. If one is not lactating, therefore, the month of pregnancy, determined in this study by using the method of Huggett and Widdas[3], suggests that the calf was lost sometime before that date. In the waterbuck, the time of occurrence of the post-partum oestrus is not known with certainty. It may be variable, although evidence suggests it to be about 21 days. Mating, followed by conception, usually occurs at this time. These variables mean that an arbitrary figure must be taken as a starting point. For the waterbuck, thirty days was considered a reasonable period. This figure was based on results obtained from slaughtered animals. Thus the month of gestation plus one month in non-lactating females tells us the age up to which the calf may have died (Table 1). Clearly if a female is four months pregnant and not lactating, the previous calf may have died at any time in the preceding five months. But the lactating animals also represent an l_x series in life table terminology, which shows the actual calf survivors alive at that time, for lactation terminates rapidly once suckling ceases. Plots of a d_x curve and an l_x curve, the former curve derived from non-lactating females and the latter from lactating, show that the curves are similar (Fig. 1). In practice, the l_x curve is the more reliable, for the only variable factor used in deriving it is the length of the post-partum delay before fertilization, whereas in the d_x curve there is the additional error in the determination of the date of death of the calf, which increases with increasing age of pregnancy.

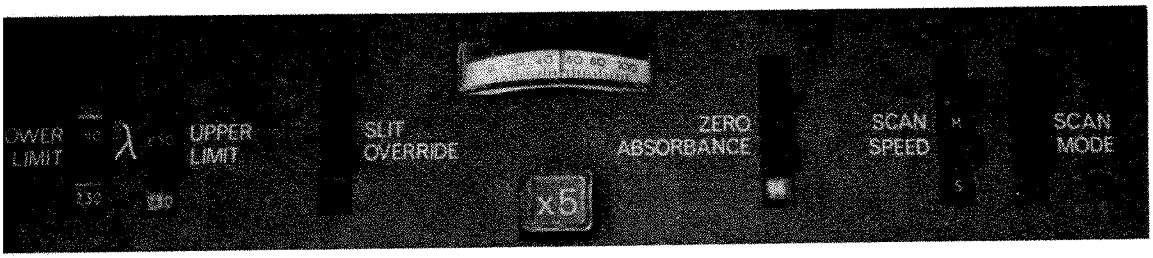
Although it is not possible to confirm the accuracy of a life table derived by this method without reference to a large number of calves marked at birth, the estimated mortality of 49 per cent in the first six months of life, which this method gave, compared with estimates of 52 per cent and 56 per cent derived from methods of direct observation.

In continually breeding mammals the application of this method eliminates much of the guesswork previously applied to this sector of the survival curve.

This work was supported by a grant from the Science Research Council, and facilities were kindly

Table 1. LACTATION STATE OF FORTY-ONE FEMALES ACCORDING TO MONTH OF PREGNANCY

Month	Lactating	l_x	Non-lactating	d_x	Month + 1
0	5	21	4	20	1
1	4	16	0	16	2
2	2	12	4	16	3
3	6	10	5	12	4
4	1	4	1	7	5
5	3	3	6	6	6



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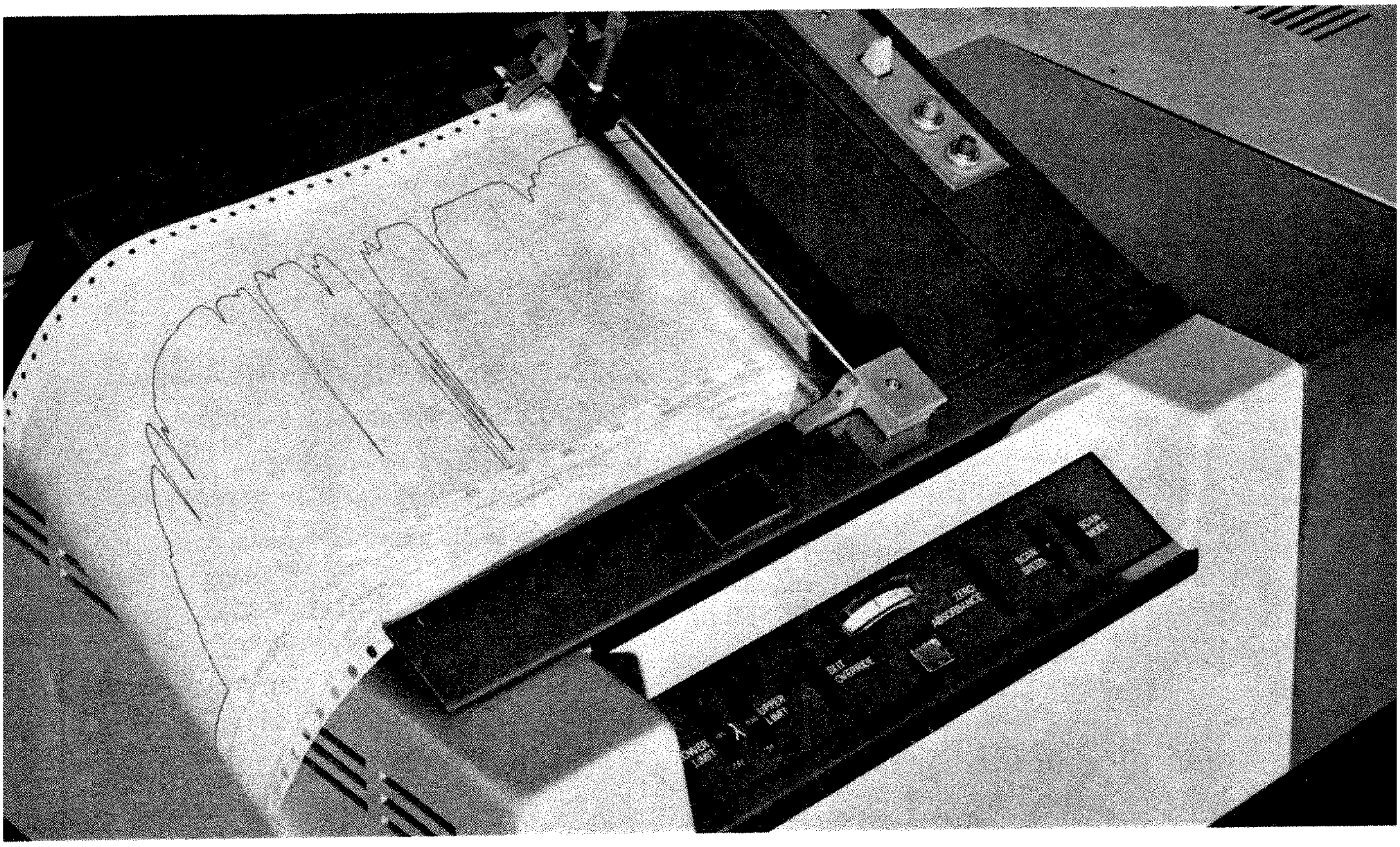

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Zoological Society of London,
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[1] Bourlière, F., in *Colloquia on Ageing*, **5** (edit. by Wolstenholme, G. E. W., and O'Connor, M.) (Ciba, London, 1959).

[2] Caughley, G., *Ecology*, **47**, 906 (1966).

[3] Huggett, A. St. G., and Widdas, W. F., *J. Physiol.*, **114**, 306 (1951).

APPLIED SCIENCE

Role of Austenite and Mineral Oil in Lubricant Failure

THE suggestion[1] that the failure of lubrication in gears is caused by the actual contact temperature reaching a critical value (150° to 200° C) at which the desorption of surface active compounds present in oils occurs has been supported[2] by evidence obtained from modified 'Bowden–Leben' friction apparatus. With mineral oils, the friction change could not be observed in this apparatus with a tool-steel friction pair, but it showed up sharply with stainless steel (18 per cent chromium, 8 per cent nickel). The mechanism of the difference between the two is the subject of this communication.

With a similar apparatus we used a steel (*EN* 39 *B*) which was chiefly bainite (hardness, Vickers pyramid number 440) but no austenite. In Fig. 1*A* the friction temperature curve, using an Admiralty turbine oil, *OEP*-90, and a tungsten carbide hemispherical slider, is shown. By gas carburizing and suitable quenching of the *EN* 39 *B* steel, the surface contained martensite and retained austenite. The friction temperature trace is shown in Fig. 1*B* for a 25 per cent retained austenite sample (Vickers pyramid number 740). A transition temperature shows up clearly, and Fig. 1*C* shows the friction temperature trace for a 5 per cent retained austenite sample (Vickers pyramid number 750). No sharp increase is found.

In carburized steel, the retained austenite content is highest just below the surface; the finish grinding of gears must often result in changes in austenite content, and the importance of this large change in the friction temperature characteristic, consequent on the presence of austenite, must be recognized in gear testing. The scuffing loads are extremely variable and such a scatter could well come from the change in austenite content. Niemann and Lechner[3] have demonstrated that alloys containing austenite reduce the load carrying capacity of gears.

The previous communication[2] did not give any reason for the transition temperature being absent in tool steel when using plain mineral oil (light stock). Mr A. Eyres suggested that the reactivity of the sulphur compounds usually present in plain oils should be studied.

We considered that sulphur compounds, for example, might react slowly with austenite but sufficiently rapidly with martensite so that, when the critical temperature was reached in the slow speed friction machine, physical desorption would be masked and a transition would not be seen. A similar mechanism would also occur for oils containing extreme pressure additives. In running machine parts, such as gear teeth, the ambient temperature is much lower than the total flash temperature achieved in the contact zone and the contact time is short; thus the reaction will not have occurred to the same extent, and a critical temperature will still be observed for martensitic steels and plain oils and, especially in the running-in period, where chemical reaction has occurred least, for oils containing extreme pressure additives.

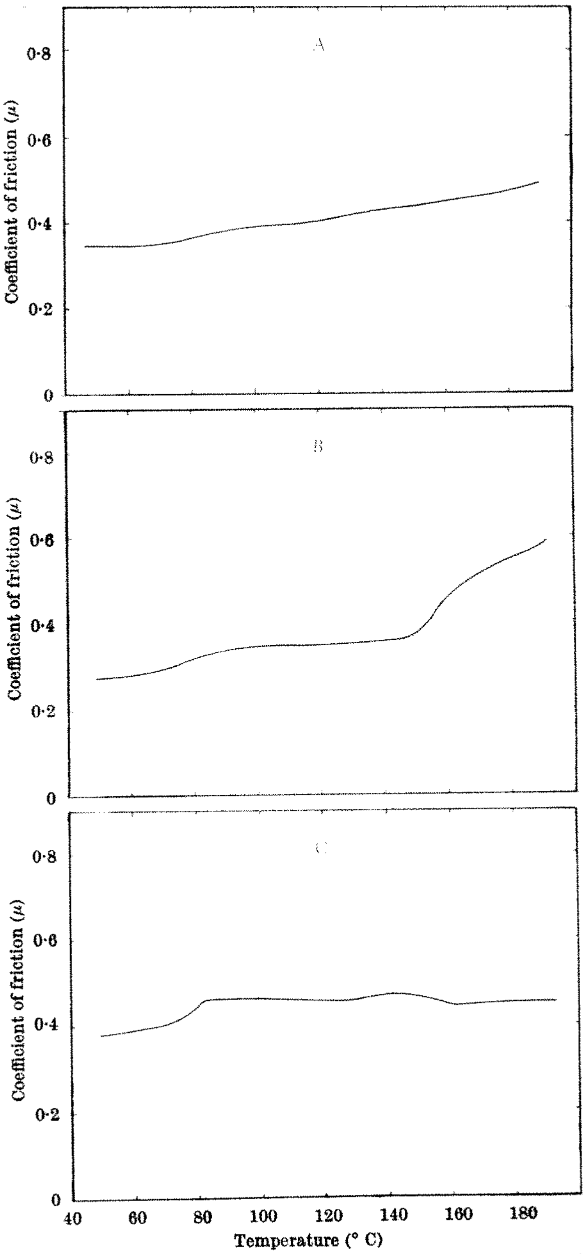

Fig. 1. *A*, Friction temperature curve using 'Bowden-Leben' machine (average of at least four traces) using a tungsten carbide slider and an uncarburized *EN* 39 *B* rider. Velocity, 0·02 cm/sec; load, 2·5 kg; turbine oil lubricant. *B*, Friction temperature curve using carburized *EN* 39 *B* with 25 per cent retained austenite; turbine oil lubricant. *C*, Friction temperature curve using carburized *EN* 39 *B* containing austenite only; turbine oil lubricant.

Groszek and Palmer[4] isolated surface active compounds present in oils by percolation through columns of activated magnesium oxide and reported that the compounds exist at a concentration of about 0·4 per cent and contain sulphur. No transition temperatures were obtained from the friction temperature curves for the 25 per cent retained austenite sample or uncarburized *EN* 39 *B* when using white oil (oil from which surface active compounds have been removed) lubricant, but when 0·59 per cent of the surface active compound was added to the white oil a quite different result was obtained for the two surfaces. The carburized steel containing retained austenite showed a critical temperature (Fig. 2), but the uncarburized steel gave no sharp critical temperature (Fig. 3). It is suggested that this effect is caused by some reactive compounds in the surfactant reacting faster with martensite than with austenite and thus excludes the possibility that the absence of a critical temperature was a result of oxidation products formed in the 'Bowden-Leben' system but not necessarily in gear and disk systems.

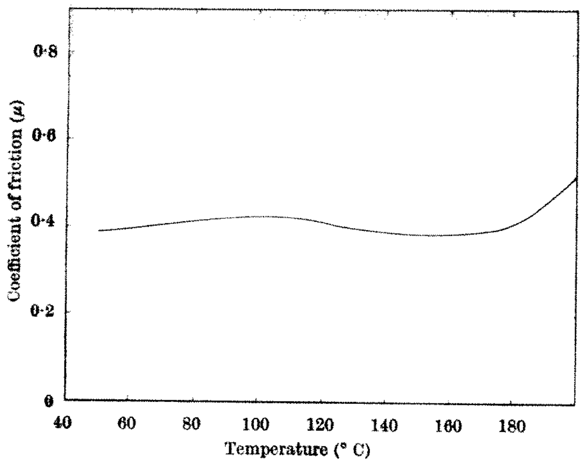

Fig. 2. Friction temperature curve using a tungsten carbide slider and a carburized *EN* 39 *B* steel rider containing 25 per cent retained austenite; lubricant, 0·59 per cent by weight surface active compound in white oil.

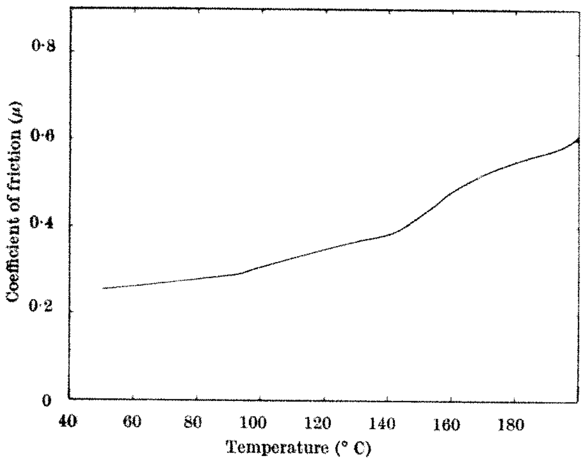

Fig. 3. Friction temperature curve using an uncarburized *EN* 39 *B* steel rider; lubricant, 0·59 per cent by weight surface active compound in white oil.

These findings are clearly very important for the choice of gear steels.

We thank A. Groszek for the surfactant, and the British Iron and Steel Research Association for carrying out the heat treatment and the X-ray determination of the austenite content. We also thank P. T. Holligan and Dr Bruce Kelley for many useful discussions. One of us (W. G.) thanks the Mobil Oil Co., Ltd., for the award of a bursary.

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Received August 31; revised November 29, 1967.

[1] Askwith, T. C., Cameron, A., and Crouch, R. F., *Proc. Roy. Soc.*, A, **291**, 500 (1966).
[2] Grew, W., and Cameron, A., *Nature*, **214**, 429 (1967).
[3] Niemann, G., and Lechner, G., *Erdöl Kohle, Erdgas, Petrochem.*, **20**, 96 (1967).
[4] Groszek, A. J., and Palmer, A. J., *J. Inst. Petrol.*, **47**, 295 (1961).

GENERAL

Test of the Alignment Hypothesis as the Reason for the Mosaic Unit Phenomenon

I HAVE reported[1,2] that ancient floor mosaic patterns tend to be certain absolute sizes (mosaic units), and I suggested[2] that the reason was practical.

There is (refs. 1–3 and unpublished work) a mathematical hypothesis—the alignment hypothesis—as follows. If particles, randomly drawn from a population of particles of sizes and shapes varied randomly within limits, are packed in adjacent rows, the probability of particles forming a line transversely through these rows is much higher at certain intervals than elsewhere (Fig. 1*A*).

We may (ref. 3 and unpublished work) have here the explanation of the mosaic unit phenomenon because the mosaic stones of ancient floors (ref. 3 and unpublished work) are usually of random varied sizes and shapes within limits, and there is varied evidence (refs. 2 to 5 and unpublished work) that the ancients usually placed stones without special selection, shaping or special spacing, usually as adjacent rows. Thus the mosaic patterns of the ancients would tend (if the alignment hypothesis is true) to turn out most regular when made to certain dimensions ("alignment intervals", see Fig. 1*B*), and they may have found this by experience.

There is close agreement (refs. 1 and 3 and unpublished work) between the typical sizes of ancient mosaic patterns and their sizes predicted from the alignment hypothesis; there is no more than 1 mm difference between the observed modal value ($\sigma = 1{\cdot}3$ mm, when $c = 1$ mm for $n > 10^4$) and the calculated value for each of the eleven most typical pattern sizes. This suggests that the alignment hypothesis works in reality.

If it does, "alignment" in the simple state of Fig. 1*A* should exist in unpatterned mosaics. I find that this is true; a frequency distribution of the distances between 257 pairs of these alignments is shown in Fig. 3. Moreover, they cluster about the commonest pattern sizes. Fig. 2*A* shows a typical case.

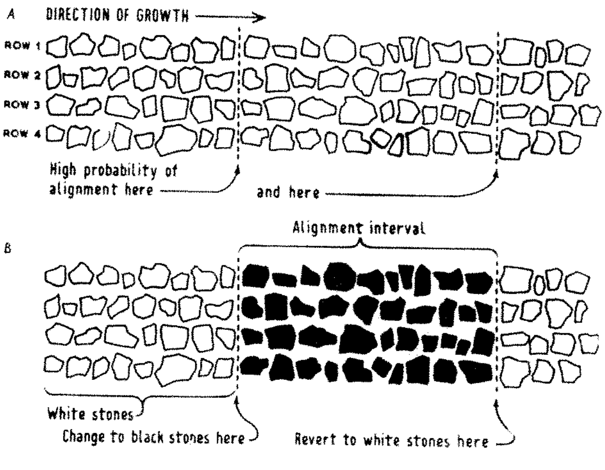
A
DIRECTION OF GROWTH
ROW 1
ROW 2
ROW 3
ROW 4
High probability of alignment here
and here
B
Alignment interval
White stones
Change to black stones here
Revert to white stones here

Fig. 1. Diagram of what is meant by alignment (*A*) showing also the use of alignments to a mosaicist (*B*).

Alignments have been demonstrated (work to be published) as a packing phenomenon in modern pebble floors, fish roes and other[2,3] materials, but a test to see if alignments occurred among cleaned ancient mosaic stones packed in the laboratory had not been attempted.

The simplest method of making this test is for a naive volunteer to place ancient mosaic stones (without special selection or spacing) in adjacent rows. Twenty-six aggregations about 50 cm long and six aggregations about 110 cm long were formed (five to ten rows wide). In twenty-eight aggregations, sixty-two alignments ran from one side to the other. The observed alignment intervals clustered at the predicted alignment intervals (Fig. 3).

I also laid stones myself (but in the dark so that I could not see the packing), using grooves to guide the production of sets of eight adjacent rows. Eighteen aggregations were about 32 cm long and two were about

55 cm long. Laying stones in daylight, I blocked my view of those already packed with a screen. Forty aggregations were about 55 cm long and eight were about 72 cm long. Removing the screen (and illuminating those done in darkness) showed alignments, passing through the full eight rows. Again, they clustered at the expected intervals (Fig. 3).

Human factors were eliminated as follows. Cleaned ancient mosaic stones were poured on to a grid of numbered squares. Volunteers moved the stones about until only one lay in each square. I then selected a random number by the conventional full technique[6] using random number tables[7,8]. The stone in the square with this number was removed and added to the rows, where it was packed tightly.

To decide which face of this stone should lie uppermost, I threw a die. If the result was one, I did not turn the stone, if two I turned the stone so that its face pointing towards the right hand side of the grid lay uppermost, and so on. (All the stones were faced to the sides of the grid by volunteers.)

The orientation of each stone was decided by tossing a coin. If "tails", it was turned clockwise through a right angle in the plane of the grid; if "heads", it was left. To decide which way around the stone should lie in this position, another toss was made. If "heads", the stone was revolved through 180° in the plane of the grid; if "tails", it was left. Twenty-five aggregations about 70 cm long resulted. Sixty-eight alignments ran through the full width of eight rows, and again clustered about the expected intervals. (There was no alignment in one aggregation.)

This method was slow, so I made a packing machine. Cleaned ancient mosaic stones fell from a hopper onto a conveyor belt, where they were constrained to form rows by strips tensioned along the length of the conveyor. They first passed under rubber flaps to make them lie flat, and were then packed by their progress being obstructed by a guillotine. Raising the guillotine cleared the channels for a fresh test.

One hundred and six aggregations were obtained (mean length 30 cm), with 234 alignments (passing through the full five rows), which clustered at the predicted intervals (Fig. 3). Fig. 2*B* shows a typical case. (There was no alignment in three aggregations.) More of the intervals agreed with the expected distances when each alignment was measured from the preceding alignment instead of from the start of the aggregation (Fig. 3). Some aggregations were short because tests were stopped if stones were forced up out of their channels, but they were always greater than 13 cm in length, and often were about 45 cm long (three ⩾ 115 cm).

These alignments occur among cleaned ancient mosaic stones packed without cement, and yet the typical

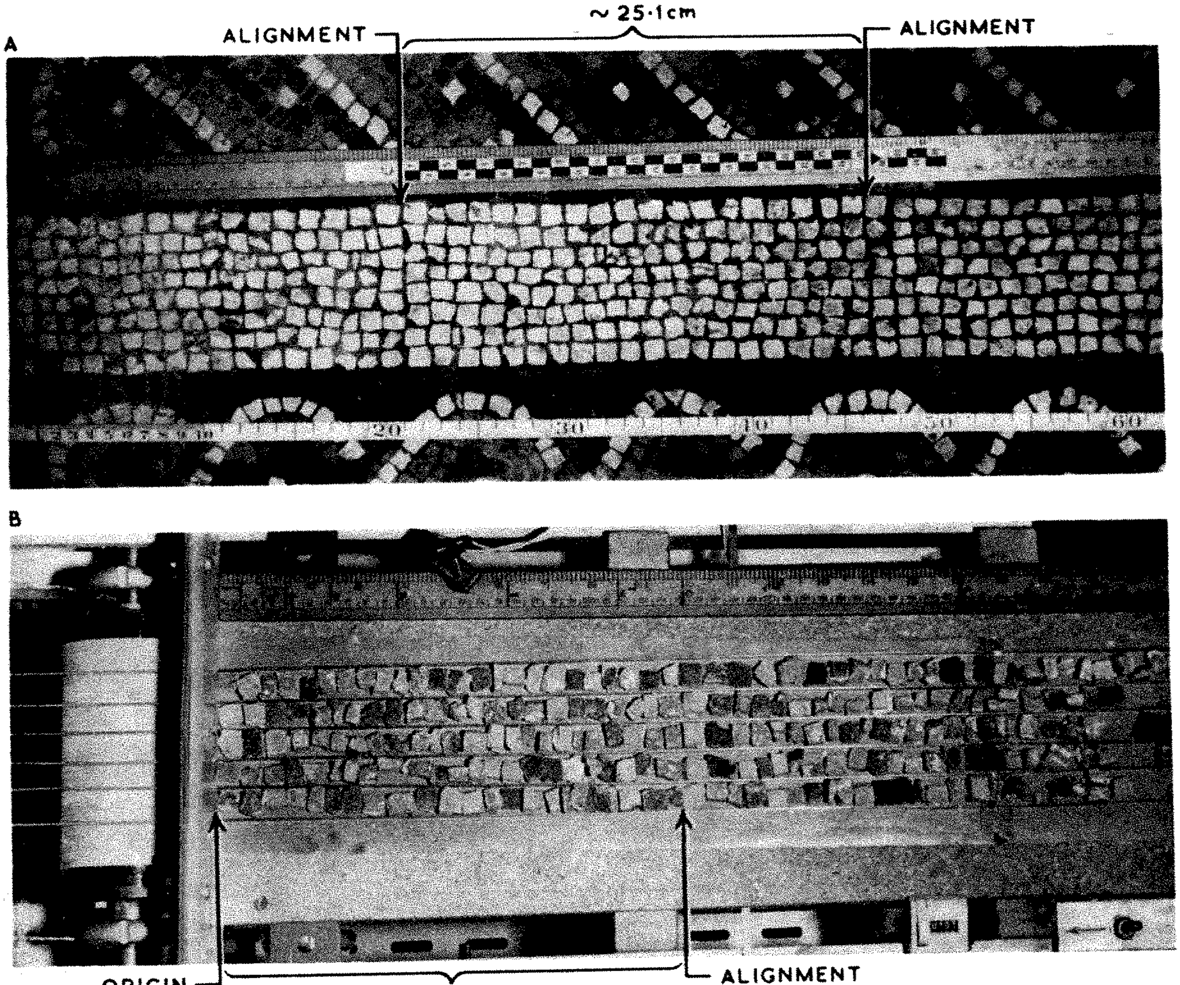

Fig. 2. Demonstration that the alignments observed in ancient mosaics can be produced experimentally, simply as a packing phenomenon. A typical interval of occurrence of an alignment occurred spontaneously among ancient mosaic stones randomly packed by machine (*B*). This coincided with a typical interval between alignments in an ancient mosaic (*A*) at Aldborough, Yorkshire (courtesy of the Ministry of Public Building and Works).

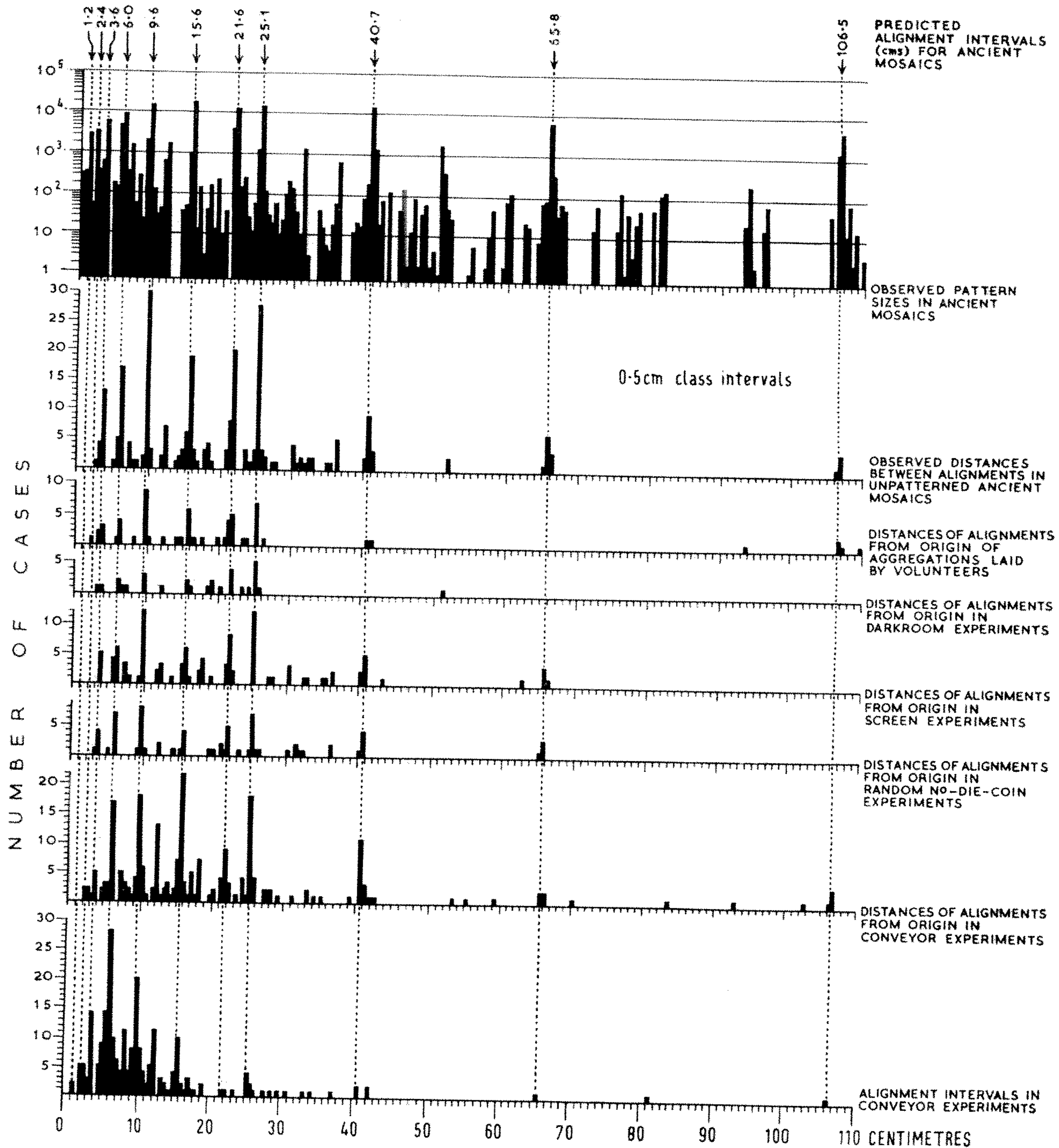

Fig. 3. Comparison of results of five experimental methods with the observed phenomena and with the intervals calculated from the alignment hypothesis.

intervals agree (Fig. 3) with the alignment intervals in ancient mosaics, in which stones are packed with cement. This supports the deduction that cement plays little part in stone packing.

While before[1-3,5], confirmation of the alignment hypothesis was observational, the existence of the alignments predicted by the alignment hypothesis has now been tested experimentally with mosaic stones and found to be true. The reason for the mosaic unit phenomenon seems to be the alignment phenomenon.

This is part of a project financed by a Leverhulme Research Award, for which I am grateful. I thank Professor Roger Warwick for support, and also Professor H. Kalmus and Professor E. R. Laithwaite. I also thank the British Museum Department of Greek and Roman Antiquities and the Ministero della Pubblica Istruzione for stones, the Goldsmiths' Company for aiding collection of specimens, and all volunteers.

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[1] Moore, R. E. M., *Bull. Inst. Classic Stud.*, **11**, 87 (1964).

[2] Moore, R. E. M., *Nature*, **209**, 128 (1966).

[3] Moore, R. E. M., *Intermedica*, 31 (Summer 1966).

[4] Moore, R. E. M., *Amer. J. Arch.* (in the press).

[5] Moore, R. E. M., *Colloquium Roman Mosaics in Britain, Inst. Arch., Univ. London* (1966).

[6] Wallis, W. Allen, and Roberts, H. V., in *Statistics* (Methuen, London, 1957).

[7] Lindley, D. V., and Miller, J. C. P., in *Cambridge Elementary Statistical Tables* (University Press, Cambridge, 1961).

[8] Fisher, R. A., and Yates, F., in *Statistical Tables for Biological, Agricultural, and Medical Research*, fifth ed. (Oliver and Boyd, Edinburgh and London, 1957).

BOOK REVIEWS

MORE NEWTON LETTERS

The Correspondence of Isaac Newton

Vol. 4 1694–1709 Edited by J. F Scott Pp xxxii + 578 + 6 plates (London Cambridge University Press, 1967 Published for the Royal Society) 231*s*

One cannot say that the documents collected in the present volume of Newton's correspondence throw much new light on the course of his career during the period it spans, although this was the time when the most considerable event occurred in his public activity In 1696, he exchanged academic life in Cambridge for a position of higher social distinction in the civil service in London About his work at the Mint, first as warden, then as master, we are well informed by Sir John Craig's authoritative study *Newton at the Mint* (Cambridge University Press, 1946), and the numerous letters and documents now published only serve to confirm and illustrate the already known picture of a conscientious, industrious, efficient, rather harsh and far from progressive administrator

From his last years at Cambridge date Newton's last creative effort in science, his work on the theory of the Moon Other scientific preoccupations, such as the preparation of the second edition of the *Principia*, the publication of the *Opticks*, his taking up the presidency of the Royal Society, have left scarcely any traces in the correspondence Newton's relations with his gifted disciples, Halley and David Gregory, were more concerned with the affairs of the Mint than with problems of mathematics and astronomy In his efforts to work out the numerous corrections to the Moon's orbit, Newton turned to Flamsteed for accurate observational data The cordial spirit in which this collaboration started soon gave way, however, to a painful succession of acrimonious recriminations, which completely spoiled the human relations between the two men

This was the more unfortunate, for they were soon drawn together again in the important and difficult task of publishing the corpus of Flamsteed's very accurate observations and the new star catalogue based on them The lamentable story of this undertaking looms large in the biographies of the two heroes, and its first part is abundantly documented, in the volume under review, by authentic texts of the relevant letters and drafts, for the climax we have still to wait until the next volume appears In a well written preface, the editor, J F Scott, passes a balanced judgment on this painful business, and after perusing the documents I cannot but concur with his plea for looking on Flamsteed's behaviour with more indulgence than on Newton's

This fourth volume of the series is the first to appear under the sole editorship of Dr J F Scott It fully keeps up the standard of excellence of the previous ones Indeed, if anything, it even improves on this standard In particular, it seems that the editor has enlisted a more extensive co-operation of experts on the special topics treated in the letters, thus providing a commentary of the greatest possible accuracy and reliability

L Rosenfeld

DIFFERENTIAL EQUATIONS

Ordinary Differential Equations

A First Course By Fred Brauer and John A Nohel Pp xvi + 457 (New York and Amsterdam W A Benjamin, Inc , 1967) $10 75

Transversal Mappings and Flows

By Ralph Abraham and Joel Robbin Pp x + 161 (New York and Amsterdam W. A Benjamin, Inc , 1967) $12 50

Ordinary Differential Equations is a good textbook for students of applied mathematics, science and engineering The subject matter is not presented as a piece of pure mathematics, with applications, but rather as a series of physical problems leading to differential equations and hence to a need for a theory of such equations, including methods for solution All the usual methods of solution are presented There are chapters on boundary value problems and on systems of differential equations There is no discussion of non-linear equations, but there are chapters on numerical methods of solution and on the use of the Laplace transform in solving equations

Transversal Mappings and Flows is a very advanced and highly specialized book originating in a series of lectures given at the University of Princeton in 1965 It deals with aspects of the qualitative theory of ordinary differential equations, especially the search for generic properties of vector fields Various results are proved by a new method, called transversality theory, which has been developed from basic ideas introduced by Thom in 1954 The first half of the book expounds the foundations of transversality technique and the second half deals with applications to flows

While the book may be of value to researchers interested in the study of dynamical systems, it must be said that it is likely only to be understood by pure mathematicians It starts with a review of the differential calculus in Banach spaces and the theory of manifolds, orientation, vector bundles and other topics belonging to differential topology Various new results or new methods of proof are presented for the first time

L S Goddard

PULSE RADIOLYSIS

Pulse Radiolysis of Water and Aqueous Solutions

By A K Pikaev Translated by Scripta Technica, Inc Translation edited by Edwin J Hart English edition revised and augmented by the author Pp xvi + 295 (Bloomington and London Indiana University Press, 1967) 75*s*

Pulse radiolysis is the study of the chemistry of the immediate short lived products of a very short pulse of ionizing radiation, the products usually being detected by their optical absorptions It developed from flash photolysis and has become a rich field of research in the last few years, with the development of the electron linear accelerator and other machines capable of delivering isolated pulses of electrons of many joules of energy and from a few microseconds long down to a few nanoseconds with corresponding rapid rise and fall times This book is the result of a very complete survey of the literature by the author as a way of getting into the field It was originally published in Russian in 1965, but this later English translation has been considerably revised to include as much as possible of the recent publications About half the book deals with the steady state radiolysis of water and aqueous solutions at high and low dose rates This, while not being strictly pulse radiolysis, gives useful background to the many reactions involved and reviews much of the earlier literature The sections on the sources of pulsed radiation and experimental techniques are the weakest part of the book The basic principles behind the experimental techniques are chiefly ignored, particularly the special design of the optical systems needed in this field This is basically a matter of using a very intense light source, getting as much as possible concentrated in the irradiation cell and then getting it down a rather lengthy light path out through a protective radiation maze and eventually through a monochromator on to a photocell The fourth chapter describes the methods for identifying the transient products and determining the reaction rates and, because it is a series of résumés of the recent literature, lacks an overall cohesion A very comprehensive summary of published experiments, however, is contained in two extensive tables Table 15 lists the relative rate constants for the reactions of OH, H and the hydrated electron with more than 200 pairs of solutes, and Table 16 the absolute rate constants for the radicals with more than 500 solutes The book provides a useful introduction to pulse radiolysis and will be a useful reference for those working in the field C W GILBERT

PHYSICAL METHODS IN BIOLOGY

Magnetic Resonance in Biological Systems

Edited by A Ehrenberg, B G Malmström and T Vänngård (Proceedings of the Second International Conference held at the Wenner-Gren Centre, Stockholm, June 1966) (Wenner-Gren Centre International Symposium Series, Vol 9) Pp viii+431 (Oxford, London and New York: Pergamon Press, Ltd , 1967) 140*s* net

A VALID reason for publication of the proceedings of a meeting in book form is to convey the results of ensuing discussions. Unfortunately, in this book any such discussion has been omitted

The forty-nine original research papers presented deal with the applications of highly sophisticated techniques of magnetic resonance, namely. NMR, EPR and Mossbauer spectroscopy, to "sub-molecular" biology, as Hugo Theorell defines it in his opening address There are also introductory papers on electron nuclear double resonance (ENDOR) and nuclear electron double resonance The biological systems range from nucleic acids to paramagnetic metal complexes

It is possible to highlight only a few of the topics covered. NMR is used to gain information about the structure of peptides, to measure fast reaction rates and to determine binding positions in metal complexes EPR is used to probe the structure of heme and non-heme iron proteins, copper-containing enzymes, metal flavoproteins and single crystals of proteins Flow techniques of EPR and their applications to the kinetics of flavin redox systems and to the conformation of labile free radicals are described

The technique of spin-labelling in which a stable organic free radical is attached to a molecule of biological interest and used as a probe is introduced in a paper by McConnell The technique is also applicable to NMR as shown by Sternlicht *et al* Here use is made of the perturbation of a protein spectrum caused by the unpaired spin of the bound radical

The group of papers dealing with Mossbauer spectroscopy is introduced by a paper briefly describing the theory and instrumentation and some applications of the method to ferredoxins In practice the method is limited to molecules containing iron-57

This is a specialist book containing a wealth of information, while the introductory papers are of wider interest The book could, however, have been improved if more papers had contained concise summaries or abstracts

The volume is well produced and contains a good subject index, but the high price reminds one, unfortunately, of the business interest in these publications

M. EBERT
N J F DODD

BIOLOGICAL CLOCKS

The Physiological Clock

By Erwin Bunning Revised second edition (Heidelberg Science Library) Pp. 167 (London · Longmans, Green and Co , Ltd , New York Springer-Verlag, 1967) 24*s* paperback

WE do some things only or especially at certain times of the day or the year At night, flowers close up, leaves droop, some animals become active, others torpid Marine animals and plants fit their activities to the tides and to the phases of the Moon We have known for some years that most of these rhythmical activities persist for a time when the environmental rhythms are eliminated and it is clearly advantageous to organisms to be able to predict, say, the dawn or the full Moon Some animals navigate by means of the Sun or the stars, some plants and animals regulate their seasonal activities by detecting the length of the night For both these purposes chronometers are needed Professor Bunning's book deals with what we know about the nature of the clock and how organisms use it His examples are drawn from plants, man and other animals His method is to present the problems and then the experiments that bear on their solution, and in many ways the book is a model for what a biological monograph should be An enormous amount of experimental data is presented within a short space, much of it in text figures, and clearly a lot of thought has gone into the arrangement of the work and the elimination of specialist jargon in order to interest all biologists The design of experiments is stressed especially because, in this field, many false conclusions have been reached in the past because of the complexity of an organism's response to its environment

The first edition of the book was published in German in 1958 and a first English edition, based on the second German edition and printed in Germany, appeared in 1964 The second English edition is a revised version of this There are a few more pages, more small print and fewer diagrams Some errors of fact have been corrected This edition retains Professor Bunning's preference for relaxation oscillation as the mechanism of the clock as opposed to harmonic oscillation Arguments on the other side are presented, but half-heartedly Similarly the argument that the circadian rhythm may be a beat phenomenon is dismissed more readily than some of us would like In a monograph by such an authority, such personal preferences are what we want, but I should like it to be more obvious that not everyone agrees

In a book of this excellence it is a pity to have to complain about presentation, but undoubtedly the author does not do himself full justice because of this The book is not easy to read This is due partly to the English style in spite of, or perhaps because of, the battery of experts who have helped the author with the translation It is also partly due to the poor printing There are many spelling and grammatical errors carried over from the old edition and new ones have appeared, although this edition is printed in America The archaic "afore mentioned" [*sic*] has replaced "above mentioned" and words are now often hyphenated at the wrong places

However, all students of biology should make the effort to read the book if they have not already read an earlier edition F A L CLOWES

WATER AND THE WORLD

The Problem of Water

A World Study By Raymond Furon Translated by Paul Barnes Pp xvi + 208 + 14 photographs (London Faber and Faber, Ltd , 1967) 42*s* net

Water, Weather and Prehistory

By Robert Raikes Pp xvi + 208 + 18 plates. (London John Baker, 1967) 45*s* net

THESE books, with a common subject, have very different objectives, but neither author seems to be quite clear what his is or how to reach it

Professor Furon is at the Faculté des Sciences in Paris The sub-title of his book—"A World Study"—seems to promise the kind of synoptic account that would be welcomed by all sorts of hydrologists everywhere, but it contains so much Paris pump hydrology that one wonders why it was translated (Mr Barnes has done a very good job) The author is obsessed with quantities, and his British publishers provide the maddening and quite unnecessary English equivalent for every continental or metric figure quoted As an example that will also reveal the scientific calibre of the book, Furon calculates an annual water use in North America on the basis of the following unsupported statement (page 163) "Each inhabitant in 1960 required daily 2,870 litres (some 650 gall) for industry, 3,210 litres (700 gall) for agriculture, and 500 litres (110 gall) for public supplies" Now all he has to do is multiply by the number of days in the year, then by the population, and out comes an estimate of the 1960 national need (Why bother with measurements when the hydrologists' job can be done as easily as this ?)

The book shows no feeling for qualities, and in spite of a formidable list of references Furon seems to be unaware that there are a few ideas in hydrology, that some of his numbers can be put into a coherent framework, and that there are criteria, not yet all good enough, for rejecting or at least questioning some of the numbers he accepts as established truth As an example he attempts to make out a water balance sheet for part of the Paris basin and, scorning anything as clear as the simple equation of continuity, he gives a long prose account that ends in chaos There is no inquest; yet a telephone call to Versailles could have produced an evaporation estimate (twice the value he used) that would have put things right His own reaction is that it is all very difficult, but it doesn't matter—the area has no economic importance as a source of water

Mr Raikes has the complementary failing of being too much concerned with qualities and not enough with quantities even the quantity that he must use frequently always has a qualitative adjective, monotonously the same (for example, "up the hill in the higher rainfall areas") His philosophy seems to be "I am a civil engineer working on water in and near Baluchistan, if I call my craft 'hydrology' it becomes a science, and I become a scientist—even omniscient As such I can disagree with any professional in any discipline, and by making the reservation (page 28) that this is not intended as a technical treatise, I can dodge the responsibility of reproducing the evidence or reasoning that I question At the end I'll put in a graceful note saying how stimulating these disagreements have been " The result is a book liberally peppered with bright ideas and clever thinking, all praiseworthy even if not all new, but completely spoiled by occasional sneers One of his targets is H H Lamb, ignored by Raikes until on page 101 he is misquoted and his reasoning dismissed as "absurd" On checking Lamb's statement it turns out to be a very cautious inference from someone else's data Absurd ? Not in the least

And now for the books rather than their authors Furon wants the nations of the world to do something about the world's water supply, but it is only in the very last lines that he mentions the International Hydrological Decade, in which more than one hundred governments are doing what he asks for

The book has many short chapters all containing a lot of facts, usually given without a source, not always the same when repeated, and not always converted by the same factor After brief reviews of water in biological material and of the properties of fresh water and sea water, a longer survey of water in agriculture adds very little to what might have been written thirty years ago, but it is here that his own special interest begins to emerge. All too short, there are delightful snippets of information on historical hydro-geology, sometimes irrelevant, but their sparkle emphasizes the dullness of the rest of the book (From one such interlude, Furon concludes that the Syrian climate has not changed in the past 3,000 years, and it is much easier to agree with him than with Raikes making the same assertion) Surveys of water out of control and of water in industry (chiefly French) follow, and then a review of supplies of drinking water comes nearest to being a "world" study After a short chapter on pollution, there is a long account of ways of finding water, with dowsing given an extended discussion that concludes "unbounded credulity is not confined to France" Modern geophysical techniques are described, usually as part of a special case study, but the technical level is low only the easy ideas are explained

The longest chapter is on development problems in arid zones and it seems to be based on an unstated axiom that to grow more food there must be more cultivated land. In the course of examining what the semi-arid regions might contribute he slips into Raikes's theme in considering how far productivity has been affected by climatic change · he concludes that the semi-arid countries round the Mediterranean have the same climate now as 2,500 years ago, but there have been changes in this period (in diary form he tells us that the fifteenth century was a "century of great disturbance caused by astronomical phenomena of intersection of perihelion of Moon with its apsis node during winter solstice"—Mr Raikes, please note) His case studies lead him to the question, "Is further irrigation in the Sahara possible ?" but in effect the answer is on a previous page "The water is worth more than the product" The final chapter—"The Thirst of the World"—contains little more than the figures for North America (already quoted), the decision that we'll not be able to exist on dew but may eventually get our fresh water from the sea

From Sir Mortimer Wheeler's fulsome preface, Mr Raikes's book should be compulsory reading for all students of man's history There are those who attribute the surges in the conflict between nomads and pastoralists to differences in their sensitivity to climatic change, particularly to the shift in extremes that would accompany a shift in a mean Raikes's thesis is that during the past 9,000 years there has been no change in world climate the early cultures that grew near the eastern end of the Mediterranean did so in a climate the same as it is now, and there has been no significant change between. His personal research contribution is based on an acceptable working hypothesis relating kind of culture to rainfall climate and from it he shows that the distribution of archaeological sites corresponds very closely to what would be inferred from present-day isohyets it is a fair deduction that climates now and then are the same This welcome piece of scholarship could be the start of a useful critical discussion, but his arguments about constancy impress neither in form nor in content

Most of the book is about the surface hydrology of semi-arid areas Near the beginning there is an outline of the scope of hydrology, and as one who later makes an earnest appeal for the avoidance of jargon Raikes tucks in a fair amount of his own, here and elsewhere A survey of water and climate as agents in changing geomorphology contains some slightly inaccurate soil physics and some half truths about erosion there is no consideration of any medium other than water for the transport of soil material Two chapters on water as a factor in plant ecology possibly put too much emphasis on the importance of ground-water and the use plants make of it Next comes the partial survey and rejection of the evidence for post-glacial climatic change, and then some interesting case studies show how the special knowledge of a thoughtful water engineer can re-interpret the evidence from archaeological digging. Finally, there is a sermon on the desirability of "The Inter-disciplinary Approach" to large-scale ecological problems, with many words to explain "inter" and none to define "discipline" Although it has all been said before, it needs repetition from time to time, and these two books show that hydrology itself would be a worthwhile subject for treatment H. L PENMAN

LIVING PRIMATES

A Handbook of Living Primates

Morphology, Ecology and Behaviour of Nonhuman Primates By J. R Napier and P H Napier Pp xiv+456 (114 plates). (London Academic Press, Inc (London), Ltd , New York Academic Press, Inc , 1967) 126*s* , $21 50

THE study of primate biology as a facet of the analysis of human origins and variation has matured during the past half century, in phase with the development of general evolutionary theory In the late nineteenth century, the standard handbook of the primates—a two volume compendium by H O Forbes, now almost a classic in the field—centred almost exclusively on the natural history (much of it anecdotal) together with the taxonomy of the order. A modern handbook is therefore required to present, in accordance with present systematic practice, not only the much expanded body of morphological data, but also that bearing, for instance, on primate physiology, ecology and genetics It is to this task that Dr and Mrs Napier have addressed themselves in the preparation of this book—the only modern handbook of the primates available today in a concise form

The first section, "Functional Morphology of Primates", presents the major structural features of the order in relation to the presumed overall trends of change discernible through its subdivisions

The second and much the largest part of the work provides a series of abbreviated "profiles" of each primate genus Each profile comprises such data as are available about, for instance, the geographical range, ecology, bodily dimensions, external and internal morphology, locomotion, genetics and serology, behaviour and social life, reproduction and development, together with some notes on longevity and breeding record in captivity Each account is followed by summary lists of references, compounded at the end of the volume into an overall bibliography of some 800 titles

The third section of the work presents a systematic list of the various primate species, correlating, for example, formal and vernacular names, and supplemented by notes relating to the taxonomy of each genus This is followed by accounts, more detailed than would have been appropriate in the second section of the volume, of such topics as the ecology of the African primates, locomotor patterns, limb proportions and associated features, together with such vital statistics of primate genera as have, to date, been gleaned

The volume is illustrated with more than 100 plates of a quality uniformly high and, at times, outstanding

A book of this type must, of necessity, be eclectic and every student of primate biology is likely to note some fact or reference that, in his opinion, ought to have been included or given a different emphasis Others may claim that Dr Napier has possibly given somewhat too great a prominence to certain aspects of primate biology to the detriment of others Again, certain of the general premises outlined in the early pages of the volume are unlikely, in all details, to meet with universal agreement Nevertheless, few will dispute that the present volume provides not only a unique compendium of modern knowledge of primate biology but also a most usable guide to the principal sources of original literature

The volume is intended as a source book, and it would be unjust to reproach the authors for not attempting to enquire into the extent to which contrasts in many of the physiological, genetical and morphological characters correlate or contrast with accepted schemes of primate systematics In many cases, the data are, as yet, insufficient to enable such studies to be carried out effectively and a stated purpose of the work is to indicate the extent of the gaps It is only as these are gradually closed that the overall body of data can be combined in a multivariate scheme summarizing the overall contrasts between extant primate groups ERIC H ASHTON

INDUSTRIAL MICROBIOLOGY

Progress in Industrial Microbiology

Vol 6 Edited by D J D Hockenhull Pp 281. (London. Heywood Books, 1967) 84*s* net

THIS sixth volume of *Progress in Industrial Microbiology* is one of a series of international reviews which each year covers a number of selected topics from the rapidly expanding field of industrial microbiology. This particular volume, edited by D. J D Hockenhull, deals with seven subjects ranging from the production of rifamycins to continuous culture, and should be of interest to workers in many disciplines

As a review it fulfils the function of providing a brief account of the history of most of the subjects, describes the latest developments in research and refers to more detailed works elsewhere

The first paper, "Production of Polyene Antifungal Agents by Streptomycetes", outlines the fungicidal activity and chemical properties of these organisms It also summarizes the toxicities of various polyenes and considers the conditions used in their production This review of Perlman's is particularly useful because the polyene group constitutes a very important group of antifungal antibiotics P. Sensi and J E Thiemann have written a detailed paper on the production of rifamycins—among the latest therapeutic agents Discovered by Sensi, Margalith and Timbal of the Lepetit Research Laboratories in Milan, an important property of rifamycin is that modification of some functional groups of the molecule yields other compounds with high antibacterial activity It seems that the production of rifamycin is favoured by a number of elements and it has also been shown that, for maximum yields of antibiotic, high aeration rates are required only at a very specific stage of the fermentation

The paper which I found most interesting is that on the bacteriology of fish spoilage and preservation by J M Shewan and G Hobbs of the Torry Research Station, Aberdeen As they point out, fish spoilage depends on a number of factors including the initial bacterial flora, the flora acquired during handling and processing, the conditions to which the fish have been subjected during storage and processing, and on the actual chemical composition of the fish Preponderant pathogenic flora are *Pseudomonas, Achromobacter* and *Flavobacterium*, but when fish are caught close to land, typical coliform and food-poisoning bacteria can be isolated Smoking and salting are both traditional methods of preservation, newer methods include freezing, use of antibiotics and irradiation

Other papers in the review are "Studies on the Mechanism of Actinomycin Production", "Recent Developments in the Production and Industrial Applications of Amylolytic Enzymes derived from Filamentous Fungi"; "Large-volume Respirometers with Particular Reference to Waste Treatment", and "Continuous Culture"

The book serves a useful purpose, but, as with all reviews, the material takes time to amass and may therefore be not quite up to date ANNE CLAYTON

BOTULISM

Botulism 1966

Edited by M Ingram and T A Roberts (Proceedings of the Fifth International Symposium on Food Microbiology Moscow, July 1966) Pp xii+531 (London Chapman and Hall, Ltd, 1967) 126*s*

THE fifth international Symposium on Food Microbiology was the first on botulism There are more than fifty papers from at least nine countries in this account of the proceedings

In the first section, on ecology, papers on spore distribution emphasize Russian, American, Scandinavian and English investigations of *Clostridium botulinum* type *E* in fish, mud and soil samples In a paper on fish and fish products associated with type *E* there is some sound commercial and public health advice about the reasonable shelf life of smoked and packed fish products, the action of inhibitors from smoke, and the dangers inherent in casual home or peasant industry production of smoked or preserved fish products

Concerning spore survival there is strong emphasis on certain aspects of food processing, and in particular the association of the concentration of sodium chloride, nitrate and nitrite with the doses of gamma radiation necessary to destroy *Cl botulinum* There is comment on the addition of sodium citrate for this purpose, although further work is clearly needed in this important field of preservation Concerning germination and growth are papers which partly support and substantiate the researches into the action of certain concentrations of sodium chloride, nitrate or nitrite in limiting or preventing growth and toxin production of types *A*, *B* or *E* There is re-emphasis of the inherent dangers of vacuum packed products held at relatively high storage temperatures

The difficulties, as well as the advanced and intensive work, concerning the establishment of precise data about the chemical constitution of botulism toxins are evident from papers in the second section, on the characterization of botulinus toxins Work on the action of some animal and fungal enzymes on toxins has a possible practical application in connexion with the high and low risk of certain foods The action of trypsin on a precursor (protoxin) considered to be practically inactive is investigated in a Japanese paper, which stresses the need for the molecular concept of research Findings from such work may have some clinical significance in the treatment of botulism when the area of maximal toxin absorption in the intestine and the criteria for activation of an inert protoxin are considered

The third section on animal botulism covers the disease in mink, gulls and duck The work on mink is an investigation of type *C* and its toxin production only when mixed with a symbiotic organism Death in gulls caused by botulism is proved, either directly or by drowning when in water, because of loss of stability It seems that a less ill bird may well recover on land but would probably die by drowning when on water

The fourth small section of special topics has a paper which reports how electrophoresis was used to examine relationships between *Cl. botulinum* and non-toxic *Clostridium* or even non-toxic mutants Results are preliminary but encouraging. Other research has suggested that the production of bacteriocine by *Cl botulinum* is not related to its toxigenic power

In the fifth section on laboratory diagnostic methods and techniques there is a specific description of the isolation of type *F* in the Pacific coast area of the United States, and a further paper outlines the problems of isolation and identification from heavily contaminated sources

The final section concerns the control of botulism and clarifies the reasons why controlling this disease needs so much precision and care, and emphasizes the public health problems associated with the difficulties many laboratories have in finding reasonable methods of detecting suspected botulism contamination. A very true reference is made to the generation of management and workers who have never been directly associated with any knowledge of a classical outbreak of botulism This is also praise for the commercial standards in the food industries, but obviously a spirit of apathy or even ignorance could develop Basic knowledge is always needed to appreciate the potential risks and problems. The need to maintain strict standards and not to deviate from proved techniques is substantiated by a description of an outbreak of botulism investigated in North America, involving a canned liver paste

I must congratulate the publishers and the editors, the result of their work is a most comprehensive and informative volume It should be of interest to many workers beyond the immediate field of food microbiology, for so much of the work reported is fundamental rather than applied I L MARTIN

A MODEST PROPOSAL

Report from Iron Mountain

On the Possibility and Desirability of Peace With Introductory Material by Leonard C Lewin Pp 141 (London Macdonald) 18*s*

MR LEWIN's work is almost a major literary event His satire on an American think-tank's treatise on the dire consequences of peace is nearly in a class with Swift's proposal for relieving famine in Ireland The beginning is brilliant John Doe, a professor at a large university in the Middle West, "telephoned me one evening last winter. . We met for lunch the next day at a midtown restaurant He was obviously disturbed . ".

The suspense grows What Mr Doe has to hand over is the report of a Special Study Group organized by the American government to prepare a contingency plan for "permanent peace" The group's first and last meetings were held at Iron Mountain, New York (a real place where corporation records are stored on microfilm in underground vaults), but otherwise it shifted from place to place to preserve secrecy Its conclusions were so shocking—that war is necessary to social stability—that both the committee and the government suppressed it. Mr Doe (a pseudonym, of course) none the less felt obliged to see the report published and handed it over to Mr Lewin, who saw to publication

The book is said to have caused great stir in America, where some people think that Professor Galbraith is the true author and others even suspect that it is the genuine handiwork of a secret governmental commission That is hard to believe The body of the book—the alleged "report"—displays the same wit and style as does the introduction, the best guess is that it is Mr Lewin's handiwork through and through His ear for the speech of the American defence pedant is perfect that blend of ponderous jargon and uncomfortable frankness: "Most of us [the committee] and that includes me, had a record of abnormally high uric acid concentrations in the blood" The trouble is that his imitation succeeds too well. The report, as it is spun out, is as dull as its real-life counterpart page-length paragraphs, cluttered with italics and footnotes and latinate neologisms

The question to be asked is whether this book is genuinely shocking People who believe in general and complete disarmament will think so and will be grateful to Mr Lewin for exposing the callousness of some American strategists What could be uglier than to calculate that war is necessary, not only as a balance to an economy otherwise regulated by supply and demand, but as a siphon to remove the ignorant young disruptive men from society ? But whether others will find it disturbing is doubtful Is war really "the principal motivational force for the development of science at every level" ? If one cannot assent to this, or similar assertions, then the thesis of Iron Mountain Boys (as they sometimes referred to themselves) loses its force

It is even possible that this book fails in its purpose to stimulate people to think about the dependence of the American economy on weapons production and of the American government on the advice of experts who suppose themselves to be objective It pretends that there is no permanent peace because societies do not find it useful, rather than because aggression is part of the human condition And it perpetuates the "we-they" myth that is what wars are often about "We" are quite different from "them" and they are wrong ("Them" in the context of this book are the think-tanks, the Rand Corporation, the Hudson Institute, and Mr Herman Kahn, the father of the mega corpse)

A modest proposal, therefore, from an admirer Mr Lewin is an excellent writer his dialogue is crisp, his mastery of the idiom faultless, his sense of showmanship superb, and could anyone have thought of a better title ? Could he not acknowledge his authorship of this book and go on to writing more fiction ? BRENDA MADDOX

University News : University College of Swansea

DR P C THONEMANN, at present chief research scientist, Division head, Experimental Division B, Culham Laboratory, UKAEA, has been appointed a professor of physics and head of the Department of Physics as from April 22

Appointments

REAR ADMIRAL JOHN E. CLARK has been appointed deputy director of the Jet Propulsion Laboratory of the California Institute of Technology

DR S G OWEN, at present reader in medicine and clinical sub-dean in the University of Newcastle upon Tyne, has been appointed second secretary to the Medical Research Council

Announcements

THE Institution of Chemical Engineers has instituted a new medal, the Council Medal, which is to be awarded annually to a person who has given exceptional service to the institution on a special project or projects The first award is to be made to Mr P L Baldwin, in particular for his work as chairman of the working party on "The Controlled Storage and Recovery of Particulate Solids" The Arnold Greene Medal, given annually for contributions to the institution's progress, has been awarded to Mr H Fossett

Meetings

ELECTROCHEMISTRY, February 19–23, Melbourne (Dr D F A Koch, Division of Mineral Chemistry, CSIRO, P O. Box 124, Port Melbourne, Victoria, Australia)

CITRUS Symposium, March 17–26, University of California, Riverside (Professor H D Chapman, Department of Soils and Plant Nutrition, University of California, Riverside, California 92502)

METALLURGICAL Developments in Low-Alloy Steels for Mechanical Engineering Applications, April 2–4, Scarborough (Mr J R Powell, BISRA, 24 Buckingham Gate, London SW1)

HIGH Pressure Research Meeting, April 9–11, Centre d'Etudes Nucléaires de Cadarache (M Rapin, CEA, Service MA, B P 61, 92 Montrouge, France)

INTERFERENCE Problems associated with the Operation of Microwave Communications Systems, April 22–24, Institution of Electrical Engineers (Conference Department, The Institution of Electrical Engineers, Savoy Place, London WC2)

AUTOMATION for Productivity, May 14–16, Institution of Electrical Engineers (Conference Department, The Institution of Electrical Engineers, Savoy Place, London WC2)

INDUSTRIAL Measurement Techniques for On-line Computers, June 10–14, Institution of Electrical Engineers (Conference Department, The Institution of Electrical Engineers, Savoy Place, London WC2)

CORRESPONDENCE

SI Units

SIR,—The issue of *Nature* of December 30 (216, 1272, 1967) quotes a draft of a document by a committee of the Royal Society on metric units and the SI Listed together with SI units are "Units to be allowed in conjunction with SI" These are described in the text as "compatible" with SI although the meaning of this is not clear in many cases it appears to be merely that they are multiples of ten times the SI unit These multiples are not, in general, in the recommended steps of one thousand

The SI at last offers a real opportunity of establishing a coherent system of units which is common to both scientists and engineers Once alternative units are allowed, coherence is lost and we will continue to have "working formulae" (using the non-SI units) which differ from the basic equations Conversion factors, the curse of applied scientists, will remain with us—and they will be unfamiliar ones It is difficult to understand why a body such as the Royal Society should advocate the retention of non-SI units such as the poise and stoke—presumably merely because they are familiar The case for the bar is little better it is of convenient size for some purposes, but is this of any real consequence ? The use of prefixes to indicate multiples and submultiples of basic units is an entirely satisfactory and well established practice for example, the μF and pF are in everyday practical use, the basic unit, the farad, being itself well outside the range of magnitude commonly encountered The SI unit of pressure, the N/m^2, is not far from the centre of the range of common pressure measurement, bearing in mind the existence of vacuum technology.

Is it too late to hope that the Royal Society will fully recognize the advantages of having one unit for one quantity, and of having coherent units, and therefore to abandon the bar, stoke, poise and gauss ? No doubt there is justification for a few non-SI units, but it does not exist for these quantities

G W. ALDERTON

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Upton Heath,
Chester

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 5

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, WC2), at 5 30 p m —Mr F E Williams, Mr F A Wilson and Mr K A T. Knox "Future Trends in the Design of Subscribers' Telephone Instruments"

Institution of Electrical Engineers, Joint Professional Group (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Specification of the British Electrical Indicating Instrument", opened by Mr L B S Golds

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W 1), at 6 p m —Mr L G Culleton Presidential Address

Society of Chemical Industry, London Section (joint meeting with the Fine Chemicals Group, at 14 Belgrave Square, London, SW1), at 6 30 p m —Professor R C Cookson "Some Aspects of Photochemistry"

Tuesday, February 6

Society of Chemical Industry, Pesticides Group—Physicochemical and Biophysical Panel (at 14 Belgrave Square, London, SW1), at 10 a m —Symposium on "The Effects of Rain on Plants and Pests"

University of London (at the Royal Veterinary College, Royal College Street, London, NW1), at 5 p m —Professor M G P Stoker "Tumour Viruses and Tumour Cells" *

Chelsea College of Science and Technology, University of London (at College Hall, College House, Manresa Road, London, SW3), at 5 30 p m —Professor Dorothy Hodgkin, OM, FRS "Molecules and Biology" (Chelsea Lecture)

Institution of Electrical Engineers, Control and Automation Division (at Savoy Place, London, WC2), at 5 30 p m —Mr K Smith "Automatic Landing of Aircraft—Control Techniques in All-weather Aircraft Operations"

Institution of Electrical Engineers, Power Division (at Savoy Place, London, WC2), at 5 30 p m —Mr K R Haigh "Electrical Cable Glands for Underwater Vehicles and Oceanographic Equipment"

Royal Institution (at 21 Albemarle Street, London, W1), at 5 30 p m —Sir Lawrence Bragg, CH, FRS "How We Hear, Speak and See" (McRobert Lecture Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties To be repeated on February 7)

University of London (at King's College, Strand, London, WC2), at 5 30 p m —Dr M. H Seevers (Michigan) "Psychological Aspects of Drug Dependence" *

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Professor A N Davison "The Myelin Sheath and Its Biochemistry" (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Society of Chemical Industry (at 14 Belgrave Square, London, SW1), at 6 p m —Dr N J L Megson "Leo Hendrik Baekeland and the Chemistry of Phenolic Resins" (Baekeland Memorial Lecture)

Wednesday, February 7

Colour Group (Great Britain) (at Imperial College, London, SW7)—Meeting on "Cosmetics" Dr T J Elliott "Colour Cosmetics", "Television Make-up" by a member of the BBC Make-Up Department

Royal Institution, History of Science Discussion Group (at 21, Albemarle Street London W1), at 1 p m —Lt Commander D W Waters "The Adoption of Marine Time Keepers at Sea" *

Society for Analytical Chemistry (at the Wellcome Foundation, Euston Road, London, NW1), at 3 p m —Meeting on "Enthalpimetry"

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, WC2), at 5 30 p m —Dr H G Lubszynski, Mr N Barford, Dr B Mayo and Mr J Wardley "A New Electrostatically Focused and Deflected Vidicon", Mr P C Ruggles, Dr N A Slark, Mr D P Mouser and Mr P H Batev "Beam Separation and Low Noise Read-out in Isocons"

Royal Microscopical Society (at the Middlesex Hospital Medical School, Cleveland Street, London, W1), at 5 30 p m —Dr A Boyde "Electron Microscopy of Surfaces of Hard and Soft Tissues"

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Professor J G Robson "Pain and Pain Suppression" (First of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation)*

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Training of Engineers for Management"

University of London (at the Institute of Archaeology, Gordon Square, London, WC1), at 6 p m —Mr I P Joliffe "Sources of Maritime Archaeology —Coastal Research in Britain" *

Thursday, February 8

Royal Society (at 6 Carlton House Terrace, London, SW1), at 9 30 a m —Discussion Meeting on "The Logical Analysis of Cerebral Functions" organized by Professor J Z Young, FRS, and Professor H C Longuet-Higgins, FRS

Royal Institution, Library Circle (at 21 Albemarle Street London, W1), at 1 p m —Mr E Howe "William Savage and the Royal Institution"

Society for Analytical Chemistry, Particle Size Analysis Group and the North of England Section of the Society for Analytical Chemistry, with the Manchester Section and Colloid and Surface Chemistry Group of the Society of Chemical Industry (at the Manchester Literary and Philosophical Society, 36 George Street, Manchester), at 2 30 p m —Meeting on "Dispersion Problems in Particle Size Analysis"

Institution of Electrical Engineers, Control and Automation Division (at Savoy Place, London, WC2) at 5 30 p m —Mr R H D Hardy "A System for Transmission and Display of Weather Information"

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Dr A M Harper "Regulation of Cerebral Circulation" (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Institution of Mechanical Engineers, Internal Combustion Engines Group, and the Diesel Engineers and Users Association (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Investigation of Diesel Engine and Turbo-Charger Interaction"

University of London (at Queen Elizabeth College, Campden Hill Road, London, W8), at 6 p m —Professor D Gabor FRS "Foreseeing and Controlling the Future" *

Arboricultural Association (in the Lecture Room Royal Horticultural Society's New Hall, Greycoat Street, London, SW1), at 6 30 p m —Mr Roland Huddleston and Mr Michael Hardy "The First Six Months of the Civic Amenities Act"

University of London (at Withersdane Hall, Wye College, near Ashford, Kent), at 8 15 p m —Professor J N Black "Energy Relations in the Management of Resource Systems" *

Friday, February 9

Federation of British Plant Pathologists (in the Lecture Theatre, British Museum (Natural History), Cromwell Road, London, SW7), at 10 50 a m —Discussion Meeting on "The Techniques and Problems in the Production and Use of Virus-tested Planting Material"

Royal Institution Photochemistry Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Dr Y V Morozov "Radiative and Radiationless Transition Probabilities in Organic Molecules" *

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, WC2), at 5 30 p m —Mr G F Craven "Evanescent Mode Filters"

Royal Institution (at 21 Albemarle Street, London, W1) at 9 p m —Professor Paul T Matthews, FRS, "Elementary Particles".

Saturday, February 10

British Society of Rheology, and the British Microcirculation Society (at the Royal College of Surgeons, Lincoln's Inn Fields, London, WC2)—Symposium on "Haemorheology and the Microcirculation"

Inner London Education Authority (at the Horniman Museum London Road, Forest Hill, London, SE23), at 3 30 p m —Professor A N Tucker "Life and Death of an East African Rainmaker" *

Monday, February 12

University of London (in the Botany Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Dr R J Benthem (Utrecht) "Conservation in Land Use Development I Reconstruction of Landscape" *

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Passenger Handling"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer (with a good honours degree in mechanical engineering or in a related subject, and preferably a particular interest in mechanical vibration and/or control systems) in the Mechanics and Mechanism Group of the Department of Mechanical Engineering, to contribute to the teaching and research work of the Department in the field of dynamics—The Secretary to the University Court, The University, Glasgow (February 10)

Lecturer or Assistant Lecturer (preferably with research interests in semi-conductors, solid state electronics and surface electronics) in the Department of Electrical Engineering and Electronics—The Registrar, The University, Liverpool, quoting Ref RV/342 (February 15)

Assistant Lecturers or Lecturers (2) in the Department of Pure Mathematics (for at least one of these posts preference will be given to applicants with special interest in functional analysis)—The Registrar, The University, Liverpool, quoting Ref RV/313 (February 16)

Lecturer (either medically or scientifically qualified candidate) in the Department of Chemical Pathology—The Registrar and Secretary, The University, Leeds, 2 (February 17)

Lecturer (with a higher qualification in medicine) in Therapeutics—The Acting Secretary, The University, Aberdeen (February 17)

Lecturers (2) (with university, commercial or industrial experience in some branch of computer science) in Computing—The Secretary, The University, Aberdeen (February 17)

Second Chair of Applied Mathematics—The Registrar, The University, Newcastle upon Tyne (February 17)

Research Fellow in Textile Engineering to investigate problems concerned with the division of heat and wear between two high speed steel rubbing surfaces—The Registrar and Secretary, The University, Leeds, 2, quoting Ref 68/38/2 (February 19)

Assistant Lecturer in Biometrics—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (February 20)

Lecturer (with a good honours degree and preferably a minimum of two years' practical experience in industry and/or research) in the Department of Civil Engineering—The Registrar, Queen Mary College (University of London), Mile End Road, London, E1 (February 23)

Assistant Experimental Officer (with a degree in a biological subject or an equivalent qualification) to take part in research on hybrid varieties of wheat and barley—The Secretary, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge (February 26)

Lecturer or Assistant Lecturer in Experimental Psychology—The Assistant Registrar (Establishment), The University of Sussex, Essex House, Falmer, Brighton, Sussex, quoting Ref 873/1 (February 29)

READER IN ANTHROPOLOGY at the LONDON SCHOOL OF ECONOMICS—The Academic Registrar (A), University of London, Senate House, London, WC1 (March 1)

READER/SENIOR LECTURER in MATHEMATICS (STOCHASTIC PROCESSES) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Brisbane, March 1)

SENIOR LECTURER, LECTURER or ASSISTANT LECTURER (preferably with a special interest in comparative vertebrate zoology or animal physiology or animal (vertebrate) ecology) in ZOOLOGY at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, WC1 (March 8)

UNIVERSITY POSTDOCTORAL TEACHING FELLOW in the DEPARTMENT OF CHEMISTRY—The Registrar, University of Nottingham, University Park, Nottingham NG7 2RD (March 29)

CHAIR OF PHYSICS, and CHAIR OF CHEMISTRY at Flinders University of South Australia—The Registrar, Flinders University of South Australia, Bedford Park, South Australia, or The Secretary-General, The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia, March 31)

GWENDOLYN MICHELL CHAIR OF MEDICINE at the University of Adelaide—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia, March 31)

CHAIR OF PHYSICS—The Institute of Physics, University of Aarhus, 8,000 Aarhus, C, Denmark (April 1)

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(not included in the monthly Books Supplement)

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Ministry of Technology Water Pollution Research 1966 The Report of the Water Pollution Research Laboratory Steering Committee with the Report of the Director of Water Pollution Research Pp xii+176+4 plates (London H M Stationery Office, 1967) 15s 6*d* net [2111

The Royal Society Scientific Research in Schools Committee—Report to Council 1967 Pp 17 (London The Royal Society, 1967) [2211

The Seams of the South Wales Coalfield By H F Adams Pp vi+20 plates (London The Institution of Mining Engineers, 1967) 42*s* [2211

University of Sussex Science Policy Research Unit—First Report 1967 Pp 22 (Brighton University of Sussex, 1967) [2311

Discovery Reports, Vol 35 Pp 1–30 Seasonal Formation of Laminae in the Ear Plug of the Fin Whale By H S J Roe (London Cambridge University Press, 1967) 26*s* net [2311

Bulletin of the British Museum (Natural History) Geology Vol 15, No 3 Burrows and Surface Traces from the Lower Chalk of Southern England. By W J Kennedy Pp 125–167+9 plates (London British Museum (Natural History), 1967) 42*s* [2311

Report of a Discussion Meeting on Data for Science and Technology held at the Royal Society, London, on 5 June, 1967 Pp 65 (London The Royal Society, 1967) [2711

Mental Health Research Fund Pamphlet No 5 The Psychosomatic Disorders Pp 15 (London Mental Health Research Fund, 1967) [2711

University of Oxford Report of the Committee on the Ashmolean Museum (Supplement No 1 to the *University Gazette*, Vol 98, November 1967) Pp 99 (Oxford The University, 1967) 10*s* [2711

Department of Education and Science Report of the Committee on Scales of Salaries for the Teaching Staff of Colleges of Education—England and Wales, 1967 Pp vii+15 (London H M Stationery Office, 1967) 2*s* 6*d* net [2711

National Institute for Research in Dairying—Report 1966 Pp 178 (Shinfield, Berkshire National Institute for Research in Dairying, Universit of Reading, 1967) 10*s* [281

The Royal Society Report of Council for the year ended 30 Septembe 1967 Pp 85 (London The Royal Society, 1967) [281

Footpaths and Bridleways a Plea for their Protection Pp 9 (London The Ramblers' Association, 1967) 1*s* 6*d* [281

Other Countries

U S Department of Commerce/Environmental Science Services Administration Winter Storms Pp 8 (Washington, D C Government Printin Office, 1967) $0 15 [271

National Museum of Canada Natural History Papers, No 35 (Septembe 30, 1967) Notes on the Biostratigraphy of Dinosaurian and Microfossi Faunas in the Edmonton Formation (Cretaceous), Alberta By Dale A Russell and T Potter Chamney Pp 22 (Ottawa Queen's Printer 1967) [2711

The Annual of Czechoslovak Medical Literature, 1965 (National Medica Library of Czechoslovakia) Pp xix+697 (Praha Czechoslovak Medica Press, 1967) [2911

India Council of Scientific and Industrial Research Annual Report o the Central Mining Research Station 1965–1966 Pp 394 (Dhanbad, Bihar India Central Mining Research Station, 1967) [2911

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The Walter and Eliza Hall Institute of Medical Research Annual Reports, 1966–67 Pp 93 (Parkville, Victoria The Walter and Eliza Hall Institute of Medical Research, 1967) [2911

Republic of South Africa Department of Commerce and Industries Division of Sea Fisheries Investigational Report No 63 The Occurrence of Phyllosomata Off the Cape with particular reference to *Jasus lalandii*. By B I Lazarus Pp 38 (Sea Point, Cape Town Division of Sea Fisheries, 1967) [2911

Annals of the South African Museum, Vol 49, Part 1 Bantu Pottery of Southern Africa By A C Lawton Pp 440+25 plates (Cape Town South African Museum, 1967) [2911

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Indian Forest Bulletin No 246, New Series (Entomology) Revision of the Indian Species of the Genus *Microcerotermes silvestri* (Isoptera Termitidae Amitermitinae) By Baini Prashas, R S Thapa and P K Sen-Sarma Pp 56 (Delhi Manager of Publications 1966) Rs 8 00 [3011

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NATURE

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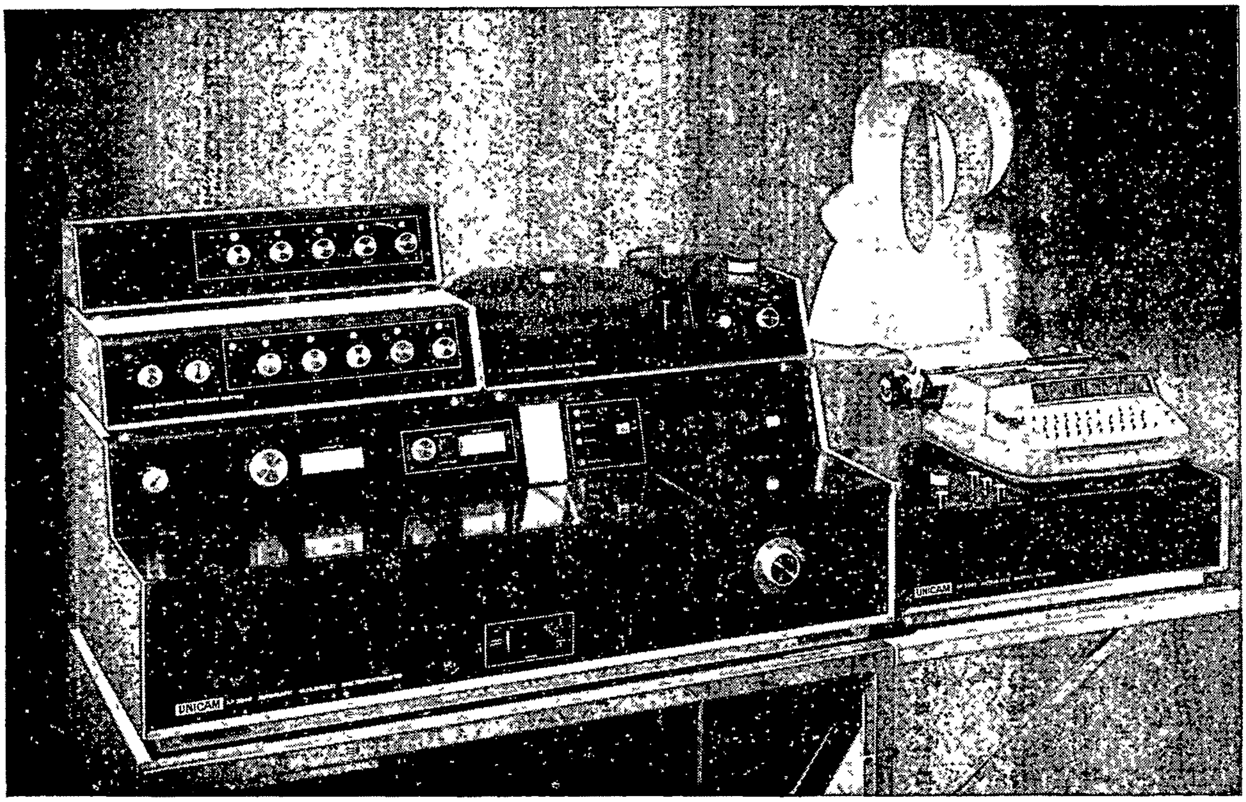

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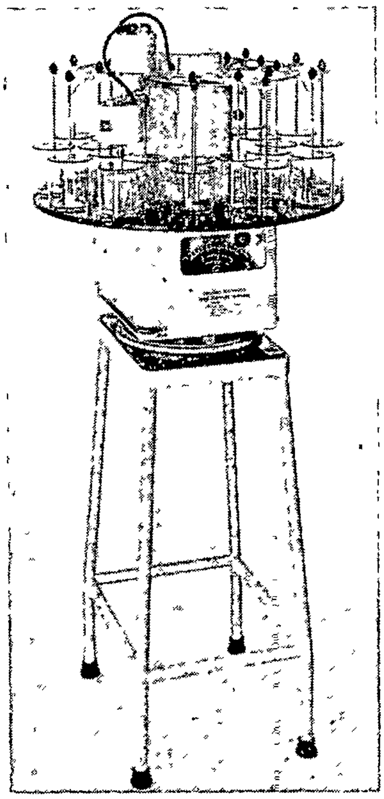

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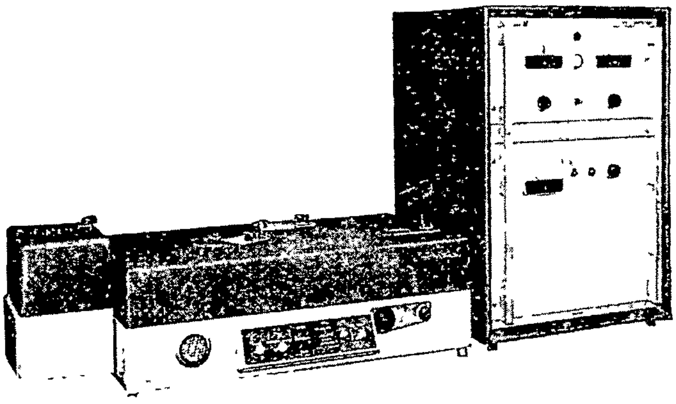

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FEBRUARY 10, 1968

Less Money to Spend

THERE is very little to cheer about in the United States budget for 1969 (see page 505), although there may be some relief among scientists that several conspicuous projects have survived more or less unscathed the wave of parsimony which has swept through the Federal Government in the wake of the Vietnam war The new proton accelerator at Weston in Illinois will be built on time (or perhaps even sooner, to judge from the vigour and the cleverness with which Dr Robert Wilson and his colleagues have embarked on the detailed design work now in hand) Expenditure on defence research will also increase, though principally because of the decisions taken in the past few months about the development of new kinds of weapons systems—the anti-ballistic missile system, for example

With economy in the air, it is unlikely that much of the extra money being laid out by the Department of Defense will spill over into other fields—indeed, there is a serious danger that the economies will actually diminish the amounts of money which the armed services have been accustomed to spend on the support of comparatively academic research in the United States Nobody should be surprised if that happens, for it has been clear for some time that over-dependence for research funds on what the military departments can afford to spare from their large budgets is potentially too dangerous for comfort, to say the best about it. In the same spirit, it is not remarkable that NASA, in its quite understandable attempt to preserve for itself as much freedom of manoeuvre as possible for the period after 1969, should have chosen to economize by cutting back on what it proposes to spend on strictly scientific work

It remains to be seen how these economies will be apportioned between the companies which build and launch satellites and the teams of people in the universities responsible for the design of instruments and the interpretation of results, but here again there is a danger that the universities will find themselves bereft of funds on which they have become accustomed to rely If next year Congress should be as eager to reduce the Administration's requests for funds as it has been in the current financial year, the consequences for American universities could be quite serious

So what, then, should be done? At this stage no purpose will be served by wringing hands about the prospect of misfortune The reasons for the financial stringency which has come on the United States are plain enough for most people to see, and much as many people would like to see them go away, there are few who would pretend that the full weight of policy-making in the United States should be exclusively determined by a concern for the health of fundamental science Moreover, it is only seemly to acknowledge that there has been no serious interruption of the tendency, which has become apparent in recent years, for the Federal Government to shoulder increasing responsibility for the welfare of higher education The consequences are most clearly to be seen in the continuing growth of financial support for university students, graduates and others In the long run, universities in the United States can only profit from still further developments along these lines, for some sharing of the cost to students and their families of four years at a university is a necessary means of making sure that all the people in need of university education eventually find their way there

By the same test, however, American universities, already jealous enough of their independence, may take recent experience in Britain as a further warning of the risks of Federal subventions for buildings and other facilities Whether state governments or the nostalgia of alumni are invariably preferable to the Federal Government as sources of support is another issue, of course, but even in the United States the entirely independent university is almost a thing of the past The point which emerges most clearly from the new budget is that the fabric of university teaching in the United States will not be unduly cramped by what has happened

The pinch will be felt in the research laboratories, and the most constructive course to follow will be to seek the kinds of structural changes in the machinery for supporting fundamental research which are likely best to serve the long-term interests of fundamental research, both in the universities and elsewhere The most urgent need is to re-examine the intervention of the mission-oriented agencies in the support of fundamental research—which does not imply that the Office of Naval Research was anything but far-sighted when it embarked on its academic programme of research after the war, that the Atomic Energy Commission is in any way deficient in its role as indulgent uncle to laboratories such as the Brookhaven National Laboratory and provider of cyclotrons to the universities, or even that the Department of Defense is malevolent in seeking to foster centres of excellence at the universities by supplying funds under what it calls Project Themis The big-spending agencies have done a splendid job in the past two decades, but there are now a great many reasons for believing that the agencies should play a smaller part The fact that science budgets tend, at present, to be marginal budgets is the immediate reason for thinking this It is, however, much more important that in present circumstances there is no ready means by which the intentions and the ambitions of the several agencies can be compared effectively with each other And in the long run the United States as a whole should profit from some machinery for making sure, for example, that a reasonable wish to embark on a programme of space research should not be followed by some of the extravagances which have marked the past few years In other words, the goal should be an independent agency able to make decisions about the commitment of resources in the whole of fundamental research and with the power to spend money as well.

This, of course, is where thoughts turn naturally to the independent agencies—the National Science Foundation and the National Institutes of Health On paper, at least, both of them seem to have done reasonably well Both of them will have a little more money, and the extra $24 million on the budget of the National Science Foundation works out at something like an increase of 5 per cent In practice, however, this means an increase of merely $14 million on what the foundation has to spend on basic research, and there is no question that the $230 million at the foundation's disposal is too small a proportion—10 per cent—of the federal money which finds its way into research at the universities This anomaly is particularly galling when it is acknowledged that the foundation spends nearly as much on the support of science teaching as on research, and that the cost of what are called supporting services—information handling and forward planning—had already passed $50 million a year ago This is not, of course, to suggest that these activities are not good works, although it is probably too late for the National Science Foundation to recapture the coordinating functions which have long since been assumed by the Office of Science and Technology There is, however, a real danger that the tail will wag the dog, or even that the dog itself will altogether disappear

But the National Science Foundation is too weak to occupy the centre of the stage This is the customary explanation of its lowly position in the pecking order The truth is that some of this weakness is internal—and remediable—and that some of it is a simple consequence of the way in which the mission-oriented agencies act as patrons of the sciences on a lavish scale The Daddario Bill, which has been hatching in Congress so long as to suggest that it may now be addled, will not tackle this problem, and it is only fair to acknowledge that a good many influential officers of the Administration, especially in the Office of Science and Technology, do not see it as a problem at all In practice, however, it will be a great misfortune if the year ahead, which may well see the retirement of Dr L Haworth, the director of the National Science Foundation, is not also taken as an opportunity for a massive strengthening of its position

Physicists and Public Issues

THE American Physical Society seems to be making heavy weather (see page 502) of the proposal by Dr Charles Schwartz that members of the society should be allowed to raise political issues at meetings and in various publications of the society It needs very little clairvoyance to know what kinds of issues Dr Schwartz and his supporters wish to raise, and it would, of course, be wrong to suggest that physicists should be less concerned about matters such as the war in Vietnam or the construction of anti-missile defences than other professional groups—sinologists, for example, or radio engineers It would also be wrong to diminish the importance of some of the issues which Dr Schwartz and his supporters have quoted as potential subjects for public discussion within the American Physical Society What is happening in south-east Asia, for example, may well become a turning point in modern history It is also inevitable that political developments of various kinds—the costing of a budget, for example—can be of vital importance for the profession of physics But this, of course, is not the point The immediate question is the extent to which great learned societies should engage in the public discussion of important public issues Although there is very little doubt that Dr Schwartz and his supporters are making an unacceptable request, the issue is interesting and also important They have done a public service in bringing it into the open

There are several interlocking questions to be decided, but the one which has most often been overlooked in the debate in *Physics Today* is the proper function of a learned society In the last resort, a society is a kind of club Its existence stems from the common interests of its members To begin with, for example, most learned societies come into existence because there is a need for channels of communication between people concerned with similar academic problems The members may have bitter disagreements among themselves about all kinds of subjects—the correctness of a theory or the size of the subscription fee—but they are bound together by their common need of some means of expressing opinions to similarly interested colleagues With the passage of time, several developments may come about There is, for example, a tendency for large societies to spawn sub-societies within themselves in response to the development of new specialized interests

It is also natural that the interests of many societies should often grow beyond the academic confines in which they are launched Physical societies naturally interest themselves in questions such as the supply of trained manpower and the salaries of professional people, for example, for these, too, are issues on which the members of the societies may fairly be said to have a common interest As committees are usually well enough aware, however, even a few dissenters are enough to bring a new venture to a halt. That is as it should be, for it would be self-destructive of a society to follow a course which alienated even a handful of serious members This is the sense in which Professor

Orear is mistaken to claim that silence implies acquiescence Often it means just busyness By the same test, it is mistaken of Dr Schwartz to think that the agreement of one per cent of the membership of a society is a sufficient mandate for new undertakings Ninety-nine per cent would be a better figure from which to open negotiations But with that reservation, there is no need artificially to restrict the scope which learned societies should choose for themselves If they wish to become pressure groups, there is no reason why they should deny themselves that luxury, and it is, of course, delightfully disingenuous that Dr Edward Teller, who has been a one-man pressure group on defence policy for several years, should now maintain that pressure groups are a nuisance Where societies are concerned, what matters is what most members want

There remains the question of how physicists in the United States should set out to tackle the kinds of questions which Dr Schwartz would like to raise within the American Physical Society The first thing is that if there is a group of physicists which feels a need to take a special interest in public issues, and if it considers that it has something special to contribute, there is no reason why it should not form a society specifically for that purpose In time it might even establish a reputation for responsibility and good sense.

Fast Reactors in Europe

The European Nuclear Energy Agency celebrated its tenth anniversary in Paris last week with the cheerful announcement of a plan to investigate the building of a fast reactor as a joint project It has been decided to set up two working parties—one technical, one more directly industrial—on fast reactors The formation of the technical group was prompted by the uneasy feeling that fast reactors have so far been developed on too narrow a front. Hitherto it has been assumed that the best method of cooling is by pumping liquid sodium around the reactor At the back of people's minds has been the feeling that perhaps gas or steam would do the job equally well Although those who have invested in fast reactor development already, including the UK Atomic Energy Authority, have presumably satisfied themselves on the point, the delegates at the recent meeting in Paris saw no harm in making doubly sure The working group will therefore be making a technical study of the two systems, and comparing them with sodium cooling The group will report back in June, and if there then seems a need to go ahead and produce some hardware, this could be set in motion by the end of the year Much will, of course, depend on the reaction of individual countries to the report

The other working party has been set up with the intention of taking early industrial soundings about possible fast reactor projects Its aim is to devise ways in which any joint reactor development undertaken by ENEA could be smoothly handed over to industry when it is complete One delegate at the ENEA meeting described the formation of this group as one of the fruits of the Dragon project Dragon is coming to an end without as much industrial follow-up as some people consider it deserves Happily, there are some signs of interest, five European companies have joined forces to consider the formation of a European organization to promote the sales of reactors using Dragon high temperature technology The companies are the Nuclear Power Group (UK), Belgonucléaire (Belgium), Gutehoffnungshutte (West Germany), SNAM Projetti S p A (Italy) and Groupement Atomique Alsacienne Atlantique (France) This is an encouraging sign of international interest in the system.

At the same time, ENEA has set up a committee whose task it will be to press forward the commercial application of heavy water reactors in Europe Although almost every conceivable variety of heavy water moderated reactor has been designed—and a number have been built—electricity utilities have tended to fight shy of them The new group will attempt to break through this consumer resistance, fortified perhaps by the knowledge that the British example of the breed, the steam generating heavy water reactor at Winfrith, reached its full design capability of 100 MW late last month The UKAEA, which designed and built the SGHWR, has its own ideas about selling it, and has prepared a contract in collaboration with Finnish industry to supply an SGHWR to Imatran Voima Oy, the Finnish electricity utility

Money for British Postgraduates

The Department of Education and Science is thinking of transferring all responsibility for postgraduate awards to central government Consultations are in progress with the Committee of Vice-Chancellors and Principals, the Association of University Teachers, the Association of Principals of Technical Institutions, the Association of Teachers in Technical Institutions, the National Union of Students and the National Society of Art Education

At present these awards are made by various departments, including the Department of Education and Science and the Scottish Education Department, as well as by research councils and local education authorities in England and Wales Although local education authorities can, in fact, make awards in any field—even to cover research leading to a doctorate of philosophy—the awards are usually provided to cover vocational courses such as diplomas in management studies, or for polytechnic courses These awards are financially similar to the ones provided for first degree courses and are assessed according to parental income

The Secretary of State for Education and Science, Mr Patrick Gordon Walker, has told local authorities that the present division of responsibility is causing increasing difficulty to applicants, academic authorities and award-making bodies In 1966, at the annual conference of the Association of Education Committees, a resolution was passed calling on the Department of Education and Science to assume full responsibility for awards for postgraduate study apart from those studies of a strictly vocational nature

Mr Gordon Walker adds that this proposal is not concerned with the total number of postgraduate awards made from public funds but simply with "the most efficient and economical way of administering them" He further maintains that until the idea of central control is completely acceptable, there is no point in considering in detail the way in which this might be brought about

Controlling Drugs

THE Medicines Bill published in Britain this week (H M S O, 10*s* 6*d*)—less than five months after the Sainsbury Committee's report on the relationship between the pharmaceutical industry and the National Health Scheme—proposes a number of controls and safeguards in the importation, marketing and retailing of medicinal products One of the principal decisions made is to appoint a Medicines Commission with eight members from medicine, veterinary medicine, pharmacy, chemistry other than pharmaceutical chemistry, and pharmaceutical industry This body will advise on matters relating to the administration of the Bill or, generally, in relation to medicinal products, but it will also be required to advise on the pattern and membership of expert committees

The Bill also follows the Sainsbury Committee in laying down that a "product licence" will be required before any medicinal product can be marketed in Britain, regardless of where it is produced Doctors, dentists and others will, however, be allowed to continue making up their own mixtures, but veterinary surgeons and practitioners will have to comply with certain standards on vaccines and sera

Applications for and renewals of licences will have to be made to the licensing authority, which will take account of factors such as safety, efficacy and quality in relation to product licences, the facilities and control arrangements in relation to manufacturers' licences, and premises and storage facilities in relation to wholesale dealers' licences Requests cannot, however, be refused on the grounds of selling price and, indeed, refusal on any grounds can only be enforced after consultation with the appropriate committee or the commission The licensing authority will be able to reserve the right to suspend licences on a number of grounds—for example, if the standards to which a product is manufactured are no longer satisfactory A product licence or certificate will also be required before clinical and field trials can be carried out, and the risk involved will have to be considered by the licensing authority Moreover, every clinical trial certificate will expire after two years but can be renewed on application The question of including medicinal products in animal feeding stuffs is also covered in the Bill, this will only be possible in accordance with the conditions in a product licence or field trial certificate Ultimately it will be for the Minister of Health to specify which medicinal products are safe enough to be sold without the supervision of a pharmacist

According to the Bill, subject to certain provisions it will now be an offence for any commercially interested party to issue advertisements that are false or misleading This provision replaces the one in the Food and Drugs Act with respect to false and misleading advertisements for drugs Furthermore, the Bill allows regulations to be made concerning the particulars in an advertisement, the form and manner of presentation, and in addition it enables the licensing authority to call for copies of advertisements Persons other than licence holders will not be able to issue advertisements without the consent of a licence holder, and a fine not exceeding £100 will be imposed on anyone contravening this restriction Copyright in the British Pharmacopoeia may pass from the General Medical Council and be assigned to Her Majesty future editions will be prepared by the Medicines Commission or an appropriate committee under its direction, and the editions will then be published by the ministers on the Commission's recommendations

Obviously the success of the Bill will depend on how strictly the new regulations are followed Those who fear that the new Bill is unduly restrictive should perhaps look at the United States where the Committee of the National Academy of Sciences is giving the Food and Drug Administration a sharper bite than it has had in the last few months

The Government Inspector

THE Comptroller and Auditor-General began his visits to British universities last week First on the list was the University of Sussex at Brighton, to be followed by the University of Lancaster In spite of the cries of horror which greeted the announcement some months ago that the universities would now be subject to an audit by the comptroller and his staff, the occasion seems to have passed off without incident

The Exchequer and Audit Department, which is responsible for carrying out the audit, seems not yet certain exactly how it is to be done "We're feeling our way," one member of the staff explained But the general pattern of visits seems likely to be that two members of the staff will visit universities for about two weeks at a time, and quite literally examine the books Surprise visits are definitely not on—"We always notify the universities in advance" It is thought that about a dozen universities will be examined each year, which means that the whole lot will be covered in about four years But more frequent visits are not ruled out—it all depends how difficult the job turns out to be A new section has been set up at the department specifically to deal with the task

Scientists Redundant

THE angry reaction to the news of AEI factory closures seems to have made the giant GEC cautious in its dealing with its research workers Following the announcement that the telecommunications laboratories at Blackheath and Harlow are to close came the statement that work would be transferred to other research establishments at Coventry and Wembley, where more technicians would be needed as well as a "substantial number of engineers and scientists" Official comment suggested that the company would be more occupied in persuading people to move with them rather than in pacifying the ones who will be redundant This may indeed be the case for some of the older men, particularly in the development engineering section, because some of them have had to move several times already, to keep up with reorganizations For the rest, it is believed that offers of employment will be made to selected staff only

The closing of the Harlow sections comes as the third and final stage of reduction Originally the laboratory served three sections of AEI with a staff of 300 working on radio, sales and telecommunication In 1963 the radio interests of the company were sold, work on semiconductors and transistors ceased and total staff fell to about 180 The cables section was withdrawn in 1966 and the remaining staff of 140 remained for telecommunications Rather than being involved in the expertise of the subject, the group has concentrated particularly on the materials science side, working on advanced materials and developing microelectronics It is thought that a few workers will be offered posts at Wembley, where fundamental work in this line is carried out, but GEC already has a well established group working on materials science There is also a group at Harlow which is just getting going onto production engineering and automation, and it is feared that the GEC is just not interested in this

It is highly likely that a similar story would emerge from the Blackheath laboratories, but GEC is trying to restrain comment The statement that the company is to build up powerful research teams, particularly at Wembley, is being received with scepticism by some of those affected

New Man at Harwell

DR WALTER MARSHALL is to succeed Dr Robert Spence as director of the Atomic Energy Research Establishment at Harwell on April 1 One of his principal tasks will be to supervise the practical and commercial assistance which the establishment hopes to give to industry in Britain

Dr Marshall, who is 35, joined the AERE in 1954 and became head of its theoretical physics division in 1960 He was appointed deputy director in 1966 Dr Spence became head of the chemistry division at Harwell in 1946 and has been director since 1964 He now goes to the University of Kent to take up the mastership of the new third college, as yet unnamed, and the chair of applied chemistry

New Man in France

THERE is great pleasure in France that the new head of the Délégation Générale de la Recherche Scientifique et Technique is Dr Pierre Aigrain, until this week in charge of higher education at the Ministry of Education Dr Aigrain is 43 and is a physicist by origin and since 1959 has been secretary-general of the French Physical Society His career has alternated spells in government departments with intervals of academic life He has, for example, held scientific positions with the French navy, the atomic energy commission (CEA) and, most recently, the army He was at the Collège de France for a brief spell in the early fifties and has been connected with the universities of Lille and Paris He has won himself a high reputation as an energetic and sensible man

Physics Information

THE American Institute of Physics has landed the first instalment of a grant from the National Science Foundation which is likely to amount to more than a million dollars in the next five years and which will be spent on the study and development of new techniques in the dissemination of information in physics Work has already begun on the project, which was described at the New York meeting of the American Physical Society at the end of January by Dr H W Koch, director of the American Institute of Physics Like the American Chemical Society, the American Institute of Physics, which is a federation of several learned societies ranging in size from the American Physical Society to the Society of Rheology (with a few hundred members), has come to occupy a central position in the publication of original material in physics Dr Koch said that the institute's journals have been publishing more than 50,000 pages in aggregate each year for several years now The total size of all its journals seems in fact to be doubling every five years The American Institute of Physics is perhaps more outspoken than other similar organizations in its belief that there would be benefits in devices which could replace formal publication

The plan for the information study now begun will cover several aspects of this problem For one thing, there is to be a thorough study of the uses of computers in the storage and retrieval of information In this sense, the Institute of Physics will be following the Chemical Abstracts Service, which is now publishing lists of titles on magnetic tape It seems, however, to be acknowledged that physicists may not be as well served by computer storage as are the chemists—for one thing the costs of tape retrieval seem to be high and informal exchanges of preprints among interested groups of physicists have been well developed But the Institute of Physics is also hoping to tackle directly the qualitative problems of the scientific literature, and the programme of work ahead is to include a serious attempt to decide what part formal articles in the scientific journals should play in the dissemination of information A convincing answer would be well worth a million dollars

Doctorates in the United States

A STATISTICAL report on the education and subsequent employment of PhD students and their equivalent in the United States has been published by the National Academy of Sciences for the Office of Scientific Personnel of the National Research Council (*Doctorate Recipients from United States Universities, 1958–1966*) The source of all the information is the Documents Records File which is compiled from extremely detailed surveys of graduates completing doctoral degrees This file goes back to 1920 and must surely be the envy of higher education authorities in other countries for its thoroughness All subjects are covered in the report, though there is no doubt about the predominance of science and technology In 1966, a total number of 17,865 graduates received doctorates, of which 6,077 (34 02 per cent) were in physical sciences and engineering, and 2,869 (16 06 per cent) were in biological sciences A further 2,666 (14 92 per cent) were awarded doctorates in the social sciences Altogether there has been an average growth rate of 9 2 per cent over the period 1958–66 Engineering has had the most spectacular increase—in 1966, four times as many doctorates were given in this field as in 1958 Corresponding to the growth in numbers of PhDs are figures for the growth in numbers of colleges and universities There is also a detailed analysis of the pattern of doctoral education—the time taken to register for higher degrees after completion of first degrees, transference from one course to another and from one institution to another during the period of study and so on

Of interest to the followers of the Swann and Willis Jackson reports on scientific and technical manpower are the analyses of postdoctoral employment Colleges and universities are the main employers of higher degree graduates in all fields and a greater percentage

Table 1 FIRST EMPLOYMENT OF HIGHER DEGREE GRADUATES IN THE USA

Field of doctorate	Year of doctorate	College and university	Elementary and secondary school	Government	Non-profit	Industry	Other	Foreign employer	Total with known employer
Total all fields	1961–63	18,877	1,722	2,410	1,305	4,319	763	2,828	32,224
	1964–66	27,362	2,082	2,921	1,760	5,499	1,127	3,961	44,712
Physical sciences and engineering	1961–63	4,643	27	598	374	3,521	345	912	10,420
	1964–66	7,259	5	845	498	4,540	597	1,338	15,082
Biological sciences	1961–63	3,096	33	640	193	442	126	808	5,338
	1964–66	4,255	4	776	289	516	184	1,135	7,159
Social sciences	1961–63	3,133	143	827	372	227	191	487	5,380
	1964–66	4,328	82	833	497	255	203	610	6,808

are attracted each year This trend is especially marked in the physical sciences and engineering Industry is the major employer of physical scientists and engineers, but it employs fewer than 10 per cent in other fields The percentage of scientists and engineers with doctorates going into industry, however, has fallen rapidly from 44 per cent in 1958–60 to 30 per cent in 1964–66 Mathematics, physics and chemistry show especially sharp declines in industrial employment Fewer than 10 per cent of the new higher degree graduates accepted employment overseas, but this figure should be balanced against the number of foreign graduate students (14 per cent) in the country Brain drain enthusiasts should have fun working on all these figures

No Merger for Engineers

THE projected merger between the Institution of Mechanical Engineers and the Institution of Heating and Ventilating Engineers is off After discussions lasting the best part of a year, the heating and ventilating engineers finally decided that they no longer wished to press on with the merger The decision has left the mechanical engineers slightly baffled "We don't know why they decided against the merger" an official said this week "It's very disappointing "

Merger talks began when the heating and ventilating engineers decided that they wanted to increase their influence The easiest way of doing this, short of a royal charter which was very unlikely to be granted, was to amalgamate with an established body The obvious choice was the Institution of Mechanical Engineers, and talks began It was first agreed that the heating and ventilating engineers should enjoy a status within the combined institution much like that enjoyed by the automobile engineers They would have been virtually an autonomous group, with their own council and their own chairman, and members in the different classes of membership in the IHVE would have been able to transfer to the equivalent classes in the combined body The IHVE would have been able to publish its own journals and hold its own conferences and meetings The mechanical engineers had agreed to set up joint working parties to study the problem of technician engineers, whose position the IHVE was anxious to protect Despite all this, the heating and ventilating engineers told the mechanicals on December 20 that they did not wish to proceed

One heating engineer suggested this week that the IHVE feared that it would be swamped when it joined the mechanicals There does seem to have been some opposition within the profession, although the mechanical engineers say that they have received several letters from individual members of the IHVE regretting the decision The Institution of Mechanical Engineers denies that it ever wanted to make a takeover bid for the IHVE, and "sincerely hopes that this is not the end of the affair The door is not closed to further talks" Meanwhile, joint meetings will continue as before Clearly the hope is that the IHVE will eventually see the folly of its ways

Safety in Space

by Angela Croome

SEVERE strictures on National Aeronautics and Space Administration (NASA) complacency, which it considers was responsible for the deaths of three astronauts in last year's Apollo fire, are contained in the Congressional report of its own inquiry into the circumstances of the accident. Some of the Senate space committee are doubtful if the shortcomings of the contractors' work would have come to light without the accident, and NASA is criticized for not passing on to Congress reservations on the performance of the Apollo main contractor, North American Aviation, which was the subject of a secret NASA report in 1965 In summary, "NASA's performance—the evasiveness, the lack of candour, the patronizing attitude exhibited towards Congress, the refusal to respond fully and forthrightly to legitimate congressional inquiries, and the solicitous concern for corporate sensitivities at a time of national tragedy—can only produce a loss of congressional and public confidence in NASA programmes"

In the meantime, extensive replacement of materials in the capsule and spacesuit to make these "almost fireproof" has been completed A quick escape hatch has been substituted for the previous design The total cost of these modifications is estimated at $100 million The changes have forced the forfeit of between 600–1,000 lb in the overall weight of the spacecraft and this in turn has required the re-design of the return parachute system to support it A fully fire-resistant material, Beta fabric, has replaced high-temperature nylon (Nomex) for the outer layer of the Apollo pressure-suit The inflammable boot-soles and helmet (polyurethane) have been replaced by carboxy-nitroso rubber and a silicone material respectively Nomex has been substituted for nylon as the material of the suit liner. The opportunity has been taken to make the suit more comfortable and durable Metallized patches cover pressure points and the outside of the gloves are equipped with artificial nails or claws to assist sample collection It is estimated that about 1,000 yards will be the walking limit of an astronaut kitted out for the first lunar excursion

On its first flight on January 23 the Apollo lunar model acquitted itself well, and NASA authorities are now considering whether the other scheduled unmanned flight could be dispensed with. Premature cut-off of propulsion firings has been attributed to over-conservative programming of the on-board guidance computer and not to malfunction.

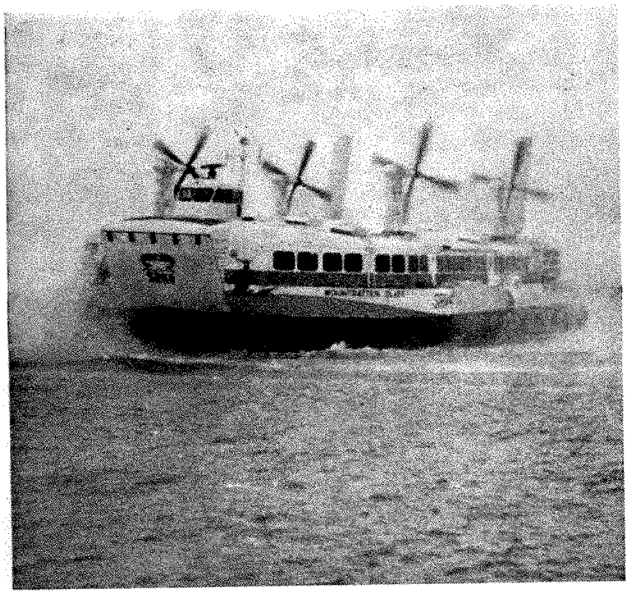
Thorough check-out of the various components of the lunar landing mission is essential, since there is considered to be no possibility of survival or rescue from an accident on or near the Moon. Dr George Mueller, director of the manned spaceflight programme, recently made clear that on the Moon there will be a 36 h oxygen supply for the two astronauts, not a large margin on the projected 22 hour stay. No rescue provisions are being made. It would take a month at least to get a relief party out there, Dr Mueller pointed out.

Nevertheless NASA has recently appointed a Safety Director whose job it will be to look into the problems of orbital rescue as well as the more conventional aspects of spacecraft and range safety. In his mid-40s, Bob P. Helgeson has had experience in atomic energy as well as with the X-15 hypersonic research aircraft programme.

Hovercraft Launched

The largest hovercraft in the world took to the water this week for its first trial. Built by the British Hovercraft Corporation at Cowes, the hovercraft is called the SRN 4, or, more grandly, the first of the Mountbatten class. It will be used as a cross-Channel ferry by British Rail and by the Swedish firm Hoverlloyd, carrying 254 passengers and 30 cars, or 600 passengers. Despite unpleasant weather conditions for the launching, the four-engined craft got away successfully, and, it seems, comparatively quietly, although so long as hovercraft are propelled by large aero engines driving propellers they are never likely to compete for quietness with muffled oars. The launching is a great moment for those who have supported the development of hovercraft in Britain, and for their inventor, Mr Christopher Cockerell, who was there to see it happen.

Sadly, it is not quite so cheerful a moment for the design staff of the British Hovercraft Corporation. The company announced this week that 40 members of the design staff working at Hitchen will be laid off over the next 2 months. The reason for this, the company says, is twofold. At the end of 1967, a number of projects which the company had hoped to start work on at Hitchen and Cowes failed to materialize. Coupled with the cuts which the Ministry of Defence has made, this has meant that design staff will have to go. Some, it is hoped, will be found work with Westland Aircraft (one of the two major shareholders in BHC). At Cowes, there should be sufficient work to sustain the design team.



The SRN4 hovercraft on its first trial.

School Mathematics

The report of the director of the School Mathematics Project for 1966–67 has a cheerful tale to tell about the acceptance of the new courses as qualifications for entry to universities. Dr Bryan Thwaites says that "with only one or two exceptions" all university departments of mathematics are prepared to accept as candidates for entry students with a pass in the Advanced level examination in what is called "SMP Mathematics". Even so, it seems, suspicions persist in the schools that a double dose of SMP is likely to be a safer means of being accepted than a single dose, and these suspicions seem to be borne out by the reaction of some of the universities to the new courses. Dr Thwaites says that "at least some university departments are going against the spirit of their formal acceptance . . ." by asking extra of intending students. It remains of course to be seen whether Dr Thwaites's exhortation will bring backsliding universities to heel.

To judge from what the report says, the publication programme is now virtually complete. This means that courses are available for students between 11 and 18. One innovation, however, is that the School Mathematics Project has turned itself into a trust which will presumably be able to superintend the further development of the teaching materials now available.

Undivided Seconds

from our Oxford Correspondent

Rather surprisingly, Congregation at Oxford has reversed its decision to divide the second class degree into upper and lower parts, a proposal accepted last term by 101 votes to 99 (see *Nature*, **216**, 631; 1967). This term's debate, on an opposition amendment that the division should be indefinitely postponed, was very much better attended. The debate, however, seemed somewhat confused, with members finding it hard to distinguish the germane from the irrelevant. There was a certain amount of doubt whether the original proposal had been to enlarge the second class as well as divide it, or merely to divide it—the two measures having rather different effects. The final vote for the amendment was 171–121, so that for some time at any rate Oxford will be making no official distinction between types of second class. What will happen, doubtless, is that fewer Thirds will be awarded, and that the Fourth Class will cease in practice to exist. So Oxford will be aligning its degree classes with those of other British universities while avoiding the difficulties of subdividing the second class candidates, most of whom would be on the borderline of the subdivision. As the second class comes to embrace all but the best and the worst, it will become more important to distinguish candidates by giving marks, and by tutorial recommendation, a system more equitable to examiner and candidate alike and one not dissimilar to the practice in universities outside Britain.

While Congregation has been discussing these moves, a group of junior members of the university has petitioned the Privy Council for changes in the procedure under which students are disciplined. At present two senior members—the Proctors—are responsible both for seeking out offenders against university regulations, and also for judging and sentencing them. The signatories of the petition are asking that the roles of police and judiciary should be separated: they propose that students should appear before a disciplinary committee to which they and the proctors would put their cases. The petition also asks that the disciplinary committee should be appointed by the Vice-Chancellor in consultation with the student council, but there is less likelihood that the authorities will accept this part of the proposal. The fact is that the university does not formally recognize junior members or their representatives, although two thousand students are graduates. It is only under an Act of Parliament, which takes a commendably tolerant attitude to their existence, that junior members are allowed to make representations to the Privy Council when new university statutes are being established. There has been no official comment on the petition from the university authorities yet.

New Man at Heriot-Watt

Dr R. A. Smith, Professor of Physics at the Massachusetts Institute of Technology, is to succeed Dr H. B. Nisbet as Vice-Chancellor of Heriot-Watt University on June 1. Dr Smith, who was born in Scotland, began a distinguished career at the Royal Radar Establishment in 1939, becoming head of the Physics Department in 1947. He took up his present chair in 1962.

At Heriot-Watt he will doubtless preside over the university's projected move from its present site in Edinburgh to Riccarton, six miles west of the city, where it will not only have room to expand but will also be less immediately under the shadow of Edinburgh University. The move to Riccarton is expected to take place in 1975, by which time the student population will have grown from 1,500 to 3,000. Whether that change will mean that people no longer ask whether Edinburgh needs two universities is another matter.

Commonwealth Universities

The United Kingdom has now replaced Canada as the Commonwealth country with most university members in the Association of Commonwealth Universities. This is revealed in the report of the association for 1966–67, in which it is announced that the admission of several new British universities brings the number of member institutions in Britain up to 43, compared with 42 in Canada.

In his introduction to the report the secretary-general of the association, Dr J. F. Foster, describes the association as mainly a service organization promoting the activities in London of its member universities in all parts of the world, including the United Kingdom. He contrasts the function of the association with that of many other international associations of universities which have as their main aim the spreading of a particular culture and encouraging studies of special questions of educational policy.

One unusual task which the association assisted during the year was the mounting of a "Retrieval Operation" by Canadian institutions. This was to persuade Canadians working in British universities to return to posts at home, and involved the sending of a party of Canadian officials to Britain. The association provided the Canadians with interviewing facilities and secretarial services in London.

The report shows that the appointments service run by the association is mostly used by members in Australia, New Zealand, Malaya and Hong Kong; this appears to be because the North American universities favour the making of an appointment after private enquiry and negotiation.

Oxbridge Admissions

A plan for studying the relationships between Oxford and Cambridge admissions arrangements and sixth form curricula in British schools has been launched with £14,000 of the Schools Council money. The operation is being financed for an initial period of two years. The investigation will be directed by Sir Desmond Lee, with a management committee consisting of Mr John Morrison, President of University College, Cambridge, Mr J. D. Mabbott, President of St John's College, Oxford, and Dr E. J. Bowen, Honorary Fellow of University College, Oxford. Sir Desmond will be retiring as headmaster of Winchester College later this year and will take up a Senior Research Fellowship at University College, Cambridge, in the autumn. In the meantime a research assistant will be appointed, and with Dr Bowen in charge of the Oxford end of the project it is hoped that work can begin in the near future.

The movement towards a broader curriculum in schools has been causing concern in Cambridge, where admission arrangements have been geared to highly specialized sixth forms, particularly those in the independent schools. A review of selection methods was thought to be necessary if the university was to continue to attract the large number of good students that it has in the past and if more entrants were to come from smaller maintained schools. The idea of a special investigation body was put forward by Mr Morrison, when the usual monthly committee on the subject was thought to be inadequate for a thorough inquiry. The scheme has grown to include Oxford because the problems of selection there are similar. Mr Morrison hopes that the investigation will reveal the extent of the problem of producing people who are prepared for the highly specialized Oxbridge courses. If there is less specialization in schools, then it will not only be the admission requirements that will need to be changed but also the content of the degree courses.

The organizers of the new project hope that the solutions which they suggest for Oxbridge may also provide something of value to other universities. Whether other universities will accept this view remains to be determined; for some years there has been some reason to complain that the existence of a special relationship between Oxbridge and the schools has set up strains elsewhere, so that an attempt to provide another special solution for the Oxbridge problem may seem a perpetuation of an existing misfortune But this may be only half the story Sir Desmond Lee is the principal architect of the Schools Council's proposal, now abandoned, that the sixth form curriculum for most British schoolchildren should consist of two principal courses of studies and a number of "minor' courses Mr Morrison, on the other hand, is an advocate of a broad curriculum based on five subjects (see *Nature*, **215**, 1329, 1967) Perhaps the Schools Council has found a way of making the principal gladiators settle their scores before tackling the more general problem of the universities as a whole

Money for Authors

AFTER years of campaigning, it looks as if British authors may yet receive royalties for their books borrowed free from public libraries So far there is no proposal for legislation, but authors have become more optimistic in the past few months The problem of paying royalties to authors—the Public Lending Right, as it is called—has been bubbling away for several years now, with occasional bursts to the surface when events help or hinder the cause Another bubble burst last week with the publication of a pamphlet, *The Arts Council and Public Lending Right* (6d , post free from the Arts Council)

This sets out the council's interest in the subject and outlines a scheme it proposes for a Public Lending Right (PLR) When the council set up its Literature Panel in 1966, one of the first steps was to appoint a special working party and its report was approved and adopted by the council in 1967 Because the money needed would have to come from public funds and government legislation would be necessary, the council has formally submitted the report to the Department of Education and Science It is anybody's guess if and when the Government will promote the necessary Bill It could possibly make the next parliamentary session, but authors fear that PLR will hardly rate among the top priorities

Authors have long been aggrieved that there is no equivalent of the Public Performing Right to cover the lending of books from public libraries Composers, publishers and authors receive fees for the performance of any work, musical or dramatic, in addition to the sale of the original publication In effect, authors believe that Public Lending has become the equivalent of Public Performance They feel that the recent development of the public libraries, which now lend about 500 million volumes a year, has taken place without anything approaching commensurate return to the producers of the books A recent survey drew attention to the plight of some authors Few earned anything like enough money to live on from their writing alone It is also argued that organized lending radically affects the economics of book publishing One library book may survive 200 issues (with one rebinding) from which the author would receive one single royalty payment and the publisher a comparable sum, from the original sale

Earlier schemes have involved payment to authors by libraries based upon the number of times the book is issued, and the money has been obtained from small charges to borrowers Librarians were, however, opposed to that scheme, two Government Bills were defeated and the subject was omitted from the 1964 Public Libraries Act Librarians argued that to charge borrowers was an abandonment of "free" public libraries, that any payment to authors by libraries would involve administrative costs to the detriment of book stocks, and that the development of public libraries helped, not hindered, book producers

In the new scheme, a system would be devised on the Danish model, using book stocks as its basis, rather than book loans as found in Sweden It is proposed that a Library Compensation Fund Committee should be established to administer a compensation fund, which would be supplied by an annual grant paid to the committee from public funds The size of the grant would be related to the total annual expenditure of Public Library Authorities on books Payments to authors and publishers would depend on the stocks held in public libraries It would be impractical for all libraries to send returns of stocks, so it is proposed that only sample libraries (three in England, for example) would send figures These stock figures would be compared with the total of stocks held by all libraries The fund committee would work out which books qualified under the scheme and it would then calculate the rate of compensation payable per volume

The scheme appears to be more easy to operate than earlier suggestions, but reservations about the scheme remain The sample of libraries, only three per year, is very small and hardly representative Only public libraries are represented, not all libraries Stock taking by the sample libraries will still be difficult, especially now that many libraries no longer keep such detailed records How will the fund committee decide which authors should benefit ?—British copyright books include a number not written by British nationals Computerization of records for administering the fund might be possible now that books published since October last year have a standard book number, but this would exclude the thousands of books published previously These points will all have to be thought out in detail by the Department of Education and Science if the scheme is to work

New Abstracts

A NEW abstracts journal, *Metals Abstracts*, has begun its monthly appearance with an issue for January 1968 The journal is published jointly by the Institute of Metals, London, and the American Society for Metals, Cleveland, Ohio It replaces the *Review of Metal Literature* and *Metallurgical Abstracts*, the respective publications of the two societies, which will now cease Overlap between them was in fact considerable, and the new journal is a rationalization which should benefit both its producers and its users

Metals Abstracts aims to provide a complete coverage of the world's metallurgical literature, and will in fact contain almost twice the number of abstracts that appeared in the British *Metallurgical Abstracts* Industrial users in Britain may regret, however, that its price to them has also increased, the former will cost £116 ($280) a year, while the latter cost £20 For libraries the cost of *Metals Abstracts* will be £41 ($100) and for members of the societies £10 ($25) Monthly and annual indexes will be provided for additional fees

The new journal is produced and printed in Britain, the index, compiled by a computer, is produced in the United States.

Physicists and Politics

The discussion of public issues by scientists is less rare in the United States than in Britain, where lips are usually sealed—in public, at least. But despite greater individual loquacity in the United States and the existence of several scientific journals which devote themselves to a discussion of public issues (*Scientist and Citizen* and *The Bulletin of the Atomic Scientists*, for example), the learned societies have so far kept their meetings and their journals free from controversial issues such as the war in Vietnam. But if Dr Charles Schwartz of the University of California at Berkeley has his way, the American Physical Society will soon change all this. He has proposed to the society an amendment which would allow discussion of any issue if one per cent of the membership of the society wished it. The suggestion was discussed at the APS meeting which has just finished in Chicago, and now goes to a full ballot of the membership of the society.

Predictably enough, the issue has roused passions on both sides. Dr Schwartz himself has had a brisk wrangle with the editors of *Physics Today*, an APS publication, about whether it should publish an account by him of the proposed amendment. His letter, eventually published along with others which give both sides of the issue, says that "there exists a whole range of issues where the technical activity of physicists gets tied up with political decision making". Unless physicists can make their opinions clear, Dr Schwartz says, they deserve to be judged by the dictum "silence implies consent". Dr Jay Orear, of Cornell University, who is chairman of the Federation of American Scientists, agrees. He thinks that scientists have a duty to protect the public from scientific hoaxes, large or small, and suggests that the establishment of an anti-missile defence system is a suitable place to start.

Dr Karl K. Darrow, however, the secretary emeritus of the APS, has other ideas. "The society has lived, thrived and done immeasurable service to physics under constitutions that limit its object to the advancement and diffusion of the knowledge of physics." Besides, opening the society to political discussions might endanger its tax-free status, he thinks. Edward Teller, from the Lawrence Radiation Laboratory, makes a more philosophical point. He says that pressure groups which exist in the United States have not been a beneficial addition to the constitution. Although, as he says, "it is a widely accepted thesis among physicists that they know best and should be consulted on every occasion", he thinks that physicists do best by keeping quiet, except on rare occasions when politicians make outrageous statements. Dr Frederick Seitz, president of the National Academy of Sciences, says that physicists have "countless avenues outside their profession through which they can express their views". He opposes the amendment, but sees no reason why *Physics Today* should not be used for some measure of social or political comment on subjects immediately related to physics.

In Britain, nobody has ever suggested that the Institute of Physics and the Physical Society (as it likes to call itself) should go in for political discussion, and Dr L. Cohen, secretary of the society, sounds enormously relieved that this is so. "There's a difference in temperament, and, of course, we don't have the Vietnam war," he says, explaining the apparent indifference of British scientists. Nevertheless, there is occasionally a need for careful management—the CERN 300 GeV machine, for example, is now as much a political as it is a scientific decision. Political discussions, he feels, would be a disruptive influence on the society, which already has to weather the tensions which result from a membership of both academic and industrial physicists.

Wellcome Foundation

For the first time the Wellcome Foundation—parent of three groups of companies: Wellcome or Burroughs Wellcome, Cooper, McDougall and Robertson Ltd., and Calmic Ltd.—is making public its full annual report and accounts, containing a ten year record. The figures show that world-wide sales for the year ending August 31, 1967, reached a total of £50·5 million, an increase of just over 15 per cent on the previous year. And Sir Michael Perrin, chairman of the foundation, records profits before tax of £6·39 million—an increase of 12·4 per cent. Furthermore, it seems that the effects of devaluation will on the whole be to the advantage of the foundation, because of its large overseas investments.

All profits received as dividends by the foundation are channelled into the support of medical research. Expenditure by the group last year on research and development was £2·86 million. One particular product of the group's commercial research during the year was the discovery of allopurinol, a drug which is used for the control of gout. A new scheme, mentioned in the annual report, will provide for all research and development within the group to be centrally controlled and co-ordinated—a scheme which, it is hoped, will extend business and increase the profitability of the group.

Research of a non-commercial nature is supported by the Wellcome Trust on a generous scale, and it has recently announced that a renewed grant of up to £36,000 is being made to two British parasitologists—Dr R. Lainson and Dr J. Shaw—to support their work in Belem, Brazil, on the epidemiology of cutaneous leishmaniasis. This is an insect-transferred disease causing extensive facial disfigurement.

The first grant of £47,500 was made in 1964, and the decision to renew the grant was prompted by the encouraging results that have been obtained. Both Dr Lainson and Dr Shaw have worked on leishmaniasis in other parts of the world, but they chose Brazil for this particular programme because of the high incidence of the disease there and because of its particularly disfiguring effects—extensive destruction of the nose and parts of the throat. Together they have confirmed an earlier suspicion that the organism *Leishmania* is transferred to humans by a particular species of sandfly of the genus *Phlebotomus* from the primary host, which they have shown to be two species of forest rodents.

Although Dr Lainson believes that it is possible for an individual to develop immunity to the disease, very little is known about the details of the immunology and it has so far proved extremely difficult to detect antibodies in the blood of immune individuals.

Cigarette Maker's Puff

BRITAIN's three million anglers are by their numbers a tempting bait for any producer of mass consumer goods. This has not escaped the attention of John Player and Sons of Nottingham, makers of Players No. 6, a brand of cigarettes.

Last week Players moved into a country hotel on the Thames to launch its "£10,000 Research Scheme to Help British Angling". The research scheme, a grandiose venture indeed, calls for a team of fisheries experts to cross the Channel and spend a whole three weeks in Holland, Belgium and Germany, where they will examine continental methods of conserving fish. On their return they will apply their findings for the benefit of British anglers. The team will be accompanied by a film unit which, to plagiarize the firm's own publicity, will make a "colour documentary film showing the work and findings of the delegation, to be available at lectures and on free loan to all angling clubs".

Some may suppose that a search of the scientific literature would with greater efficiency reveal any startling continental advances in fish conservancy. But such an endeavour would hardly make the subject of a film, prominently sponsored by Players and on free loan to all angling clubs. It is also hard at first to see why Players is contributing only £1,000 to the £10,000 "research" scheme and allowing the rest to be raised by angling competitions throughout Britain. But the prizes for all these competitions are being donated by Players, which is unlikely to take pains to conceal its generosity.

The fact that the scheme will take people out into the open air doubtless explains why the Minister of Sport sent a representative. He went as far as to say that "This is a great new development in the angling world, and Players are to be congratulated on their public spirited action in launching the scheme". Other members of the British Government, who have banned cigarette companies from advertising on television, and who intend to prevent them from giving away coupons with their products, may not feel quite so cheerful about the scheme.

Measles Vaccine

THE Ministry of Health will launch in May a campaign to vaccinate against measles all children between the ages of 1 and 15 years who have not yet contracted the disease. Trials carried out by the Medical Research Council, and experience in the United States and elsewhere, indicate that the vaccine is highly effective and that its widespread use could lead to the eradication of measles as a childhood disease. Measles is a biennial epidemic and reported cases in an epidemic year, of which next year in Britain is one, may rise to 750,000. For most people the disease is mild, but about one person in a hundred has to be admitted to hospital and in some cases the disease may be fatal; between 1962 and 1966 there were 434 deaths attributable to measles.

The cost of the campaign is expected to be about £1 million during the first year if the maximum number of susceptible children—between 3.4 and 4 million—come forward for vaccination. In each subsequent year there will be about 800,000 newborns to be vaccinated in their second year of life. The money saved from not having to treat measles in the population is expected to cover amply the costs of the vaccination campaign. It is not yet known what period of immunity is conferred by the vaccine, but it is thought unlikely that booster injections will be necessary. Antibodies have been detected in children's blood up to six years after vaccination.

Children in Britain are already offered immunization in their first year of life against diphtheria, tetanus and whooping cough—these vaccines are injected together in a single "cocktail"—and against polio, which is given orally. Vaccines for smallpox, and now measles, are given in the second year of life.

Health in Vietnam

by Anne Clayton

NOT least among the troubles of the population of Vietnam is the increasing incidence of plague, cholera, typhoid and other dengue-like diseases. These infections increase wherever there are large-scale uncontrolled movements of the population. A preliminary report on the subject has now been prepared by the World Health Organization. It indicates that there is a grave danger that infections may spread to other nations of the Pacific basin. The report, prepared during the past six months at the request of the World Health Assembly, was designed to answer several questions. Is there evidence of deterioration in the level of general health of civilian populations? Have the health services been adversely affected? Has a situation arisen in Vietnam which creates potential health hazards or problems for other countries? Any report of this nature is necessarily incomplete, but in view of the many difficulties faced by the investigators the report is a commendable effort.

For South Vietnam, the information has been provided by the official records of the WHO, WHO Epidemiological and Vital Statistics Reports, the Government of South Vietnam through the Regional Office for the Western Pacific and by reports received by the WHO quarantine unit. The account of the situation in North Vietnam is based on more limited information contained in a number of booklets which have appeared in North Vietnam together with the report of a long technical interview with the Minister of Health in North Vietnam, Dr Pham Ngoc Thach. The WHO document now issued is at pains to emphasize the incomplete character of this information, "which is not open to the usual methods of confirmation". The most recent figures in the reports refer to 1964, and the WHO report says that until official statistics are available, "the health situation in North Vietnam must remain somewhat obscure". On the basis of the comparison which it has been possible to carry out, however, it does seem that North Vietnam is less seriously afflicted by disease than the south, and that it is taking measures to prevent the situation getting out of hand.

In South Vietnam, epidemic plague, cholera and an upsurge of syphilis and gonorrhoea are distinctive features; other communicable diseases, especially tuberculosis, together with the lack of adequate sanitation and water supply, are, however, major public health problems. Plague infection has been reported to be widespread among rodents and fleas at certain ports and airports, and cases of the disease have now been recognized in 27 of the 47 provinces This situation is worsened by the large-scale movements of the civilian and military population, the prevailing environmental and sanitary conditions, the large number of vermin with their accompanying ectoparasites, the disruption of control programmes and the prevalence of antibiotic-resistant strains of *Pasteurella pestis*

The numbers of cases of typhoid and paratyphoid are also alarming, a total of 934 people were infected in 1966 Similarly, cases of cholera have risen from none in 1957 to 835 in 1966 and cases of encephalitis from 10 in 1957 to 330 in 1966 The incidence of diphtheria, poliomyelitis, malaria and rabies is also increasing at an alarming rate Reliable figures of the incidence of venereal diseases are not easy to obtain, and the problem is complicated by the presence and movement of military personnel and others living apart from their families and homes It is certain, however, that an increase of gonorrhoea reaching epidemic proportions has occurred among adolescents and young adults

To judge from the figures available, the situation in North Vietnam is surprisingly mild by comparison Plague, cholera and other dengue-like diseases seem to be absent Poliomyelitis has been virtually eradicated and tuberculosis has been substantially cut back typhoid fever is also on the way to extinction The reasons for these differences are not clear, but, as the report emphasizes, the information available for North Vietnam is not open to the usual methods of confirmation Bacterial dysentery seems to be one of the most widespread diseases and all venereal diseases are present Leprosy is also endemic

What measures are being taken to combat these large-scale infections? According to the report, vaccination and immunization are being used, but once again North Vietnam seems to have taken the lead As many as 13 million people in the north have been inoculated against smallpox and up to 18 million against cholera These compare with the figures of more than one million and more than two million respectively in the south In 1967, there were 1,184 hospitals and other establishments in South Vietnam for the care of in-patients and out-patients, and twenty-six operating theatres in provincial hospitals have been built or redesigned the number of medical personnel of the Ministry of Health has increased over the years between 1962 and 1967 Long-range planning in South Vietnam is not easy, the report maintains, because of the unsettled state of the country It has been suggested that an expert group should be appointed to study the situation and establish a national health plan, and a short-term consultant was provided by the WHO during the early months of 1967 to review the existing situation and to advise on national health planning A comprehensive study of health problems and services has also been made by a United States appraisal team which made recommendations for long-term developments

Although these findings may seem more encouraging for the North Vietnamese, the nutritional standard in the north is lower than in the south Calorie intake in the south seems to be uniformly adequate, and protein intake is ample, but there are a number of deficiencies, particularly of thiamine, riboflavine and vitamin *A* In the north, on the other hand, the inadequacy of the diet is evident, especially in children the diet is monotonous, and consists mainly of foods rich in carbohydrate and poor in protein and animal fats

Parliament in Britain

Decimal Currency

LORD BESWICK said that the Government had no intention of postponing the change to a decimal currency system Decimalization was now well advanced two out of the preparatory five years were over and much capital had already been expended Because of the real economic benefits which would accrue it was the view of the Government that it was in the best interests of the country as a whole to proceed with decimalization as planned The change was expected to be of real help to the expansion of the British export trade (Oral answer, House of Lords, January 31)

Committees of Inquiry

THE Secretary of State for Defence, Mr D Healey, gave the membership of six committees of inquiry for the Ministry of Defence appointed since October 1964 The committees and their chairmen are Sub-Committee on Research and Development for Anti-submarine Warfare (Dr J C Kendrew), Space Review Committee (Professor H Bondi), Armoured Warfare Sub-Committee (Professor J L M Morrison), Conventional Armaments Working Party (Professor B H Flowers), Personnel and Anti-Personnel Research Working Party (Professor P L Krohn), and Working Party on Materials Research (Professor A H Cottrell) The Defence Research Committee was responsible for keeping the defence research programme under review to ensure that it was geared to defence needs within the resources available Agreements with universities to carry out longer term research in aid of defence operational analysis were made wherever appropriate and two studies, involving exploratory work of interest to the study of land warfare, were at present being undertaken at Newcastle and Nottingham Universities The Defence Operational Analysis Establishment also had frequent, but less formal, contacts with university faculties Arrangements had been concluded with certain universities to create an additional post in an existing faculty to specialize in the defence aspects of its discipline Five such appointments had so far been made, at Aberdeen, Edinburgh, London (King's College), Oxford (All Souls College) and at Southampton, and two further appointments were under consideration (Written answer, January 31)

Research Establishments

THE Minister of Technology, Mr Anthony Wedgwood Benn, stated that the programmes of the Ministry's research establishments were kept under review by committees on which industry was strongly represented The ministry was encouraging the establishments to take on more work for industry on a repayment basis In defence and civil aviation, joint research programmes had been set up with the Society of British Aerospace Companies, aimed at rationalizing the location and finding of the work At the Royal Radar Establishment, an Industrial Applications Unit had been set up to act as a focal point for the application in industry of the results of the establishment's defence research Generally, the policy was that the programmes of the establishments should be closely related to the needs of industry and drawn up in collaboration with them (Written answer, January 31)

American Science Marks Time

THE budget introduced to the United States Congress by President Johnson on January 31 will have brought very little pleasure to the scientific community in the United States Scientists may, however, comfort themselves with the knowledge that the needs of the war in Vietnam have similarly curtailed the growth of expenditure in several other fields, and particularly in the development of federal programmes for the improvement of cities and of education of all kinds Everybody may, however, be pleased to see that the way in which the United States Administration has sought to draw a clear distinction between its estimates of what will be spent in the fiscal year beginning on July 1 (fiscal year 1969) and its request to Congress for new authorization, which frequently covers expenditure further ahead than the end of fiscal year 1969, has made this year's budget much easier to follow than its predecessors

The continuing decline in the expenditure of the National Aeronautics and Space Administration is perhaps the most conspicuous feature of the budget, although perhaps only because it is easily identifiable The less explicit changes of the pattern of expenditure within the budget of the Department of Defense may have a more serious effect on what happens in American universities in the year ahead For what it is worth, however, expenditure on space research and technology in 1969 is expected to work out at $4,573 million, compared with $4,803 million in the present financial year and $5,423 million in fiscal year 1967

The decline of the space budget is made possible by the fact that the Apollo programme is over the peak of its expenditure At the same time, it seems plain that NASA is being allowed to keep alive most of its hopes for programmes which will follow on Apollo even if some of these have been postponed or watered down Thus President Johnson announced in his budget message that the production of the Saturn V rocket will continue but at a reduced rate, that the development of the nuclear rocket engine will continue but that the engine will be smaller than the one first planned two years ago and that work will begin on the development of a spacecraft to put into orbit about Mars in 1973 using the Titan III rocket and not Saturn V as a launcher

In practice, expenditure on the Apollo programme as such will fall to $2,571 million in 1969 compared with $3,587 million in 1967, but there are plans to spend $422 million in the year ahead on other ventures with men put into orbits about the Earth, the President drew particular attention to a scheme for using spent rocket vehicles for various unspecified scientific purposes The growth of these schemes has, however, been accompanied by a decline in the expenditure of NASA on purely scientific work, which will account for $498 million in 1969 compared with $624 million in 1967 The development of communications satellites will take a little more The fact that NASA's expenditure on aircraft research will increase from $113 million to $120 million is partly accounted for by the beginnings of a programme to use aircraft as means of surveying natural resources from above so as to decide whether satellites would eventually be able to take over work of this kind

The Department of Defense will increase its total expenditure on scientific research, partly because of the development of anti-ballistic missile systems, strategic surveillance and what is called the "next generation of tactical aircraft" At the same time, the Department of Defense is spending more on its manned orbital laboratory, chiefly because of its concern for surveillance In effect, the advance research budget will increase from $4,600 million in 1968 to $5,100 million in 1969 It is not possible to tell from the budget how these changes will affect the amounts of money which the Department of Defense and its various subdepartments have to spend on research in American universities, but the experience of the past year seems to have been that the increasing pressure on the defence budget has curtailed the amounts of money which the services are able to spend on academic projects

The expenditure of the Atomic Energy Commission will increase along with that of the Department of Defense, and chiefly for the same reasons—the development of the anti-ballistic missile systems Within its budget, however, the AEC is planning to increase its expenditure on the development of civilian nuclear power stations from $163 million to $258 million, chiefly because of its concern to speed the development of fast reactors The basic research budget increased from $421 million to $555 million, but this is chiefly explained by the beginning of work on the Weston accelerator, which will eventually cost $250 million

For the rest, the budgets of the agencies chiefly concerned with research and development remain more or less static—few of them have increased sufficiently to make up for increasing costs (The Office of Saline Water is perhaps an exception, with a budget increased from $31 million to $41 million) At the same time, the National Bureau of Standards is receiving less as direct subvention from the Federal Government—$39 million in 1969 compared with $41 8 million in the current year A comparatively modest increase of $32 million in the billion dollar budget of the National Institutes of Health conceals an emphasis on the work of the National Heart Institute among others One cheerful feature of the budget is a plan to spend some $85 million in the coming year on the provision of buildings for medical schools and $197 million on curriculum development, staff and expansion programmes and financial assistance to medical students in medical schools

Thus the budget for the National Science Foundation will in 1969 be $480 million (compared with $456 million in the current year and $412 million in 1967) What the foundation has to spend on basic research is hardly changed ($230 million) Its institutional science programme, chiefly concerned with the development of centres of excellence, increased from $72 million to $78 million Science education will continue to take a quarter of the foundation's budget

The direct consequences of the budget for universities will be felt most seriously in the diminished ability of the Federal Government to pay for facilities on university campuses A year ago, grants towards university buildings were deliberately increased by 50 per cent to just over $300 million, now they are reduced to $230 million almost the same as in 1967

NEWS AND VIEWS

Cellular Interdependence

THE coexistence within the same animal of groups of cells with different genetic constitutions has attracted increasing attention in recent years. Immunologists, for example, recognize that the existence of mosaic individuals may help to define the conditions under which genetically different populations of cells may share the same physiological frame. Embryologists seek to know more about the circumstances under which mosaic individuals develop. The coexistence of genetically different groups of cells is then turning out to be important in the recognition of inheritable genetic defects. Now there is also the suggestion that the processes of ageing, which are known to be statistically associated with an increasing incidence of genetic abnormalities of some kinds in human reproduction, may also be linked with the increased appearance of genetic abnormalities among the body cells of living individuals. [The alternative suggestion by Dr James German, on page 516 of this issue, of the processes underlying the increasing incidence of mongolism with increasing maternal age, should not at this stage be regarded as more than what he says it is—an interesting hypothesis which deserves fuller study.] In one way and another, there has recently been a surge of interest in the coexistence of genetically different groups of cells, and it is plainly of the greatest importance to know just what factors determine the equilibrium between two such populations.

Basic biological studies by Dr D. T. Hughes of the Chester Beatty Research Institute (page 518) have demonstrated that co-operation as well as competition between cells affects the evolution of cell populations. Although interaction by cross-feeding—a situation in which cells produce substances into the culture medium which other cells take up and make use of—has previously been described in bacterial and fungal cultures, it has generally been regarded as a nuisance to be avoided, because it complicates the genetic analysis of micro-organisms. Dr Hughes, however, has shown the biological significance of interaction between cells; it is a genetically important factor in evolution, superimposed on the action of straightforward environmental selection—"survival of the fittest". The discovery of a new kind of balanced genetic polymorphism was made as a result of analysis of cell populations as a whole, carried out to determine whether there was any basis for co-operative interaction between cells. The resulting biological concept envisages a balance of cell types in which the frequencies of types of chromosomes among the cells contribute to a balance similar to that which exists in a normal cell. The situation seems to be that the balance of nature at the level of the population obeys the same laws as at the lower level of organization, within a single normal cell. Previous work on genetic polymorphism has not demonstrated this kind of balance because populations have not been considered as a whole. On the contrary, earlier work has been concerned with the frequency of characters in single cells or single organisms within a population.

It remains to be seen whether this type of polymorphism can be demonstrated *in vivo* in populations of organisms living in restricted environments analogous to the culture bottle—perhaps populations of mammals or plants living in geographically restricted environments such as islands would be interesting to investigate. There is, however, a possibility that fuller use could now be made of cultured cells as a model system for the study of evolution.

This new concept will also be of more immediate practical importance in clinical medicine. It should be important in the understanding of the development of mosaics and chimaeras, and in the study of tumour cell populations and their therapy—the idea of the pseudo-stemline may help to explain some of the difficulty encountered in killing off tumour cells.

Geomagnetic Dynamo Energy

from a Geophysics Correspondent

IT seems now generally accepted that the main geomagnetic field is produced by "dynamo" action—a magnetohydrodynamic (MHD) interaction in the Earth's liquid, conducting core which produces an essentially dipolar field whose axis coincides approximately with the rotational axis. Less firmly established is the source of the energy required to produce the fluid motions. It is fair to say that majority opinion favours thermal convection as the power source for the dynamo, although Malkus (*J. Geophys. Res.*, **68**, 2871; 1963) concluded that the driving force may well be the Earth's rotational energy operating through a differential precessional torque between mantle and core.

Stacey (*Earth Plan. Sci. Letters*, **3**, 204; 1967) has now presented a revised estimate for the electrical resistivity of the Earth's core which suggests that Malkus's theory is the more appropriate. Any MHD theory requires the core to possess a "magnetic Reynolds number" considerably greater than unity. After making the usual numerical substitutions for core scale size, magnetic diffusivity and velocity of core motions, this means that the resistivity should be substantially smaller than 4×10^7 e.m.u. Hitherto, estimates of core resistivity, based on extrapolation of the resistivity of iron to core temperatures and pressures, have been at least a factor of one hundred smaller than this figure, thereby satisfying the condition for an MHD interaction. Stacey points out, however, that such estimates have failed to take into account the effect of impurity elements which, if present in the core, will alloy with the iron.

A core containing ten per cent nickel alloying with the iron will possess a resistivity three times greater than that of pure iron Of greater importance, however, are the lighter elements which must be present to account for the fact that the core density is lower than that of iron If the core contains 15 per cent silicon, as suggested by Ringwood (*Geochim Cosmochim. Acta*, **15**, 257, 1969), its resistivity will be ten times higher than that of pure iron If it contains dissolved MgO, as suggested by Alder (*J Geophys Res* , **71**, 4973, 1966), the increase in resistivity will be even greater These considerations indicate a core resistivity of at least 3×10^6 emu (a factor of ten higher than previous estimates), but presumably less than 2×10^7 emu above which dynamo action is precluded

The magnetic Reynolds number is thus reduced to about ten, which, though still greater than unity, is sufficiently low to call in question the validity of a convective dynamo theory on the grounds that the energy requirements would be difficult to satisfy Stacey thus suggests that "we must prefer a mechanism which has sufficient [energy] in hand to operate at low efficiency", and so turns to the alternative Malkus theory. In further evidence he cites the virtual absence of field harmonics of wavelength less than 1,500 km at core level, which also indicates a lower magnetic Reynolds number, and he produces calculations to show that tidal and mantle dissipations are insufficient to account for the use of all the Earth's rotational energy. The excess energy might well be used to drive the geomagnetic dynamo.

If it is too soon to decide between the various dynamo theories, this is not only because of the difficulty of assigning accurate numerical values to core parameters on the basis of surface laboratory experience but also because of the absence of substantial laboratory data This is clearly a case where accurate measurements of the electrical resistivities of liquid iron alloys are required to narrow the range within which the core resistivity may lie In short, it is yet another example of the importance of laboratory physics to Earth science

Thermal Analysis Standards

Dr H G McAdie of the Ontario Research Foundation, Sheridan Park, Ontario, chairman of the Committee on Standardization of the International Conference on Thermal Analysis, has written as follows

Thermal methods of analysis, particularly differential thermal analysis (DTA) and thermogravimetry (TG), have achieved major importance as techniques for the study of materials [Differential thermal analysis is a technique in which the cooling rate of the material studied is compared directly with that of a standard If one junction of a thermocouple is immersed in the standard and the other in the material, any change in the cooling rate, such as might be caused by a phase change, is readily detected Thermogravimetry is a technique in which the weight of a specimen is recorded as it is heated] Such dynamic temperature methods often impose stringent requirements different from those associated with their isothermal counterparts, particularly in relation to proper experimental design based on a thorough understanding of the underlying physical principles, and to the full reporting of all pertinent experimental detail in order that the published record may be fully understood and critically evaluated

In view of the lack of essential information existing today in much of the thermal analysis literature, the Committee on Standardization of the International Conference on Thermal Analysis, established in 1965, has recently published (H G McAdie, *Anal Chem*; 543, 1967) a series of nine general and three specific recommendations concerning the necessary experimental detail which should accompany any report of information obtained from DTA or TG These recommendations are also being published in French, German, Japanese and Russian, as well as in a number of other languages, and the editors of all relevant major scientific journals are being invited to consider and adopt them so that the quality and value of the thermal analysis literature may be improved significantly Further recommendations will be issued periodically, as the methodology develops, including extensions to the newer techniques combining DTA and TG with effluent gas studies, measurement of electrical resistance and the like

The Committee on Standardization, which represents the major international areas of greatest activity in thermal analysis, is also undertaking an extensive programme to evaluate standard substances for use in the calibration of the temperature axis in DTA Subsequent programmes are planned to provide materials suitable for calibration of the enthalpy axis in DTA and the temperature axis in TG Such standard substances will not only allow the calibration of a given instrument and provide reliable systems with which to evaluate sensitivity, resolution and reproducibility, but will permit the direct intercomparison of data obtained in different laboratories involving dissimilar instrumentation

Reports of progress toward these objectives will be made to the Second International Conference on Thermal Analysis to be held in Worcester, Mass , August 18–24, 1968

Viruses and Chromosome Breakage

from our Cytogenetics Correspondent

It has been known for a number of years that viruses, including those able to induce tumours, are able to cause chromosome breakage in the cells they have infected There is a suggestion that the breaks are found preferentially at chromosomal regions that are among the last to replicate Further evidence for this has now been provided by Huang and by Kato Huang (*Chromosoma*, **23**, 162, 1967) treated cells of the rodent *Mastomys*, cultured *in vitro*, with herpes simplex virus, adenovirus-12, the mutagens 5-bromodeoxyuracil (BUdR), 2-aminopurine, and the carcinogen 7,12-dimethylbenz(*a*)anthracene All these agents caused chromosome breakage, but some chromosomes had fewer and some had more breaks than expected if breakage of chromosome regions was at random The long arm of the X-chromosome was especially sensitive and this region is known to replicate its DNA later than the remainder of the DNA The Y-chromosome is also a late replicator, yet it seems only sensitive to BUdR The Y is difficult to identify with certainty, however, so its sensitivity may have been underestimated A similar study by Kato (*Hereditas*, **58**, 221, 1967), using cultured Chinese hamster cells infected with Rous sarcoma virus, showed a high incidence of breakage located at the secondary constrictions—

regions also thought to be late in replicating their DNA Kato also recorded the sites where spontaneous breakage occurred and it turned out that these sites are the same as those broken by the virus

But what do these results indicate about the nature of the chromosome at the points of breakage or about the properties of the virus ? It seems likely that there is some qualitative difference in the structure of the chromosome that predisposes certain regions to high breakage frequencies Some years ago Hsu proposed that regions broken by certain mutagens were rich in particular DNA bases, but since then knowledge of this problem has not been much extended The position in the infected cell from which the virus directs the induction of a chromosome break is not even known with certainty Observations by Halkka (*Hereditas*, **58**, 248 and 251, 1967) may be relevant—he found that in the testes of the cricket *Acheta* infected by a rickettsia-like organism, the microbe was to be observed lying on the nuclear envelope and even to be lying on the chromosomes themselves Infection of these meiotic cells caused many lesions in the chromosomes similar to those induced by viruses If a parallel can be drawn, it is that the damage done by viruses to chromosomes may well be caused by their intimate association with the chromosome at certain preferred points, thereby impairing chromosome replication Unfortunately, viruses have not yet been located within the structure of chromosomes from higher organisms

Steroids and ATPase

from our Medical Biochemistry Correspondent

THE maintenance of the correct concentrations of sodium and potassium ions in mammalian systems is literally of vital importance. When sodium and potassium ions are moved across cell membranes against a concentration gradient, adenosine triphosphate (ATP) is split to provide the energy for the process The enzyme responsible is thought to be an ATPase which is dependent on the presence of sodium and potassium ions for its activity Bader, Post and Bond have now made preparations of this enzyme from eleven different species and six different tissues, and have shown that they are remarkably similar (*Biochim Biophys Acta*, **150**, 41, 1968) They all required the presence of sodium ions to form a ^{32}P-labelled protein from ^{32}P-labelled ATP, and dephosphorylation of the protein was always stimulated by the presence of potassium ions Although the specific activity of the preparations varied greatly, the ratio of ATPase activity to phosphoenzyme and ATPase to acyl phosphatase was always approximately the same The ^{32}P-labelled preparations were digested with pepsin or pronase and the resulting ^{32}P-labelled peptides were separated by electrophoresis The peptides all behaved identically on electrophoresis and on treatment with hydroxylamine, suggesting that the enzyme structure which becomes phosphorylated is the same in preparations of the enzyme from all these different sources

The corticosteroids produced by the adrenal gland are the hormones which regulate salt and water metabolism, and it has now been shown that the activity of the sodium and potassium activated ATPase in rat kidney varies with the plasma concentration of adrenal steroids (P L Jørgensen, *Biochim Biophys Acta*, **151**, 212, 1968) After removal of the adrenals, the activity of the ouabain-sensitive sodium and potassium activated ATPase activity of rat kidney decreased along with the sodium/potassium ratio of the plasma A sodium-rich diet delayed the appearance of both decreases The decrease in activity appeared to be due to a decrease in the amount of enzyme, as the enzyme kinetics were the same for preparations from normal and adrenalectomized animals The attachment of the enzyme to the membranes of the microsomes may be altered, as the decrease in activity was most marked when activity was measured in the presence of deoxycholate and ethylene diamine tetra-acetic acid

If adrenal steroids affect this ATPase, and these enzymes are all very similar, it is hardly surprising that adrenal steroids can affect the salt metabolism of tissues other than the kidney In a recent investigation (R Shields, J B Miles and C Gilbertson, *Brit Med J* , (i), 93, 1968) a patient with an adrenal tumour secreted four to five times as much potassium into the colon as did eight normal controls, but 3 months after operation his potassium loss was normal Since half the daily loss of potassium from this patient was in the faeces, urinary estimations alone could seriously underestimate the potassium loss in patients treated with adrenal steroids, with serious consequences

Ribosomal Proteins

from our Cell Biology Correspondent

THE chemical heterogeneity of ribosomal protein has now been established beyond question Tissières and his colleagues, for example (*Proc US Nat Acad Sci* , **57**, 1294, 1967), recently isolated and partially purified a number of proteins from *E coli* ribosomes. Given the great chemical heterogeneity of ribosomal proteins, there is every reason to expect that these proteins are functionally heterogeneous The problem then is that of defining the functions of the individual proteins and how their individual properties relate to the overall function of the ribosome In the current *Proc US Nat Acad Sci* (**58**, 2430, 1967), Traub, Hosokawa, Craven and Nomura report an analysis of the function of five basic proteins isolated from the 30*S* sub-unit of *E coli* ribosomes

Their approach was to prepare a series of ribosome derivatives, each deficient in a specific ribosomal protein and to compare the properties of their derivatives with those of normal ribosomes Having first dissociated 70*S* ribosomes into 30*S* and 50*S* sub-units, Traub *et al* further dissociated the 30*S* sub-unit into a 23*S* core which has none of the properties of a 30*S* sub-unit, and free protein—the so-called split proteins They then separated the split proteins into acidic and basic protein fractions Particles reconstituted from 23*S* cores and the acidic split proteins have none of the known properties of 30*S* sub-units, whereas particles reconstituted from cores and the basic split proteins are partially active The basic split proteins are essential for the 30*S* functions, whereas the acidic split proteins, though stimulatory, are not essential

Following up this result, Traub *et al* resolved the basic split protein into five component proteins with quite distinct amino-acid compositions To determine the function of each of these five proteins, they reconstituted 30*S* sub-units from 23*S* cores, all the acidic split proteins and four of the five basic split proteins, and then asked which 30*S* sub-unit functions the five classes of deficient particles have. Apparently two of the proteins (B_3 and B_5) and possibly a third (B_1) are essential for both amino-acid incorporation and binding of specific amino-acyl-*t*RNA. Protein B_4 is not required for these functions, but may be involved in chain initiation, and the remaining protein, B_2, has a stimulatory effect on amino-acid incorporation, but no definite conclusion about its function was reached. These preliminary experiments have certainly established that the specific activity of ribosomal protein can be assayed, and it should be possible now to unambiguously define the function of many ribosomal proteins.

Part of the function of ribosomal proteins must be to provide the two specific *t*RNA binding sites, one for peptidyl-*t*RNA and initiator *t*RNA and the other for amino-acyl-*t*RNA. Nomura *et al* (see *Nature*, **216**, 638, 1967) provided strong evidence that the peptidyl and initiator site was at the 30*S* sub-unit and that the amino-acyl site is formed by the joining of 30*S* and 50*S* sub-units. These experiments were done with *f*2 phage RNA as messenger, but the ideal messengers for such experiments are the synthetic ribopolynucleotides poly-UG and poly-AUG. Both poly-AUG and poly-UG contain codons which specify formyl methionine at chain initiation and methionine and valine and cysteine internally. Khorana's group has synthesized these polymers, and Ghosh and Khorana (*Proc US Nat. Acad Sci*, **58**, 2455, 1967) have repeated Nomura's experiments using them as messengers. Their experiments prove that two *t*RNA binding sites exist, and, furthermore, they fully confirm Nomura's scheme for the initiation and synthesis of polypeptide chains.

How Repressor Molecules Function

by
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During the past year two repressor molecules were detected, purified and some of their properties determined. The results confirm much of the theory, proposed by Jacob and Monod in 1961, for the control of gene function.

In 1961, Jacob and Monod[1] proposed their celebrated theory of how the activity of genes could be controlled. This theory, which is outlined below, followed from observations on the lactose operon of *Escherichia coli*. The lactose, or *lac*, operon is a cluster of three adjacent genes, which code for three enzymes. In order for *E coli* to utilize the disaccharide lactose, two of these enzymes are required. When *E coli* is grown in the absence of lactose, the three enzymes of the *lac* operon are present in only very small amounts; if lactose is added to the growth medium, the rate of synthesis of these three enzymes is increased about one thousand-fold and the operon is said to be "induced". Thus *E coli* is able to switch the *lac* operon on when needed: otherwise it is switched off.

The three enzymes of the *lac* operon, β-galactosidase, a membrane-bound permease and thiogalactoside transacetylase, are coded by the *z*, *y* and *a* genes, respectively (see Fig 1). Both β-galactosidase and the permease are needed to metabolize β-galactosides, such as lactose; the function of thiogalactoside transacetylase is not known. The sugar which induces the *lac* operon is called an inducer. It has been shown that the induction of the *lac* operon does not depend on the utilization of the inducer. Thus isopropylthiogalactoside (IPTG), a compound not found in nature, will act as an inducer of the *lac* operon although it cannot be metabolized. Inducers, such as IPTG, which trick the cell in this way, are called gratuitous inducers.

Close to the *lac* operon is the *i* gene which, as originally proposed by Jacob and Monod, is the structural gene for the repressor. They suggested that the repressor molecule could bind to a postulated operator site, or *o* region, adjacent to the *z* gene, thereby switching off or "repressing" the *z*, *y* and *a* genes. This repression of the *lac* operon was presumed to occur at the level of messenger RNA transcription, that is, binding of the repressor to the operator was presumed to prevent synthesis of messenger RNA from any of the three genes of the operon. In the presence of a β-galactoside inducer, it was proposed that the repressor would bind to the inducer and thereby be removed from the operator site in the DNA. According to the model, the repressor, once removed from the operator site, has no further effect on the *lac* operon; the messenger RNA corresponding to the operon is synthesized and is subsequently translated on the cytoplasmic ribosomes. The net result, of course, is the synthesis of the three enzymes.

Much of the evidence for this model has come from the study of bacterial mutants whose control of the synthesis of the *lac* enzymes is altered. These mutants fall into two classes which map genetically either in the *i* gene or the *o* region. The mutants of the *i* gene can be further subdivided into i^s and i^- mutants. i^s are super-repressed—they cannot be induced to form the *lac* enzymes even in the presence of inducer. i^- mutants, by contrast, synthesize maximal amounts of the *lac* enzymes even in the

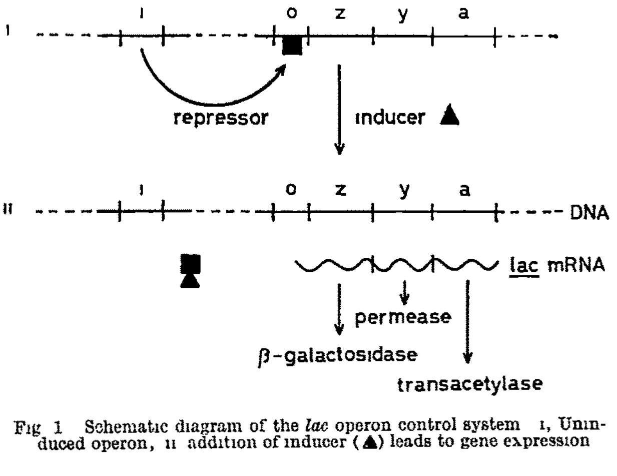

Fig 1 Schematic diagram of the *lac* operon control system. i, Uninduced operon, ii addition of inducer (▲) leads to gene expression

absence of inducer. In terms of the Jacob–Monod theory, these two types of *i* gene mutations reflect the dual nature of the *lac* repressor. In i^s mutants, the repressor is presumed to have lost all its affinity for β-galactosides and the *lac* operon is thus permanently repressed. The repressor in an i^- mutant, on the other hand, is assumed to have lost its affinity for the operator region so that, even in the absence of inducer, full synthesis of the *lac* enzymes occurs. Mutants in which the lesion maps in the *o* region are called o^c mutants and appear to be deletions of part or all of the *o* region—that is, part of the DNA corresponding to the *o* region is missing. These mutants, like i^- mutants, make large or maximal amounts of the *lac* enzymes even in the absence of inducer. According to the Jacob and Monod model, the alterations of the *o* region occurring in such mutants have rendered the *o* region incapable of binding repressor, so that synthesis of the enzymes is uncontrolled.

Evidence that the repressor is a diffusible molecule came from a study of partial diploid strains of *E. coli* which possess two complete *lac* operons. In a strain possessing an i^- gene and an i^+ gene, the i^+ gene is dominant. That is, the introduction of an i^+ gene into a cell containing an i^- gene results in the loss of the lack of control which pre-existed in the i^- cell. The *i* gene product is an active repressor and can function in a *trans* (as well as the usual *cis*) fashion and is therefore a diffusible molecule.

The predicted properties of the *lac* repressor are therefore that it can bind both to an inducer molecule and to the operator region, but it cannot bind to both at once. The *i* gene is the structural gene for the repressor.

More recently genetic evidence has shown that the product of the *i* gene is a protein molecule. This was demonstrated by the isolation of suppressible amber mutations in the *i* gene. Amber mutations have their deleterious effects during protein synthesis, so it follows that any gene in which amber mutants can be generated codes for a protein molecule.

Apart from the *lac* operon, several other control systems have been studied. The *lac* operon is induced when a small molecule, a β-galactoside, is added to the growth medium. There are other operons which are repressed when a small molecule is present. For example when *E. coli* is grown in the presence of the amino-acid histidine, the enzymes of the *his* operon which are responsible for the biosynthesis of histidine are repressed; in the absence of histidine, the *his* operon is induced. This form of control is readily fitted into the scheme for the *lac* operon presented here if the repressor for the histidine operon can only bind to its operator in the presence of histidine. In the absence of histidine, the repressor is removed from the operator, allowing messenger RNA to be copied from the *his* operon.

Of particular interest here is the control of the genes of the bacterial virus, λ. When this phage infects *E. coli*, one of two events can occur. The phage can multiply in the host cell, which it eventually kills, producing a few hundred progeny phage. The alternative event is that the λ phage enters a dormant state in the host cell. In this case the λ chromosome becomes physically integrated with the host chromosome—that is, the DNA is inserted into the *E. coli* DNA. In this latter state the phage is described as a prophage; most of the λ genes are not expressed. This repression of the λ prophage genes is mediated by a λ repressor molecule which is genetically coded by the c_1 gene of the prophage. This control system is formally similar to that of the *lac* operon. The c_1 gene codes for the λ repressor and is analogous to the *lac i* gene. The λ repressor represses the expression of the λ genome except for the c_1 gene; hence *E. coli* carrying λ as a prophage has λ repressor molecules in its cytoplasm. The effect of these repressors is two-fold. They prevent the λ genes in the prophage from being expressed. They also prevent any λ genome which might be injected into the bacterium by an infecting λ phage from expressing itself. Thus the λ prophage repressors confer immunity on the host cell from outside infection by other λ phage. The λ repressor is the "immunity substance".

During the past year, the principal advance in this field came with the isolation of the *lac* repressor by Gilbert and Muller-Hill[2,3], and the λ phage repressor by Ptashne[4,5], all at Harvard University. Some of the properties of these two repressor molecules were also determined; a summary of their findings constitutes the remainder of this review.

The *lac* repressor was detected by its ability to bind the gratuitous inducer, IPTG. Because only about ten *lac* repressor molecules exist in *E. coli* per chromosome, giving a concentration of about 10^{-8} molar in a solid pack of *E. coli* cells, and since the binding constant of the repressor for IPTG is about 6×10^{-6} molar, detection of the normal *lac* repressor would be almost impossible. Gilbert and Muller-Hill therefore isolated a "tight-binding" mutant of the repressor which they estimated had approximately a ten-fold increased affinity for the inducer, IPTG. This *lac* repressor was detected by placing a cell extract of this mutant *E. coli* inside a dialysis sack and leaving it to equilibrate with a dilute solution of radioactive IPTG. They found that slightly more radioactive IPTG was found inside the sack than outside it, thus demonstrating that a component of the extract could bind this inducer. Using this binding as an assay for the repressor, they purified the repressor and showed that the observed binding was freely reversible and that the molecule responsible for binding the IPTG is a protein with a molecular weight of about 200,000. In order to prove that this protein which binds the labelled inducer is indeed the repressor, defined as the product of the *i* gene, these authors showed that an extract of an i^s mutant (which had been purified in the same way as their "tight-binding" mutant extracts) was incapable of concentrating IPTG inside the dialysis sack.

More recently, Gilbert and Muller-Hill have purified the *lac* repressor from *E. coli* cells which had been grown on radioactive sulphate to label the proteins. Using this partially purified labelled repressor, they sought to test directly the model for control of the *lac* operon as proposed by Jacob and Monod. First, they showed that some of the radioactive protein in their purified *lac* repressor preparation became bound to pure DNA which contains the operator region of the *lac* operon. The DNA used for this experiment was isolated from a hybrid bacteriophage which carries the *lac* operon in its chromosome. The labelled repressor preparation was mixed with this DNA and was then analysed by centrifugation through a sucrose gradient. DNA molecules sediment much faster than the smaller protein molecules, and were found to carry a small proportion of the labelled protein. This labelled protein bound to the DNA carrying the *lac* operator region is the *lac* repressor. This was proved by the inability of DNA from a similar bacteriophage, but which had a defective operator region (o^c), to bind the repressor as tightly. Furthermore, when the binding of this repressor to DNA carrying an intact *lac* operator region was carried out in the presence of the inducer IPTG, no repressor was found bound to the DNA.

These experiments beautifully illustrate the mechanism of control of the *lac* operon. The repressor binds the inducer, but cannot then bind to the operator region. In the absence of inducer, the repressor binds to the operator region. How is this recognition achieved? For several years it has been imagined that the repressor is a nucleoprotein, possibly having a piece of RNA associated with it which could recognize the operator region by base-pairing. Gilbert and Muller-Hill have shown that pre-incubating this labelled repressor with ribonuclease (which would destroy a putative RNA molecule) does not affect the binding of the repressor to the operator region. Thus the repressor itself must recognize the operator DNA sequence. Furthermore, they also showed that the repressor will not bind to denatured DNA. The repressor therefore recognizes the operator, which is a unique segment of double-stranded helical DNA; just how long is this region is unknown, but at least twelve base-pairs in the DNA would probably be required in order for it to be a unique sequence in the *E coli* chromosome

The experiments performed by Ptashne on the λ repressor were all carried out by labelling the repressor with radioactive amino-acids This was necessary because there is no known assay for the λ repressor equivalent to the equilibrium dialysis against labelled IPTG which was used to detect the *lac* repressor Ptashne's aim was to infect *E coli* carrying a λ prophage with λ phages in the presence of a radioactive amino-acid, by choosing suitable conditions he hoped that a high proportion of the label incorporated into protein would be in λ repressor molecules To achieve this, Ptashne eliminated most of the protein synthesis of the host cells by strongly irradiating them with ultraviolet light to inactivate their DNA before infecting them with λ phages Second, as mentioned earlier, the λ repressor molecules present in *E coli* carrying the λ prophage will prevent expression of the infecting λ phage genes, except for their c_1 genes In order to obtain large numbers of λ repressors per cell, Ptashne infected each host cell with about thirty λ phages Because there are initially not enough λ repressors per cell to cope with thirty infecting phages, however, all the experiments were carried out with λ phages which also have a lesion in their *N* genes, this, in an unknown way, prevents most of the λ genes from functioning, but does not affect the activity of the c_1 gene

The experiment consisted, then, of heavily irradiating *E coli* containing a λ prophage with ultraviolet light and then infecting two parallel cultures of these cells either with the wild-type λ (with a defective *N* gene) or with λ phages having a defective c_1 gene (as well as a defective *N* gene) One culture was then labelled with ^{14}C-leucine, the other with ^{3}H-leucine After about an hour of growth the two cultures were mixed and an extract of the mixture prepared The mixture of proteins thus obtained was fractionated on a DEAE ion exchange column and the fractions monitored for both the ^{14}C and the ^{3}H labels The ratio of isotopes was found to be essentially constant except for a major peak corresponding to the *E coli* cells infected with the wild-type λ; this peak was absent from the cells infected with λ having a defective c_1 gene This protein peak was thus presumed to be the product of the c_1 gene Several experiments prove that this is the case, the most elegant of them made use of a mutant of the c_1 gene which was known to give the λ repressor a greater efficiency as a repressor This mutant, called ind$^-$, was shown to synthesize a repressor whose chromatographic properties on the DEAE column were different from those of the wild-type repressor

Thus, a mutation in the c_1 gene changes the properties of this protein peak, this positively identifies this protein as the product of the c_1 gene and hence as the λ repressor or immunity substance Ptashne showed that this repressor appeared homogeneous by electrophoresis on a polyacrylamide gel Its molecular weight, as judged by its rate of sedimentation in a sucrose gradient, is about 30,000 This is a sixth of the estimated size of the *lac* repressor More recently, Ptashne has shown that this labelled repressor can bind to λ DNA This was demonstrated by mixing λ DNA with labelled repressor and then analysing the mixture on a sucrose gradient Labelled repressor was found bound to the DNA As a control experiment, he showed that the repressor does not bind to the DNA of a hybrid λ phage which contains most of the λ genes but has a different immunity (and hence λ operator) from wild-type λ Ptashne also showed that the λ repressor cannot bind to denatured λ DNA

In conclusion, these experiments have served to prove several of the principal points of the Jacob–Monod theory of the control of gene expression In both cases, the repressor molecule has been shown to be a protein molecule capable of binding with great specificity to a site on the DNA It does not seem that either repressor contains a nucleic acid moiety, furthermore, the repressors bind only to helical DNA and not to denatured DNA In the case of the *lac* repressor, inducer binds to the repressor, thereby preventing the latter from binding to the operator region of the *lac* operon The next step in this exciting elucidation of the biochemistry of control mechanisms will probably be to show that when the repressor binds to DNA, it switches off the genes of the operon it controls by preventing the RNA polymerase from copying the operon into a messenger RNA

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[1] Jacob, F, and Monod, J, *J Mol. Biol*, **3**, 318 (1961)
[2] Gilbert, W, and Müller-Hill, B, *Proc. US Nat. Acad Sci.*, **56**, 189 (1966)
[3] Gilbert, W, and Muller-Hill, B, *Proc US Nat Acad Sci*, **58**, 2415 (1967)
[4] Ptashne, M, *Proc US Nat Acad Sci*, **57**, 306 (1967)
[5] Ptashne, M, *Nature*, **214**, 232 (1967).

Large-scale Density Inhomogeneities in the Universe

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There may be large scale density inhomogeneities (such as quasar clusters) in the universe, and their effect on the angular distribution of the cosmic black-body radiation could potentially be observable.

OUR aim is to discuss the possibility that observations of the angular distribution of quasars and of the cosmic black-body radiation may in the future yield important information about large-scale inhomogeneities of density in the universe There is already fragmentary evidence to suggest that this possibility may be fruitful It therefore seems desirable to clarify the theoretical situation in order to call the attention of observers to these possibilities and to prepare the way for the interpretation of the results they may obtain

The first suggestion that quasars with large red-shift may not be distributed randomly in the sky was made by Strittmatter, Faulkner and Walmesley[1], who drew attention to two prominent groupings, one in the direction of the North Galactic Pole and the other in the South Galactic hemisphere. If these groupings are real and if the quasars are at cosmological distances, the most likely explanation[2] is that quasars are clustered on a scale much larger than any previously studied scale of irregularities in the universe. This suggestion links up with the question of the angular distribution of the cosmic black-body radiation because, as was first pointed out by Sachs and Wolfe[3], large-scale irregularities would influence the temperature of radiation which passes through them. A possible example has been discovered recently by Wilkinson and Partridge[4] and has encouraged us to carry out further calculations of the effects involved. We shall give a simplified description of our results here, reserving for a separate publication by one of us (M. J. R.) a more detailed account of the calculations.

Scale of Possible Quasar Clusters

The angular size of a possible quasar cluster is a non-trivial question because, at large red-shifts, important model-dependent relativistic effects come into play. If a cluster expands at the same rate as the background universe, and if its present diameter is d_0, then at earlier times corresponding to a red-shift z, the diameter would be

$$d = \frac{d_0}{1+z}$$

If d_0 is much smaller than the present "radius" of the universe c/H_0 (where H_0 is the present Hubble constant), the angular diameter at various red-shifts can readily be calculated in terms of d_0H_0/c. We shall restrict our attention to Robertson–Walker models with zero cosmical constant which can be characterized by the density parameter

$$\sigma_0 = \frac{4\pi}{3} G\rho_0 c^2/H_0^2$$

in which ρ_0 is the present mean density. If the cosmical constant is zero, σ_0 is equal to the deceleration parameter q_0. Numerical values for the angular diameter are given in Table 1 for three values of σ_0.

Table 1 ANGULAR SIZE OF A FREELY EXPANDING CLUSTER AS A FUNCTION OF ITS RED-SHIFT z

z	Angular diameter (units of d_0H_0/c)		
	$\sigma_0=\frac{1}{2}$	$\sigma=1/10$	$\sigma_0=1/100$
0.5	2.72	2.41	2.40
1	1.71	1.34	1.33
1.5	1.36	0.99	0.96
2	1.18	0.80	0.75
3	1.00	0.59	0.53
∞	0.50	0.10	0.01

The case $\sigma_0=\frac{1}{2}$ corresponds to the Einstein–de Sitter model, the case $\sigma_0=1/100$ to roughly the lowest permitted value for the mean density ($\rho_0\sim 4\times 10^{-31}$ g cm^{-3} if $H_0\sim 100$ km sec^{-1} Mpc^{-1}) and $\sigma_0=1/10$ to an intermediate case.

We note that for a given present size d_0, the smallest angular scale occurs at the largest red-shift (though this would not be the case in closed models with $\sigma_0>\frac{1}{2}$). In fact, a cluster would not expand at the same rate as the rest of the universe: if it had developed from a perturbation of small-amplitude, it would be separating out, so that the angular diameter would depend somewhat less steeply on z than in Table 1. Deviations from free expansion would be more important in a higher-density universe.

We now ask what linear size to expect for a quasar cluster. According to Sandage and Luyten[5], there are about 0.4 quasars (quasi-stellar radio sources and quasi-stellar galaxies combined) per square degree down to $B=18^{\mathrm{m}}$. Thus the minimum detectable angular scale of a cluster would be a few degrees. In fact only about 200 QSOs have so far been discovered, and these would be too sparsely distributed over the sky to reveal any clustering on an angular scale less than 10 or 20 degrees. But from Table 1, a freely expanding cluster with a red-shift of, say, 1.5 and subtending an angle of about 20 degrees, would have linear dimensions ~ 750 Mpc at the present epoch in an Einstein–de Sitter universe. This is an appreciable fraction of the "radius" of the universe ($\sim 3{,}000$ Mpc), and so the question arises: is it reasonable to consider that irregularities may exist on such a large scale? This is a difficult question because we do not yet understand the origin of the known small-scale irregularities in the universe. It may, however, be helpful to consider what is known, or reasonably conjectured, about these small irregularities. This information is summarized, in very round figures, in Table 2, where we have assumed that $\rho_0\sim 10^{-30}$ g cm^{-3}.

Table 2 OBSERVED CLUSTERING IN THE UNIVERSE

Scale	Object	Density contrast $\left(\frac{\delta\varrho_0}{\varrho_0}\right)$
30 Kpc	Galaxy	10^6
1 Mpc	Galaxy cluster	10^3
30 Mpc	Supercluster	10
1,000 Mpc	?	?

It does not seem out of the question that on a scale $\sim 1{,}000$ Mpc, there should be quasar clusters in which $\delta\rho_0/\rho_0$ is of the order of unity. If the rate of formation of quasars depends on some power of the ambient density as seems to be the case with stars where the power involved is ~ 2 (ref. 6), or if the lifetime or brightness of quasars is enhanced by the higher density, the concentration of quasars would help to delineate the density distribution and quasar clustering might be detectable.

Further insight into the possibility that there may be large-scale irregularities in the universe can be gained from a consideration of the behaviour of perturbations with various scales during the expansion of a "hot big bang" universe. During the earliest stages of such a universe, the energy density of the radiation would exceed the rest-energy of the matter, and the matter and radiation would be strongly coupled together. Taking the present temperature of the primeval black-body radiation to be $\sim 3°$ K and H_0 to be ~ 100 km sec^{-1} Mpc^{-1} (so that $\rho_0\sim 4\times 10^{-29}\sigma_0$ g cm^{-3}) the radiation-dominated phase continues until the temperature falls to $T_c\sim 8\times 10^4\sigma_0°$ K (at a time t_c, say). During this period the Jeans length is essentially the radius of the observable universe. Another critical stage in the expansion occurs when $T=T_d\sim 3{,}000°$ K (at time t_d). At this time the matter almost completely recombines and subsequently becomes almost transparent to radiation, at least until it is reheated. In a low density universe, there is a third important time, t_e, corresponding to a red-shift $(1+z)\sim 1/2\sigma_0$. Before t_e the universe behaves like the Einstein–de Sitter model, but afterwards the deceleration parameter decreases towards zero.

We define $r(t)$ to be the present length-scale of a perturbation which would roughly fill the observable universe at time t. Then

$$r(t_c)\sim 4\times 10^6\sigma_0^{-1}\ \mathrm{pc}$$

and

$$r(t_d)\sim\begin{cases}2\times 10^7\sigma_0^{-\frac{1}{2}}\ \mathrm{pc} & (\sigma_0\gtrsim\frac{1}{25})\\ 10^8\ \mathrm{pc} & (\sigma_0\lesssim\frac{1}{25})\end{cases}$$

For $t>t_e$, $r(t)$ is roughly the radius of the presently observable universe.

We shall discuss only "adiabatic" perturbations in which the densities of matter and radiation are both perturbed so that the entropy per baryon is the same as in the unperturbed state. We also confine ourselves to perturbations which, in the absence of pressure gradients, would be of the "relatively increasing" kind. We can visualize these as regions of the universe which start expanding with a kinetic energy different from their surroundings, in contrast to "relatively decreasing" perturbations, which can be thought of as starting to expand at a slightly different time from the rest of the universe, but with the same energy.

Perturbations of any given scale r exceeded the horizon size at sufficiently early times. At those times they evolved independently of the rest of the universe. If $r < r(t_c)$, the perturbations start to interact with other parts of the universe during the radiation-dominated phase ($t < t_c$), and will then oscillate until t_d. Any density or—more important—velocity perturbations which survive at $t > t_d$ will initiate the growth of condensations, for the matter can then condense unimpeded by the radiation and the gas pressure is about 10^{-8} times the radiation pressure or less. Adiabatic expansion causes the oscillations to diminish in amplitude between t_c and t_d. Furthermore, there will be significant damping due to radiative viscosity during this period (for the mean free path of the radiation, though much less than the horizon distance, is by no means negligibly small). Even while the gas is still ionized, adiabatic perturbations with r up to a few Mpc would have been damped out[7] during the recombination phase, all scales up to $r(t_c)$ would suffer appreciable damping. Initial perturbations with $r > r(t_c)$, on the other hand, never undergo an oscillatory phase nor are they subject to radiative damping.

If the initial amplitudes of these large-scale perturbations are sufficiently large (and always if $\sigma_0 \geqslant \frac{1}{2}$), they will eventually develop into bound systems. However, if $\sigma_0 < \frac{1}{2}$, smaller-amplitude perturbations may still have positive energy. They will undergo relative growth, but eventually self-gravitation will become negligible even within the perturbation and the density contrast will subsequently remain constant during the free expansion. If the density within an unbound cluster eventually exceeds the background density by a factor x, say, the separating out will be almost completed by a time $t \sim x\, t_e$.

The initial spectrum of irregularities in the universe is a mysterious question, and it is, of course, possible that perturbations on scales $\gg r_c$ and r_d have much smaller initial amplitudes than the smaller scale amplitudes which presumably developed into galaxies, galaxy clusters and superclusters. There is no *a priori* reason to rule them out, however, and we have seen that they would in fact grow faster than smaller-scale perturbations of similar initial amplitudes.

In the remainder of this article we discuss the types of observation which may, in the future, determine whether very large scale irregularities do actually exist in the universe.

Observational Evidence

The following lines of evidence are at present relevant the angular distribution of (*a*) quasars, (*b*) the black-body radiation and (*c*) the radio source counts.

(*a*) *Quasars*. The angular distribution of the 103 quasars with red-shifts known to us does at least suggest that there may be clustering at large red-shifts on an angular scale ~ 20 degrees. The number of quasars is still too small for us to be able to decide whether the effect is significant, especially because of the effects of selection.

(*b*) *Black-body radiation*. We turn next to the interesting question of the isotropy of the primeval black-body radiation. This problem was discussed by Sachs and Wolfe[3], who calculated the amplitude of the temperature fluctuations which would be caused by a distribution of perturbations (with small $\delta\rho_0/\rho_0$, but arbitrarily large length-scale) in an Einstein–de Sitter universe. In that analysis, the temperature anisotropies can be attributed to the perturbation in which we are situated and to perturbations so distant that the radiation underwent its last scattering when passing through them. We are here, however, especially interested in the effects on the black-body radiation of particular irregularities with $z \sim 1$–3 which may be delineated by observed concentrations of quasars. The radiation would have undergone its last scattering long before passing through such an irregularity, so that Sachs and Wolfe's work is not directly relevant. Moreover, the amplitude of the density fluctuations will not be small—in fact, no quasar clustering would be evident unless $\delta\rho_0/\rho_0 \gtrsim 1$—and we wish to discuss open universes with $\sigma_0 < \frac{1}{2}$ as well as the Einstein–de Sitter model. We shall therefore describe a somewhat different type of analysis, the aim of which will be to determine the order of magnitude and sign of the temperature variations induced by the gravitational field of a single perturbation.

In a homogeneous isotropic universe with negligible pressure, the material within a co-moving sphere would expand in exactly the same way if the remainder of the universe were removed. If $\sigma_0 < \frac{1}{2}$, the sphere would have positive energy and its radius R (taking the usual Schwarzschild radial co-ordinate) is related to the proper time t of an observer moving together with the boundary by

$$\left.\begin{aligned} R &= 2\left(M\,\frac{(1-v_\infty^2)}{v_\infty^2}\right)\sinh^2 y \\ t &= M\left(\frac{(1-v_\infty^2)}{v_\infty^2}\right)^{3/2}(\sinh 2y - 2y) \end{aligned}\right\} \qquad (1)$$

(c and $G = 1$) where v_∞ is the asymptotic velocity, which will be a function of σ_0 and the gravitational mass M. The radius R is simply proportional to the scale factor of the universe.

The remainder of the universe would expand in identical fashion if the matter within the co-moving spherical boundary were replaced by any other spherically symmetrical distribution of matter with the same value of M. The type of perturbation which we shall consider will be one in which the matter within a co-moving sphere is replaced by a uniform sphere of the same mass but lower kinetic energy. If the total energy is still positive, the expansion of the sphere will be given by equation (1) with v_∞ replaced by $v_\infty (< v_\infty)$. Initially the universe would be almost uniform, but a spherical condensation would gradually separate out, the density contrast finally attaining a value $x \sim (v_\infty/v_\infty)^3$. The expansion of the spherical perturbation would mimic a universe with a different radius of curvature.

Expressions analogous to equation (1) can be written down for bound clusters which eventually contract and for clusters separating out of an Einstein–de Sitter universe.

A perturbation of this type is illustrated schematically in Fig. 1*a*. Any inhomogeneous universe must contain clumps of enhanced density surrounded by regions of density less than average. Such a universe can be simulated by a "swiss cheese" model in which spherical perturbations like that shown in Fig. 1*a* are distributed through space. The perturbations would exert no gravitational influence on one another, but would each develop as though they were embedded in an otherwise uniform and isotropic universe. The absence of any significant 12 h or 24 h anisotropy in the radiation temperature[8] confirms that the overall expansion of the universe is in fact accurately isotropic.

Before we discuss how the black-body temperature would be perturbed by an irregularity of the type shown in Fig. 1*a*, it will be helpful to consider the frequency shift induced by passage through a uniform expanding sphere, centre O, surrounded by empty space. Suppose that light is emitted at a point X (at rest relative to O and far outside the sphere), passes through O, and is received at a distant point Y. The presence of the sphere would increase the time light takes to travel from X to Y

The passage of such a light ray is traced schematically in Fig. 2. At first sight one might think that, if X and Y are equidistant from O, there would be no frequency shift between the two points. Though this would be true if the sphere were not expanding, the expansion in fact causes a blue-shift. This effect can best be understood by imagining that monochromatic light is being emitted from X. The time delay caused by the sphere diminishes as the sphere expands, so that each wave-crest is subject to a shorter delay than its predecessors. The crests are thus received at Y with a frequency higher than the emitted frequency (see Fig. 2). An alternative interpretation may make this effect clearer: the light falls towards O down a steep potential gradient but, because the sphere is expanding as the light goes through, it climbs up against a more gradual potential gradient and achieves a net gain of energy from the gravitational field.

Suppose that a particular ray enters the sphere at a point A at time t_A and emerges from the other side at t_B (see Fig. 2). Since the sphere can be regarded as part of a homogeneous universe, the ratio of the wavelengths observed by co-moving observers at A and B is

$$1 + z = \frac{R(t_B)}{R(t_A)} \qquad (2)$$

This red-shift can be interpreted as the sum of three contributions: (i) the *blue*-shift already discussed, due to the expansion; (ii) a straightforward Doppler red-shift, due to the fact that A and B are both receding from O when the radiation passes through them; and (iii) a gravitational red-shift

$$(1-2M/R(t_B))^{1/2}/(1-2M/R(t_A))^{1/2}$$

When the sphere is part of a homogeneous universe, these three contributions combine to give the same net red-shift as equation (2). When, however, we come to consider inhomogeneities, this is no longer true and it is then helpful to treat the three contributions separately.

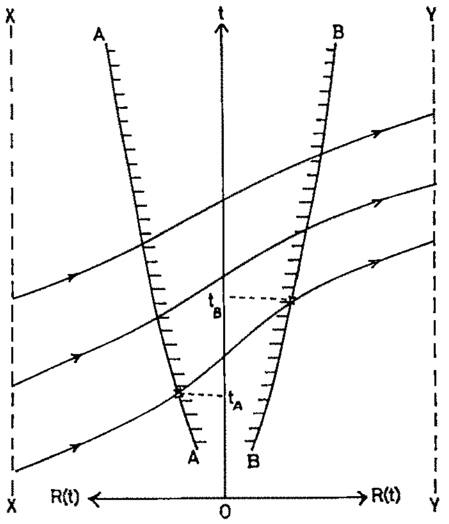

Fig. 2. Diagram tracing the paths of light rays through an expanding sphere surrounded by empty space. These rays experience a time delay which decreases as the sphere expands, so they would be observed at Y blue-shifted relative to X.

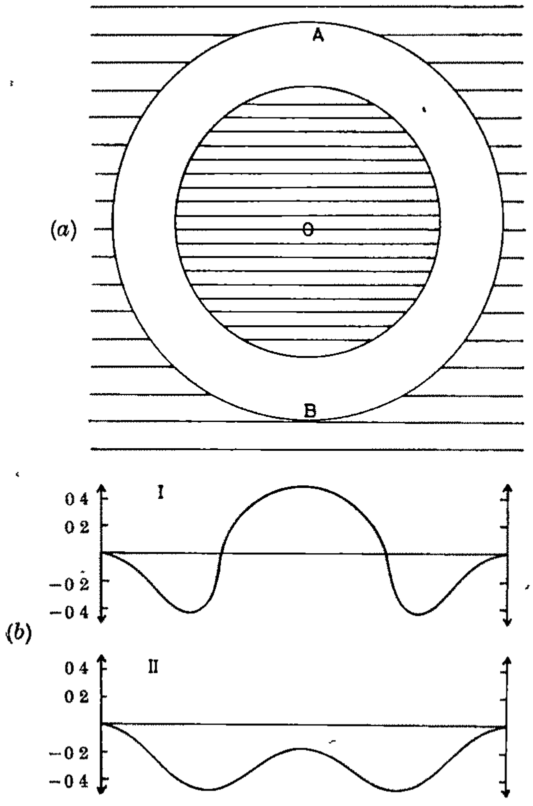

Fig. 1. *a*, Illustration of the type of spherically symmetric perturbation discussed in this article. *b*, Temperature profiles across a cluster with ~3 times the background density. $\Delta T/T$ is expressed in units of GMv/c^2R, where v and R are the velocity and radius of the unperturbed boundary. The profiles are drawn for (I) an undecelerated background universe and (II) an Einstein–de Sitter universe.

The accurate calculation of the temperature perturbations is rather complex, even for spherically symmetric density irregularities. We shall present here a simplified treatment which is strictly valid only for irregularities very much smaller than the observable universe. It should, however, be good enough to give the sign and magnitude of the temperature variations due to clusters of the size in which we are interested (up to ~1/4 of the radius of the universe). This simple treatment may also elucidate more clearly the physical interpretation of the effects involved.

We assume that the expanding sphere had radius R' and expansion velocity v', when the radiation which we now receive passed through it, whereas if the same mass M were expanding with the rest of the universe, its radius and velocity would be R and v respectively. If the cluster were small compared with the horizon size, these quantities would change only by small amounts during the passage of the radiation. We can also assume $R' \gg M$, and v and $v' \ll 1$. v' and R' cannot be specified independently, because they are related through equation (1). Suppose that there are no other irregularities along the line of sight through O, and that the black-body radiation is not scattered after passing the point A.

The changing gravitational field within the sphere of radius R' will (through effect (i)) contribute a blue-shift $2Mv'/R'$. If, however, the matter were smoothed out there would be a blue-shift $2Mv/R$. If the cluster were separating out from the background $v'/R' < v/R$, so that effect (i) makes a *negative* contribution to $\Delta T/T$.

The cluster will create a deeper potential well at O than would be present if the material were smoothed out, and its existence will thus increase the time radiation takes to get from A to B. This extra time delay tends to increase the black-body temperature (an effect which we shall call (iv)) for the following reason. The radiation reaching B at t_B must have passed A at the instant t'_A ($<t_A$). The temperature at A, being proportional to R^{-1}, would have been higher at this earlier time. The extra time delay amounts to

$$\sim 2M \log (R/R') \qquad (3)$$

so

$$R(t_A) - R(t'_A) \sim 2Mv \log (R/R') \qquad (4)$$

and the consequent fractional temperature increase due to (iv) is

$$\frac{2Mv}{R} \log (R/R') \qquad (5)$$

The alterations in (ii) and (iii) caused by the extra time delay expression (3) make negative contributions to $\Delta T/T$ which, however, are $\sim M/R$ times smaller, and so can be neglected In this approximation, the net temperature change caused by the irregularity (and attributable solely to effects (i) and (iv)) is therefore

$$\frac{\Delta T}{T} \sim 2M \left\{ \frac{v'}{R'} - \frac{v}{R} \left(1 - \log \left(\frac{R}{R'} \right) \right) \right\} \qquad (6)$$

In a very low density universe where the cluster has already separated out completely, $v'/R' \sim v/R$ so that expression (6) would then be positive The same is true for any unbound cluster in a universe with $\sigma_0 < \frac{1}{2}$ If the density contrast is small, and $v' = v - \delta v$, $R' = R - \delta R$, the magnitude of $\Delta T/T$ is proportional to $\delta R/R$ If we apply equation (6) to an Einstein–de Sitter universe, we find that $\Delta T/T$ is negative because v'/R' is then smaller than v/R by a sufficient amount to make (i) more important than (iv). In this case, however, the effect is proportional to $(\delta R/R)^2$ when the density contrast is small This is because $\delta v/v = 2\delta R/R$, so that the first order terms in equation (6) cancel out This is in agreement with Sachs and Wolfe's first-order analysis, according to which a perturbation of this type in an Einstein–de Sitter universe would have zero effect on the black-body temperature

One can similarly calculate $\Delta T/T$ for radiation which is intercepted by a cluster but does not pass through the centre and thus determine the expected temperature profile across a cluster For irregularities of the type considered here, it is found that $\Delta T/T$ is negative near the edge for all values of σ_0, though (as we have seen) it becomes positive at the centre for a cluster of positive energy The profiles are shown in Fig 2b for a cluster with ~ 3 times the mean density, for models with two different values of σ_0 The exact profiles should not be taken too seriously, because they obviously depend on the detailed density distribution across the irregularity It is, however, interesting that even the simple type of cluster considered here can cause a ΔT of either sign

Radiation which does not pass through O will suffer a small angular deflexion due to the gravitational field of the cluster Liouville's theorem[9] ensures that the magnitude of this deflexion is exactly what is required to produce the black-body radiation intensity appropriate to the perturbed temperature

A cluster with $d_0 \sim 750$ Mpc, contrast density ~ 3, and $z \sim 1\ 5$ in an Einstein–de Sitter universe would give rise to a decrease of $\sim 0\ 3$ per cent in the radiation temperature in the direction of its centre and $\sim 0\ 9$ per cent somewhat away from the centre The angular extent of the profile would be ~ 20 degrees A similar cluster in a low-density universe with $\sigma_0 \sim 1/10$ would give rise to a decrease in temperature of $\sim 0\ 2$ per cent near the edge, but a comparable increase at the centre Wilkinson and Partridge[4] observed a possible decrease in temperature of $\sim 0\ 5$ per cent in the direction $R\ A \sim 2$ h, $\delta \sim -8°$ If this is attributable to a quasar cluster in the same general direction, we conclude that either the universe has roughly the Einstein–de Sitter density or, if the density is lower, the strip of sky scanned by Wilkinson and Partridge did not pass through the centre of the cluster

We must next try to establish that the temperature anisotropies attributable to clusters with $z \sim 1$–3 will not be swamped by the effects of more distant clusters[10] Since seven-eighths of the material within the particle horizon of an Einstein–de Sitter universe (and an even larger fraction in the case of a lower density universe) lies at $z > 3$, it would be unreasonable to suppose that no such clusters exist Formula (6) can be applied provided that the radiation has not been scattered since entering these clusters, and provided also that they are then smaller than the observable universe We find that if $(1+z) \lesssim 1/\sigma_0 x$, where x is the terminal value of the density contrast, $|\Delta T/T|$ increases in proportion to $(1+z)$, for larger z, it decreases roughly as $(1+z)^{-\frac{1}{2}}$ for the universe, then approximates to an Einstein–de Sitter model These very distant clusters do not therefore pose a problem except in a very low density universe ($\sigma_0 \lesssim 1/20$)

Consider now the clusters which lie across the surface where the last scattering occurred (at a red-shift $z \sim z_s$) If $(1+z_s) \lesssim 1/x\sigma_0$, the effect will be of the same order as formula (6) However, if $(1+z_s) \gg 1/x\sigma_0$, we can apply the results of Sachs and Wolfe, since not only would the universe then approximate to an Einstein–de Sitter model at $z \sim z_s$, but $\delta\rho/\rho$ would have been small We find that, if the universe were now filled with clusters of dimensions ~ 750 Mpc, with $\delta\rho_0/\rho_0 \sim 1$ the temperature fluctuations would be ~ 2 per cent This conclusion is independent of σ_0· in a low density universe the clusters separate out more slowly, and the larger value of $\delta\rho/\rho$ at $z \sim z_s$ compensates for the lower mass of each cluster. The angular scale of these irregularities would be $\sim 20\,\sigma_0$ degrees These temperature variations would be ~ 10 times higher than the upper limits permitted by observation[4,11] The value of ~ 2 per cent deduced here may be a very severe overestimate, however, because the clusters need not occupy the whole of space (perhaps no more than 10 per cent) and because Sachs and Wolfe's calculations assumed that the last scattering occurred at a well defined instant whereas the universe would in fact gradually become transparent during a time of the order of the expansion time scale Therefore unless the irregularities are much larger than the horizon size at z_s (that is, unless $z_s \gg 10\sigma_0^{-1}$ if the irregularities have a present scale ~ 750 Mpc), the temperature variations in the Rayleigh–Jeans part of the spectrum due to clusters with $z \sim z_s$ would be smeared out by depth effects

Finally, we must consider whether we ourselves lie within a cluster Unless we were positioned exactly at its centre, our own cluster would produce a 24 h anisotropy in the black-body radiation Though this would be partly due to the gravitational field of the cluster, the main contribution would be essentially a Doppler effect, arising from the fact that the cluster would be expanding more slowly than the overall Hubble velocity we would have a peculiar velocity towards the centre. This effect would be too great to be consistent with the observations unless $\sigma_0 \lesssim 1/100$ In our previous paper[2] we supposed that we were in a cluster, and that σ_0 was indeed very small We have seen, however, that a higher density is permissible without the temperature fluctuations caused by other clusters being too large Since (as we shall see) the isotropy of the radio source counts is harder to reconcile with the existence of clusters if σ_0 is very low, it is perhaps more likely that we are not within a cluster

(c) *Source count isotropy* The radio source counts have provided no evidence for any anisotropy in the distribution of radio sources The permitted limits would appear to be $\lesssim 25$ per cent for sources stronger than 2 flux units at 178 MHz, on scales ~ 20 degrees square[12,13], and $\lesssim 10$ per cent at 0 2 flux units on the same angular scale[13] This figure is based not on direct counts but on a statistical analysis of the measured radio noise Is the hypothesis of large-scale clustering compatible with these data ? We believe so, for the following reasons

(i) Because of the large spread in the intrinsic power of radio sources, a sample chosen at a given flux density will include powerful sources with $z \gtrsim 4$ as well as weak local sources In fact, according to Longair's[15] analysis of the counts, the anisotropy limits at ~ 2 flux units are likely to be more crucial in this regard because it is at this level that the largest contribution comes from very distant sources (It is, however, worth pointing out that interpretations of the source counts, and of magnitude red-shift relations for quasars, would need modification if clustering occurs on the scale envisaged here) Even at ~ 2 flux units, however, sources with $z \sim 1$–2 will be diluted by both nearby and even more remote sources

(ii) The clusters may not have fully separated out at $z \sim 4$ Accordingly we would not expect any large aniso-

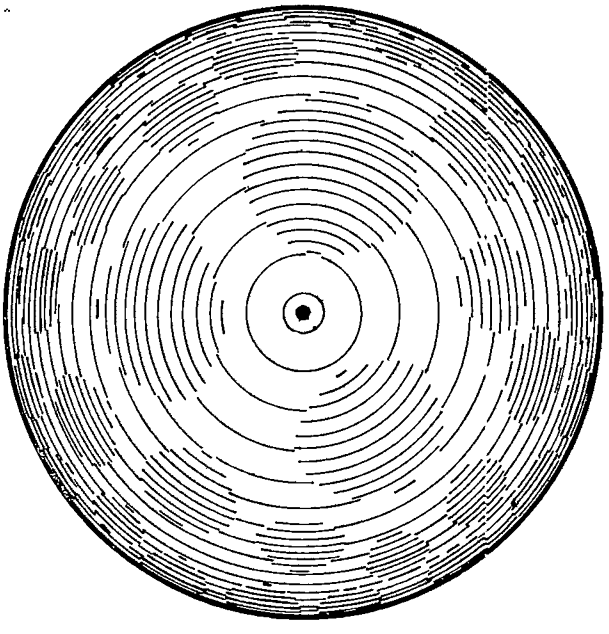

Fig 3 A possible distribution of large scale clusters as seen by an observer at the centre The contrast between clusters and the background would be less marked in the past (though the mean density was then higher) because the clusters would not have fully separated out The shading is meant to illustrate this Very remote objects (for example, radio sources) need not therefore appear anisotropically distributed

tropies in the distribution of the most distant sources The separation would only in fact have been completed by $z \sim 4$ in a very low density universe for which $1/\sigma_0 \tau \gtrsim 5$. We attempt to illustrate this effect in Fig 3 The contrast between the density inside and outside clusters would be less marked at large z, and moreover we would be observing a large number of clusters at these great distances In Fig 3 we are not depicted as being in a cluster ourselves, for the reason already given

Clustering on a scale of $1-2 \times 10^8$ pc could in principle be revealed by a study of the distribution of radio galaxies (and such clustering would be expected theoretically if the even larger quasar clusters exist, except perhaps in a universe with $\sigma_0 \leqslant 1/25$) However, in practice their detection would be difficult because of the number (~ 10) of such clusters which would be expected along a typical line of sight

In this article we have attempted to stress two points The first is that the spectrum of irregularities in the universe may well contain large-scale components of appreciable amplitude The second is that such components, if they exist, may be amenable to systematic observational study by a detailed analysis of the angular distribution of quasars and of the cosmic black-body radiation We would therefore urge that the necessary observational studies be undertaken

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[1] Strittmatter, P A, Faulkner, J, and Walmesley, M, *Nature*, **212**, 1661 (1966)

[2] Rees M J, and Sciama, D W, *Nature*, **213**, 374 (1967)

[3] Sachs, R K, and Wolfe, A M, *Astro J*, **147**, 73 (1967)

[4] Wilkinson, D T, and Partridge, R B, *Nature*, **215**, 719 (1967)

[5] Sandage, A, and Luyten, W J, *Astro J*, **148**, 767 (1967)

[6] Schmidt, M *Astro J*, **129**, 243 (1959)

[7] Silk, J, *Nature*, **215** 1155 (1967)

[8] Partridge, R B, and Wilkinson, D T, *Phys Rev Lett*, **18**, 557 (1967)

[9] Lindquist, R, *Ann Phys*, **37**, 487 (1966)

[10] Wolfe, A M, *Astro J* (in the press)

[11] Conklin, E K, and Bracewell, R N, *Phys Rev Lett*, **18**, 614 (1967), *Nature*, **216**, 777 (1967)

[12] Holden, D J, *Mon Not Roy Astro Soc*, **133**, 225 (1966)

[13] Payne, A D, *Aust J Phys*, **20**, 291 (1967)

[14] Hughes, R G, and Longair, M S, *Mon Not Roy Astro Soc*, **135**, 131 (1967)

[15] Longair, M S, *Mon Not Roy Astro Soc*, **133**, 421 (1966)

Mongolism, Delayed Fertilization and Human Sexual Behaviour

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Decreasing frequency of coitus accompanying advancing age and prolongation of marriage, predisposing to delayed fertilization, may explain the correlation of increased incidence of mongol births with advancing maternal age

MONGOLISM (Down's syndrome)[1] is the developmental consequence of trisomy of chromosome 21 (ref 2) Its cause has remained obscure, but the increased incidence among offspring of mothers more than 35 years old suggests that the chromosomal non-disjunction usually occurs during female rather than male gametogenesis At what stage in oogenesis is such an error particularly likely to occur ?

The maturation of the mammalian egg consists of a first division, which is completed at about the time of release of the primary oocyte from the ovarian follicle, and a second division which begins immediately thereafter The division of the secondary oocyte, however, becomes suspended in metaphase and progresses to completion only after a spermatozoon penetrates it Without such penetration, which usually occurs in the peritoneal cavity[3] or upper portion of a uterine tube, disruption of the alignment of the second division metaphase chromosomes sets in, and generalized cellular deterioration occurs The human secondary oocyte suspended in metaphase has been estimated to be fertilizable for 1 day or less[3-5] In other mammalian species the period of fertilizability varies, it is usually between 6 and 30 h[6] Fertilization toward the end of this brief period often has serious embryological consequences in many species[6-13]

It has been clearly established that delayed fertilization of mammalian eggs may have unfavourable consequences This was recognized in 1934 by Hammond, who detected a decrease in both fertility rate and litter size in rabbits[7] If the oocyte lies unpenetrated in the uterine tube for several hours, its capacity for being fertilized decreases Should it be fertilized late, however, the incidence of abnormal or unsuccessful cleavages, of structurally abnormal embryos, and of embryonic reabsorption or abortion has been observed to increase in several non-human species in experimental conditions[6,8,9] Abnormal eggs containing either one or three pronuclei (including the male) have been observed[6,9], as a result of abstriction of either two or of no secondary polar bodies, that is, both or neither female 1c complements, at the second maturation telophase Hyperdiploid, hypodiploid and mosaic embryos have been found in increased numbers after delayed fertilization[13,14], and cytogenetic examination of the oocyte which has remained unfertilized in the tube for several hours has shown scattering of chromosomes from the metaphase plate of the arrested second maturation spindle[11,13] The various errors in division observed experimentally may be attributed to a disturbance of chromosomal segregation of the maturing egg existing outside the ovary and suspended in metaphase II a few hours too long before a spermatozoon becomes available to penetrate it and cause syngamy There is no evidence that metaphase II of oogenesis is less vulnerable in man than in other mammals examined so far Blandau and Young proposed that delayed fertilization might be a cause of early abortion in the human female[8] Edwards[15] more recently emphasized the desirability of beginning embryonic development "with a pair of fresh gametes"

Human Fertilization

In many mammals, coitus-induced ovulation or periods of oestrus are among the mechanisms ensuring fusion of fresh gametes only In the human female, however, ovulation is not coincident with as clearly demarcated a period of heat Delayed fertilization in human females seems therefore to be a possibility if coitus occurs only sporadically or if it occurs regularly but relatively infrequently—that is, at intervals greater than that of the fertilizing potential of spermatozoa, which is perhaps 48 h[4,5] With regular coitus at intervals shorter than this, it would become relatively impossible for an egg to loiter in the upper tube for long before being met by one of the continuously present, capacitated spermatozoa A decline in the regular frequency with which spermatozoa are deposited in the lower genital tract of a sexually active woman therefore theoretically permits a perilous situation to arise This situation could lead to fertilization of a cell released from its ovarian follicular environment for too long and, as a consequence, errors in chromosomal segregation at the second maturation division could arise. Embryonic triploidy, trisomy, chromosomal mosaicism or other aneuploid states are some of the results possible Many aneuploid embryos would doubtless be aborted or reabsorbed promptly, but a small number would be viable for longer periods, some until birth Those affected by mongolism and the other two autosomal trisomy syndromes would be among those sometimes capable of surviving intrauterine life What might predispose to delayed fertilization in man, and how might it be detected ?

Direct evidence for the occurrence of delayed fertilization in man is difficult to obtain Certain considerations and deductions, however, may lead to meaningful experiments and observations substantiating or contravening it Thus, (*a*) spermatozoa are continually present, and forestall delayed fertilization, only if the intervals between coitus are shorter than the span of time a spermatozoon maintains fertilizing ability, this is possibly two days, (*b*) coital frequency usually decreases with advancing age[16], and, therefore, (*c*) the incidence of abnormal embryos including trisomics might be expected to be greater in older mothers, (*d*) an increased frequency and regularity of coitus in older mothers, however, should reduce theoretically the incidence of abnormal embryos by decreasing the frequency times when the tube contains no spermatozoa As a result the possibilities for delayed fertilization will be reduced Surveys comparing the incidence of mongolism in either old or young groups having sporadic or infrequent coitus with that in similar groups having regular and frequent coitus could provide evidence for or against this expectation It would be useful to make a study of the incidence of mongolism in older mothers recently married, who might as a group be assumed to be sexually more active than mothers of the same ages married for a long time The incidence of mongolism among the offspring of unmarried mothers, who as a group engage in coitus sporadically rather than systematically, could also be determined In addition, careful recording of the frequency of the coitus about the time of conception in both young and old women having mongol children might provide interesting data

Previous Investigation

An assumption that is probably valid is that the mean frequency of marital coitus is negatively correlated with the duration of marriage This assumption is based partly on personal clinical case histories and is also supported by data recorded in the report by Kinsey *et al* [16] According to that report, frequency of marital coitus in women declines at a constant rate from a median of 2 5 times weekly by the age of 25 to 1 3 times by the age of 45 In the age group 41–45 in that survey, 40 per cent of married women had coitus less than 1 0 times weekly, in contrast to 15 per cent in the age group 21–25 The frequency of marital coitus reportedly[16] reaches a maximum in the first 1–2 years of marriage and then steadily decreases Studies correlating age, duration of marriage and coital frequency are not available

The durations of marriage in women before their deliveries of mongols have been investigated as part of a survey aimed at a more complete biological and sociological characterization of women having mongols Because mongolism occurs so much more commonly among offspring of older women, it seemed possible that causative factors might differ in the young and the old The mothers therefore were grouped by age The distribution of the time intervals between marriage and the delivery of a mongol in 201 mothers is shown in Fig 1 About half of the women were 35 or older at the time the mongol was born The mean duration of marriage in the young group was only 4 94 yr, whereas in the older group it was 12 08 yr A unimodal normal or Poisson distribution was found in the older group, there was a striking lack of marriages of short duration Only 17 per cent of mothers past 34, and only 12 per cent of those past 38, had been married 6 yr or less None of the fourteen mothers delivered of a mongol when older than 42 had been married less than 8 yr It is apparent that most women older than 34 delivered of mongols have, in addition to their longevity been married a long time That is, as a group they are not women who are bearing children late because of marriage late in life, as is the case for 30 per cent of all women aged 39–45 being delivered of babies

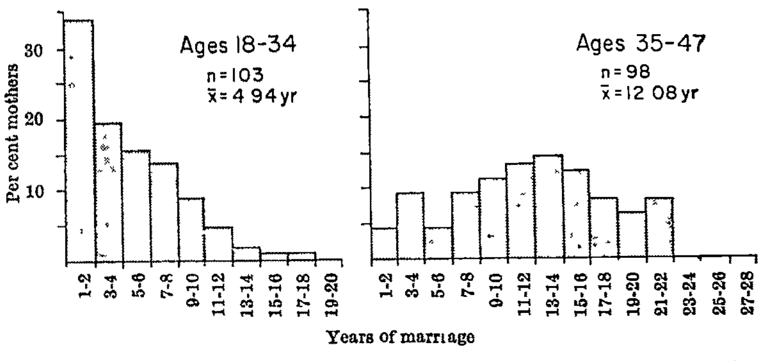

Fig 1 Distributions of durations of marriage before delivery in 201 mothers of mongols, grouped by age This population is composed of (*a*) all married mothers known to have been delivered of mongols at the New York Hospital during a 10 year period (1957–1966), (*b*) the married mothers of all mongols currently institutionalized at the North Jersey Training School and of all mongols who have died in the nursery of that institution whose medical records recorded the date of the mother's marriages

at the New York Hospital (unpublished data for the year 1960) For this second group a bimodal distribution of durations of marriage exists, with a prominent early mode composed of women who married at an advanced age, a mode not present in the older mothers of mongols investigated here

The two distributions in Fig 1 are, as would be expected, very different and are shown partly to emphasize that older women having mongols are different from the younger in ways other than age alone Is mongolism therefore correlated causally with biological old age itself, or is it connected with some superimposed attribute or behavioural pattern characteristic of old age? Consideration of the two distributions in Fig 1 suggests that if delayed fertilization subsequent to infrequent coitus is a cause of mongolism, the circumstances predisposing to it in the young are different from those in the old, the former being a group who in general are much more active sexually because of youth and short duration of marriage This does not necessarily imply, however, that the cause in the young is other than delayed fertilization, although multiple causes certainly might exist Mongolism is very infrequent in young women, common in old Infrequent, unusual and varied circumstances in the young, for example, illness or brief absences from the home of one of the marriage partners, might result in occasional periods of infrequent or sporadic coitus, but these periods could be significant should they coincide with the time an ovum becomes available for fertilization In contrast, the predisposing circumstance—actually a continual threat—for the long-married would be the decline in frequency of coitus usually characteristic of increasing age and adjustment to married life.

Hypothesis drawn from these Data

The following hypothesis is proposed, based on the experimental evidence in animals and on a consideration of man's sexual habits and the known correlation of mongolism and advancing maternal age Mongolism may be the result of delayed fertilization consequent to sporadic or decreased frequency of coitus

I suggest that human disease, genetic in this instance, may sometimes be the result of a disturbance of a natural biological phenomenon resulting from an accepted and seemingly normal pattern of social behaviour rather than from the damaging effects of some inhospitable environment, or from a seemingly spontaneous error of nature The observations suggest a novel medical view of the old mother, an individual in many ways unaltered anatomically, biochemically or as a result of her interaction with some exogenous noxious agent, but different in her sociology and behaviour The hypothesis to explain a cause of sporadic mongolism is attractive not only because it can be tested further but also because it implies that the incidence of the disorder could, in an informed society, be decreased

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[1] Penrose, L S and Smith, G F, *Down's Anomaly* (Little, Brown and Co, Boston, 1966)

[2] Lejeune, J, Gautier, M, and Turpin, R, *CR Acad Sci*, 248, 602 (1959)

[3] Horne, H W, and Thibault, J, *Fertil Steril*, 13, 135 (1962)

[4] Arey, L B, *Developmental Anatomy*, sixth ed (W B Saunders Co, Philadelphia, 1954)

[5] Patten, B M, *Foundations of Embryology* (McGraw-Hill, New York, 1958)

[6] Marston, J H, and Chang, M C, *J Exp Zool*, 155, 237 (1964)

[7] Hammond, J, *J Exp Zool*, 11, 140 (1934)

[8] Blandau, R J, and Young, W C, *Amer J Anat*, 64, 303 (1939)

[9] Chang, M C, and Fernandez-Cano, L, *Anat Rec*, 132, 307 (1958)

[10] Austin, C R, *The Mammalian Egg* (Blackwell Scientific Publications, Oxford, 1961)

[11] Hancock, J L, *J Reprod Fertil*, 2, 307 (1961)

[12] Adams, C E, and Chang, M. C, *J Exp Zool*, 151, 155 (1962)

[13] Austin, C R, *Nature*, 213, 1018 (1967)

[14] Butcher, R L, and Fugo, N W, *Fertil Steril*, 18, 297 (1967)

[15] Edwards, J H, in *New Directions in Human Genetics, Birth Defects, Original Article Series*, 1 (No 2), 82 (The National Foundation, New York, 1965)

[16] Kinsey, A C, Pomeroy, W B, Martin, C E, and Gebhard, P H, *Sexual Behavior in the Human Female*, 348, Fig 63, Table 93 (W B Saunders Co, New York, 1953)

Cytogenetical Polymorphism and Evolution in Mammalian Somatic Cell Populations *in vivo* and *in vitro*

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Co-operation as well as competition between cells affects the evolution of cell populations Thus studies on chromosomal variation in relation to age, environment and mechanisms of cytogenetical instability have led to the discovery of a new kind of balanced chromosomal polymorphism.

GENETIC polymorphism has been defined as the occurrence together in the same habitat of two or more discontinuous forms of a species, in such proportions that the rarest of them cannot be maintained merely by recurrent mutation[1] Polymorphic populations can, however, evolve by the action of selection on mutations[2] The study of variation in individual mammalian somatic cells has so far revealed only a few distinct genetical markers, these features include morphological variations in nuclei[3], sex chromatin[4] and chromosomes[5], cytochemical variations in the relative amount of DNA in each nucleus[6] and in each chromosome[7] and the presence or absence of enzymes such as alkaline phosphatase[8] The present communication is primarily concerned with types and frequencies of morphological variations in chromosomes

Essentially the same mechanisms of chromosome mutation (caused by aberrations in chromosome replication and their subsequent segregation at cell division) are responsible for both congenital chromosomal variations in populations of mammals and also within micropopulations of somatic cells in particular tissues Thus similar types of variations in the characteristic pattern of chromosomes in each cell (karyotype) have been found to form the basis of congenital malformations in populations of mammals and also within micropopulations of their somatic cells in particular tissues, indicating the operation of basic mechanisms of chromosome mutation Several genetic and environmental factors are now known to affect the stability of the karyotype and consequent evolution of somatic cells Thus a congenital predisposition for the formation of chromosome anomalies may be inherited, for example, the sticky gene in maize, a recessive human gene associated with breakage of mitotic chromosomes and increased incidence of tumours, a human familial tendency towards chromosome meiotic non-disjunction and congenital malformations[9]. Abnormal individuals caused by such congenital chromosome anomalies seem to have consequently unstable somatic cells, thus Klinefelter's syndrome mosaics and Down's syndrome have a significantly higher incidence of leukaemia, and 31 per cent of Turner's syndrome were reported to have ovarian tumours. There is also a circumstantial association between an increased incidence of spontaneous chromosome anomalies with the ageing of somatic tissues. Three categories of environmental factors affecting karyotype stability can be distinguished: (1) biological, for example, outbreaks of viral infection in North America, Sweden and Japan associated with damage to the chromosomes of somatic cells and an increased incidence of congenital malformations due to chromosomal anomalies, (2) chemical agents such as benzene associated with leukaemia in industry, (3) physical agents, for example, atomic bomb radiation inducing chromosomal congenital malformations, neoplasms and leukaemia in Japan.

Environmental factors in addition to inducing such anomalies also act during the evolution of somatic cell populations. It is now known that, in addition to natural selection of the cellular phenotype in the manner proposed by Darwin, there is an important level of selection of chromosomal mutations within the cell that must be compatible with limiting patterns of chromosome structure[10], thus the types that arise are not entirely the result of natural selection of competing cells. Sometimes a balance of chromosomal types follows selection (*a*) during differentiation of embryos containing two or more chromosome anomalies, resulting in mosaics, (*b*) following exchange of cells across the placenta to give chimaeras, both situations often exemplifying a state of balanced polymorphism. Most information about evolutionary changes in somatic cell populations has, however, been derived from the study of neoplasms[11] and the heteroploid transformation of cultured diploid cells *in vitro*[12].

The present studies were directed at examining the stability of diploid cell populations *in vitro* in standard environmental conditions, to be followed by further experimental studies on the action of one environmental agent at a time on the evolution of somatic cells. As a control, cells from the same species were studied *in vivo* (where this information was not already available) to determine the extent of karyotype stability in normal conditions of animal ageing.

Ageing in Human and Hamster Tissues *in vivo*

Although it had been known for a long time that older mothers produce a higher incidence of Down's syndrome, it was not until 1963 that an increased incidence of chromosome anomalies was also found to be associated with advancing age of normal somatic tissues[13] and this was later confirmed by other studies. No reports are available about the extent of chromosomal anomalies in ageing experimental animals.

In the present study, bone marrow cells were examined in Syrian hamsters ranging in age from 4 weeks to 16 months. No anomalies were detected at 4 weeks, tetraploid cells occurred at a frequency of 0.4 per cent by 11 weeks. Aneuploidy was only found in the oldest of the available animals at 16 months. For example, in a male, chromosome counts in 506 cells showed a mode of 70.4 per cent diploid cells (forty-four chromosomes), with 29.0 per cent aneuploid chromosome numbers (including 12.3 per cent greater than diploid) and 0.6 per cent tetraploid. Karyotype analysis of fifty modal cells showed that 20 per cent were pseudodiploid, as a result of abnormal combinations of chromosome types within the same normal chromosome number (forty-four chromosomes). (The normal male karyotype is conveniently subdivided into five groups according to chromosome size and type: A9, B10, C13, D8, E4 (see Fig. 1).)

Analysis of aneuploid karyotypes of chromosome numbers 43, 45 and 46 showed that they also had abnormal combinations of chromosomes per group within the karyotype, in addition to the expected simple loss or gain of one or two chromosomes. Some aneuploid karyotypes were replicated, indicating the early formation of clones. The most frequent example was a forty-five chromosome karyotype with an extra D group chromosome in ten cells. Thus karyotype instability was found in old, seemingly normal animals, presumably as a result of ageing. These observations provided information on the extent of karyotype variability in somatic cell populations growing in their natural environment, as a "control" for experimental studies *in vitro*.

Ageing and Evolution *in vitro*

If recent work on cultured somatic human cells[14] is of wider validity, as is likely, then other normal mammalian somatic cells will also have a limited lifetime *in vitro*. The number of cell doublings possible *in vitro* was related to the age of human donor tissue. Thus cultured diploid cells could only complete the limited number of cell generations which they had not undergone *in vivo*. Furthermore the frequency of chromosome anomalies was

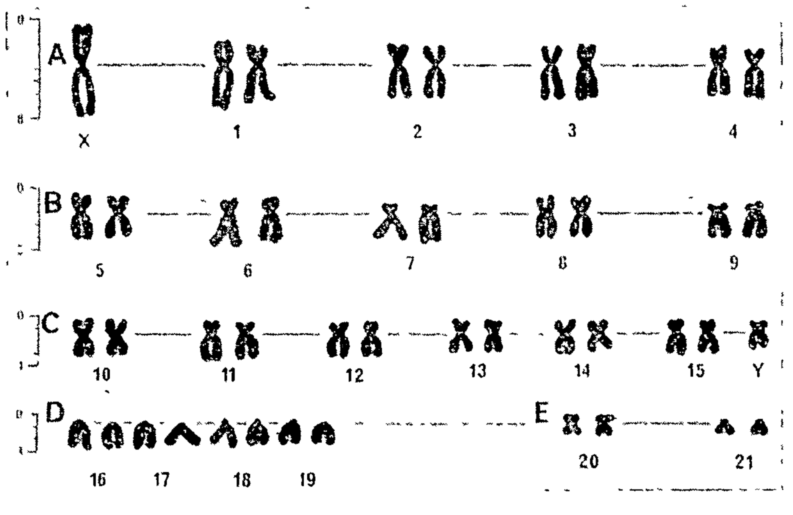

Fig. 1. Karyotype of normal male Syrian hamster (*Microcricetus auratus*)

found to increase as the cells approached the last few generations of their finite lifetime—as was also found in the ageing Syrian hamster bone-marrow in the present study Ageing cultures, however, just before degeneration at the time of onset of increased chromosome anomalies, were sometimes found to undergo malignant transformation. Such transformed cells were found to be capable of indefinite growth *in vitro,* and seemed to be immortal

It was therefore desirable to study the stability of diploid hamster and human cells *in vitro* to obtain further information about the extent of stability of somatic cell populations

Capstick *et al* [15] reported that a diploid cell strain of baby hamster kidney (BHK), established by Macpherson and Stoker, had a 92 per cent diploid mode (forty-four chromosomes) after 110 monolayer transfers and 66 days in suspension culture (a total of 386 cell doublings) Because this length of time was far in excess of a finite lifetime of 50–60 cell generations expected from previous studies, a sample of Capstick's P1 BHK 21/13 was obtained It was aimed to determine by more detailed karyotype analysis if such cells had retained a normal diploid constitution after a long time *in vitro*

The BHK cells were cultivated in suspension in the same way as that described by Capstick *et al* During the logarithmic phase of growth, samples were fixed directly for the study of mitosis and others were fixed after colcemid and hypotonic pretreatment for the study of metaphase chromosomes Two analyses were carried out, 1 month apart, when the cells had grown for 451 and 480 cell generations, respectively A diploid modal chromosomal number was found to be maintained during this time, with a frequency of 29 9 per cent in the first sample followed by 25 0 per cent in the second The later culture (at 480 cell generations) was karyotyped and analysed in detail with the aid of photomicrography The morphological components of the karyotype were grouped into five groups within which certain chromosomes could be identified individually (compare Fig 1) normal chromosomes *X*, 5, 10, 14, 16–19, 20, 21, abnormal chromosomes consisting of a metacentric in group A larger than *X* and another smaller than *X*, also a characteristic submetacentric and a dicentric On this basis it was found that all the diploid cells (at 480 cell generations) were far from normal, they had individually different karyotypes These could be distinguished by (*a*) differences in the combinations of chromosomes per group (A–E) adding up to forty-four chromosomes per cell, or (*b*) even by combinations of the presence or absence of singly identifiable chromosomes alone (see Table 1) In addition, karyotype analysis of aneuploid chromosome numbers (making a total of 100 karyotypes) failed to show two cells with the same karyotype If the sample that was analysed was fully representative of the whole cell population, then the observations indicate not only that the karyotypes were highly unstable but that chromosome mutation probably occurred at most cell divisions to produce different daughter cells

In order to obtain further information concerning the rate of chromosome mutation, the frequency of structural anomalies was determined in 40,000 cells from culture there were 0 2 per cent distinctly abnormal interphases with micronuclei, excessively small or giant nuclei and binucleate cells, 19 2 per cent of anaphases had abnormalities such as bridges and lagging chromosomes, only 4 per cent of 1,000 well spread metaphases (colchicine pretreated) had aberrations of chromosome structure including dicentrics, rings, chromatid interchanges, minute and broken chromosomes The rates of occurrence of structural defects that were observed cannot, it seems, account for the findings of every analysable karyotype in one hundred cells being different

In order to throw further light on this phenomenon, younger diploid cultures were required in order to make serial observations *in vitro* to find out more about karyotype stability in relation to duration in culture Because Burkitt lymphoma cultures recently established by Epstein and Barr were claimed to be diploid, samples of three cell lines, EB1, EB2 and EB3 were obtained for study The culture conditions and cytogenetical studies on chromosomal ploidy levels in the earliest samples have already been described in our previous report in conjunction with the kinetics of these cell lines[16]

Table 1 GAINS OR LOSSES IN INDIVIDUALLY IDENTIFIABLE CHROMOSOMES IN PSEUDODIPLOID BHK KARYOTYPES

	Normal							Abnormal				Deviations/cell		
								M						
Cell Nos	*X*	5	10	14	D	20	21	< *X*	> *X*	SM	DC	Gain	Loss	Total
1									+1			1	0	1
2			−1							+1	1	1	1	2
3						−1		+1				1	1	2
4		+1		−1	−1							1	2	3
5			+2					+1				3	0	3
6						−1	+1			+1		2	1	3
7	−1				−1					+1	+1	2	2	4
8		−1	+1		−1		+1					2	2	4
9			+2		−2							2	2	4
10					−1	+1	+1			+1		3	1	4
11	+1			+1	−2	−1						2	3	5
12	+1					−1	+1			+2		4	1	5
13			+1	−1	−1				+1	+1		3	2	5
14			+1		+1	−1				+1	+1	4	1	5
15	+1	+1			−2	−1	+1					3	3	6
16			+1	+1	−1	−1				+2		4	2	6
17	+2		+2		+1	−1				+1		6	1	7
18	−1		−1	−1		−1		+3				3	4	7
19		−1	+2	−1	−1	−1			+1			3	4	7
20			+1	−1	+2	−1		+1		+1		5	2	7
21		+1		+3	−1	+1					+2	7	1	8

D, Chromosomes 16–19, M, group A metacentric chromosomes, smaller than *X* (< *X*) or larger than *X* (> *X*), DC, dicentric, SM, abnormal group A submetacentric chromosome bigger than 1

When first received these cell lines had been in culture for different periods of time, between 5 and 16 months since establishment from tumour biopsies (EB1 and EB2 from females and EB3 from a male)

Fig 2 summarizes the distributions of chromosome numbers in seven samples studied over a period of 28 weeks For convenience, the chromosome numbers are grouped into ploidy levels Euploid chromosome numbers (2*n*, 3*n*, 4*n*) are shown in black and the aneuploid groups of eleven chromosomes are shaded It can be seen that the bimodal distribution in EB1 at 67 weeks changed to a mostly hyperdiploid population at 90 weeks, after it had been grown in a different culture medium (containing calf instead of human serum) This shows the influence of environment superimposed on age Another example of the effect of an *in vitro* environment is provided by EB2, which remained diploid when cultivated in America[17] but had become hypotetraploid by the time that it had been received from its laboratory of origin in the present study The effect of age in a standard environment was then shown by the youngest cell line, EB3

Fig 2 shows that a drastic change in the number of chromosomes occurred after 34 weeks culturing The apparent stability of chromosome numbers before 34 weeks is, however, misleading, because karyotype anomalies were found to occur in cells of the diploid mode as early as 22 weeks This shows the unreliability of chromosome numbers alone as indications of the genetic structure of a cell population

Each karyotype was photographed and analysed for 265 characteristics using a punch card system in order to sort out complicated variations among 350 karyotypes of the lymphoma cultures The analyses have been considered in relation to the age of the cultures (1) The two populations of the youngest cell line EB3, karyotyped in detail, showed that the modal diploid cells were predominantly pseudodiploid at 34 and 50 weeks (61 per cent and 31 per cent, respectively) The frequency of apparently normal karyotypes dropped from 22 2 per cent of the culture at 34 weeks to 9 6 per cent at 50 weeks Altogether, the frequency of any replicated karyo-

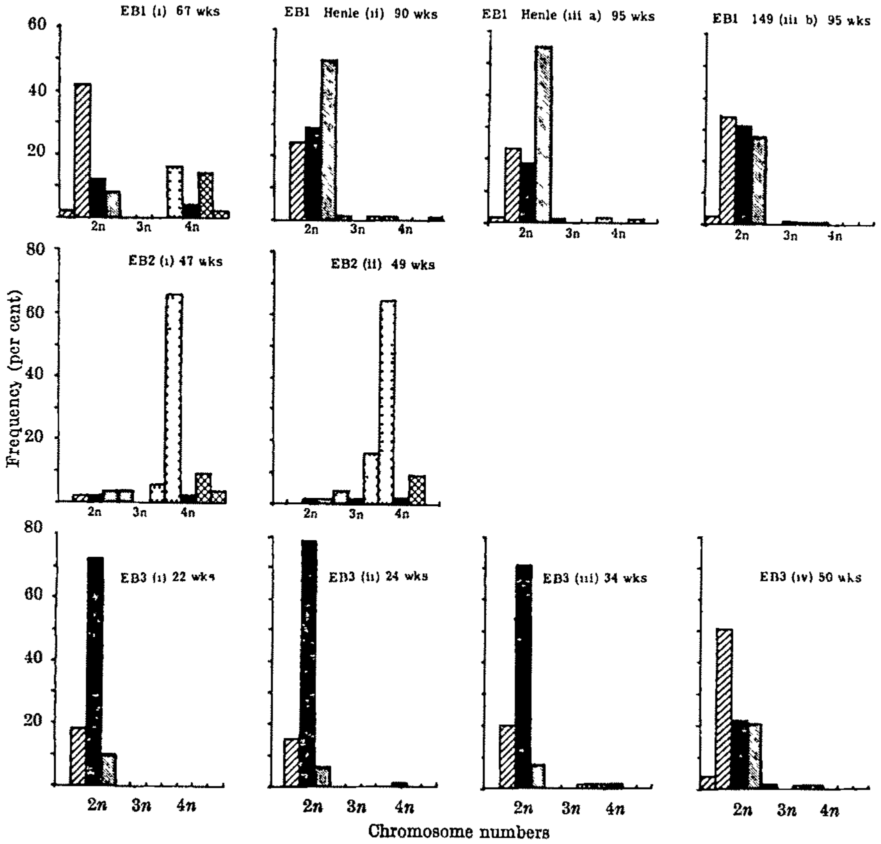

Fig 2 Ploidy level distributions of chromosome numbers in cultures of Burkitt lymphomata

types (euploid and aneuploid) dropped from 22 2 per cent at 34 weeks to 9 6 per cent at 50 weeks (2) The older cell lines EB1 and EB2 were also unstable and had a much reduced frequency of replicated karyotypes 16 5 per cent in EB1 ii at 90 weeks, 18 2 per cent in EB1 iii *a* at 95 weeks, none in EB1 iii *b* at 95 weeks, and also none in the hypotetraploid EB2 ii at 49 weeks Thus in two out of six lymphoma cultures all the cells had different karyotypes, like the situation in the old BHK culture with a seemingly high chromosome mutation rate

Again, like the BHK, the frequency of structurally abnormal chromosomes was too low to account for the observed high frequency of different karyotypes in these lymphoma cultures, ranging from 0 4 per cent of total chromosomes in EB3, to 0 9 per cent in EB1, and 1 4 per cent in the hypotetraploid EB2 (see Table 2) These anomalies included deviations in chromosome size, centromere position, dicentrics, chromatid interchanges and breakage

Mechanism of Karyotype Instability

Cytological mechanisms leading to the formation of abnormalities in chromosomal constitution are still far from being well understood The observations described in this report can, however, be interpreted with the aid of two hypotheses which propose ways in which chromosomes come into close proximity and stick together, followed by non-disjunction at cell division or structural changes such as translocation between chromosomes (1) the nucleolar mechanism[18] was indicated by cytological evidence that nucleolar organizers of different chromosomes tend to form common nucleoli, bringing these chromosomes into close proximity and liable to stickiness, (2) a heterochromatin mechanism[19] was suggested on the basis of autoradiographic evidence and the assumption that late replicating allocyclic segments of autosomal chromosomes consist chiefly of heterochromatin (subsequently demonstrated[20]) Aberrations in the heterochromatin are also known to cause chromosomes to stick together

Neither of these hypotheses alone can explain the observations on hamster and human karyotypes in the present study It is likely that both mechanisms operate in the same cells at the same time Thus deviations from the normal karyotype were not restricted to, nor invariably most frequent in, chromosomes concerned with nucleolar organization in the hamster and human cell populations In the Burkitt lymphoma cultures, karyotype analysis of 350 karyotypes showed a disproportionate gain in group C chromosomes compared with other groups, as was previously found in human tumours[21,22], but the loss deviations were distributed throughout the remainder of the karyotype Groups D and G would have been preferentially affected in each population if the nucleolar mechanism had predominated

The frequency of observed aberrations in chromosome structure was too low in both hamster and human cell populations (as already described) to account for the great diversity of karyotypes Because chromosome interchanges (somatic crossing over) were rare and other obvious structural changes were not frequent enough, the majority of observed anomalous karyotypes were probably caused by deviations in whole chromosomes (without concurrent structural change) This could come about by differential replication of certain chromosomes as found during the evolution of a leukaemia cell population[23] A sequence of gains of particular chromosomes could best be explained by selective endomitotic duplication[24] The evolution of the present cell populations does not seem to follow the same pathway, however, because the karyotypes exhibit both gain or complementary loss of chromosomes Here the most likely mechanism is non-disjunction two daughter chromosomes going to one cell and none to the other at anaphase It is proposed that in the pseudodiploid BHK and lymphoma cells non-disjunction probably occurred simultaneously in different

Table 2 SUMMARY OF KARYOTYPE ANALYSES OF CULTURED HUMAN LYMPHOMA CELL POPULATIONS

Cell line		Normal male cell	EB3 iv	Normal female cell	EB2 ii	EB1 ii	EB1 iii *a*	EB1 iii *b*
Age of cell line in weeks			50		49	90	95	95
Total metaphases karyotyped			104		30	103	66	22
(*A*) Pattern of total chromosome constitution of whole culture samples compared with karyotype of normal cells								
Percentage distribution of total chromosomes*/culture, among the groups A–G of the human karyotype	A	13 0	12 9	13 0	11 5	12 6	12 7	12 7
	B	8 7	8 4	8 7	7 5	8 0	8 3	8 3
	C	32 6	33 2	34 8	31 1	35 7	35 8	33 6
	D	13 0	13 2	13 0	13 4	12 5	12 4	12 3
	E	13 0	12 6	13 0	9 5	12 8	12 6	12 5
	F	8 7	8 5	8 7	7 8	8 5	8 0	8 6
	G	10 9	10 3	8 7	8 1	8 9	8 8	10 6
Distribution of chromosome groups per culture, compared with normal karyotype	Correlation coefficient		0 99943		0 99092	0 99908	0 99968	0 99958
	t test		20 9		16 5	52 1	88 3	77 1
	P		< 0 001		< 0 001	< 0 001	< 0 001	< 0 001
(*B*) Deviations from the normal pattern of chromosomes/karyotype group (A–G) per cell within each culture								
(*a*) Total chromosomes*/culture	Expected		4,784		2,760 (4*n*)	4,738	3,036	1,012
	Observed		4,746		2,527	4,943	3,053	1,012
Normally distributed chromosomes/group/cell			4,287		1,377	4,463	2,788	883
Chromosomes deviating from normal No /group			459		1,150	480	265	120
$\chi^2_{(1)}$ of deviations from normal			51 6		693 0	16 0	20 3	16 4
	P		< 0 001		< 0 001	< 0 001	< 0 001	< 0 001
(*b*) Structurally abnormal chromosomes/culture								
Percentage of total chromosomes			0 4		1 4	1 0	0 9	1 2
Percentage of total deviations from normal karyotype groups			3 9		3 0	10 0	10 2	9 3

* Calculated for structurally normal chromosomes only

chromosome groups, that is, the loss of a chromosome in one group being compensated by a gain in another group, while retaining the normal diploid number of chromosomes per cell Furthermore, a widespread occurrence of anomalous distribution of chromosomes during cell division in both euploid and aneuploid cells can be deduced from the very high percentage of cells with entirely different karyotypes, in the various cell populations (from 78 per cent of EB3 ii to 100 per cent of EB1 iii *b*, EB2 ii and BHK samples)

It is then rather puzzling to find that, in spite of all this variation in karyotypes, modal and other chromosome numbers were nevertheless maintained In some cultures the frequency of replicates was so low that all that modal cells (such as pseudodiploid) had in common was the same chromosome number, the latter having selective advantage by some unknown mechanism Karyotype instability may be an inherent feature of these cell populations, for example, by genetic variability in the efficiency of the centromeres of different chromosomes

The finding of extensive karyotype instability within an apparently stable modal chromosome number requires assessment in relation to the stemline concept

Pseudo-stemline Concept

Cytogenetical studies on rat ascites tumours led Makino[25] to conclude that the genetic behaviour of tumour cell populations is determined by the modal cells (because they are in the majority and single-cell clones of modal cells could reproduce tumour characteristics) Such modal cells were termed stem-cells and could be recognized by having the same karyotype The evolution of new stemlines during tumour progression then explained changes in the character of transplanted tumour cell populations

Present studies conflict with the stemline concept as defined because no true stemlines were found in either the hamster or human lymphoma cell populations during a period of consecutive observations adequate for selection of new stemlines. There was such a wide variation in karyotypes that modal cells could only be distinguished in terms of chromosome number alone Nevertheless, because these cells formed the majority of consecutive subcultured populations they were chiefly responsible therefore for growth, like stem cells If so, the stemline concept should be defined in terms of what the cells do, rather than what they look like We can then extend the concept to another category of stem cells It is proposed that when modal cells can only be characterized by having the same chromosome number, but not by their karyotype, then they should be called a "pseudo-stemline" Such a situation has also been found in current studies on HeLa cultures The modal chromosome number has remained stable over several months, although the modal cells had very unstable karyotypes so that no two cells were found to have identical karyotypes

Indications of the possible significance of the extensive karyotypic variation (found within pseudo-stemlines and other chromosome numbers) were obtained by further analysis of karyotypes per population

Balanced Cytogenetical Polymorphism

In order to determine whether there is any chromosomal evidence for interaction between cells, the populations were analysed as a whole and compared Table 2*A* shows the distribution of chromosomes in the human karyotype groups A–G in Burkitt lymphoma cultures (including all chromosome numbers per culture), compared with the normal karyotype It can be seen that the proportions of chromosomes belonging to groups A–G in each population in the cultured neoplasms are highly significantly correlated with that of the corresponding normal male or female karyotype Reference to Fig 2 shows that the similar balance of chromosomal types within each population (exemplified in Table 2*A*) occurs despite wide variation in chromosome numbers per cell within and between cultures The most striking example is population EB2 ii, with chromosome numbers ranging from 46 to 166 and a mode of 86, that has a chromosome group distribution which does not depart significantly from that of a normal female karyotype

Table 2*B* (*a*) also shows that the close correlation with the normal balance of chromosome group distribution occurs in cell populations that significantly deviate from the number of structurally normal chromosomes/karyotype group/culture, expected in a normal cell population In addition, Table 2*B* (*b*) shows that these significant deviations from normal cannot be the result of the frequency of structurally abnormal chromosomes because they form too low a percentage of the observed deviations Non-disjunction is then considered to be the chief source of the observed balanced cytogenetical polymorphism, like the situation described by Barber in *Uvularia* pollen grains in 1941 (ref 26) except that here it is on a population scale It is suggested that this balance is maintained as follows (*A*) Selection by the *in vitro* culture environment of those karyotypic variants that will not upset the balanced distribution of chromosomes/group/population It is anticipated that such a balance of karyotype groups will only be recognizable in the early stages of heteroploid transformation and evolution of cell populations, when non-disjunction of structurally normal chromosomes is the chief source of polymorphism. Subsequent structural changes of chromosomes would obscure the possible presence of any balance in the distribution of physiologically active chromosomal material in the population as a whole. (*B*) Interaction by crossfeeding between physiologically inadequate mutant cells, in the same way as the well known examples of syntrophism in bacteria[27] could form a physiological basis for the observed chromosomal balance in mammalian cell populations. The well known feeder layer phenomenon, whereby certain mammalian cells can only grow in the presence of other cells[28], is supporting evidence. Furthermore, recent biochemical studies have indicated that metabolic co-operation can also occur between cultured mammalian cells (BHK)[29] by transfer of metabolic information.

The frequency of survival of physiologically inadequate chromosome mutations could then depend on the available supply of metabolic products from other cells.

Evolution

These observations indicate that co-operation as well as competition between cells affects the evolution of cell populations. This is a simple principle which has often been overlooked. Thus the balance of cell types obtained during the differentiation of chromosomal mosaics and chimaeras may be the product of intercellular interactions involving more co-operation than competition.

Because *in vitro* cell populations provide us with a model system for the study of evolution on a highly condensed time scale (where the average life cycle of the cell is 1 day rather than 20 yr for the whole organism, as in man) we may now venture to obtain further fundamental information about the stability of cytogenetical mechanisms in a shorter time.

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[1] Ford, E. B., in *The New Systematics*, 493 (Oxford, 1940).
[2] Ford, E. B., in *Genetic Polymorphism* (Faber and Faber, London, 1965).
[3] Ruddle, F. H., *J. Nat. Cancer Inst.*, **28**, 1247 (1962).
[4] Moore, K. L., *The Sex Chromatin* (Saunders, Philadelphia, 1966).
[5] Moorhead, P. S., in *Approaches to the Genetic Analysis of Mammalian Cells* (edit. by Merchant, D. J., and Neel, J. V.) (Univ. Michigan Press, 1962).
[6] Atkin, N. B., *Cytogenetics*, **1**, 113 (1962).
[7] Carlson, L., Caspersson, T., Foley, G. E., Kudynowski, J., Lonnaka, G., Simonsson, E., and Sören, L., *Exp. Cell Res.*, **31**, 589 (1963).
[8] De Carli, L., Maio, J. J., Nuzzo, F., and Benercetti, A. S., *Cold Spring Harbor Symp. Quant. Biol.*, XXIX (1964).
[9] Hauschka, T. S., Hasson, J. E., Goldstein, M. N., Koepf, G. F., and Sandberg, A. A., *Amer. J. Hum. Genet.*, **14**, 22 (1962).
[10] Lima-de-Faria, A., *J. Theoret. Biol.*, **2**, 7 (1962).
[11] Hsu, T. C., *Intern. Rev. Cytol.*, **12**, 69 (1961).
[12] Chu, E. H. Y., *Nat. Cancer Inst. Monograph*, **7**, 55 (1961).
[13] Jacobs, P. A., Brunton, M., Court Brown, W. M., Doll, R., and Goldstein, H., *Nature*, **197**, 1080 (1963).
[14] Hayflick, L., *Exp. Cell Res.*, **37**, 614 (1965).
[15] Capstick, P. B., Telling, R. C., Chapman, W. G., and Stewart, D. L., *Nature*, **195**, 1163 (1962).
[16] Cooper, E. H., Hughes, D. T., and Topping, N. E., *Brit. J. Cancer*, **20**. 102 (1966).
[17] Kohn, G., Mellman, W. J., Moorhead, P. S., Loftus, J., and Henle, G., *J. Nat. Cancer Inst.*, **38**, 209 (1967).
[18] Ohno, S., Trujillo, J. M., Kaplan, W. D., and Kinosita, R., *Lancet*, ii, 123 (1961).
[19] Stalder, G. R., Buhler, E. M., and Buhler, U. K., *Humangenetik*, **1**, 307 (1965).
[20] Lima-de-Faria, A., and Jaworska, H., *Nature*, **217**, 138 (1968).
[21] Levan, A., *Hereditas*, **55**, 28 (1966).
[22] Wakonig-Vaartaja, R., and Hughes, D. T., *Europ. J. Cancer*, **3**, 263 (1967).
[23] Lejeune, J., in *Les Chromosomes Humaines* by Turpin, R., and Lejeune, J., 208 (Gauthiers-Villars, Paris, 1965).
[24] Grouchy, J. de, and de Nava, C., in *Progres en Hematologia* (Flammarion, Paris, 1966).
[25] Makino, S., *Intern. Rev. Cytol.*, **6**, 25 (1957).
[26] Barber, H. N., *J. Genetics*, **42**, 223 (1941).
[27] Hayes, W., in *The Genetics of Bacteria and their Viruses* (Blackwell, Oxford, 1964).
[28] Puck, T. T., and Marcus, P., *Proc. US Nat. Acad. Sci.*, **41**, 432 (1955).
[29] Subak-Sharpe, H., Burk, R. R., and Pitts, J. D., *Heredity*, **21**, 342 (1966).

Histochemical and Biochemical Study of Synaptic Lysosomes

by

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Synthesis and degradation of protein occur in isolated "synaptosomes". Evidence for this is based on histochemical studies of cerebral cortical tissue which show the presence of lysosomes at the pre-synaptic ending of the neurone, and on biochemical studies.

EVIDENCE for protein synthesis at the synapse is conflicting. *In vivo* studies involving injected radioactively labelled amino-acids indicate that protein is synthesized almost exclusively in the perikaryon[1], and that significant radioactivity cannot be shown in protein from axonal or synaptic areas for a long time after the injection of labelled amino-acids[2]. Even amino-acid analogues which are not incorporated into protein do not produce measurable radioactivity in the axonal and synaptic areas after injection into the ganglion[3]. Furthermore, electron photomicrographs of neurones show identifiable images of ribosomes only in the perikaryon[4]. These results are consistent with the proposal of Weiss[5] that the proteins found in distal portions of the neurone arrive there by somato-axonal flow of moieties which were originally synthesized in the cell body.

In vitro experiments, on the other hand, indicate a potential for protein synthesis in both the axon and the pre-synaptic area. Sections of the axon of the Mauthner neurone of the goldfish fix labelled amino-acids into protein at a substantial rate *in vitro*[1]. It has also been reported that the "synaptosome" fraction derived from brain homogenates incorporates labelled amino-acids into protein when appropriately supplemented and incubated[6]. Brain mitochondria, including those present in the synaptic area, have been shown to synthesize protein at a rate which is at least as great as that of mitochondria isolated from other organs[7,8]. Finally, there are reports of the *in situ* formation of acetylcholinesterase in structures which bound the synapse[9].

It was suggested that these apparent contradictions would be resolved if protein was synthesized in distal portions of the neurone as follows[8]. Injected radioamino-acids are preferentially transported into the neurone at the cell body. Here, a very active machine for protein synthesis exists which, it seems, is not different from that found in other cells[10]. Protein is rapidly formed and slowly transported to distal portions of the neurone by the intraneuronal micro-circulation. Triggered by some unknown mechanism, the protein which is being transported is hydrolysed by lysosomes into its constituent amino-acids; these amino-acids are re-employed by local systems for the formation of characteristic protein. (A similar function has been suggested for multivesicular bodies in insects[11]. Here, however, there is evidence that lysosomes also transport the proteins to be degraded).

Thus in any experiment in which radioactivity is injected and sites of protein synthesis are identified by measuring radioactivity, it would seem that all significant synthesis occurs in the perikaryon because protein synthesis in more distal regions would be proceeding from amino-acids which were made up into protein at some time before the radioamino-acid was injected. Direct proof of this hypothesis is difficult to obtain, but the following consequences should be amenable to experimental verification: first, sub-cellular organelles which have the morphological appearance of lysosomes and which can be shown to contain acid hydrolases should be found in axonal and synaptic areas; second, it should be possible to obtain evidence for the hydrolysis of endogenous protein and the subsequent utilization of the derived amino-acids for *de novo* protein synthesis by structures found in synaptic areas.

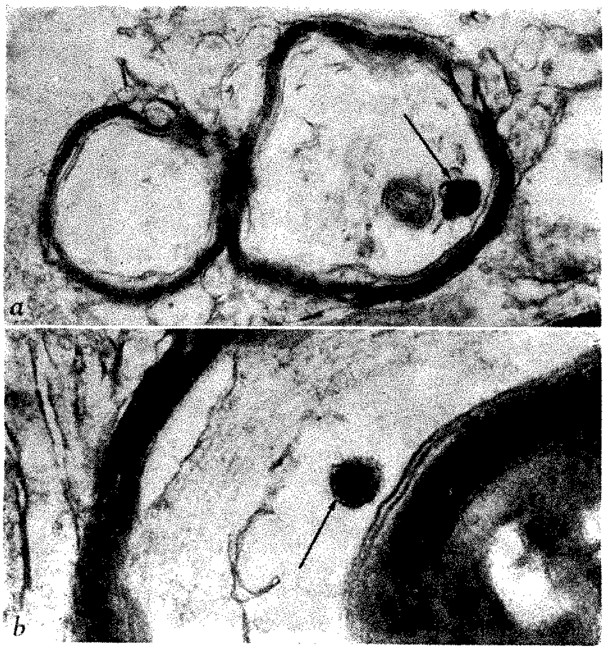

Fig. 1. *a* and *b*, Rat cortex incubated for the cytological demonstration of acid phosphatase. *a*, Transverse section of axon showing lysosome (arrow) containing reaction product. Organelle near lysosome is a mitochondrion. (× *c*. 16,575.) *b*, Longitudinal section of axon containing a lysosome. The latter is surrounded by a clearly visible membrane (arrow) and contains reaction product. (× *c*. 21,850.)

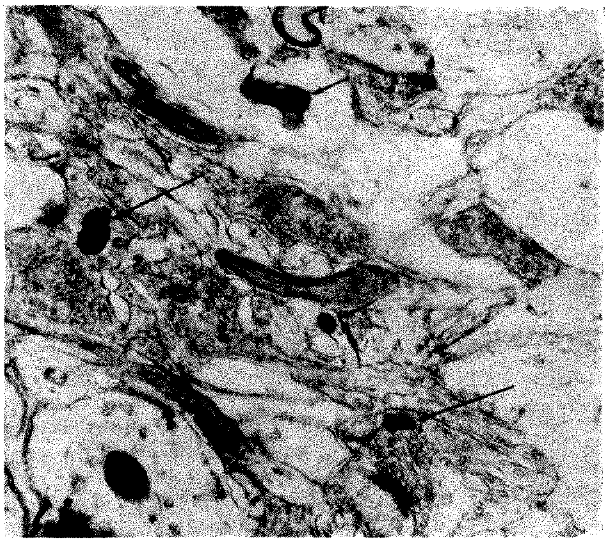

Fig. 2. Two of the numerous synapses in this section of cortex contain lysosomes on the pre-synaptic side (long arrows). Reaction product resulting from incubation of tissue for the cytological demonstration of acid phosphatase is present not only in these lysosomes but also in two others apparently located in dendrites (short arrows). (× *c*. 10,870.)

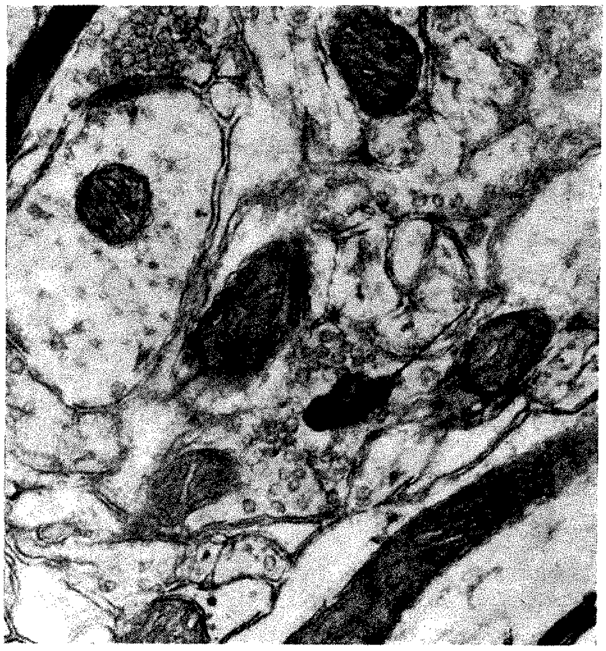

Fig. 3. Coarse and fine reaction product (arrow) resulting from histochemical incubation for acid phosphatase is clearly visible in a lysosome surrounded by pre-synaptic vesicles. (× *c*. 21,740.)

Histochemical Studies*

Cortical tissue for histochemical procedures was used from rat brains fixed by perfusion for 10 min with 3·5 per

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* Experiments conducted at the Department of Pathology at Yale Medical School by M. K. Gordon and K. G. Bensch.

Table 1. ISOLATION PROCEDURES

Fraction 1: Ten per cent homogenate of rat brain cortex in 0·44 M sucrose +0·05 mM EDTA. Debris and nuclei were removed by centrifugation at 4,000*g* for 10 min in the *SS*-34 rotor of a Sorvall *RC*-2 refrigerated centrifuge. The supernatant was centrifuged at 17,300*g* for 10 min. The supernatant was discarded and one pellet was resuspended in 0·44 M sucrose. All procedures were carried out with sterile solutions and sterilized glassware.

Procedure *A*	Procedure *B*
Fraction 2: Centrifuged at 12,000*g* for 10 min Supernatant discarded Pellet resuspended in 0·44 M sucrose	Diluted to 0·32 M sucrose and 1 ml. layered over gradient consisting of equal volumes of 1·2 and 0·8 M sucrose Centrifuged at 37,000 rpm for 75 min in the Spinco *SW*-39 rotor
Fraction 3: Centrifuged at 11,000*g* for 10 min Supernatant discarded Pellet resuspended in 0·44 M sucrose	Interface layer ("synaptosomes") from *B*-2 diluted with five volumes of 0·32 M sucrose
Fraction 4: Centrifuged at 10,000*g* for 10 min Supernatant discarded Pellet resuspended in 0·44 M sucrose	Pellet ("heavy mitochondrial fraction") from *B*-2 resuspended in 0·44 M sucrose
Fraction 5: Centrifuged at 9,000 rpm for 10 min in swinging bucket type *HS* rotor of Sorvall. Supernatant discarded, pellet resuspended in 0·44 M sucrose	Fraction *B*-3 centrifuged at 100,000*g* for 30 min in *S*-50 rotor of Spinco. "Synaptosome" fraction
Fraction 6: Centrifuged at 9,000 rpm for 10 min	
Fraction 6 (Ca^{++}): Prepared identically to above except that sucrose contained 1 mM calcium chloride instead of 0·05 mM EDTA	

Table 2. SOME PROPERTIES OF BRAIN MITOCHONDRIAL FRACTIONS

Preparation	^{14}C-Leucine incorporation[1]		Acid phosphatase activity[2]		Incorporation of ^{14}C-leucine / Acid phosphatase activity		Malic dehydrogenase activity[6]
	μμmoles[3] / g original tissue	μμmoles / mg protein[4]	*OD* / g original tissue	*OD* / mg protein	/g original tissue	/mg protein	/mg protein
Procedure *A*[5]							
Fraction 1	96·6	3·0	91·1	3·8	1·06	0·79	476
Fraction 2	95·2	6·1	34·0	3·1	2·80	1·97	488
Fraction 3	77·7	9·2	16·3	3·1	4·77	2·97	1,147
Fraction 4	39·0	13·3	5·4	1·1	7·22	12·1	1,533
Fraction 5	21·8	17·9	2·9	0·57	7·52	31·5	1,530
Fraction 6[7]	18·3	26·0	1·95	0·38	9·38	68·4	3,040
Fraction 6 (Ca^{++})	18·7	2·3	18·8	0·98	1·0	2·35	—
Procedure *B*[5]							
Fraction 5[7]	50·0	10·4	34·5	1·67	1·45	6·22	828

[1] Incubations performed in the Dubnoff metabolic shaker at 37° C for 30 min. Total volume is 0·6 ml. and contains 0·167 M sucrose; 0·02 M *tris* at *p*H 7·6; 0·01 M *tris* phosphate at *p*H 7·6; 0·01 M magnesium acetate; 0·005 M α-ketoglutarate; 0·002 M ADP; 0·006 M mercaptoethanol; 0·03 μCi [^{14}C] L-leucine (specific activity averaged 250 mCi/mM); 0·04 M potassium chloride; and 0·1 M sodium chloride.

[2] Acid phosphatase activity = *OD* (410 mμ) as a result of *p*-nitrophenol being released after 30 min at 37° C (ref. 19).

[3] Radioactivity measured in Packard liquid scintillation counter.

[4] mg Protein determined by method of Lowry *et al.*[20].

[5] See Table 1 for isolation procedures.

[6] Malic dehydrogenase activity measured as the rate of NADH produced. Activity reported in fluorometer units/min/mg protein as measured in the Aminco-Bowman spectrophotofluorimeter[21].

[7] Bacteria counted on blood-agar pour plate after 3 days of incubation at 37° C. Counts averaged forty colonies/mg protein.

cent glutaraldehyde, and buffered to *p*H 7·2 with 0·05 M cacodylate. The tissues were washed in cacodylate buffer containing sucrose for several hours before immersion in the incubation medium. Very small blocks, no larger than 1 mm³, were incubated in the Gomori medium for acid phosphatase[12] at *p*H 5·5 for 1 and 2 h. Control tissue was incubated in complete medium containing 0·01 M sodium fluoride as inhibitor and in medium without substrate. The incubated tissue was subjected to osmosis, dehydrated and embedded in DER 'Maraglas'.

Deposits of lead phosphate were seen in membrane bound organelles in all portions of the neurone, the cell body, dendrite, axon and pre-synaptic endings (Figs. 1–3).

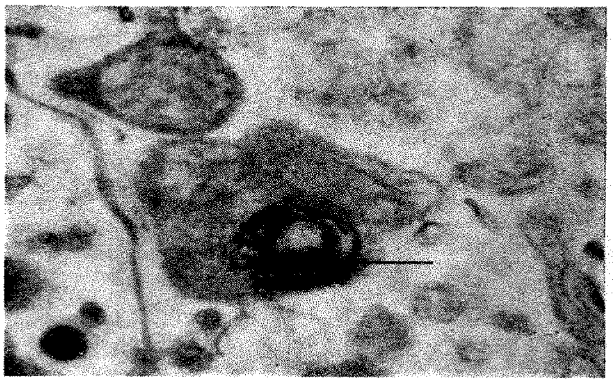

Fig. 4. A synaptosome from a pellet incubated for the demonstration of acid phosphatase showing reaction product (arrow) in an irregular vacuole containing membrane-like structures. (× *c*. 16,575.)

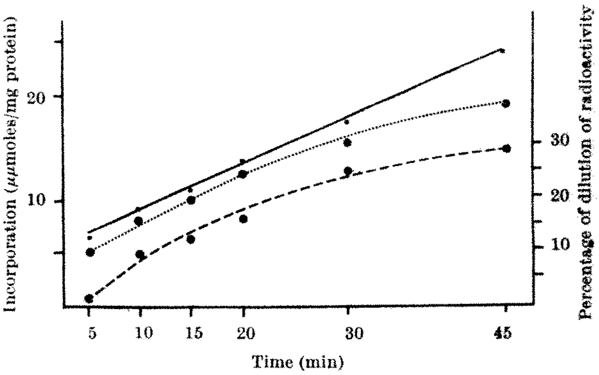

Fig. 5. Protein synthesis by brain mitochondria. Incubation medium and conditions were identical to those described in Table 2 (footnote 1) except that 1·4 mμmoles ^{14}C-tyrosine (specific activity 367 Ci/min) was added in place of ^{14}C-leucine. A pellet enriched with mitochondria was equivalent to 0·9 mg Lowry protein (average of four experiments). Endogenous tyrosine averaged 0·4 mμmoles/mg protein determined on TCA supernatant by method of Udenfriend[16]. This analysis for total free tyrosine was repeated at each of the time intervals designated in the figure. Incorporation of ^{14}C-tyrosine: ———, corrected for isotope dilution; . . . . , assuming constant specific activity. – – – –, Percentage of dilution of radioactivity.

These had the typical morphological appearance of lysosomes. In some organelles, in the pre-synaptic ending, deposits of reaction product were localized on the surface of membranes of what seemed to be multivesicular bodies. In the present study, lysosomes in the pre-synaptic ending and in the axon differed from those in the perikaryon in that they were uniformly smaller, had irregular contours and did not usually have the same complex structure seen in the larger perikaryal lysosomes (Figs. 1–3). It is assumed that pre-synaptic lysosomes are formed in the cell body[13] and that they undergo alteration as they migrate to the distal portions of the cell. On the other hand, the question has been raised[14] as to whether all lysosomes are modifications of the same organelle, or whether they differ in the kinds of enzymes which they contain. It is possible that the lysosomes which migrate are a different species and not variations of the lysosomes which reside in the cell body.

Histochemical evidence for acid phosphatase activity in preparations of synaptosomes which were used in the biochemical studies reported here was also obtained (Fig. 4).

Biochemical Studies*

Pellets were obtained by centrifugation of brain homogenates as described in Table 1. Acid phosphatase was found at all stages of mitochondrial enrichment (Table 2),

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* Experiments conducted in the Department of Biochemistry at the Institute of Living by G. G. Deanin and M. W. Gordon.

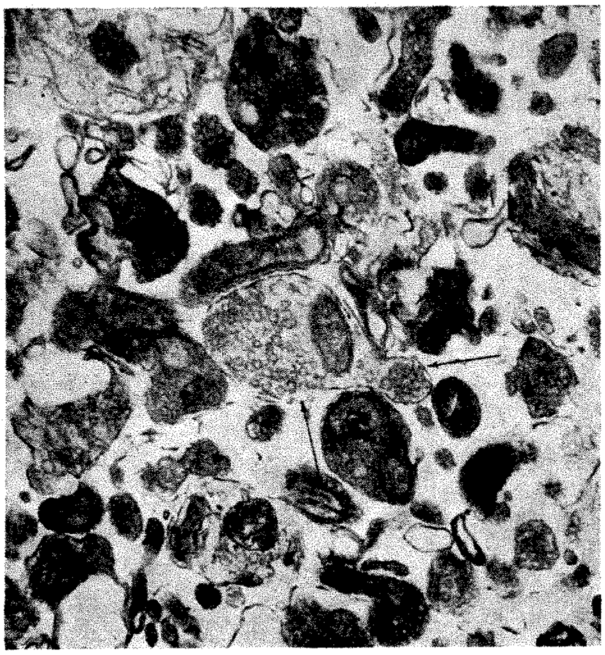

Fig. 6. Fraction from cortex of rat brain consisting almost exclusively of synaptosomes (arrows). The synaptic vesicles as well as mitochondria are well preserved. (× c. 7,720.)

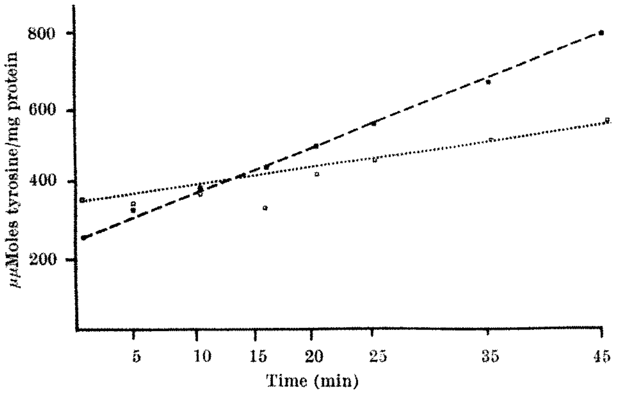

Fig. 7. Protein hydrolysis by synaptosomes. The analysis for total free tyrosine was performed on a TCA supernatant aliquot of a synaptosomal preparation for each of the indicated time periods and temperatures (. . . , 20° C; - - - - 37° C) by the fluorometric method of Udenfriend[16].

even in those preparations containing the highest malate dehydrogenase specific activity. These pellets can incorporate ^{14}C-leucine into protein (Table 2). Confirmation that protein synthesis, not N-terminal labelling, accounts for the acid-precipitable radioactivity was obtained by treating incubated pellets with Sanger reagent[15], hydrolysing with hydrochloric acid, extracting the hydrolysate with ether and counting the organic extract in a scintillation counter. Less than 1 per cent of the acid-precipitable activity was ether soluble. Furthermore, bacterial contamination was so low as to be negligible (Table 2).

In other experiments using preparations enriched with mitochondria (fraction *A*-6), the rate of incorporation of ^{14}C-tyrosine, an amino-acid for which a very sensitive method of detection exists[16], was followed as a function of time (Fig. 5). In addition, at each of the time intervals measured for incorporation of radioactivity, the total concentration of tyrosine in the medium was estimated. This estimate of the total tyrosine permitted a calculation of its continuously changing specific activity. Assuming instantaneous equilibration of the medium tyrosine with that incorporated into the acid-precipitable Sanger reagent negative fraction, a re-calculation of the net incorporation of tyrosine was made. With this assumption, the fixation of tyrosine into protein is a linear function of time for 45 min (Fig. 5). Bacteria-free mitochondria isolated from liver also synthesize protein at a linear rate for approximately this length of time[17].

Direct evidence for proteolytic activity was also obtained from synaptosomal preparations (fraction *B*-5, Fig. 6). Incubations of synaptosomes were carried out at both 20° and 37° C in a 'Dubnoff' metabolic shaker. Samples were removed at intervals and the amount of tyrosine formed was estimated by a fluorometric method[16]. Fig. 7 shows the increase of tyrosine with time at both temperatures.

The evaluation of the kinetics of protein synthesis by "synaptosomes" seems to require the consideration of additional variables. Like nuclei[18], "synaptosomes" rapidly concentrate amino-acids from the medium but fail to retain them. Furthermore, the rate of loss seems to be a more sensitive function of temperature than is the rate of accumulation of the amino-acid. At 20° C in the presence of 2 μM tyrosine the synaptosomal concentration

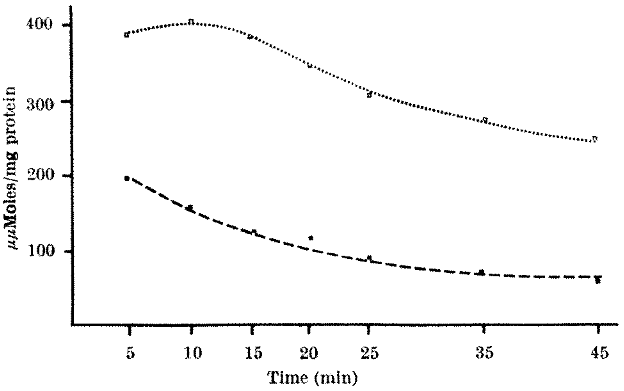

Fig. 8. Transport of ^{14}C-tyrosine into synaptosomes. Incubation medium and conditions as in Table 2 (footnote 1) except that 2·2 mμmoles of ^{14}C-tyrosine (specific activity 367 Ci/min) replaced the ^{14}C-leucine. After incubation for the indicated times and temperatures (. . . . , 20° C; - - - - , 37° C) an aliquot was removed into an ice-cold solution of 0·32 M sucrose, centrifuged for 10 min at 12,000*g* and washed twice with the same concentration of sucrose. The pellet was suspended in ice-cold 0·3 M TCA, centrifuged and an aliquot of the supernatant was counted in Bray's solution in a scintillation counter. Protein was determined by the method of Lowry[20].

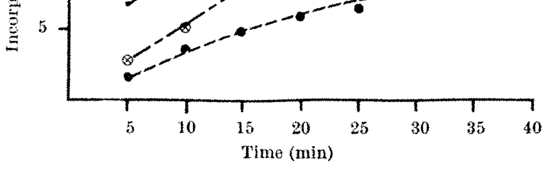

Fig. 9. Protein synthesis by synaptosomes. Conditions and methods identical to those of Fig. 1 except that the experiment was run at 20° C using concentrations of ^{14}C-tyrosine as indicated below. Isotope dilution values were estimated by direct measurement of tyrosine[16] at each time interval.

■——■, 8·5 μM tyrosine } Uncorrected incorporation
● - - - ●, 2·3 μM tyrosine }
□ . . . □, 8·5 μM tyrosine } Corrected for isotope dilution
⊗— — —⊗, 2·3 μM tyrosine }

of amino-acid is greater than at 37° C for all equivalent time periods examined (Fig. 8). Studies of protein synthesis were therefore carried out at 20° C where the possible effects of a rapidly changing internal concentration of tyrosine would be less marked. Nevertheless, correction for isotope dilution linearized the data at low concentrations of medium tyrosine for much shorter time periods than with mitochondrial pellets (Fig. 9). If the application of the isotope dilution corrections is a legitimate procedure, however, the same experiment run at a higher medium concentration of tyrosine should result in a longer period of linearity in uptake because the effect of isotope dilution should be minimized. This proved to be the case (Fig. 9).

Explanations for the reduction in the rate of uptake after only 20 min of incubation compared with 45 min for mitochondria have not been thoroughly worked out. It is possible, however, that the internal concentration of free tyrosine in the "synaptosome" is the variable which accounts for this more rapid fall-off in uptake.

The natural substrates for lysosomal activity at the synapse are not known. As mentioned earlier, there are two possible routes by which protein can reach the distal portions of the neurone: protein can be incorporated in multivesicular bodies or lysosomes and transported by these organelles[11]; or alternatively, because there is a net flow of cytoplasm from the cell body to the distal end of the axon[5], it is interesting to consider that lysosomes may digest portions of the cytoplasm in the pre-synaptic ending. If this were so, such lytic activity could in turn be a factor in promoting somato-axonal flow. Whatever the source of protein for proteolysis, the presence of synaptic lysosomes, which have been demonstrated both histochemically and biochemically, may create the amino-acid resource for protein synthesis at the pre-synaptic ending.

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[1] Edström, A., *J. Neurochem.*, **13**, 315 (1966).

[2] Barondes, S. H., *J. Neurochem.*, **13**, 721 (1966).

[3] Ochs, S., Johnson, J., and Ng, M.-H., *J. Neurochem.*, **14**, 317 (1967).

[4] Palay, S. L., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **1**, 69 (1955).

[5] Weiss, P., in *Regional Neurochemistry* (edit. by Kety, S. S., and Elkes, J.), 220 (Pergamon Press, New York, 1961).

[6] Austin, L., and Morgan, I. G., *J. Neurochem.*, **14**, 377 (1967).

[7] Campbell, M. K., Mahler, H. R., Moore, W. J., and Tewari, S., *Biochem.*, **5**, 1174 (1966); Bachelard, H. S., *Biochem. J*, **100**, 131 (1966).

[8] Gordon, M. W., and Deanin, G. G., in *Proc. Intern. Conf. Nutr. Behav. and Learn.* (in the press).

[9] Koenig, E., and Koelle, G. B., *J. Neurochem.*, **8**, 169 (1961); Koenig, E., *J. Neurochem.*, **12**, 343 (1965).

[10] McIlwain, H., *Biochemistry and the Central Nervous System* (third ed.), (Little, Brown and Co., Boston, 1966).

[11] Locke, M., and Collins, J. V., *Science*, **155**, 467 (1967).

[12] Gomori, G., *Microscopic Histochemistry* (Chicago University Press, 1952).

[13] Gaban, P. B., *Intern. Rev. Cytol.* (edit. by Bourne, G. H., and Danielli, J. F.), **21** (Academic Press, New York, 1967).

[14] de Duve, C., *Ciba Found. Symp., Lysosomes*, 1 (Little, Brown and Co., Boston, Mass, 1963).

[15] Sanger, F., *Biochem. J.*, **45**, 563 (1949).

[16] Udenfriend, S., *Molecular Biology* (edit. by Kaplan, N. O., and Scheraga, H. A.), 132 (Academic Press, New York, 1962).

[17] Beattie, D. S., Basford, R. E., and Koritz, S. B., *J. Biol. Chem.*, **242**, 3366 (1967).

[18] Allfrey, V. G., Meudt, R., Hopkins, J. W., and Mirsky, A. E., *Proc. US Nat. Acad. Sci.*, **47**, 907 (1961).

[19] Hunter, G. D., and Millson, G. C., *J. Neurochem.*, **13**, 375 (1966).

[20] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[21] *The St. Louis Method* (edit. by Schulz, D., and Passonneau, J.) (Dept. Pharmacology, Washington University of Medicine, St. Louis, Missouri).

Adjuvanticity of Vitamin A

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Vitamin A may act as an adjuvant through its effect in damaging lysosomal membranes, in this way stimulating cell division. Possibly this stimulation of cell division at the time when antigen is available in the cell leads to the induction of immunity and not paralysis.

BOVINE gamma G protein is a non-immunogenic antigen in mice, in contrast to bovine gamma A protein. A particle-free solution of the gamma G protein will induce a specific state of immunological paralysis if injected into mice in amounts greater than 10 µg. Combination of the non-immunogenic antigen with Freund's adjuvant, *Bordetella pertussis*, or adsorption of the protein onto a particulate substance such as aluminium phosphate, makes the protein immunogenic so that, on injection into mice in one of these forms, a powerful immune response is started[1,2]. The gamma A protein is defined as immunogenic because it will immunize as a particle-free solution without an extrinsic adjuvant being necessary; the gamma A is considered to possess its own "adjuvanticity"[3]. The property of adjuvanticity possessed by, or added to, an antigen could therefore be considered as a switch controlling one of two possible cellular responses to antigen which are shown.

This paralytic effect of bovine gamma G protein antigen in the absence of adjuvanticity can be used to assay various substances for adjuvant properties in mice[3]. The degree of adjuvanticity can be measured by estimating the degree of immunity induced to the injected particle-free gamma G, or alternatively it can be measured by the decrease in the degree of paralysis induced by the same protein. The definition of adjuvanticity used here is restricted by comparison with the accepted definition of an adjuvant as a substance which increases the degree of an immune response to an immunogenic antigen[4]. Most adjuvants are complex substances, such as Freund's adjuvant[4] or endotoxins such as that of *B. pertussis*[5]; these substances also possess adjuvanticity in the sense used here. The complexity of these substances lies both in their chemistry and in the fact that they are antigens in their own right: it is for this reason that an effort was made to find some simple non-antigenic adjuvants. Certain non-ionic detergents and fatty acids were found which acted in this way[3]. Other studies have shown the adjuvant properties of aliphatic nitrogenous bases in guinea-pigs and mice[6]. Both studies agree with the view that damage to cellular membranes could be one of the first effects of some adjuvants. Vitamin A is known to have detectable effects on biological membranes[7]. This communication reports experiments which show that vitamin A has adjuvant properties.

Effects of *B. pertussis* and Vitamin A on Mice

CBA male mice, 4–5 months old, were used in these experiments. *B. pertussis* (Glaxo) were injected in saline in the numbers indicated in Fig. 1, and vitamin A alcohol

('Retinol', Koch-Light), dissolved in 0·5 ml. of liquid paraffin (BP), was injected in the amounts indicated in Fig. 2. The vitamin A and the *B. pertussis* were injected subcutaneously in the dorsal region 1 day before the intraperitoneal injection of 0·5 mg or 5·0 mg of a particle-free (RCF = 30,000*g*, 25 min) bovine gamma globulin (BGG—Armour and Co.). Commercial bovine gamma globulin contains 80–90 per cent gamma G protein and 8–16 per cent gamma A protein (unpublished data). The degree of immunity induced by the injected gamma G protein was measured one month later by the antigen-elimination test[2]. There were six or seven mice in each group for which the geometric mean ($\log_2$) response was estimated by the published method[2].

Fig. 1 shows the effect of injecting different amounts of *B. pertussis* into mice subsequently injected with either 0·5 mg or 5·0 mg of particle-free bovine gamma globulin. The degree of immunity obtained is dependent both on the amount of adjuvant and at least to a certain extent on the amount of antigen. Fig. 2 shows that a similar effect is obtained when increasing amounts of vitamin A are injected into mice injected with 0·5 mg of particle-free bovine gamma globulin. Table 1 shows that mice which failed to show immunity in the adjuvant assay were immunologically paralysed.

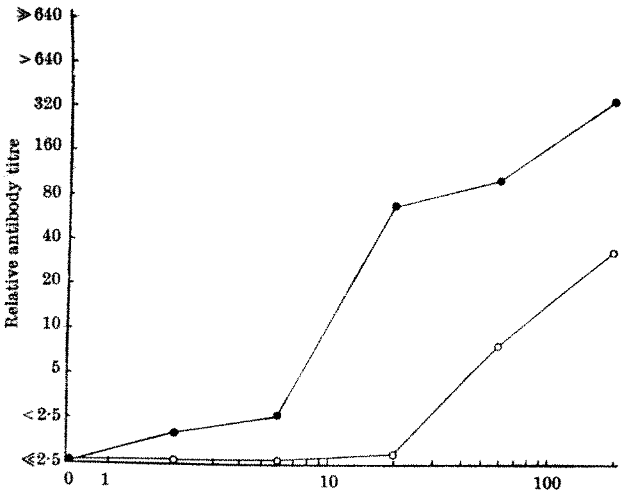

Fig. 1. Dose response effect of *B. pertussis* injected subcutaneously 1 day before the intraperitoneal injection of 5 mg (●) or 0·5 mg (○) of particle-free bovine gamma globulin. The abscissa is the number of pertussis organisms. The ordinate is the antigen elimination response 1 month after the initial treatment.

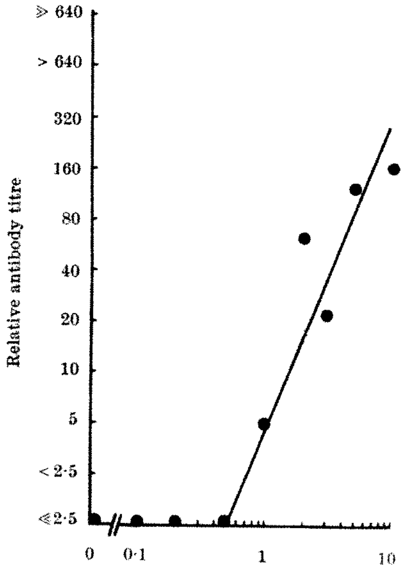

Fig. 2. Dose response effect of vitamin A alcohol in 0·5 ml. liquid paraffin, injected subcutaneously 1 day before the intraperitoneal injection of 0·5 mg of particle-free bovine gamma globulin.

Table 1. EFFECT OF RECHALLENGING GROUPS OF SEVEN MICE USED TO ASSAY THE ADJUVANT EFFECT OF 2 × 10^8 PERTUSSIS ORGANISMS INJECTED INTRAPERITONEALLY AT DIFFERENT TIMES AFTER THE INJECTION OF 0·5 mg OF PARTICLE-FREE BOVINE GAMMA GLOBULIN. ELIMINATION TEST CARRIED OUT 4 OR 5 WEEKS AFTER THE TREATMENT

	First treatment				
	Bovine gamma globulin and pertussis	Days after bovine gamma globulin injection when pertussis given	First elimination test*	Second treatment (1 month after first)	Second elimination test*
1	Yes	1	320	Not done	—
	Yes	1	Not done	Freund's, 1 mg BGG	> 640
2	Yes	15	≪2·5	Not done	—
	Yes	15	Not done	Freund's, 1 mg BGG	6·3
3	No	—	—	Freund's, 1 mg BGG	> 640

* See the ordinate of Figs. 1 and 2 to interpret the values in these columns. Geometric means ($\log_2$).

Possible Reasons for These Effects

A dose response effect of both a complex adjuvant (*B. pertussis*) and a simple adjuvant (vitamin A) has been shown. The antigen dose effect seen in Fig. 1 is at a level about 10 to 100 times higher than when alum precipitated bovine gamma globulin is used (unpublished data). This could be caused by the relative efficiencies of antigen uptake and it may indicate a role of macrophages in the response to a particulate antigen. Recent experiments[8] (and unpublished data) show that increased phagocytosis is unlikely to be the only factor accounting for the action of adjuvants. It is clear, however, that the stimulation of macrophages to "pre-process" antigen[9], or to release such material more freely, has not been ruled out as one possible mechanism of adjuvant action.

At the cellular level, immunological paralysis as defined by the response of the whole animal may result because all the lymphoid cells are paralysed, whereas an immune response by the animal could be caused by relatively few cells producing antibody at the same time that the majority is paralysed. It would be misleading therefore to extrapolate from results of experiments on whole animals to possible cellular mechanisms, and in the context of this communication it is clear that any conclusion concerning the action of adjuvants (adjuvanticity) may only apply to a restricted population of cells.

The portion of an immunogenic antigen which is responsible for adjuvanticity may in certain cases be identical with antigenic determinants and in most cases is at least closely associated with antigenic determinants on the same molecule or cell. The response to this property of adjuvanticity may be the result of one particular cellular response to stimuli from a wide range of different kinds of substance. For instance, substances with known adjuvanticity include toxins, cellular antigens, carbohydrates, non-ionic detergents, aliphatic nitrogenous bases and vitamin A. Gall[6], on the basis of his experiments with aliphatic nitrogenous bases, has suggested that the action of adjuvant in increasing the degree of an immune response is indirectly caused by damage to lymphoid cell membranes. It is now further suggested that one result of this damage, either directly or in some cases through damage to macrophages, is a stimulation of cell division by immunologically competent cells. Recent work[10] shows that the increased number of peripheral lymphocytes induced in mice after the injection of *B. pertussis* does not arise by cell division. This finding restricts the population of cells where adjuvant stimulated division could occur.

The osmotic sensitivity of lysosomes has been shown to be increased by a non-ionic detergent ('Triton *WR* 1339')[11] and it has also been shown that vitamin A increases the permeability of lysosomal membranes[12]. Earlier experiments[13] showed that vitamin A increased the rate of cell division of chick fibroblasts *in vitro*. On the basis of these and their own observations, Allison and Mallucci[14] suggested that lysosomes are concerned with the start of cell division, and Weissman[15] has speculated on the possible connexion between lysosomal stability and various autoimmune diseases. It seems possible that some adjuvants such as vitamin A or non-ionic detergents could act through their effect in damaging lysosomal membranes, and in this way stimulate cell division. It is possible that this stimulation of at least one cycle of cell division at the time that antigen is available in the cell leads to the induction of immunity rather than paralysis. Post-inductive proliferation of pre-antibody-producing cells is irrelevant here. Such a definition of adjuvanticity would be compatible with the "recognition mechanism" which it was suggested acted as a paralysis/immunity "switch" in a proposed cellular mechanism of immunological paralysis[2,16].

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[1] Dresser, D. W., *Immunology*, **5**, 378 (1962).
[2] Dresser, D. W., *Immunology*, **9**, 261 (1965).
[3] Dresser, D. W., *Nature*, **191**, 1169 (1961).
[4] Freund, J., in *Nature and Significance of the Antibody Response* (edit. by Pappenheimer, jun., A. M.), 46 (Columbia Univ. Press, 1953).
[5] Farthing, J. R., *Brit. J. Exp. Path.*, **42**, 614 (1961); Pieroni, R. E., and Levine, L., *Nature*, **211**, 1419 (1966).
[6] Gall, D., *Immunology*, **11**, 369 (1966).
[7] Lucy, J. A., and Dingle, J. T., in *Metabolism and Significance of Lipids* (edit. by Dawson, R. M. C., and Rhodes, D. N.), 383 (John Wiley, London and New York, 1964).
[8] Golub, E. S., and Weigle, W. O., *J. Immunol.*, **98**, 1241 (1967).
[9] Frei, P. C., Benacerraf, B., and Thorbecke, G. J., *Proc. US Nat. Acad. Sci.*, **53**, 20 (1965); Mitchison, N. A., in *Regulation of the Antibody Response* (edit. by Cinader, B.) (Thomas, Springfield, Ill., in the press).
[10] Morse, S. I., and Riester, S. K., *J. Exp. Med.*, **125**, 401 (1967).
[11] Wattiaux, R., Wibo, M., and Baudhuin, P., in *Ciba Foundation Symposium on Lysosomes*, 176 (1963).
[12] Dingle, J. T., in *Ciba Foundation Symposium on Lysosomes*, 384 (1963).
[13] Lasnitsky, I., *Exp. Cell Res.*, **8**, 125 (1955).
[14] Allison, A. C., and Mallucci, L., *Lancet*, ii, 1371 (1964).
[15] Weissman, G., *Lancet*, ii, 1373 (1964).
[16] Dresser, D. W., *Immunology*, **6**, 345 (1963).

Kinetics of Myofilament Activation in Potentiated Contraction: Staircase Phenomenon in Human Skeletal Muscle

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Staircase potentiation in skeletal muscle depends on an intensification of the twitch "active state" early in the contraction cycle. A working hypothesis of this is proposed.

THE isometric twitch elicited by a single electrical stimulus delivered either to the motor nerve or directly to the muscle is usually discussed in terms of Hill's model[1] of a two-component system—for example, contractile elements arranged in series with passive elastic elements. The latter seem to be partly located in the tendon and partly distributed along the muscle fibres[2]. The "active state" which is the capacity of the muscle to shorten or develop tension obviously has a very limited duration after a single stimulus. Even if the contractile elements could develop full tension instantly, the twitch force recorded at the tendon would rise rather slowly as the series elastic elements were being stretched; furthermore, there would not be enough time for the twitch tension to approximate tetanic tension. In his model, Hill postulates that the "active state" rises rapidly to its maximum, even in a single twitch. Twitch potentiation by drugs is then ascribed to prolongation (rather than intensification) of the "active state" whereby twitch force is enabled to approximate tetanic force more closely because more time is available for stretching the series elastic elements[3-5]. Such a view is challenged by the present findings that in staircase potentiation the activation of myofilaments seems to be intensified but not at all prolonged.

"Active State" Intensity in a Single Twitch

That the series compliance interacts with an "active state" of rapid onset and subsequent slower decay to determine the form of the isometric twitch is well substantiated, for example, by the fact that added[6] or reduced[1] series compliance will decrease or increase twitch tension more than tetanic tension. The subsidiary proposal, however, that the "active state" would be established to full intensity even after a single stimulus seems both unnecessary and inadequately substantiated. One argument for this latter view is that when a frog or toad muscle at 0° C is quickly stretched after a single stimulus, thereby reducing the series compliance, the contractile elements can bear, for a short time, a tension which is larger than the normal twitch tension[1]. It should be appreciated, however, that the increased tension does not always precisely match, and indeed sometimes exceeds, the tetanic tension. Furthermore, a muscle forcibly stretched during tetanus will sustain for a short while a greater tension than the maximum tension actively developed by the same muscle when it is extended to the same length before the tetanus[7-9]. Indeed, the intensity of the active tension-generating process in the sliding filament system cannot be equated with the capacity of the system to resist applied stretch[10,11].

Contrary to several statements in the literature, other methods in use provide no direct evidence that a single stimulus would elicit a plateau of maximum "active state". When an isometrically contracting muscle is released a fixed distance (0·5–3 mm) at various times after a stimulus, the tension recorded falls abruptly and is then redeveloped in proportion to the level of the (decaying) "active state" still present at that time[12]. In frog muscle at 0° C there still occurs some redevelopment of tension as late as 0·9 sec after a single stimulus[11]. The "active state" decay is further prolonged during nitrate potentiation[4]. While the quick-release analysis discloses the time course of the decay of "active state" from its early peak, however, it provides no evidence on the absolute level of activation which is achieved during this early peak. Also the experiments cannot be easily performed on the much faster mammalian muscle twitch.

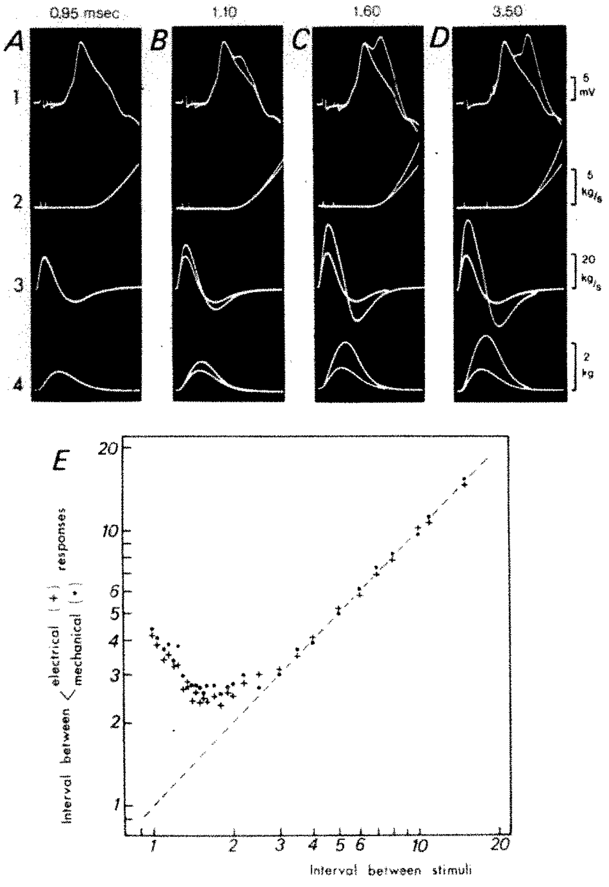

Fig. 1. Normal adult man. Intramuscular temperature 37° C. *A–D*, Cathode-ray oscillograms of responses of the adductor pollicis muscle *in situ* to supramaximal electric pulses delivered to the ulnar nerve at the wrist. In each frame the response to a single shock is superimposed on the response to two shocks at the interval indicated on the top. The two upper traces present on a fast sweep the belly-tendon electrical response (1) and the first differential of the isometric myogram (2). The two lower traces present on a slower sweep the same first differential (3) and the isometric myogram (4). *E*, Relation between the interval of the two shocks delivered to the motor nerve (abscissa) and the separation interval of the muscle responses elicited by each of the two shocks (ordinate). Notice the similarity of the latter for the electrical and the mechanical responses.

According to another method the minimum frequency of repetitive stimulation necessary to achieve tetanic fusion[12,13] or maximum rate of tension development[14] is evaluated and the critical intervals necessary to either maintain or establish full activation of the myofilaments are thought to correspond to the duration of the postulated "active state" plateau. Using mechanical transducers of high resolution one must stimulate at rates as high as 600/sec (ref. 15) or 425/sec (ref. 16) in experiments on fast mammalian muscles. The same feature can be tested more simply with only two stimuli[14], for the contraction elicited by two stimuli should only separate from that elicited by a single stimulus when the latter becomes able to add an increment of "active state". Differentiation of the tension record increases the resolution of the method and shows that the two curves separate after 44 msec in frog muscle at 0° C (ref. 14). In mammalian fast muscles at body temperature the separation occurs as early as 4 msec (ref. 9, 17) or even 2–3 msec (refs. 15, 16). The question is whether the minimum interval is determined by the duration of the "active state" plateau or by some other factor.

Data such as those shown in Fig. 1 indicate that it is, in fact, the duration of the refractory period for electrical excitation which sets the lower limit to the interval for eliciting an increment of mechanical activation in double shock experiments[15]. The tests are performed on normal adult men. Four cathode-ray oscilloscope traces are simultaneously recorded onto 35 mm film: from below upwards, the isometric myogram of the adductor pollicis muscle (trace 4) and the rate of tension change obtained by electronic differentiation of the myogram (trace 3) on a slow time-base sweep; the same differential function with amplification increased ten times horizontally and four times vertically (trace 2) and the belly-tendon electrogram of the adductor pollicis muscle on a similar time-base (trace 1)[18]. Supramaximal electric pulses are delivered through needles to the ulnar nerve at the wrist, at the intervals indicated in msec on the top of records *A–D* and in the abscissa of graph *E*. In each record the response to double-pulse stimulation is photographically superimposed on the response elicited by the first pulse alone. The intramuscular temperature checked with a thermistor needle is 37° C. The electronic differentiation is linear up to four times the maximum rates of tension changes recorded, even when tested with ramp functions of only 2 msec duration.

In the experiment illustrated, the reponses elicited by two pulses at 0·95 msec or less (*A*) are identical to that for a single pulse. At intervals of 1·00 or 1·10 (*B*) msec a small hump appears on the electrogram about 4 msec after the beginning of the response to the first pulse. The rate of tension change for single shock indicates a mechanical latency (including nerve conduction and neuromuscular transmission) of 7·5 msec; the trace for two pulses at 1·10 msec separates about 4 msec later (*B*, 2). As the interval increases to 1·60 msec the separation interval decreases to about 2·50 msec, both for the electrical and mechanical responses (*C*). This curious effect suggests that the second volley now propagates faster in the motor nerve as the relative refractor state left by the first response dissipates, and that it also acquires a greater transmission potentiality at the motor endplate. For greater separation of the two stimuli the interval between the muscle response to the first and second volleys increases and actually equals the corresponding interval between the two shocks. This appears in graph *E* in which the interrupted line is drawn through the origin with a slope of one. The muscle is thus irresponsive for about 2·5 msec, but two stimuli delivered to the motor nerve at shorter intervals, down to about 1·0 msec in this instance, can still elicit a second muscle response because the nerve functions as a time buffer through slowing of conduction of the second volley. In the present argument the principal point stressed is that the earliest separation intervals are equal throughout the test for the electrical and for the mechanical responses of the muscle.

The significance of this double-shock test turns out to be quite different from the one proposed by those who failed to record simultaneously the electrical response of the muscle[14,16,19–21], for they overlooked the fact that the refractory state of the muscle electrogenic membrane is the true limiting factor at short intervals. In other words, the second stimulus delivered before the critical interval fails to increase the rate of tension development beyond that in a single twitch, not because the "active state" generated by the preceding stimulus would then be maximum (and therefore impossible to increase any further, as argued by MacPherson and Wilkie[14]), but simply because the second stimulus is ignored by the still irresponsive muscle membrane. Such evidence implies that a single stimulus cannot possibly elicit a maximum plateau of "active state" because: (1) activation of myofilaments is not abrupt but takes a finite time to develop[2,10], and (2) as soon as 2·50 msec after the muscle has started developing tension (the minimum separation interval in Fig. 1, *E*) the intensity of activation can be further augmented. This conclusion is in line with present views[22,23] that myofilaments are activated by calcium release from sarcoplasmic reticulum (SR) during depolarization of the muscle membrane beyond a level called the "mechanical threshold"[24] and that subsequent sequestration of the myoplasmic calcium in a sarcoplasmic

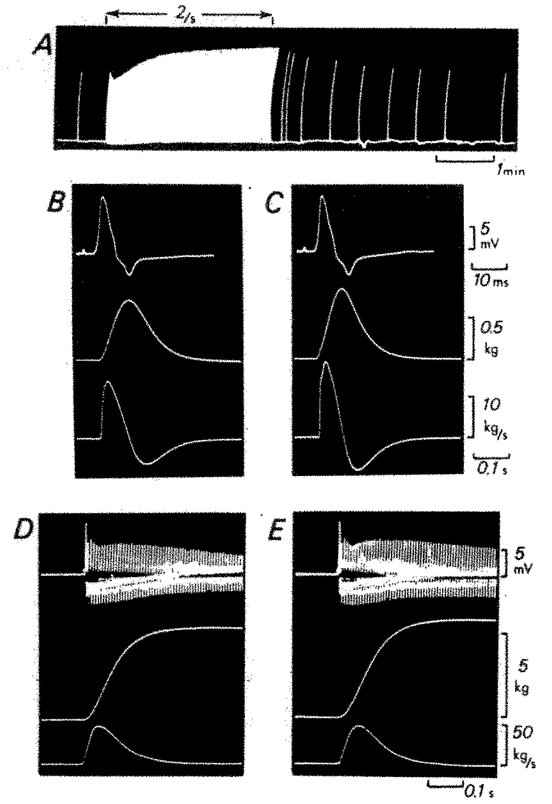

Fig. 2. *A*, Normal adult man. Continuous slow-speed polygraph record of the isometric myogram of the adductor pollicis muscle stimulated infrequently through its motor nerve, except for a series at 2/sec. *B–E*, Normal adult woman. Cathode-ray oscillograms of responses to the first (*B*) and the 200th (*C*) supramaximal shocks in a continuous series at 2/sec. From above downwards, the belly-tendon electrical response (on a faster sweep), the isometric myogram and its first differential. *D*, Tetanic response to supramaximal shocks at 125/sec, elicited at the peak of the staircase potentiation of the twitch. *E*, Tetanic response elicited in the same muscle after dissipation of the staircase phenomenon. Notice that the maximum rate of tension development (third trace) is similar in *D* and *E*.

calcium sink causes relaxation[23,25,26] and determines its actual speed[27]. With two or more stimuli at close intervals each muscle action potential triggers the intracellular release of an additional amount of calcium at a time when the SR sink has not yet removed all the free calcium liberated by the preceding spike. Because the contractile activation depends on the concentration of myoplasmic calcium over about a ten-fold range from 0·2 μM (refs. 23, 26 and 28) the "active state" must be subject to gradation in its intensity as well as in its duration.

Twitch Potentiation

In chemical potentiation such as produced by caffeine, nitrate, zinc and so on, the twitch tension is increased but the tetanic tension is not, while the contraction time and the relaxation time of the twitch are both increased[3,30]. The latter changes are accounted for by a slowing of the decay of "active state", as is indeed evidenced by quick-release experiments[3–5]. This slowing allows the twitch tension to approximate the tetanic tension more closely because the contractile elements have more time for stretching the series elastic elements. The potentiation is indeed smaller when the twitch tension P_t is closer to the maximum tetanic force P_0 (ref. 31). At 0° C the decay of "active state" is also slowed down by the chemical potentiators, but little actual increase of P_t occurs because the ratio of P_t to P_0 is close to unity[4,5]. Recent studies using a more sensitive recording technique disclosed yet another feature of the potentiated twitch, namely, an increase in the rate of tension development[32–34]. This suggests that the potentiators not only prolong the decay of the "active state", but also increase the intensity of myofilament activation earlier in the contraction cycle. The two effects occur together in chemical potentiations, but they can be dissociated from each other. The following evidence indicates that staircase potentiation is unique in its dependence on the intensification factor only.

Staircase Potentiation in Human Skeletal Muscle

When a rested muscle is stimulated at a rate of 0·5–3/sec, the twitch force gradually increases to reach a new steady state level. This is the classical staircase phenomenon first described in the frog heart[35] and also studied in the skeletal muscles of lower mammals[36,37] and of man[38]. In Fig. 2*A*, the isometric myogram of the adductor pollicis muscle of a normal adult man is recorded on a slow-moving polygraph. Supramaximal electric pulses are delivered to the ulnar nerve at infrequent intervals, except for an uninterrupted series at 2/sec for 3 min. During the series, the force P_t decreases progressively in the first ten–twenty twitches (negative staircase) and thereafter increases steadily (positive staircase) to reach a plateau equal to 136 per cent of the initial P_t. When the 2/sec stimulation is interrupted, P_t returns to control value in a few minutes. In the twenty-one normal adult muscles included in this study the mean initial negative staircase is $-8·7$ per cent and the mean maximum staircase potentiation is $+24·5$ per cent. Characteristic features are illustrated by the cathode-ray oscillograms of the first and two hundredth responses in a 2/sec series (Fig. 2*B* and *C*). The latency of the muscle electrical response does not change. The first negative phase of the potential shows no significant change in its voltage, but its mean duration is decreased to -7 per cent. The mean voltage of the second positive phase is increased by $+18$ per cent. The latter two effects are recorded rather early in the series and are not parallel to the twitch potentiation: they are probably related to an acceleration of the conduction of the muscle spike[18]. Changes in neuromuscular transmission or repetitive discharges are excluded. The two hundredth twitch of the series is not only stronger than the first but its time course is accelerated. The mean contraction time *CT* decreases by -14 per cent and the mean relaxation

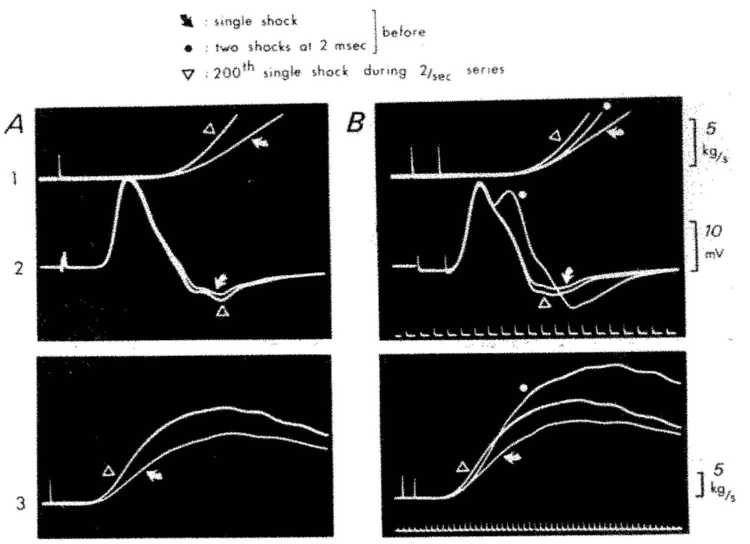

Fig. 3. Normal adult man. Cathode-ray oscillograms of responses to single or double supramaximal shocks delivered to the ulnar nerve at the wrist. From above downwards, the first differential of the isometric myogram (1) and the belly-tendon electrical response (2) on a fast sweep, and the first differential of the myogram (3) on a slower sweep. Horizontal calibrations in milliseconds. Photographic superimpositions of traces as indicated by the symbols (see text).

time $T\frac{1}{2}R$ decreases by -13 per cent. The mean maximum rate of tension development which we call R_t, given by the upward peak of the first differential of the myogram, is increased by $+37$ per cent. The mean maximum rate of relaxation S_t, given by the downward peak of the first differential, is also increased by $+34$ per cent. The consistent shortening of the time course of the potentiated twitch rules out the current explanation[39] which postulates a prolongation of the decay of "active state" in staircase. On the other hand, it is interesting that the maximum capability to generate force is not much affected by the staircase phenomenon. The maximum tetanic force P_0 recorded just after 3 min of uninterrupted 2/sec series (Fig. 2*D*) is equal to, or slightly less than, the P_0 recorded in the same muscle after the staircase effect has dissipated (Fig. 2*E*). The maximum rate of tension development in tetanus R_0, given by the upward peak of the first differential, is equal in both records (Fig. 2*D* and *E*, third trace). Thus the maximum intrinsic velocity of the muscle is not increased by staircase. Because the potentiated twitch is shortened rather than prolonged, the phenomenon can only be explained by an increase of the "active state". The evidence of Fig. 3 is relevant on this point. The first and two hundredth responses are superimposed on the same photographs using fast oscilloscope sweeps. The electrical response shows the slight changes described earlier (*A*2). The first derivative of the myogram presents not only a larger peak (*A*3) but also a very early increase (*A*1) so that the mechanical latent period appears shorter for the two hundredth twitch. Fig. 3*B* presents a similar display but with the further superposition of the responses to two shocks at 2 msec delivered before the 2/sec series. For such closely spaced double stimulation the rate of tension development separates from the curve for a single shock after 2·5 msec at the earliest (compare Fig. 1), and there is no evidence for any shortening of the mechanical latent period. Later in the contraction cycle the curve for double shocks (dot) crosses the curve for the potentiated single twitch (triangle) and it reaches a larger peak (Fig. 3, *B*3). These recordings on a fast sweep thus emphasize a unique feature of the potentiated contraction, namely, a mechanochemical change which occurs very early in the contraction cycle and which is not elicited by closely spaced double shocks. Our working hypothesis is that the prolonged stimulation at low rate induces two changes: (1) a lowering of Hodgkin and Horowicz's mechanical threshold[24,40,41] whereby a roughly unchanged muscle action potential elicits an earlier and larger calcium release from sarcoplasmic reticulum and the twitch "active state" is intensified; and (2) an acceleration of the subsequent process of uptake of myoplasmic calcium by the reticular calcium sink which shortens the relaxation of the potentiated twitch.

While this evidence is at variance with current interpretations of twitch potentiation, it also emphasizes that, in this respect, skeletal muscle is not as different from heart muscle as is usually believed. In heart muscle the increase of frequency of contraction (positive inotropy) augments the contraction force and rate of tension development and decreases the contraction time[42-44]. A shortening of latency of contraction is also described[44]. Clearly the staircase potentiation in heart muscle is related, not to prolongation of "active state", but either to intensification of the "active state" or to a shift of the force–velocity relation[43,46]. Sonnenblick[44,45] favours the latter hypothesis for heart muscle in which he recognizes a peculiar "ability to modulate its intrinsic velocity", in contrast to skeletal muscle[44]. It is difficult to evaluate the two possible mechanisms, however, because heart muscle cannot be tetanized and therefore its maximum force and rate of contraction cannot be measured[46,47]. The difficulties associated with the time-dependence of activation do not occur in skeletal muscle and we find that the maximum rate of tension development for a maximum tetanus is not increased at the time the single twitch discloses staircase potentiation (Fig. 2*D* and *E*). Thus the force–velocity relation is not affected by the staircase phenomenon which should essentially involve the intensity of activation of the myofilaments.

In conclusion, the kinetics of contraction can be analysed rather consistently and quantitatively in human muscle *in situ*. Besides their obvious importance in human muscle diseases (unpublished work of J. E. Desmedt and B. Emeryk) such studies are useful because they are concerned with muscles in entirely normal physiological conditions and thus offer a point of view which is complementary to that of *in vitro* biophysics of muscle sub-systems. The present work was prompted by the finding[38] that the twitch time course is shortened, rather than prolonged, in staircase potentiation elicited by 2/sec stimulation. This ruled out the current explanation based on a prolongation of the "active state" decay. On the other hand, the force–velocity relation of the muscle is not modified because the maximum rate of tension development in tetanus is unchanged (Fig. 2*D* and *E*). The staircase phenomenon must therefore depend on an intensification of the twitch "active state", early in the contraction cycle (Fig. 3), and a working hypothesis is proposed. This conclusion is contrary to the current view that the "active state" would be maximum in the single twitch[1], but it is argued that this postulate of Hill's model was not in fact adequately substantiated and can be abandoned on the basis of new evidence.

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[1] Hill, A. V., *Proc. Roy. Soc. Biol.*, **136**, 399 (1949).
[2] Jewell, B. R., and Wilkie, D. R., *J. Physiol.*, **143**, 515 (1958).
[3] Kahn, A. J., and Sandow, A., *Ann. NY Acad. Sci.*, **62**, 137 (1955).
[4] Ritchie, J. M., *J. Physiol.*, **126**, 155 (1954).
[5] Hill, A. V., and MacPherson, L., *Proc. Roy. Soc.*, B, **143**, 81 (1955).
[6] Hill, A. V., *Proc. Roy. Soc.*, B, **138**, 325 (1951).
[7] Abbott, B. C., and Aubert, X. M., **117**, 77 (1952).
[8] Walker, S. M., *Amer. J. Physiol.*, **172**, 37 (1953).
[9] Walker, S. M., *Amer. J. Phys. Med.*, **39**, 191 (1960).
[10] Huxley, A. F., *Prog. in Biophysics*, **7**, 257 (1957).
[11] Jewell, B. R., and Wilkie, D. R., *J. Physiol.*, **152**, 30 (1960).
[12] Ritchie, J. M., *J. Physiol.*, **124**, 605 (1954).
[13] Mauriello, G. E., and Sandow, A., *Fed. Proc.*, **12**, 123 (1953).
[14] MacPherson, L., and Wilkie, D. R., *J. Physiol.*, **124**, 292 (1954).
[15] Buller, A. J., and Lewis, D. M., *J. Physiol.*, **176**, 337 (1965).
[16] Wilander, B., *Acta Physiol. Scand.*, **68**, 1 (1966).
[17] Norris, F. H., *Amer. J. Physiol.*, **200**, 667 (1961).
[18] Desmedt, J. E., *Acta Neurol. Belg.*, **58**, 977 (1958).
[19] Bleckmann, I., Jurna, I., and Rummel, W., *Pflüg. Arch.*, **277**, 422 (1963).
[20] Brust, M., *Amer. J. Physiol.*, **210**, 445 (1966).
[21] McRorey, H. L., Gale, H. H., and Alpert, N. R., *Amer. J. Physiol.*, **210**, 114 (1966).
[22] Huxley, A. F., and Huxley, H. E., *Proc. Roy. Soc.*, B, **160**, 433 (1964).
[23] Hasselbach, W., *Prog. in Biophysics*, **14**, 169 (1964).
[24] Hodgkin, A. L., and Horowicz, P., *J. Physiol.*, **153**, 404 (1960).
[25] Ebashi, S., and Lipmann, F., *J. Cell. Biol.*, **14**, 389 (1962).
[26] Weber, A., Herz, R., and Reiss, I., *J. Gen. Physiol.*, **46**, 679 (1963).
[27] Podolsky, R. J., and Costantin, L. L., *Fed. Proc.*, **23**, 933 (1964).
[28] Weber, A., Herz, R., and Reiss, I., *Proc. Roy. Soc.*, B, **160**, 489 (1964).
[29] Schädler, M., *Pflüg. Arch. Ges. Physiol.*, **296**, 70 (1967).
[30] Sandow, A., *Arch. Phys. Med. Rehabil.*, **45**, 62 (1964).
[31] Isaacson, A., *Nature*, **196**, 381 (1962).
[32] Sandow, A., and Seaman, T., *Life Sci.*, **3**, 91 (1964).
[33] Johnson, D. D., and Loomis, T. A., *Life Sci.*, **4**, 2309 (1965).
[34] Sandow, A., and Brust, M., *Biochem. Z.*, **345**, 232 (1966).
[35] Bowditch, H. P., *Arbeit. Physiol. Leipzig*, **6**, 139 (1870).
[36] Rossbach, M. J., *Arch. Ges. Physiol.*, **15**, 1 (1877).
[37] Walker, S. M., *Amer. J. Physiol.*, **149**, 7 (1947).
[38] Desmedt, J. E., and Hainaut, K., *CR Acad. Sci.*, **264**, 363 (1967).
[39] Ritchie, J. M., and Wilkie, D. R., *J. Physiol.*, **130**, 488 (1955).
[40] Etzensperger, J., and Gasciolli, A. M., *CR Soc. Biol.*, **157**, 1776 (1963).
[41] Sandow, A., Taylor, S. R., and Preiser, H., *Fed. Proc.*, **24**, 1116 (1965).
[42] Niedergerke, R., *J. Physiol.*, **134**, 569 (1956).
[43] Abbott, B. C., and Mommaerts, W. F. M. J., *J. Gen. Physiol.*, **42**, 533 (1958).
[44] Sonnenblick, E. H., *Amer. J. Physiol.*, **202**, 931 (1962).
[45] Sonnenblick, E. H., *J. Gen. Physiol.*, **50**, 661 (1967).
[46] Mommaerts, W. F. H. M., and Langer, G. A., *Amer. Rev. Med.*, **14**, 261 (1963).
[47] Brady, A. J., *Fed. Proc.*, **24**, 1410 (1965).

LETTERS TO THE EDITOR

PLANETARY SCIENCE

Eliminating False Alarms when detecting Seismic Events Automatically

ONE of the principal difficulties of using the full capabilities of a seismic array and a digital computer for the automatic detection of seismic events is the number of false alarms produced by spurious noise spikes on single channels That this is a problem is shown by the fact that the usual dynamic range between the background noise level and saturation is normally in excess of 40 dB Large noise impulses on a single channel will therefore predominate on a phased sum of the array seismometer outputs unless the number of sensors is very large

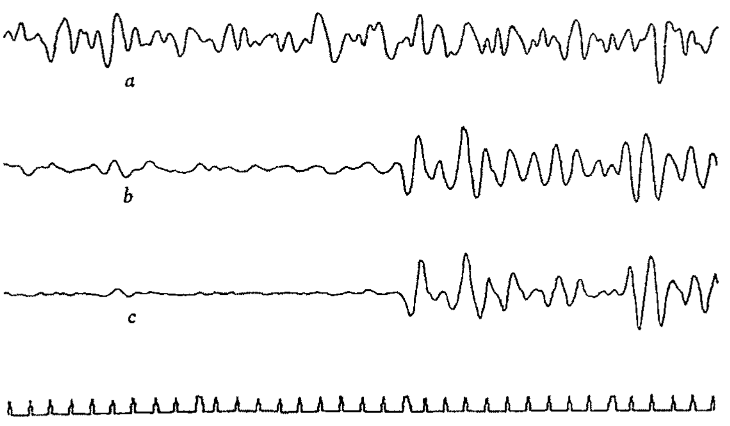

Fig 1 Origin 52 9 S , 28 1 E , depth 33 km, mag 4 7, azimuth 215 8°, J B phase velocity 21 9 km/sec Origin time October 5, 1966, 23 h 23 min 53 2 sec *a*, Single seismometer, *b*, phased sum, *c*, process described in this communication

Various methods of overcoming this problem have been given in the literature Birthill and Whiteway[1], for example, have adopted a correlation and averaging process using two partial sums from the phased array Although an improvement in noise rejection is gained, it is limited by the short duration of the averaging time (because of the small time during which a seismic event is coherent) as well as being very demanding in computer time[2]

I have tried several different techniques in an effort to overcome these deficiencies, and have found one which not only reduces susceptibility to this type of noise but gives evidence of improving the signal to noise ratio as well as the array resolution The particular process which was used is to take the *n*th root of the absolute value of each individual signal, with the original sign preserved before phasing and summation For $n=2$ this corresponds to

$$E_i(t) = \text{signum}\,[S_i(t)+N_i(t)] \times |S_i(t)+N_i(t)|^{\frac{1}{2}}$$

where $S_i(t)$ is the coherent signal component and $N_i(t)$ is the noise output of the ith seismometer at time t If after summation the result is squared, then in the case when $S_i(t) \gg N_i(t)$ this process is identical to the direct summation process, except for a constant factor depending on the number of sensors used

A comparison of this method, using a value of $n=2$, with that of the normal phased summation technique is shown in Fig 1 and reveals a signal to noise ratio improvement for an event which is hidden in the background noise on a single seismometer output A further comparison on two events with similar azimuths and phase velocities (Fig 2) shows an enhancement of the smaller event when the array is correctly phased and greater rejection, particularly in the coda, when the array is phased for the second event

I am not able to give any formal mathematical reason for these results, however, I suggest that the character of seismic background noise is not stationary for

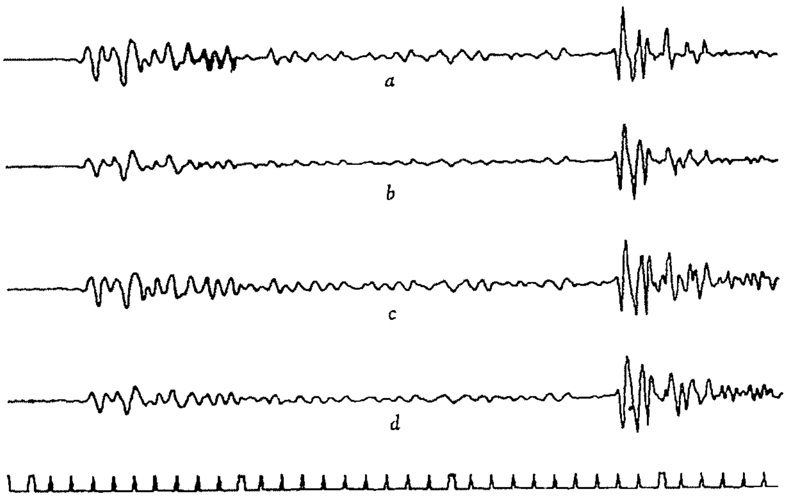

Fig 2 Event 1 origin 15 1 S , 173 5 W , depth 33 km, mag 4 8, azimuth 93 2°, J B phase velocity 14 5 km/sec Origin time October 14, 1966, 2 h 32 min 31 8 sec Event 2 origin 23 2 S , 179 8 W , depth 456 km, mag 4 8, azimuth 102 9°, J B phase velocity 14 0 km/sec Origin time October 14, 1966, 2 h 34 min 34 1 sec *a*, Process described in this communication, array phased to first event and *b* to second event *c*, Normal delay and sum, array phased to first event and *d* to second event

small intervals of time and that my process tends to equalize the variance of this noise.

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[1] Birthill, J W , and Whiteway, F E , *Phil. Trans Roy Soc* , 258, 421 (1965)
[2] Weichert, D H , Manchee, E. B , and Whitham, K , *Geophys J Roy Astro Soc* , 13, 277 (1967)

Palaeomagnetism of a Stratiform Intrusion in the Pensacola Mountains, Antarctica

A LARGE stratiform mass of pyroxene gabbro interlayered with smaller amounts of anorthosite and pyroxenite and capped with granophyre makes up most of the northern ranges of the Pensacola Mountains (Fig 1)[1] These layered rocks are at least 2,000 m thick in both the Dufek Massif and the Forrestal Range, and are believed to belong to a single sheet-like body with an areal extent of at least 8,000 km² The age of the body is not known with certainty, but it intrudes folded rocks as young as Permian (personal communication from J M Schopf) and therefore is probably Mesozoic or younger

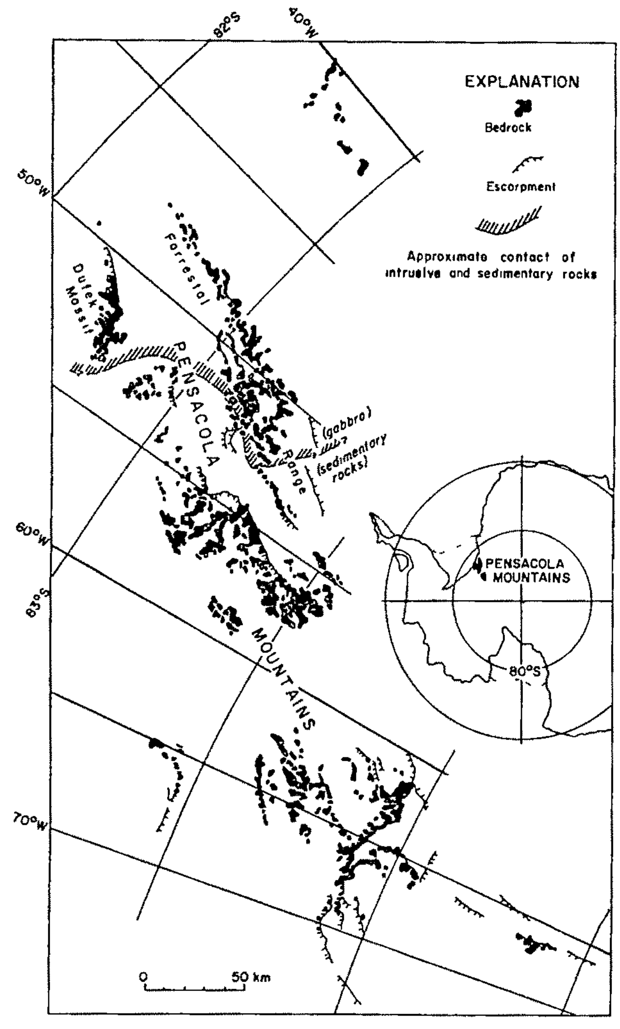

Fig 1 Index map showing the location of the Dufek Massif and the Forrestal Range

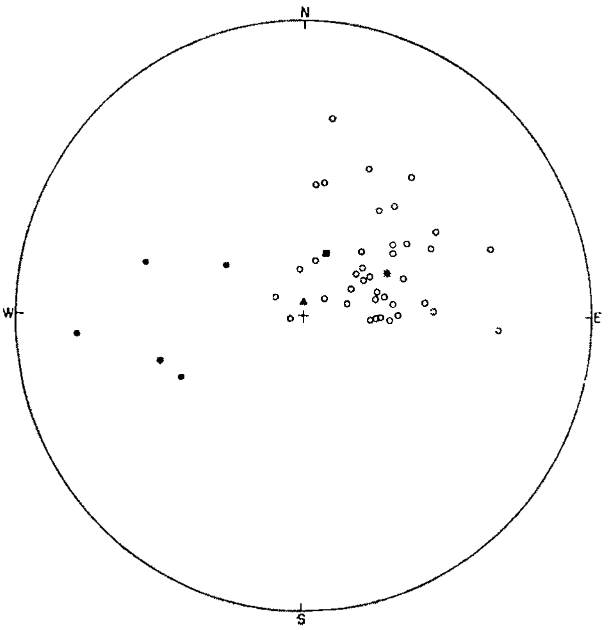

Fig 2 Equal-area projection of site-mean directions of remanent magnetization for the Dufek Massif Open circles are site-mean directions plotted on the upper hemisphere, solid circles are plotted on the lower hemisphere Mean direction for the Dufek Massif (computed after the lower hemisphere points were "reversed" by inversion through the centre of the projection) indicated by * The direction of the present geomagnetic field in the sampling area is shown by the square (upper hemisphere), while the axial dipole direction is shown by the triangle

As a preliminary investigation of the palaeomagnetism of the Dufek Massif, natural remanent magnetization of fifty-seven samples from forty-three sites scattered throughout much of the available exposure has been measured. The results are summarized in Table 1 and Fig 2 Magnetic stability is indicated by divergence of the average direction of magnetization from the direction of the present geomagnetic field and by rough consistency of direction between samples of opposite polarity

Table 1 AVERAGE DIRECTION OF MAGNETIZATION AND PALAEOMAGNETIC POLE POSITION FOR THE DUFEK MASSIF

Direction of magnetization				Palaeomagnetic pole		
N	D	I	k	α_{95}	λ	φ
43	61 5	−63 0	14 7	3 0°	47 5° S	164 0° W

N, No of sites, D, declination east of north, I, inclination positive downward, k, precision parameter (ref 2), α_{95}, radius λ, latitude, and φ, longitude, of the palaeomagnetic pole

To determine the effect of alternating field magnetic cleaning, a suite of twelve specimens was chosen at random and demagnetized in fields ranging up to 300 oersteds Although the directions of individual specimens showed some change during this process (average change < 8°), neither the mean direction of the suite nor the precision of the mean altered to any significant extent

The pole position listed in Table 1 is near, but not in, the cluster of palaeomagnetic poles which have been calculated for rock units assigned to the Ferrar Dolerites of Harrington[3] (see, for example, poles 9 37, 9 38, 9 42 and 9 56 in ref 4) The Ferrar Dolerites are believed to be Jurassic, and the palaeomagnetic data indicate that the gabbros of the Dufek Massif also originated then The slight difference in pole position may indicate that the two are not precisely contemporaneous

The five reversely magnetized sites shown in Fig 2 are located in a definite zone, and similar reverse polarity zones have been encountered in the gabbros of the Forrestal Range This arrangement could be explained by reversal of the Earth's magnetic field during cooling of the stratiform gabbros or by mineralogically controlled self-reversal If the Earth's magnetic field reversed its polarity one or more times while the gabbro body was in the process of cooling through the range of temperatures in which it acquired its magnetization, the result would be a system of polarity zones parallel to the isothermal surfaces in the gabbro On the other hand, polarity zones parallel to compositional layering could arise if (*a*) certain portions of the stratiform complex possess self-reversing properties[5] by virtue of the composition of their iron oxide minerals, and (*b*) these iron oxide minerals vary systematically throughout the complex (share in the cryptic layering[6]) Mineralogical studies are in progress to resolve this question

The gabbros of the Forrestal Range are much more strongly magnetized than those of the Dufek Massif This is shown in Fig 3, where intensity of remanent magnetization is plotted against magnetic susceptibility. Such a diagram brings together the two principal variables controlling total magnetic intensity—specifically those variables which are most closely related to other rock properties[7] Systematic differences in position on such a plot should reflect systematic differences in gross composition, mineralogy, texture or other rock properties In this case it tends to substantiate the view (Ford's unpublished results) that the gabbros of the Forrestal Range represent part of the upper portion of the stratiform complex, while those of the Dufek Massif formed near its base

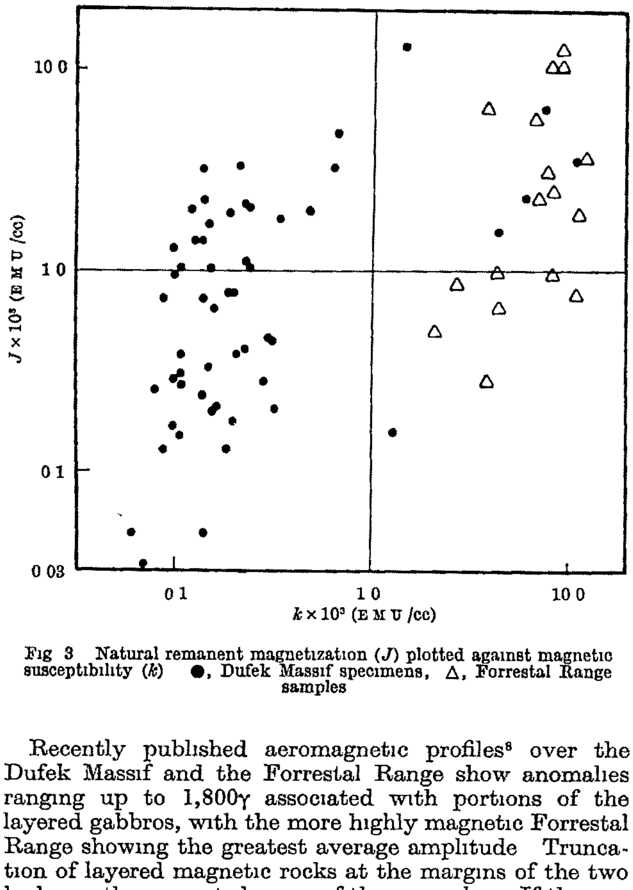

Fig 3 Natural remanent magnetization (*J*) plotted against magnetic susceptibility (*k*) ●, Dufek Massif specimens, △, Forrestal Range samples

Recently published aeromagnetic profiles[8] over the Dufek Massif and the Forrestal Range show anomalies ranging up to 1,800γ associated with portions of the layered gabbros, with the more highly magnetic Forrestal Range showing the greatest average amplitude Truncation of layered magnetic rocks at the margins of the two bodies is the suggested cause of the anomalies If the size and geometrical configuration of the polarity zones are favourable, reversals of polarity could make an important contribution to these anomalies by producing a source of strong magnetic contrast within the gabbro bodies In the Dufek Massif, where the ratio of remanent to induced magnetization is almost uniformly high, this factor should be especially important

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[1] Aughenbaugh, N B, *Intern Geophys Year World Data Center A, Glaciology, Glac Rep*, 4, 155 (1961)
[2] Fisher, R A, *Proc Roy Soc*, A, 217, 295 (1953)
[3] Harrington, H J, *Nature*, 182, 290 (1958)
[4] Irving, E, *Paleomagnetism and its Application to Geological and Geophysical Problems*, Appendix (1964)
[5] Uyeda, S, *Jap J Geophys*, 2, 1 (1958)
[6] Wager, L R, and Deer, W A, *Medd Gronland*, 105, No 4 (1939)
[7] Beck, jun, M E, *US Geol Surv Prof Paper*, 550-*D*, *D*109 (1966)
[8] Behrendt, J C, Leister, L, and Henderson, J R, *Science*, 153, 1373 (1966)

Genesis of Concordant Deposits of Base Metal Sulphides: an Experimental Approach

Despite the great economic importance of the stratigraphically concordant massive sulphide deposits which occur in regionally metamorphosed rocks of pre-Cambrian to Palaeozoic ages in all continents, little is known of their origin Writers on these deposits, which include those of Mount Isa[1], Broken Hill, the Scandinavian Caledonides[2], the Bathurst–Newcastle area[3] and the Rhodesian Copperbelt, have tended to accept one of two apparently conflicting views Some maintain that the deposits are syngenetic[4], representing original sedimentary or volcanic sulphides, while others consider them to be epigenetic[5], and formed by precipitation from hydrothermal solutions of igneous or metamorphic origin

In a study of the Caledonian chalcopyrite–pyrite–arsenopyrite veins of the English Lake District, one of us concluded[6] that the mineralizing solutions were derived from the dehydration of rocks undergoing metamorphism in the deeper levels of the Caledonian geosyncline Significant metal contents have since been noted in brines of metamorphic origin from the Salton Sea area in California[7], and pockets of hot zinc-rich solutions of presumed volcanic origin have been located in the Red Sea[8] Prolonged contact between such solutions and pyrite deposits of sedimentary or volcanic origin could well produce massive sulphide deposits with features characteristic of both syngenetic and epigenetic origins, and so we have conducted experiments to determine the effectiveness of pyrite as a scavenger of base metals, and the extent to which pyrite deposits would be dispersed by metamorphism

The scavenging properties of pyrite were tested by holding mixtures of pyrite and pyroxenite, pyrite and carbonaceous shale, and pyrite and synthetic limestone at 500°–600° C, 1–2 kbars for 10 days, in the presence of appropriate mineralizers (Table 1) The pyrite was

Table 1 RUN CONDITIONS

Run No	Reactants	Time (days)	T (° C)	Pressure (kbars)	Mineralizer
3	60% pyroxenite–40% pyrite	10	600	2 0	50% HCl
4	70% limestone–30% pyrite	10	500	1 0	4M–NH_4Cl
5	70% shale–30% pyrite	10	600	2 0	50% HCl

Table 2 TRACE ELEMENT CONTENTS OF REACTANTS (P P M)

Sample	Co	Ni	Cu	Zn	Pb
Pyrite	100	150	300	250	850
Black shale	75	200	250	250	500
Pyroxenite	80	800	100	150	400
Synthetic limestone	500	500	500	500	500

extracted by grinding the recrystallized charge to 150 mesh, followed by flotation using a collector of potassium amyl xanthate, with boral as the frother Sodium silicate was used as a depressant for calcite in the runs containing synthetic limestone Pyrite fractions of high purity were obtained by this method, and analysed by X-ray fluorescence (for cobalt, nickel, zinc and lead) and colorimetric methods (copper), with the results shown in Table 3 The experimental enrichment factors were then recalculated to eliminate the effects of variations in the initial trace element concentrations and rock to pyrite ratios (Table 4)

Table 3 EXPERIMENTALLY DETERMINED ENRICHMENT FACTORS (FINAL/INITIAL CONCENTRATION) FOR THE PYRITE FRACTION

Run No	Rock type	Co	Ni	Cu	Zn	Pb
3	Pyroxenite	2 0	2 0	1 3	1 4	1 1
4	Limestone	6 0	3 7	1 7	1 2	1 5
5	Black shale	1 5	1 7	1 5	1 4	1 0

Microscopic examination of the products indicated that reaction was incomplete, and these enrichment factors are therefore lower than those which would be observed at equilibrium The relative concentrations in this Table are considerably more accurate than the absolute values in Table 2

Table 4 ADJUSTED ENRICHMENT FACTORS

Run No	Rock type	Co	Ni	Cu	Zn	Pb
3	Pyroxenite	12 3	4 3	3 3	1 7	1 3
4	Limestone	8 0	5 7	3 1	1 5	2 4
5	Black shale	2 0	2 2	2 0	1 7	1 0

The adjusted enrichment factor is $(p_f/r_f)/(p_i/r_i)$, where p_i, p_f are the initial and final concentrations of a given element in the pyrite fraction, and r^i, r_f are the initial and final concentrations in the rock fraction

Cobalt and nickel, which form pyrite-type sulphides, and copper, which is moderately soluble in the pyrite structure, were strongly fractionated

$$M^{2+} + \text{excess } FeS_2 \rightleftharpoons (Fe,M)S_2 \text{ solid solution} + Fe^{2+}$$

but lead and zinc, which have very limited solubility in pyrite, showed much weaker initial fractionation, possibly involving reactions such as

$$Zn^{2+} + 2Fe^{2+} + FeS_2 \rightleftharpoons 2(Fe,Zn)S \text{ solid solution} + 2Fe^{3+}$$
$$Pb^{2+} + 2Fe^{2+} + FeS_2 \rightleftharpoons PbS + FeS + 2Fe^{3+}$$

In the experiments on dispersion, finely ground mixtures of pyrite and rock powder were held at 600° C, 2 5 kbars for 12 days in the presence of mineralizers which included 4 molar ammonium chloride, 50 per cent hydrochloric acid, saturated sodium chloride solution and distilled water Recrystallization of pyrite was effected in all runs except those containing distilled water, but there was no detectable migration of pyrite along the temperature gradient of about 10° C/cm maintained in the gold sample capsules In similar conditions, but with no rock powder to hinder convection, pyrite was rapidly transported to the cold end of the tube It is concluded that all the elements studied will be concentrated in the pyrite bodies during the metamorphism of a mixed pyrite–silicate sequence, the final concentration in the sulphide body depending on the fractionation constants under the pressure–temperature–composition conditions of metamorphism, the initial trace element contents in the pyrite and its country-rock, the effective rock to pyrite ratio, and the time available for reaction

It also seems that pyrite deposits in a compact rock mass would not suffer significant dispersion during metamorphism, unless there was a flow of metamorphic fluid through the pyrite-bearing rock Dispersion, however, may become more important at high metamorphic grades, when solubilities increase with pressure and temperature At low grades any such tendency will probably be outweighed by mechanical concentration of the easily deformed sulphides in regions of low stress, such as fold crests

The experimental results thus support our initial hypothesis that the mineralogy and structure of some syngenetic pyrite bodies could be modified so profoundly during subsequent diagenesis, metasomatism and metamorphism as to render meaningless the distinction between "syngenetic" and "epigenetic" or "hydrothermal" deposits We should instead expect to find all gradations between the two extremes

Accepting this as a working hypothesis, two economically important results follow First, the search for further ore deposits of this type should be concentrated in areas of low to medium grade regional metamorphism (low dispersion), in which strongly deformed country rocks containing high initial concentrations of thiophile elements (mechanical and chemical concentration) are associated with pyrite bodies Second, the possibility of tapping the metamorphic fluids themselves as sources of base metals should not be overlooked, for fluids of the Salton Sea[7] and Red Sea[8] types may well occur within reach of the surface in many of the younger volcanic and orogenic belts of the world

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[1] Solomon, P J , *Econ Geol* , **60**, 737 (1965)
[2] Vokes, F M , *Econ Geol* , **57**, 890 (1962)
[3] Boyle, R W , *Econ Geol* , **60**, 1529 (1965)
[4] Sales, R H , *Econ Geol* , **57**, 721 (1962)
[5] Garlick, W. G , *Econ Geol* , **58**, 447 (1963)
[6] Strens, R G J , thesis, Univ Nottingham (1962)
[7] White, D E , Anderson, E T , and Grubbs, D K., *Science*, **139**, 919 (1963)
[8] Manheim, F T , Hathaway, J C , Degens, F T , McFarlin, P F , and Jokela, A , *Geol Soc Amer Program Abstracts*, 100A (1965)

PHYSICS

Existence of a Surface Tension Discontinuity at a Liquid Free Surface

EXPERIMENTS have shown that, in certain conditions, a free water surface can exist in equilibrium with different values of surface tension in different regions So far, only two such regions have been distinguished in any one water surface and their common boundary can be seen clearly in reflected light, after a little practice, as an unbroken sinuous line One region appears to be chemically clean while the other is obviously contaminated with a surface film This situation remains stable only if the water has a low velocity (less than 23 cm/sec) which acts—see Fig 1—in such a way that the contaminated surface region is "contained" by the shear stresses set up by the flow against the underside of the surface film If the water

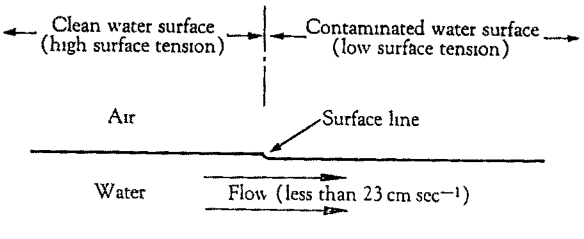
Clean water surface (high surface tension)
Contaminated water surface (low surface tension)
Air
Surface line
Water
Flow (less than 23 cm sec⁻¹)

Fig 1 Water surface profile perpendicular to surface line

velocity dies away the contaminated zone spreads out to cover the whole water surface It is the line marking this boundary which is the subject of the present study and it will be referred to here as the "surface line" This topic is of interest in chemical engineering where the results might be used to control the separation of minerals and other compounds by surface flotation

A reflected light Schlieren system was developed for this study from an earlier form of the apparatus described elsewhere[1] Fig 2 shows the lay-out of the optical components of the system in relation to the "hydraulic" apparatus The water tank used had a free surface measuring 60 × 90 cm with a depth of 60 cm Untreated mains water was introduced through a system of baffles and screens, covering the full width of the tank, which was designed to ensure two-dimensional circulation of the water in the tank proper, as indicated in Fig 2 When steady conditions had been established, water left the tank by a full width sharp edged weir, running the full width of the tank, placed at the opposite end to the inlet

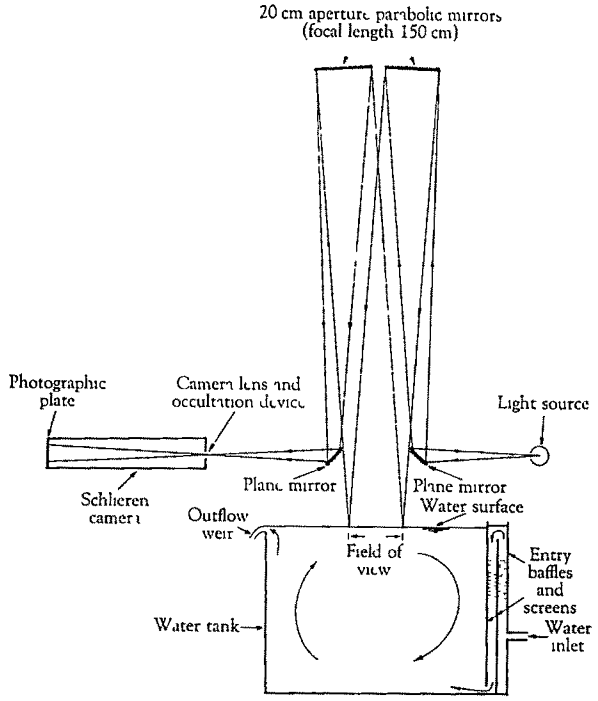

Fig 2 Experimental apparatus shown in elevation

The conditions near the free surface were similar to those shown in Fig 1 Water moved slowly from left to right, the surface contamination being trapped by the inlet baffles at the right, the free surface overflow at the left ensured that a clean water surface was maintained in this region by the rising current there

Before any measurements were made it was apparent that the "strength" of the surface line (in the visual sense) was dependent to some extent on the degree of initial cleanliness of the water surface The inevitable contamination from the atmosphere of the laboratory, however, ensured that the line could always be detected after a brief search The position of the line was variable and depended on the water velocities and the surface contamination level The optical apparatus was arranged on a rigid framework that could be traversed as a whole relative to the water tank so that the surface line once detected could be "moved" into the field of view if necessary The two 20 cm parabolic mirrors gave a parallel beam of light that was reflected at the water surface with an incidence angle of 5° This was as close to normal reflexion as could be obtained and gave a slightly elliptical field of view (20 1 × 20 0 cm) If the angle of incidence became large (say, greater than 8°), optical and geometrical distortions became significant

The optical system, which consisted of plane and parabolic face-silvered mirrors, gave an image of the point light source close to the lens and on the axis of the Schlieren camera This image was small (slightly larger than the light source) only when the reflecting water surface was plane, any distortion of the water surface in the field of view partially defocused the image It is this effect which was used in combination with a suitable occultation device (Fig 3) to give a Schlieren image of the water surface on the camera plate The camera lens was not essential, but its use improved the quality of the final image in a system where small optical imperfections were present

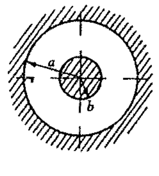

Fig 3 Occultation device used in all experiments Light accepted falling in annulus $b < \text{radius} < a$

Fig 4 shows a Schlieren photograph of the surface line for which the occultation device was set with a narrow acceptance annulus In these conditions two approximately equidistant lines appeared, one brighter than the other This showed that there existed two points of known water surface slope on any perpendicular to the surface line By taking a series of photographs while systematically varying the dimensions of the acceptance annulus it was possible to build up quantitative data on the surface line The phenomenon was unsteady in that the spacing between the two lines on the plate in Fig 4 was seen to vary slightly both with distance and time. The space variable could be eliminated to some degree by averaging a number of values taken from each photograph and this was done, but the time variable was more difficult to remove In the current tests it was partially dealt with by curve smoothing at a later stage of the analysis

The difficulty of relating results from the individual photographs in a series was overcome by fixing one of the acceptance annulus dimensions for that series (either a or b) The relationship between the radius of this annulus and the water surface slope was established by direct calibration, an optical flat mounted on a tilting stage being substituted for the water surface The sensitivity of the apparatus was found to correspond to a water surface slope of 0 00277 radians for each mm of the annulus radius. It can be seen from this that the annulus being of finite thickness will accept light from water surface slopes lying

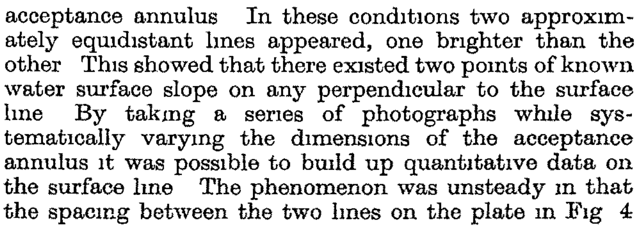

Fig 4 Schlieren photograph of surface line ($a = 12$ mm, $b = 10$ mm).

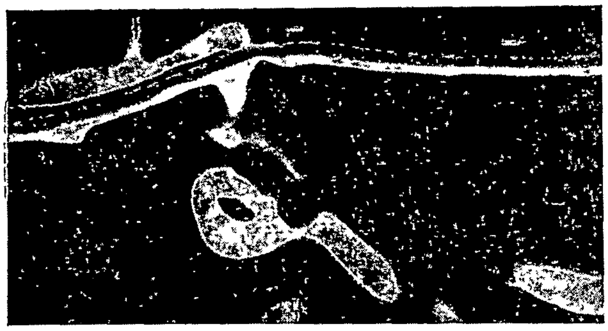

Fig 5. Schlieren photograph of surface line (test *C*–4)

within a certain range It is therefore possible by using wider annuli and by measuring the widths as well as the separation of the photographic traces to build up more complete data of the surface line Three such series were carried out (tests *A*, *B* and *C*) from which Fig 5 is an example Table 1 gives the dimensions of the acceptance annuli used in these tests Because in each test one dimension remains constant it was possible to check the amount of time variation present It was also possible to plot a graph of water surface slope, dz/dx, against perpendicular horizontal distance, x, and to include results from the four photographs in each test on one graph A smooth curve was drawn through these points and numerical integration of this curve gave the mean profile across the line (z—x relationship) These are shown plotted in Fig 6 It must be remembered that the slopes of these profiles and therefore also the curvatures are distorted by the × 25 exaggeration of the vertical scale In spite of this it is clear that the convex (upwards) portion of each profile has a much smaller radius of curvature than the concave part This result corresponds to the point mentioned, that of the two lines shown in Fig 4—and on all such photographs—the upstream trace is always weaker than the other one

Table 1. VALUES OF ACCEPTANCE ANNULI DIMENSIONS (mm) CORRESPONDING TO RESULTS SHOWN IN FIG 6

Test	Annulus radius	1	2	3	4
A	*a*	15 0	15 0	15 0	15 0
	b	4 5	6 0	8 0	10 0
B	*a*	12 5	12 5	12 5	12 5
	b	4 5	6 0	8 0	10 0
C	*a*	4 5	7 0	10 0	12 5
	b	3 5	3 5	3 5	3 5

The interpretation of the slope data to give profiles of the type shown in Fig 6 was assisted by a simple knife-edge test that showed that all water surface slopes attributable to the surface line face the same way It followed from this that the surface line must have a monoclinal step profile rather than that of a solitary wave or cusp The height of this step was obtained from the numerical integration process and was for tests *A*, *B* and *C*, 0 157, 0 183 and 0 185 mm, respectively Mean flow conditions for these three tests—discharge, water level and mean velocities—were the same, the only uncontrolled variable was the surface tension on the contaminated (lower) side of the line.

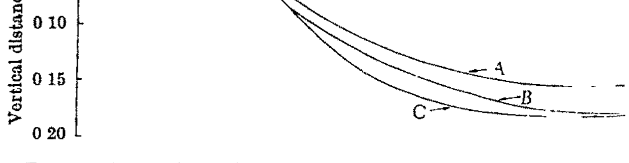

Fig 6 Mean water surface profile perpendicular to the surface line for three tests (vertical scale exaggerated × 25)

In the experiments described, the surface line clearly defined the edge of a contaminated portion of the water surface From this it was suggested that it marked a change in the surface tension value of the free surface and attempts were made to measure this on either side of the line Such measurements were difficult because it was essential that they did not interfere with the surface film formed by the contamination The capillary wave method promises to give the best results but so far has not yielded any reliable data

Observation of this surface film showed that it possessed only second order random velocities and that its mean velocity in the direction of flow (see Fig 1) was zero There must therefore be a boundary layer in the fluid below the film which exerts a shear stress against the underside of the film, acting in the direction of flow It seems probable that this shear stress provides the mechanism for balancing the unequal horizontal surface tension forces on either side of the line, but it also follows that there will be no sharp change in surface tension value at the line

The change in water level measured at the surface line introduces small out of balance hydrostatic pressure forces which cannot readily be explained by hydrodynamic reasoning It is hoped that any relationship between these and surface tension values will be revealed by further experiments in which it is planned to vary the surface tension values systematically while making measurements of these and the corresponding differences in water surface elevation at the line

I thank the Queen's University of Belfast for laboratory facilities and equipment and Professor T. M Charlton for his help and encouragement I also thank the Laboratoires de Mécanique des Fluides of the University of Grenoble for facilities

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[1] Sellin, R H J , *J Sci Instrum* , 40, 355 (1963)

Wavelengths and Relative A-Coefficients for the Forbidden Lines of Atomic Sulphur

THE multipole lines from atomic sulphur are of considerable astrophysical interest Moreover, a direct measurement of their wavelength is the best and most direct method of fixing the positions of the metastable levels involved Furthermore, a measurement of the relative intensity of magnetic dipole and electric quadrupole lines from the same upper level gives the relative transition probability, which is a valuable check on the theoretical predictions

In an earlier note[1] we reported the first laboratory excitation of the forbidden lines of SI and gave the approximate wavelengths This communication presents the results of relative intensity and accurate wavelength measurements on these lines

The lines were excited in a high voltage a c discharge through a π-shaped tube 100 cm long and 3 cm in diameter with tungsten electrodes in the side arms The tube was filled with krypton to a pressure of a few mm of mercury after some sulphur had been distilled in under vacuum After running for a time impurities such as carbon monoxide were cleaned up Krypton was used as the filler gas rather then xenon (which we used before), so that additional population of the $^1S_0$ state would result through the resonance reaction

$$\mathrm{Kr}(^3P_2)+\mathrm{S}_2(^3\Sigma)\rightarrow \mathrm{S}(^1S_0)+\mathrm{S}(^1S_0)+\mathrm{Kr}(^1S_0)$$

This approximately doubled the intensity of the forbidden lines

Optimum conditions for the emission of the lines were achieved when the tube was surrounded by an oven kept at 45° C and the tube current was 12 mamp

For the wavelength measurements the discharge was observed end on through an optically flat window using a 3 5 m Fastie–Ebert echelle grating spectrograph The $^1S_0$–$^3P_1$ transition was observed in the thirteenth order with a plate factor of 0 33 Å/mm and the $^1S_0$–$^1D_2$ transition was observed in the eighth order where the plate factor was 0 49 Å/mm The effective aperture of the instrument was approximately *f*/30 and it was kept in a constant temperature room to minimize shifts during the long exposures which were necessary Kodak II *L* and *Oa F* plates were used to record the lines Krypton, impurity xenon and allowed sulphur lines were used to provide reference wavelengths Using these, the wavelength of the $^1S_0$–$^3P_1$ transition was found to be 4589 261 ± 0 007 Å and that of the $^1S_0$–$^1D_2$ transition 7725 04 ± 0 01 Å

Toresson[2] has made measurements on the vacuum ultraviolet spectrum of SI and from these has fixed the term values of the low lying levels including the $^1S_0$ and $^1D_2$ metastable levels Using his values for these levels, one can compute the wavelengths of the $^1S_0$–$^3P_1$ and $^1S_0$–$^1D_2$ transitions as 4589 61 Å and 7725 8 Å, which are in disagreement with the values given here Toresson's positioning of both the $^1S_0$ and $^1D_2$ levels is, however, based on single transitions in the vacuum ultraviolet and may therefore be liable to some error On the other hand, his values for the $^3P_{0,\,1,\,2}$ states are based on a considerable number of transitions and can therefore be regarded as more accurate Assuming his values for the $^3P_{0,\,1,\,2}$ levels, and using our measured wavelengths as already given, a revised term system can be drawn up as given in Table 1 Also shown are the values given by Moore[3]

Table 1 REVISED SI LEVELS

Term symbol	Moore	E cm^{-1} Toresson	Present results
$3s^2\ 3p^4\ ^3P_2$	0 00	0 00	0 00
$^3P_1$	396 8	395 56	395 56
$^3P_0$	573 6	573 19	573 19
$^1D_2$	9,239 0	9,237 76	9,238 10
$^1S_0$	22,181 4	22,177 82	22,179 46

Bowen[4] has observed a faint line at 7726 5 ± 0 3 Å on one spectrum from a nebula and has suggested that it might be caused by the $^1S_0$–$^1D_2$ transition This now seems unlikely in the light of the present results Swings (personal communication) has observed a line in the solar spectrum at 7725 04 ± 0 01 Å in agreement with our observed value and which, from width considerations, could be caused by a forbidden transition of SI

Measurements of relative intensity were made on the two lines with a Jarrell-Ash 0 5 m Ebert grating spectrograph and an EMI 9558 *QB* photomultiplier The relative quantum efficiency of the detecting system in the two wavelength regions was obtained using a tungsten filament lamp calibrated by the National Physical Laboratory A value of 5 1 ± 0 7 was obtained for the ratio of the A-coefficients which compares with Czyzak and Krueger's[5] theoretical value of 5 09 This agreement between theory and experiment is better than that reported earlier[6] for the equivalent lines of atomic oxygen.

We thank Professor D J Bradley of this department for putting his high resolution spectrograph at our disposal One of us (J A K) wishes to acknowledge the award of a postgraduate studentship from the Government of Northern Ireland, Ministry of Education.

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[1] McConkey, J W , Burns, D J , and Herman, L , *Nature*, **204**, 1180 (1964)

[2] Toresson, Y G , *Ark for Fys* , **18**, 417 (1960)

[3] Moore, C , *Atomic Energy Levels*, 1, 467 (Nat. Bur Stand Circ , 1949)

[4] Bowen, I S , *Astrophys J* , **121**, 306 (1955)

[5] Czyzak, S J , and Krueger, T K , *Mon Not Roy Astro Soc* , **126**, 177 (1963)

[6] McConkey, J W , Burns, D J , Moran, K A , and Emeleus, K G , *Phys Lett* , **22**, 416 (1966)

THE SOLID STATE

Crystal Structure of Ethylenebidiguanide Silver (III) Nitrate

WHILE trying to prepare co-ordination compounds of ethylenebidiguanine with bivalent silver analogous to Cu(II) and Ni(II) by oxidation of a silver salt by sodium persulphate, Ray and Ghosh[1] found that a complex compound was formed in which silver exhibited an oxidation state of three instead of the expected two Tervalent silver is isoelectronic with Pd(II) Ray and Ghosh proved the trivalency of silver by showing experimentally the diamagnetic character of silver (III) ethylenebidiguanide complex salts, and on the basis of this evidence they proposed a square-planer configuration (dsp^2 hybridization) for silver in these compounds, with the silver coordinated to four nitrogen atoms of the ligand I have analysed the crystal structure of silver ethylenebidiguanide nitrate to confirm this proposal, and also because complete crystallographic work on a trivalent silver compound has not yet been reported

Silver ethylenebidiguanide nitrate crystallizes into orange red needles, elongated in the (001) direction Preliminary work carried out on a *GE-XRD*-6 diffractometer established that the crystals belong to a triclinic system and the centrosymmetric space group $P\bar{1}$ was assigned This was consistent with the subsequent structure analysis The cell dimensions are $a=8\ 283\pm0\ 004$ Å, $b=12\ 748\pm0\ 004$ Å, $c=8\ 530\pm0\ 004$ Å, $\alpha=104\ 12°$, $\beta=96\ 64°$, $\gamma=99\ 73°$, $D_{\mathrm{obs}}=2\ 03$ g cm^{-3}, $D_{\mathrm{calc}}=2\ 03$ g cm^{-3}, $Z=2$.

Three-dimensional intensities were collected on a *GE-XRD*-6 diffractometer with a single crystal orienter using copper $K\alpha$ radiation, cobalt–nickel balanced Ross filters and a scintillation counter A needle shaped crystal (0 1 × 0 1 × 0 3 mm) was used The 2,555 independent reflexions within a range of $2\theta=0°$–$120°$ were examined,

Table 1 LEAST-SQUARES PLANES AND THE OUT-OF-PLANE DISTANCES OF THE PLANE DETERMINING ATOMS

Plane No	Atoms defining the plane	Equation of the plane	Out-of-plane distances of the plane determining atoms
1	Ag, N_1, N_2, N_3, N_4	$0\ 899P+0\ 131Q+0\ 314R=2\ 86$ Å*	Ag = 0 186, $N_1=-0\ 259$, $N_2=0\ 963$, $N_3=-0\ 289$, $N_4=-0\ 229$ Å
2	N_2, C_1, N_5, N_6, C_2, N_7, N_3, Ag	$0\ 787P+0\ 565Q+0\ 248R=2\ 284$ Å	$N_2=-0\ 056$, $C_1=0\ 011$ $N_5=0\ 031$, $N_6=0\ 002$, $C_2=0\ 032$, $N_7=-0\ 116$, $N_3=0\ 163$, Ag = −0 075 Å
3	N_{10}, C_6, N_1, N_4, C_5, N_8, N_9, Ag	$0\ 962P+0\ 094Q+0\ 259R=2\ 32$ Å	$N_{10}=0\ 126$, $C_6=-0\ 071$, $N_1=0\ 271$, $N_4=-0\ 221$, $C_5=-0\ 086$, $N_8=-0\ 46$, $N_9=0\ 576$, Ag = −0 121 Å
4	Ag, N_4, C_4, C_3, N_2	$0\ 766P+0\ 552Q+0\ 330R=2\ 304$ Å	Ag = 0 007, $N_4=-0\ 177$, $C_4=0\ 125$, $C_3=-0\ 405$, $N_3=0\ 450$ Å

* The coefficients of *P*, *Q* and *R* are the direction cosines of the normal to the plane with respect to the axes $a(a\times b)\times a$ and $(a\times b)$, respectively

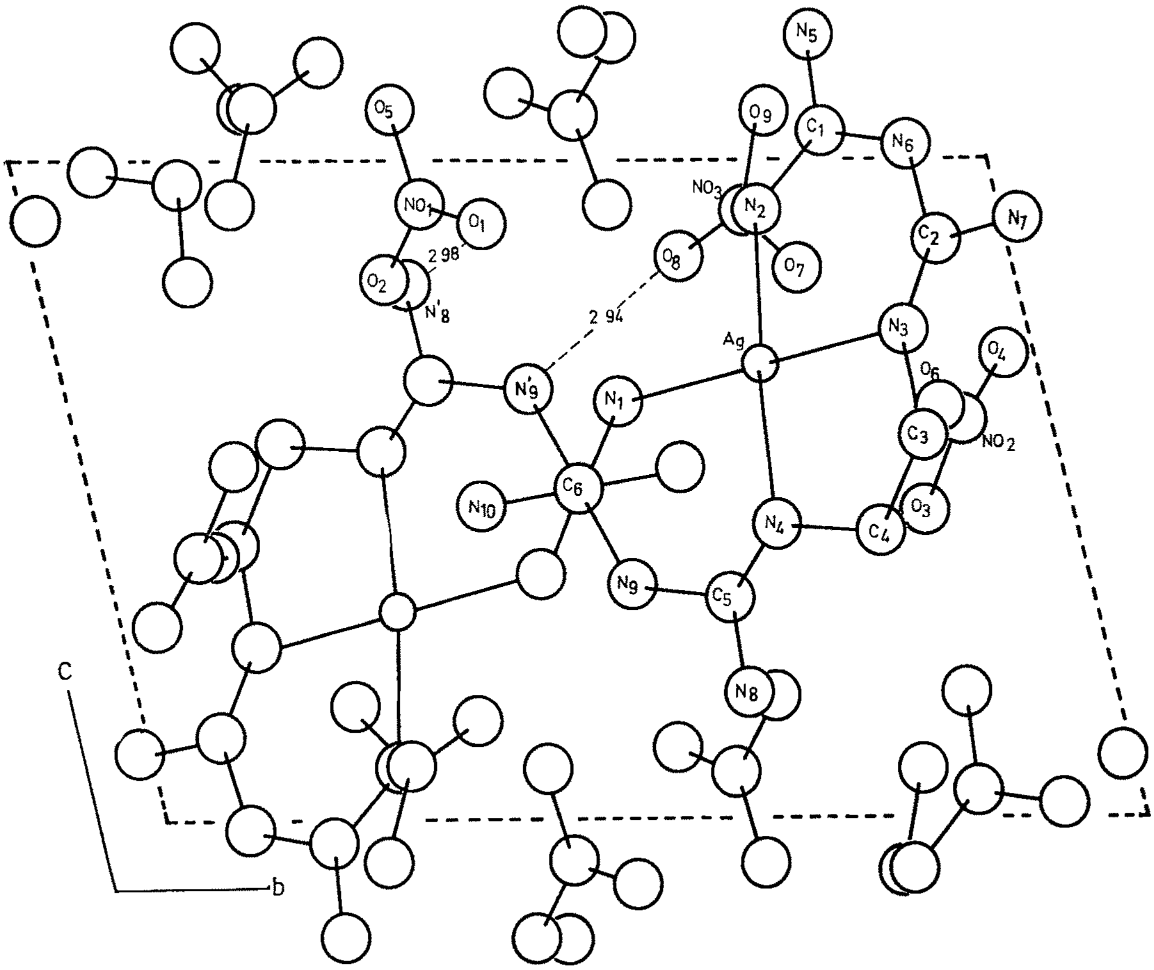

Fig. 1. A diagrammatic representation of the structure projected down the *a* axis

of these ninety-seven were too weak to be measured. The stationary crystal–stationary counter technique was used for the intensity measurements. Absorption and the usual $1/LP$ corrections were applied.

To solve the structure, the co-ordinates of silver were obtained from a 3-D Patterson synthesis. A silver-phased 3-D Fourier synthesis then carried out revealed the positions of all the light atoms. The structure was then refined in three cycles by the least-squares method with individual anisotropic temperature factors and a block diagonal approximation. The final agreement index R was 0.105 for all observed reflexions.

A diagrammatic representation of the structure, along the *a* axis, is shown in Fig. 1, and the various bond lengths and angles are illustrated in Fig. 2. The estimated standard deviations in the various bond lengths and angles are Ag—N = 0.012 Å, C—N, C—C and N—O are 0.02 Å and 0.8° for various angles. A molecule of ethylenebidiguanine acts as a tetradentate ligand and forms two co-ordinate and two covalent bonds through the four nitrogen atoms. This results in one five-membered and two six-membered rings forming a chelate compound. Table 1 shows the results of calculations to test the planarity of these rings.

Table 1 shows that the five-membered and the two six-membered rings are planar. The angle between plane 2 and 3 is 138.3°, between 2 and 4 is 146.4° and between 3 and 4 is 150.8°. So the molecule is not planar as in the analogous copper complex[4] and as expected from the previous chemical work[1]. The non-planarity of the three rings results in the distortion of the square-planar configuration of silver as can be seen in Table 1.

All three independent nitrate ions are planar and their dimensions have normal values. The two nitrate ions are hydrogen bonded to the silver complex ion (Fig. 1).

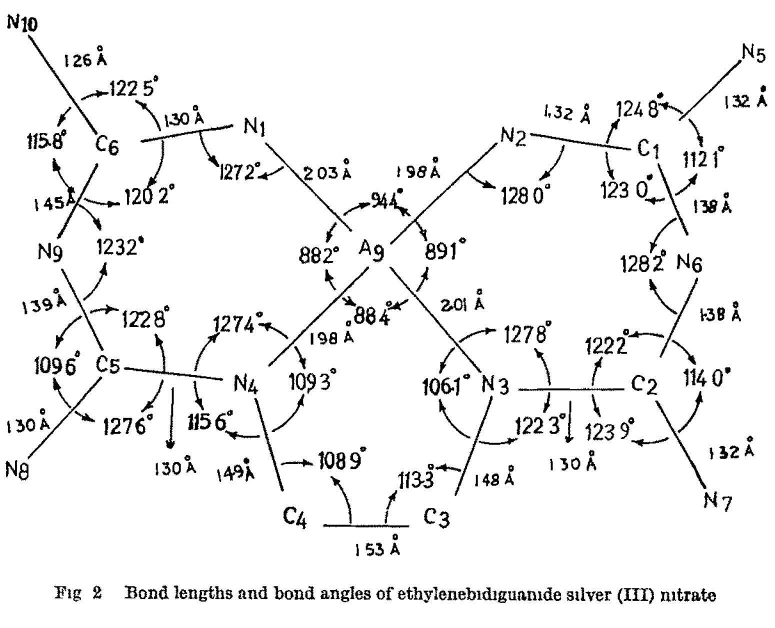

Fig. 2. Bond lengths and bond angles of ethylenebidiguanide silver (III) nitrate

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[1] Ray, P , and Ghosh, J *Indian Chem Soc* , 20, 291 (1943)
[2] Ray, P , and Chakravarty, J *Indian Chem Soc* , 21, 47 (1944)
[3] *International Tables for X-ray Crystallography*, 3 (Kynoch Press, Birmingham, 1962)
[4] Kunchur, N R , and Mathew, M , *Chem Commun* , 86 (1966)

Crystal Structure and the Lower Phase Transition in Solid Deuterium Bromide

The study of crystal structure and phase transition in hydrogen and deuterium chlorides reported in two previous communications[1,2] has now been extended to cover the temperature range 4°–150° K A comprehensive communication reporting on these investigations is in preparation Meanwhile, the structure investigations are continuing to cover the remaining hydrogen and deuterium halides

It has been known from specific heat measurements[3,4] that solid deuterium bromide undergoes two second-order phase transitions, both spread over temperature ranges of several degrees (lambda transitions) The lower transition is completed at about 93 5° K, the upper transition at about 120 3° K This behaviour is markedly different from that of hydrogen bromide[5], which has three second order transitions in the solid state The two upper transitions are very close to one another, the one being completed at about 113 6° K, the other at about 116 9° K

X-ray powder photographs taken of solid hydrogen bromide by different researchers have been interpreted differently Ruhemann and Simon[6] suggested that hydrogen bromide has a face-centred orthorhombic lattice over the entire solid range Natta[7], however, claimed that in addition to the face-centred orthorhombic phase there is also a face-centred cubic phase restricted to a narrow temperature range between the two upper phase transitions We think that both conclusions were erroneous The suggestion of Ruhemann and Simon was based on the presence of a few persistent low angle lines which were probably caused by impurities On the other hand, Natta's claim regarding the temperature range of the face-centred cubic phase can be attributed to the large uncertainty of his temperature estimates and to his apparent lack of X-ray data above 120° K These early X-ray studies revealed only the location of the bromine atoms but gave no clue regarding the location of the hydrogen atoms

Subsequent experimental investigations using dielectric constant[8–11], optical[12], spectroscopic[13–24], nuclear magnetic resonance[25], and inelastic neutron scattering[26] techniques led to the conclusion that below the lowest transition region, both hydrogen bromide and deuterium bromide have an ordered structure composed of hydrogen-bonded zigzag chains Various structural models of this type were put forward, those consisting of antiparallel chains being more favoured, following earlier theoretical predictions The higher phases were thought to be associated with various kinds of disorder in the orientation of the molecules Though the type of orientational disorder could not be specified unambiguously, it was generally agreed that the molecules could not rotate freely in any of the solid phases No suggestion has been put forward to account for the two upper transitions in hydrogen bromide

To resolve these ambiguities, the crystal structure of solid deuterium bromide was investigated by X-ray and neutron powder diffraction methods using the experimental techniques described in previous communications[1,2] X-ray powder photographs taken at 86°, 92°, 95°, 107° and 118° K showed that the bromine atoms form a face-centred orthorhombic lattice over the entire 86° K to 118° K temperature range, that is, right up to the end of the upper transition region The volume of the face-centred orthorhombic unit cell increases with the temperature over the entire range investigated The total volume increase over the lower transition region (stretching from about 74° K to about 93 5° K) is approximately 1 4 per cent and it is strongly anisotropic only the a and b parameters increase, the c parameter decreases A conspicuous feature of all X-ray powder photographs taken in the 86°–118° K range is the blurring of the medium and high angle lines They are markedly different in appearance from the much sharper X-ray powder lines recorded above the upper transition region

The unit cells of deuterium bromide derived from the neutron powder diffraction patterns recorded at 84° K and 107° K agreed in size with the face-centred orthorhombic unit cells derived from the X-ray powder photographs, but their symmetries were different at both temperatures The space group of deuterium bromide at 84° K was found to be $Bb2_1m$—the same as that of the ordered low temperature phase of deuterium chloride[1,2] On the other hand, the space group of deuterium bromide at 107° K was found to be $Bbcm$ Neutron diffraction patterns recorded at 88° K and at 91° K showed a gradual change from space group $Bb2_1m$ to space group $Bbcm$ This was particularly conspicuous in the case of the (101) peak the intensity of which decreased rapidly with increasing temperature until it disappeared altogether above the lower transition region This is illustrated in Fig 1 where the absolute values of the $F(101)$ and $F(111)$ structure factors observed at four different temperatures are plotted The specific heat of deuterium bromide[3,4] over the lower temperature region is shown in Fig 2

These findings can be interpreted as follows Deuterium bromide below the lower transition region is isomorphous with the low temperature phase of deuterium chloride[1,2], that is, it has an ordered crystal structure composed of parallel plane zigzag chains The results of the least-squares refinement of this model are summarized in Table 1

The crystal structure of deuterium bromide in the "middle phase" lying between the two transition regions is disordered The disorder is orientational each molecule has two equilibrium orientations, one is the same as in the ordered phase, the other opposite to it The unit cell of this disordered middle phase is shown in Fig 3 The results of the least-squares refinement of this model are summarized in Table 2

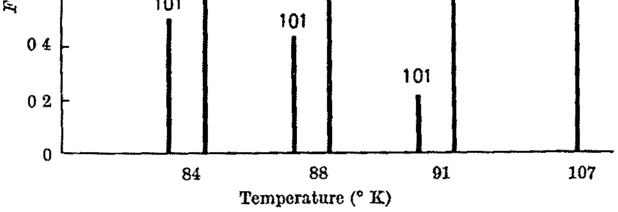

Fig 1 Plot of the absolute values of $F(101)$ and $F(111)$ structure factors for deuterium bromide observed at 84° K, 88° K, 91° K and 107° K

Table 1. STRUCTURAL DATA OF DEUTERIUM BROMIDE AT 84° K

Lattice parameters

$a=5.44\pm0.02$ Å, $b=5.614\pm0.005$ Å, $c=6.120\pm0.005$ Å

Space group *Bb*2$_1$*m*, No. of molecules in the unit cell 4

Fractional positional parameters of the atoms

	x	*y*	*z*
Deuterium	0.075 ± 0.002	0.179 ± 0.003	0
Bromine	0.25	0	0

Isotropic temperature factors of the atoms (*B* in Å^2)

Deuterium	5.0 ± 0.5	Bromine	2.1 ± 0.4

D–Br bond length 1.38 ± 0.03 Å
Br–Br–Br angle in the chain 91° 48′ ± 10′
R factor after six cycles of isotropic refinement $R_1=4.5$ per cent

Table 2. STRUCTURAL DATA OF DEUTERIUM BROMIDE AT 107° K

Lattice parameters

$a=5.559\pm0.005$ Å, $b=5.649\pm0.005$, $c=6.095\pm0.005$ Å

Space group *Bbcm*, No. of molecules in the unit cell 4

Fractional positional parameters of the atoms

	x	*y*	*z*
Deuterium	−0.172 ± 0.006	+0.177 ± 0.004	0
Bromine	0	0	0

Isotropic temperature factors of the atoms (*B* in Å^2)

Deuterium	8.4 ± 0.8
Bromine	4.4 ± 0.8

D–Br bond length 1.38 ± 0.04 Å
Br–Br–Br angle in the chain 90° 56′ ± 4′
R factor after six cycles of anisotropic refinement $R_1=6.1$ per cent

Because of the small difference between the lattice parameters *a* and *b* in the middle phase, the powder peaks considerably overlap in this region. This explains the somewhat higher value of the *R* factor and the great uncertainties of the temperature factors. The positional parameters, however, are expected to be accurate within the limits stated.

In the lower transition region, deuterium bromide forms a mixed crystal the two components of which are the ordered low temperature and the disordered middle phases. The composition of the mixed crystal varies continuously with temperature and the two components are miscible in all proportions. In other words, the crystal structure of deuterium bromide in the lower transition region is a substitutional solid solution of two "types" of deuterium bromide molecules. One type has a single equilibrium orientation, characteristic of the ordered low temperature phase, the other type has two equilibrium orientations, characteristic of the disordered middle phase. The two types of molecules form a continuous series of solid solutions, which follow Vegard's law and whose composition varies continuously with temperature. The volume requirements of the two types of molecules differ by less than 1.4 per cent which does not prevent the formation of an apparently homogeneous phase over the lower transition region; nevertheless it is sufficient to give rise to mechanical strains in the specimen. This explains the blurred character of the X-ray powder lines in this region. It also suggests that the distribution of the two types of molecules among equivalent lattice sites is not entirely random. It is more likely that molecules of the same type form small domains dispersed over the entire specimen.

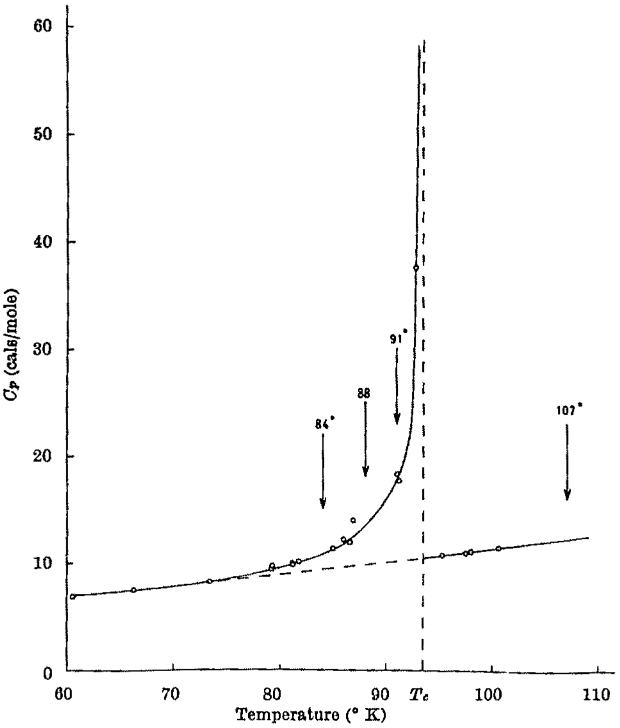

Fig. 2. The specific heat of deuterium bromide over the temperature range 60° K to 100° K. The arrows indicate the temperatures at which the structure factors in Fig. 1 were recorded. The vertical dashed line marks the end of the lower transition region. The slightly sloping dashed line gives an idea of the temperature range in which the specific heat is "anomalously" high.

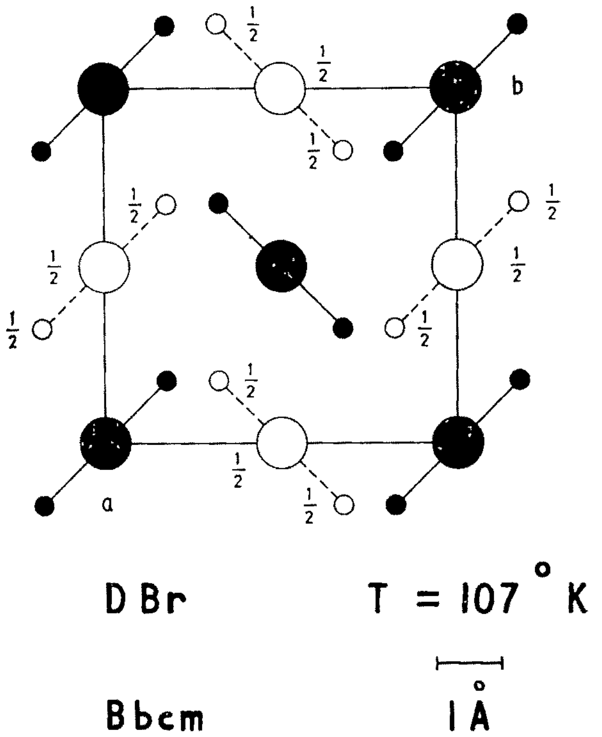

Fig. 3. The unit cell of the disordered middle phase. ●, The equilibrium position of bromine atoms in the plane of the page, ○, the equilibrium position of bromine atoms at $z=c/2$, •, the equilibrium position of deuterium atoms in the plane of the page, and ∘, the equilibrium position of deuterium atoms at $z=c/2$.

Because in the disordered middle phase $F(101)=0$, the composition of the solid solution can be estimated by taking the ratio of the absolute value of $F(101)$ observed at a given temperature to the absolute value of $F(101)$ calculated for the ordered low temperature phase. This ratio is expected to give the ratio of the number of deuterium bromide molecules in the ordered low temperature phase to the total number of deuterium bromide molecules in the specimen. The values of this ratio at 84°, 88°, 91° and 107° K, expressed in percentages, are shown in Fig. 4. The dashed curve in Fig. 4 should only be considered as indicative of the composition of the solid solution in the lower transition region for two reasons. First, the number of observations is too small, second, deuterium bromide is expected to show thermal hysteresis in the lower transition region similar to that observed in hydrogen bromide[27]. Thus the composition of the solid solution may depend not only on the temperature but also on the direction in which the lower transition region is traversed. The data in Fig. 4 were obtained when the region was traversed in the direction of increasing temperature. It is interesting to compare the lower transition of deuterium bromide with the single transition in deuterium

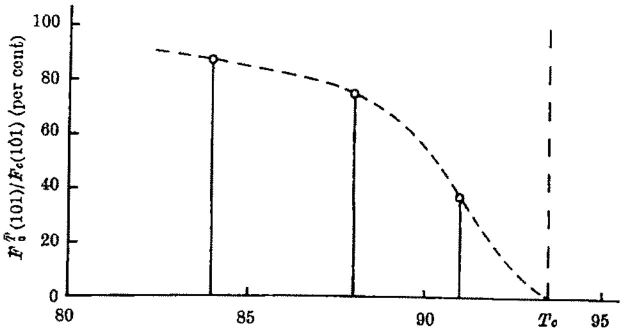

Fig 4 The ratio of the absolute value of $F(101)$ observed at a given temperature to the absolute value of $F(101)$ calculated for the ordered low temperature phase The ratio should give the ratio of the number of deuterium bromide molecules in the ordered low temperature phase to the number of deuterium bromide molecules in the specimen The ratio is expressed as a percentage Temperatures 84° K, 88° K, 91° K and 107° K

chloride[1,2] On the molecular level both transitions are discontinuous—the molecule is either in one state or in another—and both are accompanied by a discrete change in the volume requirement of the molecule But while in deuterium chloride the transition from the low temperature phase with a single equilibrium orientation into the high temperature phase with twelve equilibrium orientations is accompanied by a volume change of about 1 9 per cent (in preparation) and the transition takes place within a temperature region much narrower than 1° K, in deuterium bromide the transition from the low temperature phase with a single equilibrium orientation into the middle phase with two equilibrium orientations is accompanied by a volume change less than 1 4 per cent and the transition of all molecules in the specimen is spread over a temperature range of some 20° K

Although the X-ray and neutron diffraction work described in this communication refers to deuterium bromide, the available experimental evidence suggests that the results obtained are applicable to the lower transition region of hydrogen bromide as well The investigation of a possible hysteresis effect both in the orthorhombic lattice parameters and in the composition of the solid solution over the lower transition region is in progress, so is the study of the crystal structure of both hydrogen and deuterium bromides in the upper transition regions

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[1] Sándor, E , and Farrow, R F C , *Nature*, 213, 171 (1967)
[2] Sándor, E , and Farrow, R F C , *Nature*, 215, 1265 (1967)
[3] Clusius, K , *Z Naturforsch* , 1a, 142 (1946)
[4] Clusius, K , and Wolf, G , *Z Naturforsch* , 2a, 495 (1947)
[5] Giauque, W F , and Wiebe, R , *J Amer Chem Soc* , 50, 2193 (1928)
[6] Ruhemann, B , and Simon, F , *Z Phys Chem* , B, 15, 389 (1932)
[7] Natta, G , *Gaz Chim Ital* , 63, 425 (1933)
[8] Smyth, C P , and Hitchcock, C S , *J Amer Chem Soc* , 55, 1830 (1933)
[9] Damkohler, G , *Ann Phys* , [5], 31, 76 (1938)
[10] Powles, J G , *J Phys Rad* , 13, 121 (1952)
[11] Brown, N L , and Cole, R H , *J Chem Phys* , 21, 1920 (1953)
[12] Kruis, A , and Kaischew, R , *Z Phys Chem* , B, 41, 427 (1938)
[13] Callihan, D , and Salant, E O , *J Chem Phys* , 2, 317 (1934)
[14] Hornig, D F , and Osberg, W E , *J Chem Phys* , 23, 662 (1955)
[15] Hornig, D F , and Hiebert, G L , *J Chem Phys* , 27, 752 (1957)
[16] Hiebert, G L , and Hornig, D F , *J Chem Phys* , 28, 316 (1958)
[17] Anderson, A , Walmsley, S H , and Gebbie, H A , *Phil Mag* , 7, 1243 (1962)
[18] Friedrich, H B , and Person, W B , *J Chem Phys* , 39, 811 (1963)
[19] Friedrich, H B , Yamada, H , and Person, W B , *J Chem Phys* , 43, 4180 (1965)
[20] Anderson, A , Gebbie, H A , and Walmsley, S H , *Mol Phys* , 7, 401 (1964)
[21] Savoie, R , and Anderson, A , *J Chem Phys* , 44, 548 (1966)
[22] Brunel, L C , and Peyron, M , *J Chim Phys* , 63, 181 (1966)
[23] Brunel, L C , and Peyron, M , *CR Acad Sci* , *Paris*, 264, 930 (1967)
[24] Arnold G M , and Heastie, R , *Chem Phys Lett* , 1, 51 (1967)
[25] Alpert, N L , *Phys Rev* , 75, 398 (1949)
[26] Boutin, H , and Safford, G J , in *Inelastic Scattering of Neutrons*, 2, 393 (1965)
[27] Eucken, A , and Gottner, W , *Nachr Ges Wiss* , *Gottingen, Math Phys Kl* Fachgr II, 167 (1937)

CHEMISTRY

Activation of Small Molecules by Transition Metal Ions and their Complexes

A QUALITATIVE, symmetry based view of the nature of the bonding in so-called π-complexes of unsaturated organic molecules with transition metals was put forward by Dewar[1] and Chatt[2] Simultaneous charge transfer from filled ligand orbitals to unfilled metal orbitals and from essentially non-bonding metal orbitals to antibonding ligand orbitals forms the basis of a model which successfully explains, for example, the change in the C—O stretching frequency of co-ordinated carbon monoxide compared with that of the free ligand The relative importance of "forward donation" and "back-donation" is not a matter for generalization; a theory of the bonding in Zeise's salt suggests that they are comparable[3] even for such a relatively poor acceptor as ethylene

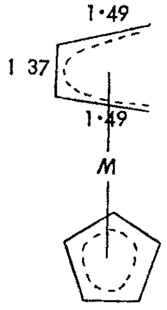
Largely as a result of crystal structure determinations during the past year or so, the geometries of co-ordinated ligands are now much better known As such, the implication of the Chatt–Dewar model—that the wave function describing the bonding in metal complexes will contain functions representing excited states of both the metal and ligands—can be examined in some detail by citing some of the structural data on carbon disulphide, acetylene, butadiene and oxygen complexes of transition metals in low valence states

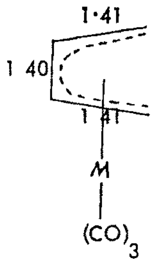
The structural analysis, by X-ray diffraction methods, of bistriphenylphosphine platinum carbon disulphide shows[4] the co-ordinated carbon disulphide to be non-linear, the S—C—S bond angle being 136°, the mean C—S bond length in the co-ordinated molecule is 1 63 Å Carbon disulphide, in its electronic ground state, is linear with a C—S bond length of 1 55 Å; the lowest excited state ($^3A_2$) of CS_2 is bent—the angle being 135° and the mean C—S bond length 1 64 Å (ref 5) These values are identical, within experimental error, to those found in the platinum complex A qualitative view of the change in geometry of carbon disulphide on excitation follows from Walsh's rules, the principal assumption of which is that a molecular orbital of an AB_2 system has lower energy if, on changing the bond angle at the central atom A, the molecular orbital changes from being built up from a p-type atomic orbital on A to one built up from an s-type atomic orbital on A (ref 6) That bending of the molecule lowers the π^* energy level by an electron volt or so presumably has an equivalent in the metal complex in so far as overlap of metal d-orbitals with this modified antibonding orbital is larger

Ingold and King[7] were the first to recognize that the geometry of a molecule in its excited state can be very different from that in its electronic ground state They found that the ^1A_u excited state of acetylene was *trans*-bent with a C—C—H bond angle of 120° and a C—C bond length of 1 38 Å, the *trans*-bent geometry is stabilized to some extent, compared with a *cis*-bent structure, through the minimization of non-bonded repulsion interactions. In both bistriphenylphosphine platinum diphenylacetylene[8] and hexacarbonyldiphenylacetylenedicobalt[9] the diphenylacetylene is *cis*-bent, the C—C—Ph bond angle averaging 140° and the C—C bond length 1·39 Å. In these complexes, non-bonded interactions with the metal will favour *cis*-bending. Kroto and Santry[10] have suggested that a *cis*-bent excited state of acetylene has a bond angle of 142° and a carbon–carbon bond length of 1·38 Å. These values are again identical, within experimental error, to those observed in the metal complexes.

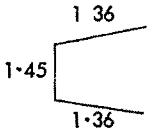
Unfortunately, there is no structural analysis available of ethylene co-ordinated to a transition metal which has the accuracy of the other determinations discussed here. This is a pity because there is some evidence that an excited state of ethylene has a geometry in which the molecule distorts into a pyramidal configuration for the atoms surrounding each carbon atom. This is the essential stereochemistry of the co-ordinated carbon atoms in bistriphenylphosphine platinum tetracyanoethylene[11] and racemic iron tetracarbonylfumaric acid[12].

The geometry of butadiene or quasi-butadiene complexes of metals has recently been reviewed[13]. The molecules fall into two distinct classes in which the butadiene fragment is respectively *trans* to the cyclopentadienyl or to the tricarbonyl group. The mean dimensions (±0·03 Å) of the butadiene ligand in these two examples are

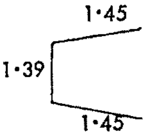




The Huckel molecular orbital predictions of the geometry of butadiene in its ground and lowest excited states are



Ground state

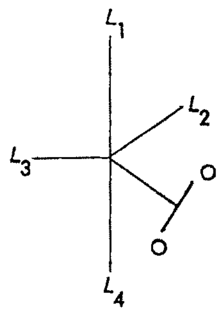


Excited state

Excitation leads to a change in bond alternation which is also found in the π-cyclopentadienyl metal butadiene complexes; the butadiene ligand *trans* to the tricarbonyl group has an intermediate structure, the geometry being roughly one corresponding to a superposition of the electronic ground and lowest excited states of the free ligand. These results are in keeping with the fact that the carbon monoxide ligand is a much better acceptor than π-cyclopentadienyl and are a useful reminder of the importance of the nature of *trans*-ligands in a complex (see later).

Discussion of the bonding of molecular oxygen to transition metals is not new, but four recent and precise structural investigations seem to add a good deal to the understanding of the problem.

The oxygen molecule in decammine-μ-peroxo-dicobalt monosulphate *tris*-(bisulphate)[14] bridges the cobalt atoms so that the Co—O—O—Co group is planar, the O—O distance being 1·31 Å. This is very close to the bond length in the O_2^- ion (1·28 Å) and a good deal less than that of the peroxide ion (1·49 Å). A quite different mode of bonding is found in some mononuclear iridium complexes[15–17]. The oxygen molecule occupies an equatorial



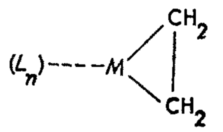
position in a trigonal bipyramidal arrangement around the iridium, the oxygen–oxygen bond length varying considerably. It is 1·30±0·03 Å when $L_1=L_4=Ph_3P$, $L_2=Cl$ and $L_3=CO$, 1·51±0·03 Å when $L_1=L_4=Ph_3P$, $L_2=I$ and $L_3=CO$ and, finally, in a chelating phosphine complex $L_1L_2=L_3L_4=Ph_2PCH_2CH_2PPh_2$, the O—O distance is 1·65±0·02 Å. This last distance, in particular, points out very clearly the shortcomings in discussions of the co-ordinated oxygen molecule in terms of $O_2{}^{n-}$ in this type of complex. If the bonding of oxygen is similar to those in the other π-complexes we could imagine that its structure would approximate one corresponding to the excitation

$$\pi^4\ \pi^{*2} \rightarrow \pi^3\ \pi^{*3}$$

in the free molecule. The precise amount of electron density transferred to antibonding orbitals on the oxygen through the intermediacy of the metal will depend on the remaining ligands in the complex. The two identified excited states arising from the $\pi^3\ \pi^{*3}$ configuration are the $^3\Sigma_u^{\pm}$—the weak Herzberg and stronger Schuman–Runge systems. The O—O distance in the $^3\Sigma_u^+$ state is 1·42 Å but is 1·60 Å in the $^3\Sigma_u^-$ state[18]; this latter value is close to the value in the bis-chelating phosphine complex. The variation in the O—O distance reflects the presence of the strong π-accepting carbonyl ligand in two of the complexes while the difference between the chloro- and iodo-complexes is a comment on their different donor and acceptor relations with the metal. Correlation effects are largely responsible for the difference in geometry between the $^3\Sigma_u^+$ and $^3\Sigma_u^-$ states of oxygen, but other correlation effects must intervene in the metal complexes—they are diamagnetic—and it is not obvious why it is the $^3\Sigma_u^-$ state which seems to approximate the geometry in the metal complex. The $^1\Sigma_u^-$ excited state of oxygen must have a similar internuclear distance to that of the $^3\Sigma_u^-$ state, but emphasis on the precise spin and orbital character of the co-ordinated ligand is misplaced to some extent. In all complexes, the metal ligand–bond energy must be more than comparable with ligand correlation energies.

It is interesting to examine the possible reasons for the two extreme types of bonding of oxygen—the σ-bonded species in binuclear complexes and the π-bonded arrangement in mononuclear systems (the number of mononuclear complexes with "unidendate" oxygen is likely to be very small indeed). When the transition metal ion is in a relatively high oxidation state, *d* orbitals will be considerably contracted compared with that in the zerovalent state; overlap with antibonding π* levels on the oxygen will be small and a σ-bonded arrangement of the kind found in the dicobalt peroxo-species will be favoured. In complexes such as the iridium ones mentioned earlier and bistriphenylphosphine nickel—and platinum—oxygen, the π-bonded arrangement will be energetically preferred.

The correspondence between a simple valence bond description of the structure of, say, ethylene complexes



where one imagines localized metal–carbon σ-bonds, the formation of a three membered ring and rehybridization of the carbon atoms, and that which emerges from a molecular orbital model, has been emphasized for the particular example of butadiene complexes[19] The valence bond model is less satisfactory because it is not useful in predicting the changes of geometry consequent on the variation of the *trans* ligands and/or substituents in the ligand itself

The ligand geometries can be discussed in terms of their polarization by the electric field of the metal ion, concepts such as the "softness" of the ligand can also be introduced but are not considered to be particularly helpful

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[1] Dewar, M J S , *Bull Soc Chim France*, **18**, C71 (1951)

[2] Chatt, J , *J Chem Soc* , 2939 (1953)

[3] Moore, J W , *Acta Chem Scand* , **20**, 1154 (1966)

[4] Baird, M , Hartwell, G , Mason, R , Rae, A I M , and Wilkinson, G , *Chem Commun* , 92 (1967)

[5] Kleman, B , *Canad J Phys* , **41**, 2034 (1963)

[6] Walsh, A D , *J Chem Soc* , 2260 (1953)

[7] Ingold, C K , and King, G W , *J Chem Soc* , 2702 (1953)

[8] Glanville, J O , Stewart, J M , and Grim, S O , *J Organometall Chem* , **7**, P9 (1967)

[9] Sly, W G , *J Amer Chem Soc* , **81**, 18 (1959)

[10] Kroto, H W , and Santry, D P , *J Chem Phys* , **47**, 792 (1967)

[11] Belluco, U , Giustiniani, M , and Uguagliati, P , *Abst Symp on Valence and Reactivity*, Oxford, p 56 (1968)

[12] Corradini, P , Pedone, C , and Sirigu, A , *Chem Comm* , 341 (1966)

[13] Churchill, M R , and Mason, R , *Adv Organometallic Chem* , **5**, 93 (1967)

[14] Schaefer, W P , and Marsh, R E , *Acta Cryst* , **21**, 735 (1966)

[15] LaPlaca, S J , and Ibers, J A , *J Amer Chem Soc* , **87**, 2581 (1965)

[16] McGinnety, J A , Doedens, R J , and Ibers, J A , *Science*, **155**, 709 (1967)

[17] McGinnety, J A , and Ibers, J A , *Abs Amer Cryst Assoc* , 24 (1967)

[18] Herzberg, G , *Spectra of Diatomic Molecules*, 559, 560 (Van Nostrand, 1950)

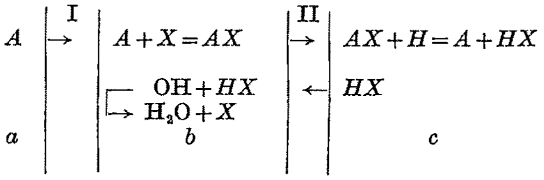
[19] Kettle, S F A , and Mason, R , *J Organometallic Chem* , **5**, 97 (1966)

Transport of Ions against their Concentration Gradients in an Analogue System for Active Transport using Charged Membranes

To transport an ion against its concentration gradient requires energy, which in the biological membrane is supplied by metabolic or chemical reactions By the Curie–Prigogine principle of non-equilibrium thermodynamics, a vector flow of ions resulting from chemical coupling can only be achieved in an asymmetric membrane system[1-3] This is a consequence of the different tensorial order of the chemical and diffusional thermodynamic forces, which are scalar and vectorial, respectively Any membrane system which will transport ions against their electrochemical potential gradients using a chemical reaction as a driving force must therefore be asymmetric in the direction of active flow containing, for example, two membranes of different character through which the ions must pass

A common assumption in the theories of active transport is that the ion transported is at some stage attached to an activated carrier molecule which, on passing the membrane, is deactivated and releases the free ion The most remarkable feature of biological systems is the extremely high specificity of the active transport or pump mechanism for a particular ion, sodium, calcium and other ion pumps are well known In the simple physical model system described here, no attempt could be made to parallel such specificity or to use an ion for which a known biological pump exists The object was simply to construct a pump mechanism for an ion, using a known chemical reaction as a driving force, a specific carrier mechanism and charged membranes of known characteristics This exercise was thought to be useful because known biological pumps occur in very complex systems, where the physical nature of the membrane, the carrier mechanism and the metabolic driving reaction are not sufficiently well known

In outline the model pump consists of a two membrane system and contains three solutions, a, b and c Membrane I is a simple cation exchange membrane which is effectively impermeable to anions The cation to be pumped, A, is in the simple aquo or uncomplexed form in solutions a and c In b, however, it is complexed by the anion of a weak acid with which it forms an uncharged species AX There is thus set up a concentration gradient for the A ion across membrane I, driving this ion into solution b When A passes into solution b it is complexed by an excess of the ligand X so that effectively no free A is present Membrane II is designed to allow AX to pass through into solution c while retaining in b the anionic complexing ligand X, it is therefore a cation exchanger on the side facing solution b On passing through II, by diffusion, into solution c the complex AX is destroyed by strong acid, releasing the free ion A and associated weak acid HX To prevent back diffusion of A and free hydrogen ion, membrane II is an anion exchanger on the side facing solution c. The membranes are so designed that they will act as one way "valves" allowing only those ions involved in active transport to pass, retaining in b the complexing ligand and in c the transported A and hydrogen ion As this system operates, a concentration of associated HX will build up in solution c and back diffusion of this acid into b will become important Again we assume that membrane II is impermeable to ions but not to neutral species Because b contains the salt of the weak acid, it is alkaline If we therefore consider a system in which the pHs of b and c are maintained, a steady state will be reached such that the concentrations of HX in c and anion X in b will remain constant In such a system the net chemical reaction will be the reaction of hydrogen and hydroxyl ions to form water



In principle also it may be noted that the role of the weak acid may be replaced by a suitable redox system in which the anionic ligand is created in b and destroyed in c by the action of oxidants in b and reductants in c

In the simple physical system described here cupric ion was chosen as the ion to be actively transported, using as carrier the neutral acetate complex CuAc$_2$ In solutions a and c the copper ion is in the uncomplexed form in a medium of sodium nitrate Solution c, however, contains nitric acid initially 0 1 molar and acetic acid equal to that required in solution b The central compartment b contains the complexing acetate, present as sodium acetate, of equal concentration to the sodium nitrate of a and c plus 0 02 molar acetic acid to prevent copper precipitation as hydroxide Solution b has pH 9 The concentration of acetate was chosen as 0 46 molar to give maximum concentration of the complex $CuAc_2$ (ref. 4) which is in practice close to 50 per cent

Membrane I is a commercial cation exchanger (AMF *C* 103, supplied by American Machine and Foundry Co) and is highly impermeable to anions Membrane II is a supported membrane of hydrous zirconia held in the pores of a plastic PVC frit Zirconia is an amphoteric ion exchanger which has cation exchange properties in base and anion exchange in acid solutions and negligible exchange characteristics in neutral solutions[5] The bathing solutions *b* and *c* are, respectively, basic and acidic, so the zirconia behaves as a sandwich of cation and anion exchangers It was chosen in preference to a pair of commercial exchangers because of its rather more open structure Such a double membrane will effectively present a barrier to both anions and cations while allowing "normal" permeability to uncharged species, this permeability being some function of the membrane structure and independent to a first degree on the membrane charges

The experimental apparatus consisted of a 'Perspex' cell with three compartments, *a*, *b* and *c*, provided with mechanical stirrers, and with working volumes of 115 ml each. These compartments were separated by membranes I and II, which had cross-sectional areas of 5 cm^2 Experiments were carried out at 20°±3° C Copper ion was analysed spectrophotometrically in 2 molar ammonia solution at a wavelength of 6300 Å[6], sodium by flame-photometry and total cation content by ion exchange for hydrogen ion and titration

Two preliminary experiments were carried out on the reduced system solution *a*, membrane I, solution *b*, and solution *b*, membrane II, solution *c* In the first experiment, the total copper concentrations in *a* and *b* were equal at 0 02 molar Analysis showed that cupric ion left solution *a* and entered *b* at a rate which was almost steady during a period of days, while sodium was transported in the opposite direction, from *b* to *a*, in amounts equivalent to those of copper ions This was verified by a constant total cation analysis of solution *a* during the experiment, indicating that transport of one copper ion from *a* was accompanied by transport of two sodium ions into *a* These results are summarized in Table 1 and Fig 1

In the second experiment, a small increase of 3 per cent in the total copper ion in *c* was observed after a period of 2 days and no net sodium ion transport was detected Analysis of the acid content of *c* showed that twenty times as much acid was used up in this period as would be required to destroy the copper complex transported, but it was equal to the decrease in the base concentration of *b*. This consumption of acid and base results from the interaction of these ions to form water within the zirconia membrane

To achieve an almost steady state concentration of copper ion in *b* during experiments on the complete cell, the initial total concentration of copper in *b* was increased to 0 04 molar Over a period of 7 days there was copper transport from solution *a* to *c*, during which the *p*Hs of *b* and *c* were maintained approximately constant by the addition of 20 mequiv. of sodium hydroxide or nitric acid in a total volume of 2 ml added in small amounts over the period of the experiment After 5 days, solution *a* was considerably depleted of copper and was replaced with fresh original solution The total copper concentration in *b* remained constant in the last 2 days of the experiment so that the number of copper ions leaving solution *a* equalled those reaching *c* in the same period Analysis for sodium again showed that sodium ion transport from *b* was coupled with copper transport in the opposite direction The removal of copper ion from solution *a* leaves that solution negative with respect to *b* and sets up an electrical potential gradient across the membrane I The gradient can only be reduced by sodium transport with the electrical gradient from *b* to *a* because this cation exchanger is effectively impermeable to anions Analysis of the sodium contents of *b* and *c* shows that there is a small sodium flow from *c* to *b* by salt diffusion through membrane II These results are summarized in Table 2 and Fig 1.

Table 1

Time (days)	Copper ions transported (mequiv)		Sodium ions transported (mequiv)	
	a	*b*	*a*	*b*
0 89	−0 712	+0 666	—	—
3 90	−2 42	+2 56	−2 5	−2 5

Table 2

Time (days)	Solution (ml)	Concentrations of copper (molar)			Copper ions transported (mequiv)		
		a	*b*	*c*	*a*	*b*	*c*
0	115	0 0200	0 0400	0 0200	0	0	0
4	115	0 0100	0 0450	0 0245	−2 30	+1 15	+1 04
5	110	0 0080	0 0470	0 0255	−2 74	+1 59	+1 26
6	105	0 0183	0 0480	0 0265	−3 17	+1 79	+1 47
7	100	0 0166	0 0480	0 0275	−3 44	+1 79	+1 67

In this simple system it is therefore possible to use a net chemical reaction to cause the directed flow of cupric and sodium ions : copper ion by virtue of its ability to form a neutral acetate complex and sodium by non-selective electric coupling with the copper ion flow The section membrane I, solution *b*, membrane II, parallels the function of the biological membrane, at least in principle, and contains the essential asymmetry required by the Curie–Prigogine principle In the case of the frog epithelium, which actively transports sodium ion, the bathing solutions need not be other than chemically identical so that the membrane itself must contain all the ingredients necessary for the specific active transport of sodium ion and so include within itself the whole metabolic and carrier chemistry Unpublished results of H H Ussing have shown that the frog epithelium has the property of a degree of electrolytic rectification , it conducts electric current more easily in one direction than in the other This is also a general property of a sandwich of cation and anion exchangers

In this simple analogue system zirconia plays the same part because absorbed salt depletion or concentration within the membrane causes its resistance to increase or decrease according to the direction of current In the initial solution conditions of the experiment on the com-

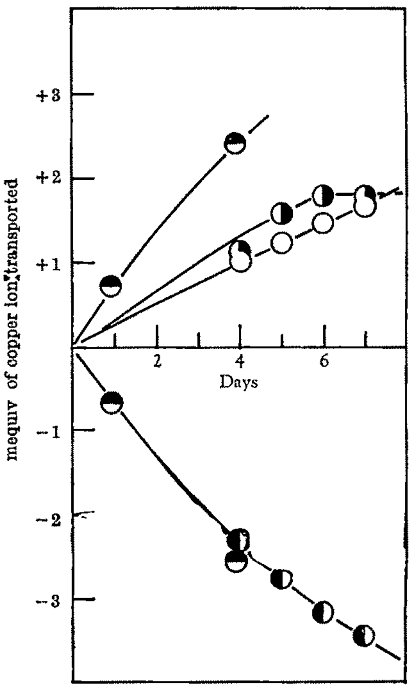


Fig. 1 ◓, Experiment I, ◐, *a* experiment III, ◑, *b* experiment III, ○, *c* experiment III posite membrane system a current of 18 or 26 mamp flowed between copper electrodes in *a* and *c* under a constant 6 V supply dependent on the direction of the applied field This analogy may point to the more general validity of this type of ion barrier in the biology of active transport

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[1] Curie, P, *Oeuvres*, 129 (Gauthier-Villars, Paris, 1908)

[2] de Groot, S R, and Mazur, P, *Non Equilibrium Thermodynamics* (North-Holland, Amsterdam, 1962)

[3] Fitts, D D, *Non Equilibrium Thermodynamics* (McGraw-Hill, New York, 1962)

[4] Sillén, L G, and Martell A E, *Stability Constants*, Spec Pub Chem Soc No 17 (1964)

[5] Kraus, K A, Phillips, H O, Carlson, T A, and Johnston, J S, *Proc Second Intern Conf Peaceful Uses of Atomic Energy*, 3, 28 (1958)

[6] Fronaeus, S, *Acta Chem Scand*, 5, 859 (1951)

Detection and Location of N-Methylamino-acid Residues in N-Acyloligopeptide Methyl Esters by Mass Spectrometry

IN a previous communication[1] we described a procedure for complete N-methylation of peptide –CONH– groups in oligopeptide derivatives A mixture of methyl iodide and silver oxide in dimethylformamide was used as the reagent This reagent is also capable of methylating –OH or –SH groups It was shown that the N-acyloligopeptide methyl esters exhibited enhanced volatility after methylation, and that the permethylated products were more suitable for mass spectrometric sequential analysis of their amino-acid residues

It has not been possible to detect and locate with certainty the presence of an N-methylamino-acid residue in a peptide by mass spectrometry because the mass number as well as the elemental composition of a given amino-acid and its N-methylated lower homologue (for example, leucine and N-methylvaline) are the same We describe here a method of solving this problem, making use of the mass spectrometric shift technique[2]

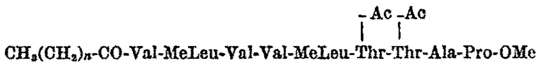
A comparative study of the mass spectra of an N-acyloligopeptide methyl ester and its permethylated derivative provided information on the presence as well as the location of originally existing N-methylamino-acid residues in oligopeptide derivatives (1) The increase in molecular weight after complete N-methylation indicates the number of methyl groups introduced in the molecule and thus the number of peptide –CONH– groups (together with –OH, –SH groups, if present) in the oligopeptide derivative before methylation (2) Each amino-acid residue which had gained a methyl group revealed itself by an increase of 14 mass units recognizable from the peaks arising by peptide bond cleavage (3) An amino-acid residue with its peptide nitrogen originally methylated or fully substituted showed no change in mass number after methylation (unless it contained other methylatable functional groups), and thus its position could be determined

To illustrate the foregoing approach we have chosen fortuitine (I) as a model compound This naturally occurring peptidolipid[3] is composed of nine amino-acid residues including two N-methylleucines and one proline

The sequence of nine amino-acids in fortuitine was deduced by mass spectrometry and was reported previously[4] Comparison of the mass spectra of fortuitine (I) and its permethylated derivative (II) discloses that the molecular weight has increased by 84 mass units on methylation This corresponds to six (6 × 14, Val_3Thr_2 Ala) methyl groups being incorporated in the molecule, in agreement with the proposed structure of fortuitine. It is evident from the mass spectra (see Table 1 and Fig 1) of fortuitine and permethylfortuitine (II) that only the mass of each newly N-methylated amino-acid residue has increased by 14 mass units after methylation This therefore allows us to locate exactly the two N-methylleucine units in fortuitine (I)

Table 1 FRAGMENTATION OF FORTUITINE (I), PERMETHYLFORTUITINE (II) AND PERDEUTERIOMETHYLFORTUITINE (III)

(I)



$$CH_3(CH_2)_n\text{-CO-Val-MeLeu-Val-Val-MeLeu-}\overset{\overset{\text{–Ac}}{|}}{\text{Thr}}\text{-}\overset{\overset{\text{–Ac}}{|}}{\text{Thr}}\text{-Ala-Pro-OMe}$$

$n = 18$ or 20 (M^+ 1,359 for $n = 20$)*

$$\Big\downarrow -2CH_3COOH\ (2 \times 60 = 120)$$

(Ia)

Mass units†		99	$\underline{127}$	99	99	$\underline{127}$	83	83	71	$\underline{128}$
	$CH_3(CH_2)_n$-CO	-Val	-MeLeu	-Val	-Val	-MeLeu	-Thr $-H_2O$	-Thr $-H_2O$	-Ala	-Pro-OMe
m/e‡		422	549	648	747	874	957	1040	1111	1239 (M-120)
For $n = 20$										

(II) Permethylfortuitine (molecular weight 1443 for $n = 20$)

$$\Big\downarrow -2CH_3COOH\ (2 \times 60 = 120)$$

(IIa)

Mass units†		113	$\underline{127}$	113	113	$\underline{127}$	97	97	85	$\underline{128}$
	$CH_3(CH_2)_n$-CO	-MeVal	-MeLeu	-MeVal	-MeVal	-MeLeu	-MeThr $-H_2O$	-MeThr $-H_2O$	-MeAla	-Pro-OMe
m/e‡		436	563	676	789	916	1013	1110	1195	1323 (M-120)
For $n = 20$‡										

(III) Perdeuteriomethylfortuitine (molecular weight 1461 for $n = 20$)

$$\Big\downarrow -2CH_3COOH\ (2 \times 60 = 120)$$

(IIIa)

Mass units†		116	$\underline{127}$	116	116	$\underline{127}$	100	100	88	$\underline{128}$
	$CH_3(CH_2)_n$-CO	d_3 -MeVal	-MeLeu	d_3 -MeVal	d_3 -MeVal	-MeLeu	d_3 -MeTd $-H_2O$	d_3 -MeThr $-H_2O$	d_3 -MeAla	-Pro-OMe
m/e‡		439	566	682	798	925	1025	1125	1123	1341 (M-120)
For $n = 20$										

The mass spectra were obtained with an AEI-*MS*9 instrument equipped with a direct inlet system and operating at 70 eV

* Fortuitine (I) gave a molecular ion peak at m/e 1359 ($n = 120$)[4] Under our conditions fortuitine (I) or permethylfortuitine (II and III) did not give the molecular ion peaks but instead showed the M-120 peaks corresponding to the loss of two molecules of acetic acid

† The numbers underlined represent the nominal mass of amino-acid residues which are not methylatable

‡ Peaks for $n = 18$ are observed 28 mass units lower

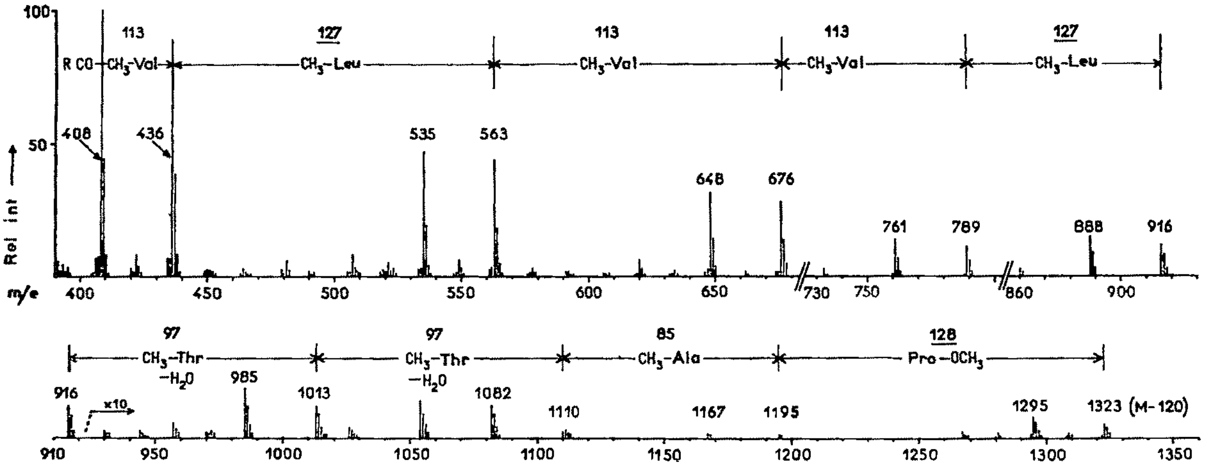


Fig 1 Mass spectrum of permethylfortuitine $R = CH_3(CH_2)_n$—where $n = 18$ or 20

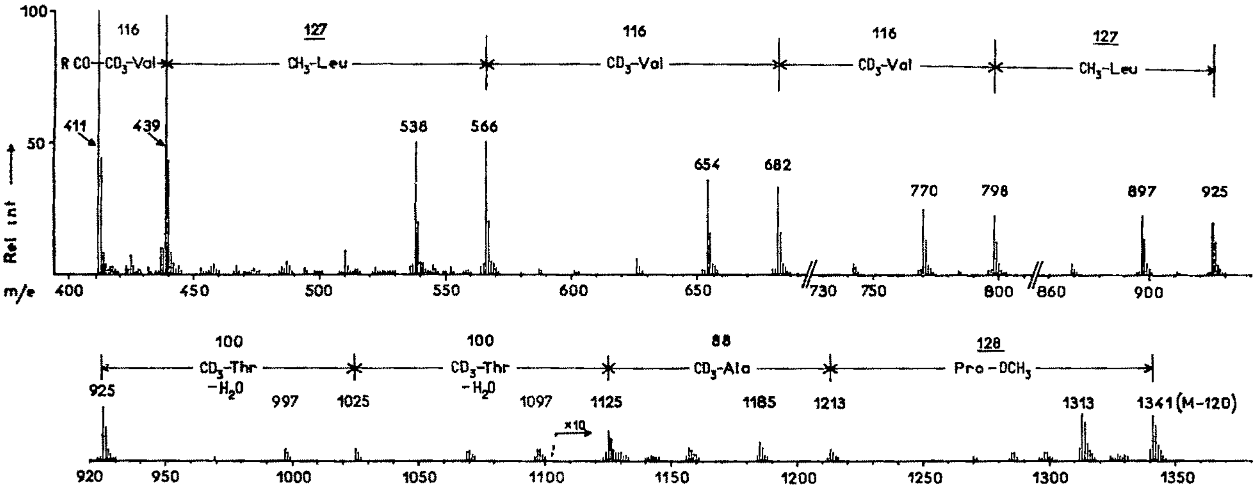


Fig. 2. Mass spectrum of perdeuteriomethylfortuitine $R = CH_3(CH_2)_n$—where $n = 18$ or 20

This procedure cannot, however, be used for oligopeptide derivatives which do not give mass spectra before methylation, for example, the heptapeptide derivative[1]

$$\text{Ac-Gly-Phe-Tyr-Thr-Pro-}\underset{\underset{\text{Ac}}{|}}{\text{Lys}}\text{-OMe}$$

In such cases, the N-acyl peptide methyl ester may be treated with a mixture of trideuterio methyl iodide and silver oxide in dimethylformamide to obtain the corresponding perdeuteriomethylated product Comparison of the mass spectra of the permethylated and the perdeuteriomethylated derivatives would indicate a shift of 3 mass units (CH_3 against CD_3) for each N-deuteriomethylated amino-acid residue Such a shift would not be observed if the amino-acid residue did not originally contain any methylatable hydrogen

In order to check this approach we prepared perdeuteriomethylfortuitine (III) The observed increase of 18 mass units (3 × 6) in the molecular weight of (III) [as compared with (II)] established the incorporation of six deuteriomethyl (CD_3) groups The amino-acid sequences, including the position of the original two N-methylaminoleucine residues as well as one proline, can now be unequivocally recognized from the mass spectrum (see Table 1 and Fig 2) of perdeuteriomethylfortuitine (III)

We wish to point out that it may be possible to find the location of the N-methyl residues originally present from the mass spectrum of the perdeuteriomethylpeptide derivative alone Nevertheless, a comparison with the mass spectrum of the permethylpeptide derivative makes it simpler to recognize the position of the original N-methylamino-acid residue in the peptide chain

We trust that this method will find successful application to natural compounds of unknown structures

We thank Mme E. Vilkas of this Institute for the gift of fortuitine which made this study possible.

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[1] Das, B C, Géro, S D, and Lederer, E, *Biochem Biophys Res Commun*, 29, 211 (1967)

[2] Biemann, K, in *Mass Spectrometry*, chap 8 (McGraw-Hill, New York, 1962)

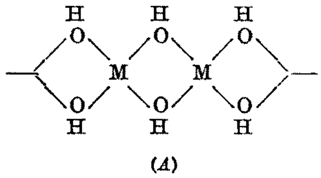
[3] Vilkas, E, Miquel, A M, and Lederer, E, *Biochim Biophys Acta*, 70, 217 (1963)

[4] Barber, M, Jollès, P, Vilkas, E, and Lederer, E, *Biochem Biophys Res Commun*, 18, 469 (1965)

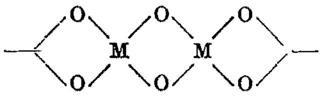
Mechanism of Bonding in Aqueous Nickelous Nitrate–Magnesium Oxide Cements

THE low temperature cementation effects in the nickelous nitrate–water–magnesium oxide system have only recently been reported[1] When aqueous solutions of nickelous nitrate are added to magnesium oxide powder, a basic salt with the idealized formula of $Ni_2(OH)_3NO_3$ forms and bonds the magnesium oxide aggregate together. A mechanism for the formation of the basic salt and its subsequent bonding to the magnesium oxide grain surfaces is suggested

When the salts of metallic elements are dissolved in water or in other solvents, aggregation of the cations will occur in suitable conditions[2] This process ultimately leads to the formation of polynuclear complexes, and in those formed in aqueous solution the metal ions are linked by means of hydroxide bridges, as in (*A*), or oxide bridges, as in (*B*)



(*A*)



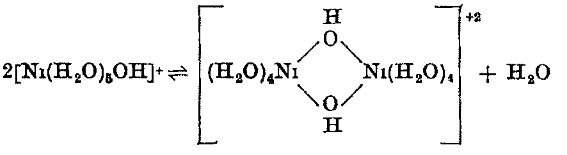
(*B*)

In aqueous solutions, the conditions necessary to bring about the formation of these complexes involve increasing the *p*H of the solution by the addition of OH^- ions, or increasing the concentration of metallic cations in the solution[2]

When an aqueous solution of nickelous nitrate is added to the magnesium oxide aggregate, the water reacts with magnesium oxide to form magnesium hydroxide at the surface of the grains This reaction increases the *p*H of the cement solution causing nickel hydroxide polymers to form

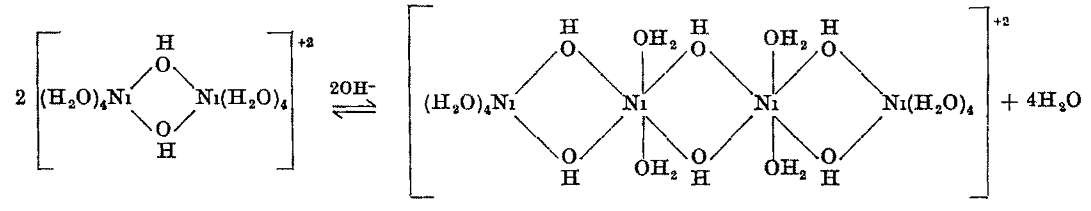
Cuta *et al* [3] and Veprek-Siska[4] have found that solutions of nickel salts are capable of such aggregation Veprek-Siska gave the following equations for aggregation of nickel in aqueous solutions

$$[Ni(H_2O)_6]^{+2} + H_2O \rightleftharpoons [Ni(H_2O)_5OH]^+ + H_3O^+ \qquad (1)$$



(2)

This process can be extended so that nickel hydroxide polymers of infinite length can be obtained A continuation of reaction (2) is shown below in reaction (3)



(3)

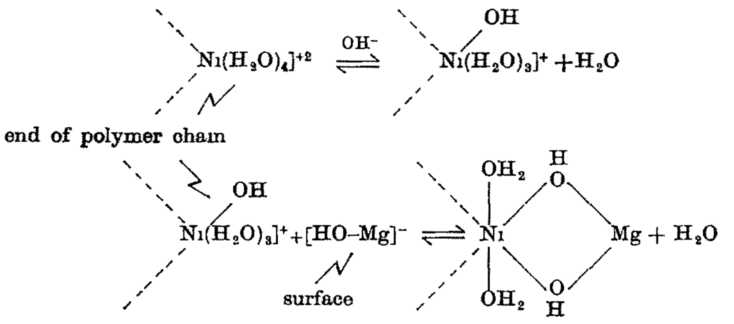
The idealized reactions (1)–(3) lead to the formation of nickel hydroxide in solution The same reactions would be applicable to the formation of basic nickel nitrate

All the known basic nickel nitrates consist of layers of nickel hydroxide interleaved with nitrate ions[5] Then the nickel hydroxide polymers would form as shown in reactions (1)–(3) with the nitrate ions positioned between these hydroxide polymers This conclusion is also consistent with what is known about the structures of the hydroxyhalides of nickel which are considered to be $Ni(OH)_2$ layers with halide ions and water molecules randomly arranged between them[6]

The water in the cement solution is either bound on the surface of the magnesium oxide or held in the co-ordination sphere of the nickel ion The product of reaction (3) contains only four nickel ions This polymer can be extended as long as nickel ions are available in the solution

The bonding of the basic nickel nitrate to the surface of the magnesium oxide grains can be ideally visualized as follows First, water in the cementing solution reacts with magnesium oxide to form magnesium hydroxide on the surfaces of the grains Then, as this reaction proceeds, the basic nickel nitrate precipitates from solution and reacts with the magnesium hydroxide that is forming

Consider the end of a nickel hydroxide polymer chain from reaction (3) The end of this molecule reacts with the newly formed magnesium hydroxide surface layer in the following manner



(4)

(5)

Thus this surface reaction stops the polymerization of the salt at the surface of a magnesium oxide grain and bonds the basic nickel nitrate to the magnesium oxide This process repeated in many places in three dimensions causes the cementing action

To confirm the presence of a magnesium hydroxide layer between the bonding phase and the aggregate, single crystals of magnesium oxide were reacted with the cementing solution Later the resultant hardened cement phase was stripped off the single crystals to expose the magnesium oxide–cement interface These interfaces were then examined using X-ray diffraction techniques It was found that a magnesium hydroxide layer was present between the magnesium oxide matrix and the cement phase

Reactions (1)–(3) show how nickel hydroxide or basic nickel nitrate polymers can form in solution It is thought that the heat given off by the magnesium oxide + water reaction at the aggregate surface causes the precipitation of the basic salt and its subsequent bonding to the magnesium oxide grains[1] If magnesium oxide powder is treated with water, dried and then mixed with the nickelous nitrate solution, no cementation occurs If the resultant mixture is dried in an oven at 250° F, $Ni_2(OH)_3NO_3$ will form, as determined by X-ray diffraction analysis These dried samples, however, could easily be crushed by hand pressure indicating that the magnesium oxide grains are simply mechanically entrapped in the basic salt matrix For cementation to occur, the nickelous nitrate solution must be added to unreacted magnesium oxide grains

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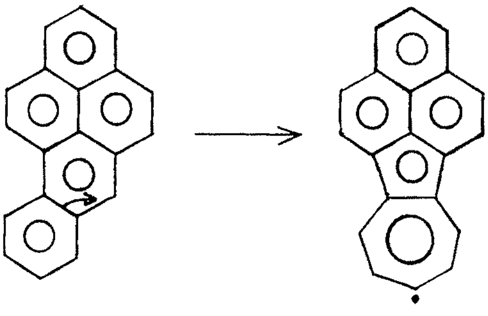
[1] Williams, E L, and Wilson, H H, *J Amer Ceram Soc*, **50**, 629 (1967)
[2] Gimblett, F G R, in *Inorganic Polymer Chemistry* (Butterworths, 1963)
[3] Cuta, F, Ksandr, Z, and Hejtmanek, M, *Chem Listy*, **50**, 1064 (1956)
[4] Veprek-Siska, J, *Chem Listy*, **49**, 10 (1955)
[5] Feitknecht, W, *Fortschr Chem Forsch*, **2**, 670 (1953)
[6] Wells, A F, in *Structural Inorganic Chemistry*, third ed (Oxford, 1962)

Possible Formation of an Azulene-type Radical obtained on Heating 3,4-Benzpyrene

IN an earlier communication[1] we reported that 3,4-benzpyrene (I), an agent known to cause cancer and one of the components of tobacco smoke condensate, when heated to about 200° C gives rise to free radicals, which give well resolved electron spin resonance spectra This communication describes further work on these free radicals

The relevant spin resonance spectrum (Fig 1*a* and *b*) could be completely simulated (Fig 1*c*) if the following splitting constants were assumed $a_1 = 1\ 135$ gauss (two protons), $a_2 = 1\ 562$ gauss (two protons), $a_3 = 2\ 362$ gauss (two protons), $a_4 = 5\ 196$ gauss (two protons), $a_5 = 5\ 469$ gauss (two protons), $a_6 = 8\ 271$ gauss (one proton) The less well resolved spectrum, reported previously (see Fig 3 of ref 1), could also be simulated using the same splitting constants but using a larger linewidth (200 mgauss)

The similarity between Fig 1*b* and *c* shows that the two proton splitting constants in each of the five pairs of coupling constants are equal to within at least half the linewidth (0 025 gauss) A species, which possesses five pairs of equivalent interacting protons, must have appreciable symmetry Limiting the search for a likely species to radicals which do not contain other than five, six or seven-membered rings, the data indicate the formation of radical II (derived from azuleno[1,2,3-cd]phenalene) which may be formed by a single bond rearrangement as follows



I II

Alternative structures are species in which the azulene system is fused to the benzene ring through its seven-membered ring (azuleno[5,6,7-cd]phenalene) and a structure, similar to II, in which the unpaired electron is located at the other end of the molecule Such species, however, are considered less likely than structure II.

The possible formation of an azulene-type radical is of interest because some simple azulenes readily afford radicals[1] and also because some azulenes have been reported to possess anti-inflammatory[2], anti-pyretic[3], anti-irritant[4] and, possibly, growth inhibiting[2,5] properties The radicals formed seem to be fairly stable in vacuum at temperatures between room temperature and 300° C, however, in the presence of other substances they may react further For example, the radicals formed from benzpyrene combine with air to form a different radical, as judged by a drastically altered electron spin resonance spectrum Confirmation of the proposed structure of the radical, as well as the evaluation of its biological activity, would be made possible by a synthesis of the parent hydrocarbon (II), it is hoped that this communication will encourage the appropriate synthetic work.

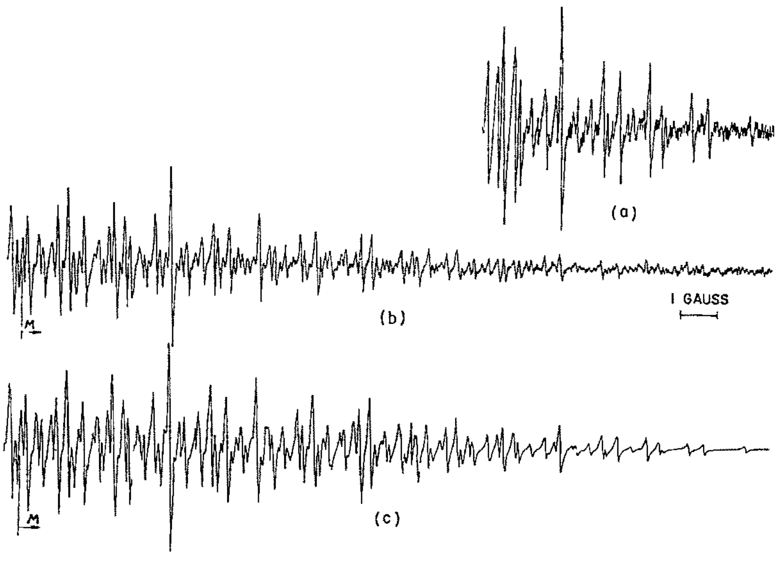


Fig 1 Experimental (*a*, wings under higher gain, *b*, half-spectrum, *M* shows midpoint of spectrum) electron spin resonance spectrum of 3,4-benzpyrene, heated in vacuum to 220° C The computed spectrum, *c*, was obtained with the parameters given in the text and assuming a linewidth of 50 mgauss

The spectra were determined by standard methods on a Varian *E*-3 spectrometer To eliminate the possibility that an impurity might be responsible for the observed spectrum, 3,4-benzpyrene was obtained from several suppliers (Aldrich and Baker) and a zone-refined sample was also used Similar spectra were obtained for each compound on heating

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[1] Forbes, W F , and Robinson, J C , *Nature*, **214**, 80 (1967)
[2] Gordon, M , *Chem Rev* , **50**, 127 (1952)
[3] Maksimenko, G N , *Farmakol Toksikol* , **27**, 571 (1964)
[4] Goldemberg, R L , *J Soc Cosmet Chem* , **16**, 317 (1965)
[5] Barton, H , *Acta Biol Med* , **2**, 555 (1959)

BIOCHEMISTRY

Urinary Hydroxyproline Excretion in Phosphate Depleted Rachitic Rats

THE increased output of hydroxyproline peptides in the urine of patients with osteomalacia[1] has been found to increase still further when the rickets are cured with phosphate[2] Studies using ^{14}C-proline indicate that the hydroxyproline in the urine of growing rats is derived both from recently synthesized soluble collagen and from the catabolism of the mature insoluble collagen fibres[3] The experiments reported here were designed to discover whether the increased excretion of hydroxyproline in healing rickets comes from the synthesis of new collagen.

Weanling Wistar strain male white rats 4 weeks old were divided into two groups, the control group continued on a normal diet and the other was fed a low phosphate diet deficient in vitamin D (ref 4) for 4 weeks The growth rates of both groups were comparable (body weight at 8 weeks normal rats 210 g, rachitic rats 140 g) and florid rickets developed without severe stunting of growth which in itself may induce changes in hydroxyproline output[5] The rachitic rats were divided into two pairs, one pair was re-fed with a diet supplemented with phosphate and the other was maintained on the depleted diet After 2 days on this regime, pairs of normal, re-fed and depleted rats were given 50 μc of ^{14}CU-proline intraperitoneally, placed in metabowls and the expired labelled carbon dioxide and radioactive urine were separated from faeces collected at intervals during the next 48–72 h The animals were then killed, blood was obtained for chemical estimation, and bone and skin were dissected out for analysis The urine was hydrolysed with 6 normal hydrochloric acid at 126° C for 3 h and the chromogens were absorbed onto charcoal, evaporated over potassium hydroxide in a vacuum and chromatographed to separate proline from hydroxyproline[6] The hydroxyproline was extracted and the specific activity estimated[7] in three separate fractions, the mean value being plotted in Fig 2

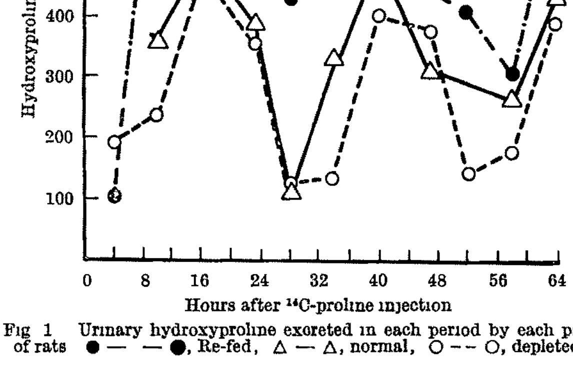

Fig 1 Urinary hydroxyproline excreted in each period by each pair of rats ● — — ●, Re-fed, △ — △, normal, ○ - - ○, depleted

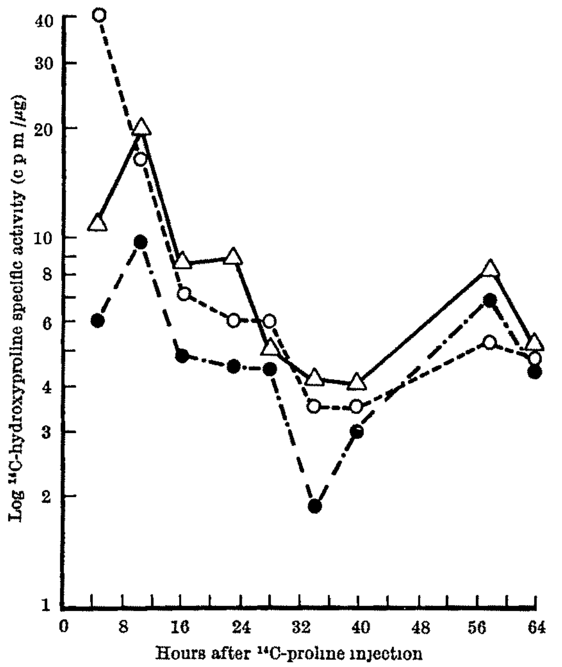

Fig 2 Specific activity of urinary hydroxyproline in each period for each pair of rats. ● — — ●, Re-fed, △ — △, normal, ○ - - ○, depleted

Fig 1 shows the mean excretion of hydroxyproline for each pair of rats during each period (the normal excretion corrected for body weight) and the specific activity of the hydroxyproline excreted in the urine per period is shown in Fig 2 The amounts and specific activity of the labelled carbon dioxide from each group of rats are shown in Table 1 The concentration of calcium in the plasma was normal for all three groups, and low concentration of phosphate in the plasma of the rachitic group was completely corrected in the re-fed group

Table 1 $^{14}CO_2$ EXPIRED BY THE THREE GROUPS OF RATS ABSORBED INTO SODIUM HYDROXIDE

		Carbon dioxide (μmoles/ml)	^{14}C (c p m /ml)	Specific activity (c p m /μmole)
Run I	Rachitic	18 5	360	19 5
	Re-fed	19 6	528	27 0
	Normal	21 0	620	29 5
Run II	Rachitic	15 5	293	18 9
	Re-fed	17 7	319	18 0

The output of hydroxyproline was comparable in the rachitic and normal rats (corrected for body weight) The maintained output in phosphate deficient rats suggests an increased turnover of collagen in this state, which could be linked with the increased number of new osteoblasts formed along osteoid seams[8] On the other hand, secondary hyperparathyroidism, which may occur in osteomalacia, could also increase the resorption of mature collagen[9], however, in our experiments the serum calcium was normal and hyperplasia was not found[10] The higher specific activity of urinary hydroxyproline in the rachitic rats during the first two periods suggests that the urinary output comes from the degradation of recently synthesized collagen, this has also been recorded *in vitro* with chick bone deficient in vitamin D (ref 11) In the re-fed rats the serum chemistry of which had returned to normal and the wet bone weight of which had increased, the specific activity of urinary hydroxyproline scarcely differed from that of normal rats in the first stages, despite the two or three-fold increase in the output of hydroxyproline It seems that increased synthesis is matched by increased resorption in equal proportions Later in the experiment, however, a lower specific activity was recorded as resorption of non-labelled collagen dominated, but fresh synthesis continued from an ever-decreasing pool of labelled proline There was no evidence of different sized proline pools, for total excretion of labelled carbon dioxide derived from proline metabolism did not differ significantly in the three groups of animals Analysis of the tibial metaphyses (Table 2) showed that the specific activity of the collagen 2 days after a single injection of labelled proline was higher in the rachitic than in the re-fed animals, which suggests that the higher formation rates in the latter lead to more labelled collagen after 48 h This suggestion is supported by the higher counts per microgram of decalcified tissue It seems that after 72 h resorption of mature unlabelled collagen in the re-fed rats has raised the specific activity

Table 2 SPECIFIC ACTIVITY OF HYDROXYPROLINE IN TIBIAL BONE 48 AND 72 H AFTER A SINGLE INJECTION OF ^{14}CU-PROLINE

(c p m /μg of hydroxyproline ± *S D*)

Time	Normal	Re-fed	Rachitic
48 h	—	2 4±0 3	3 5±0 3
72 h	5 6±1 9	5 4±0 3	2 5±0 7
c p.m /μg of decalcified tibial metaphyseal tissue			
48 h	—	154±14	310±30

Thus there is evidence of increased collagen turnover in rickets, particularly during healing by phosphate repletion when increased synthesis and resorption take place in almost equal proportions

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[1] Crabbe, P , and Isselbacher, K J , *Gastroenterology*, **48**, 307 (1965)
[2] Nagant de Deux Chaisnes, C , and Krane, S M , *J Clin Invest* , **44**, 1078 (1965)
[3] Prockop, D J , *J Clin Invest* , **43**, 453 (1964)
[4] Harrison, H E , and Harrison, H C , *J Biol Chem* , **185**, 857 (1950)
[5] Widdowson, E M , and Whitehead, R G , *Nature*, **212**, 683 (1966)
[6] Flanagen, B , and Nichols, G , *J Biol Chem* , **237**, 3686 (1962)
[7] Prockop, D J , and Udenfriend, S , *Anal Biochem* , **1**, 228 (1960)
[8] Frost, H M , *L'Osteomalacie* (edit by Hioco, D J), 3 (Masson and Cie, Paris, 1967)
[9] Avioli, L V , and Prockop, D J , *J Clin Invest* , **46**, 217 (1967)
[10] Copp, D H , and Suiker, A P , *Radioisotopes and Bone* (edit by McClean, F , Lacroix, P , and Budy, A M), 1 (Blackwell, Oxford, 1963)
[11] Paterson, C R , and Fourman, P , *Fifth European Symposium on Calcified Tissues* (1967)

Effect of Phenylhydrazine and Hydrolysed Glucose Cycloacetoacetate on Adenosinetriphosphatase Activity in Liver Mitochondria

ALTHOUGH freshly prepared liver mitochondria are unable to hydrolyse ATP at a perceptible rate[1], damaging liver mitochondria is known to bring about ATP hydrolysis[2] Deleterious effects of phenylhydrazine on liver have also been reported[3,4]

It has been suggested that the hydrolysed product of glucose cycloacetoacetate plays a beneficial part in the regeneration of hepatic cells damaged by phenylhydrazine[5] This product has been reported to affect alkaline phosphatase activity and the phosphatases involved in glycolysis[6,7] Glucose cycloacetoacetate has also been reported to prevent fatty infiltration of liver[8], and the association of increased ATPase activity with fatty livers is well known[9] We have therefore tried to study the *in vitro* effect of glucose cycloacetoacetate on the ATPase activity of normal mitochondria as well as those incubated with phenylhydrazine hydrochloride

Glucose cycloacetoacetate was prepared by the method of West[10] as modified by Nath *et al* [11] The hydrolysed product was obtained by the procedure of Nath and Nath[12] A solution of recrystallized phenylhydrazine hydrochloride was prepared in redistilled water

The mitochondria from rat liver were isolated in ice-cold isotonic sucrose–versene (0 25 molar sucrose, 0 005 molar versene) by the method of Schneider and Hogeboom[13]. The reaction medium used for the assay of ATPase activity contained 0 075 molar potassium chloride; 0 05 molar sucrose; 0 05 molar *tris* hydrochloric acid buffer (*p*H 7 5), 0 001 molar EDTA, and 0 005 molar ATP The reaction was allowed to continue for 20 min at 26° C and was stopped by the addition of 10 per cent trichloroacetic acid Inorganic phosphate was determined in a sample of the solution by the method of Fiske and Subba Row[14] The results are presented in Table 1

Table 1 EFFECT OF PHENYLHYDRAZINE AND GCAh ON ATPase ACTIVITY OF NORMAL MITOCHONDRIA *in vitro*

Substances added	ATPase activity (μmoles of inorganic phosphate/mg of protein/ 20 min and *SEM*)	Activity increase (per cent)
(1) None	22±0 2	—
(2) Phenylhydrazine	84±0 1	282
(3) GCAh	16±0 4	−27
(4) Phenylhydrazine plus GCAh	39±0 5	77

GCAh, glucose cycloacetoacetate.
All estimations were carried out in triplicate The concentration of phenylhydrazine and GCAh in the final reaction medium was 3 mg The mitochondria were incubated with the assay medium for 30 min before addition of substrate (ATP)

These results show that the activity of ATP in normal hepatic mitochondria is enhanced by phenylhydrazine, which adversely affects the liver This may enhance the enzyme activity like other liver damaging substances such as carbon tetrachloride[9] Furthermore, data show that hydrolysed glucose cycloacetoacetate markedly lessens the enhanced activity of the enzyme caused by phenylhydrazine The hydrolysed product of glucose cycloacetoacetate has also been found to lower the activity of ATPase in normal mitochondria Nath and Prasannan had suggested that glucose cycloacetoacetate is involved in the regeneration of hepatic cells damaged by phenylhydrazine[5]

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[1] Kielley, W W , and Kielley, R K , *J Biol Chem* , **200**, 213 (1953)
[2] Myers, D K , and Slater, E C , *Nature*, **179**, 363 (1957)
[3] Chanutin, A , Lentz, E A , and Ludewig, S , *Amer J Physiol* , **173**, 474 (1953)
[4] Damodaran, M , and Vijayraghavan, P K , *Curr Sci* , **12**, 115 (1943)
[5] Nath, M C , and Prasannan, K G , *Biochem J* , **73**, 729 (1959)
[6] Brahmankar, D M , Meghal, S K , and Nath, M C , *Ind J Biochem* , **1**, 167 (1964)

[7] Krishnamurthi, V , Nayudu, S. G , and Nath, M C , *Proc. Soc. Exp Biol. and Med* , 117, 861 (1964)
[8] Nath, N , Yawalkar, V D , and Nath, M. C , *Nature*, 208, 1001 (1965)
[9] Artizzu, M , Baccino, F M , and Dianzani, M U , *Biochim Biophys Acta*, 78, 1 (1963)
[10] West, E S , *J Biol Chem* , 74, 561 (1927)
[11] Nath, M C , Chitale, R P , and Belavady, B , *Nature*, 170, 545 (1952)
[12] Nath, M C , and Nath, N , *Ind J Med Res* , 53, 1010 (1965)
[13] Schneider, W C , and Hogeboom, G H , *J Biol Chem* , 183, 123 (1950)
[14] Fiske, C H , and Subba Row, Y *J Biol Chem* , 66, 375 (1925)

Conversion of δ-Amino Laevulinic Acid to Porphobilinogen by Different Tissues of the Polychaete Annelid *Neoamphitrite figulus*

THE heart-body, a small spongy mass of tissue within the dorsal vessel of the terebellid polychaete *Neoamphitrite figulus* (Dalyell), is almost certainly concerned with synthesis of the haemoglobin of the blood The coelomocytes also contain haemoglobin, but this is different Kennedy and Dales[1] have shown that the heart-body tissue and the coelomocytes in this species converted porphobilinogen (PBG) to uroporphyrin Tissues were incubated with purified PBG suspended in sea water and at room temperature for varying periods and the products identified after extraction and purification by paper chromatography Some similarly performed experiments using δ-amino laevulinic acid (ALA) did not give clear results Subsequent work, using the method of McRae[2], whereby the conversion of ALA to PBG is identified against suitable controls by the positive reaction PBG gives with Ehrlich's *p*-aminobenzaldehyde reagent[3], has, nevertheless, given quite clear results McRae's method was followed in detail except that to quantify the results the tissues were merely dried with acetone and weighed, for it was desired only to compare the relative ability of different tissues to convert ALA to PBG McRae used homogenates of whole animals (*Dugesia*) Tissues were deep frozen and homogenized on thawing in M/15 phosphate buffer, *p*H 7 0, the ALA being added to provide a concentration of 400 μg/ml of homogenate Homogenates, suitably controlled, were incubated at 37° C for 2 5 h The colour of the Ehrlich reaction was measured at 553 mμ after 15 min with a 'Unicam *SP* 500' spectrophotometer. While the activity of each tissue is expressed in arbitrary units, their relative activities in one typical experiment are shown in Table 1 The experiment was repeated five times with similar results The tissues of five worms were pooled in each experiment Table 1 shows that the heart-body is ten times more active than any other tissue determined The body wall muscle is colourless and if it contains any myoglobin this is in a relatively low concentration and any ALAase present is insufficient to produce enough PBG to be detected by the method On the other hand, Kennedy and Dales[4] detected coproporphyrin III, which must have been formed during haem synthesis, in the body wall, whether this is formed in the skin or is deposited there, as this negative result suggests, is being investigated further The hind stomach or gizzard has muscles of a deep red colour and some ability to convert ALA to PBG is not surprising The ability of the coelomocytes to do so is also expected, confirming the hypothesis that they synthesize their own haemoglobin from ALA and do not receive compounds later in the biosynthetic pathway to haem The high activity of the gut (fore stomach plus intestine) from which the contents had carefully been removed is surprising, especially because Kennedy and Dales[4] found no free porphyrins in these tissues It may be that the coelomocytes, which contain haemoglobin, arise from the coelomic epithelium of the gut wall. This is being investigated further

Table 1 CONVERSION OF ALA TO PBG BY DIFFERENT TISSUES OF *Neoamphitrite figulus*

Tissue	Weight of tissue (mg)	Activity (arbitrary units/ mg of tissue)
Heart-body	1 8	89 00
Coelomocytes	64 9	8 50
Fore stomach + intestine	39 6	7 82
Hind stomach (gizzard)	58 0	4 08
Body wall	177 5	0 00
Blood	7 9	0 00

I wish to thank Miss Jennifer Pell for collecting the specimens used

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[1] Kennedy, G Y , and Dales, R P , *J Mar Biol Assoc UK*, 48 (in the press, 1968)
[2] McRae, E K , *J Exp Zool* , 142, 607 (1959)
[3] Mauzerall, D , and Granick, S , *J Biol Chem* , 328, 1141 (1958)
[4] Kennedy, G Y , and Dales, R P , *J Mar Biol Assoc UK*, 37, 15 (1958)

Measurement and Significance of Weight Heterogeneity in Some Protein and Polysaccharide Preparations

MOST of the molecular weight values reported for macromolecules have been obtained by methods based on ultracentrifuge measurements Many biologists and biochemists now have access to an analytical ultracentrifuge and their requirements can be satisfied by sedimentation velocity experiments

Although macromolecules may sediment at similar rates and produce sharp boundaries, it is sometimes forgotten that this does not necessarily imply homogeneity of the system Thus serum transferrins produce a single symmetrical boundary in ultracentrifuge experiments whereas under electrophoresis the proteins may be resolved into two or more discrete fractions, depending on the animal species The transferrins may also exhibit some degree of association, for reported molecular weights of the transferrins[1,2] of several species are between 66,000 and 90,000 The discrepancy in the reported molecular weights is of some clinical importance because it affects the reliability of iron binding measurements Polysaccharides such as glycogen and amylopectin produce sharp boundaries as a result of the very large concentration dependence of the sedimentation coefficient[3] These polysaccharides possess a very broad distribution with respect to sedimentation velocities Several methods have been proposed in the past twenty years for the quantitative measurement of weight heterogeneity In 1944, Gralén[4] proposed that the width of the sedimenting boundary was an index of heterogeneity More recently, Baldwin[5,6] and other workers have made detailed analyses in which the complete distribution curve of the sedimentation coefficients is evaluated (compare Schachman[7]) Other more basic factors are often ignored, regardless of the method selected for quantitation of heterogeneity Quite often the criteria of purity applied to the preparation are not sensitive enough to justify detailed mathematical analysis of the boundaries The preparations may also undergo physicochemical changes during the experiment[8] so that control experiments are essential

We have been studying changes in the catabolic heterogeneity and weight heterogeneity of serum 7*S* gamma globulins in mouse and man after immunization with various antigens, including malignant tumours Electrophoretically distinct fractions of 7*S* gamma globulin are broken down at different rates in the mouse[9,10] and signi-

Table 1 MOLECULAR WEIGHT AVERAGES AND HETEROGENEITY OF GAMMA GLOBULINS FROM NORMAL AND IMMUNE MICE

Immunizing tumour	M_w	M_z	M_z/M_w
None	162,000	179,000	1 10
Leukaemia	194,000	317,000	1 63
Epithelioma	221,000	349,000	1 58
Mammary	157,000	239,000	1 52

ficant changes in these catabolic rates have been observed in mice immunized with transplantable tumours[9] These findings are clinically important because there is now evidence that heterogeneity of both function and antigenicity in the 7*S* gamma globulins of certain experimental animals may correlate with immunological tumour enhancement[11]

Ultracentrifuge experiments showed an increased weight heterogeneity of mouse 7*S* gamma globulin following immunization with transplantable homologous tumours when compared with 7*S* gamma globulins from non-immunized mice, measured under identical conditions[12] In these experiments, short columns of 7*S* gamma globulin solutions were centrifuged to sedimentation equilibrium in double sector cells The values of the molecular weight averages M_z and M_w were computed by the method of Van Holde and Baldwin[13] and the ratio of these values, M_z/M_w, provided a measure of the weight heterogeneity in the system Immune preparations gave significantly greater M_z/M_w ratios than those for normal gamma globulin preparations (Table 1), indicating that they were much more heterogeneous The immune gamma globulins contained a proportion of molecules with weights greater than 400,000, suggesting that those were aggregated forms of 7*S* gamma globulin There may be a correlation between increased weight heterogeneity and changes in catabolic heterogeneity of mouse 7*S* gamma globulin The recent observation[14] that human 7*S* gamma globulin possesses catabolic heterogeneity, apparently analogous to that of mice, suggests that similar weight heterogeneity changes might also be observed

Reported molecular weight values of the 7*S* gamma globulins of several species range from 130,000 up to 195,000 (ref 15) Pain[16] has recently investigated the problem of gamma globulin heterogeneity using horse 7*S* gamma globulin He found that the preparation was essentially homogeneous according to size, but his experiments did not include 7*S* gamma globulin from immunized animals

A study of the molecular weight distributions in human 7*S* gamma globulin samples from normal and pathological subjects might well yield information of clinical importance The technique of measuring weight heterogeneity in terms of the relative M_z and M_w average molecular weights is, however, unsatisfactory in view of the large number of measurements which would have to be made for these observations to have statistical significance A simple method is required which can be used routinely and which exposes the protein to the least physical change *in vitro* An obvious method seemed to be to measure the degree of departure from the theoretical Gaussian curve of the observed Schlieren diffusion pattern, a method which was first reported by Gralén[4], who measured D_M and D_A for various derivatives of cellulose and used the ratio D_M/D_A as a measure of heterogeneity The diffusion coefficients D_M and D_A were obtained from plots of $\sigma_M{}^2$ (the square of the standard deviation of the experimental curve) and $\sigma_A{}^2$ (determined by the height area method) plotted respectively against time

The behaviour of mixtures of albumin monomer and dimer was examined in order to determine the limitations of this technique experimentally The monomer and dimer samples used were obtained by fractionation of a commercial albumin preparation (Behringwerke A G , Marburg, Lahn) through 'Sephadex *G* 150'

Diffusion experiments were carried out in synthetic boundary cells in an MSE analytical ultracentrifuge (Measuring and Scientific Equipment, Ltd , London) Boundaries were produced at slightly higher speeds (15,000 r p m , 16,000*g*) than those normally used for diffusion experiments, in solutions containing 6–7 mg of protein/ml Photographs of the diffusion patterns were taken on 35 mm film at suitable intervals of time, and tracings of enlargements (× 24) of these were then made on graph paper Values of $\sigma_M{}^2$ and $\sigma_A{}^2$ were computed from measurements of the ordinates at suitable intervals on either side of the maximum point (the mode) of individual enlargements

Although the diffusion patterns of the original albumin preparation showed considerable asymmetry, presumably due to the presence of tetramers and pentamers, mixtures of monomers and dimers showed no asymmetry Further, the plots of $\sigma_A{}^2$ against time were linear, indicating homogeneity. Replacement of dimer in the mixture with an equal amount of 7*S* gamma globulin produced marked asymmetry of the diffusion curve and produced positive deviation from linearity of the $\sigma_A{}^2$ against time plots

The diffusion coefficients showed a roughly linear relationship to the composition of the mixture, but the ratio, D_M/D_A, provided little evidence of heterogeneity in the monomer–dimer systems (Table 2) Monomer–dimer interactions in 7*S* gamma globulin systems would be expected to be analogous to albumin, and so the method is unlikely to be useful for measuring heterogeneity in these systems

Table 2 RELATIONSHIP BETWEEN MOLAR COMPOSITION OF THE ALBUMIN MONOMER–DIMER MIXTURE AND DIFFUSION CHARACTERISTICS

Percentage monomer	$D_{20,w}$ (× 10^7 cm² sec⁻¹)	D_M/D_A
100	5 80	0 98
70	5 40	0 98
20	4 90	0 95
10	4 37	0 94
0	4 33	0 96
Commercial albumin	4 11	0 81

The D_M/D_A index seemed to be of some value in preliminary studies made of relative molecular distributions in glycogen preparations from various tissues These glycogens, which have $S^0{}_{20,w}$ values of 70–80*S*, show appreciable sedimentation velocity at 15,000 r p m so that the analysis is really of the sedimentation boundary Du Pont de Nemours (Wilmington, Delaware) have recently introduced an analogue computer, the 310 'Curve Resolver', for resolving overlapping peaks This instrument, which analyses the experimental curve in terms of its simplest composition using any preset curve distribution, may be of value for the rapid analysis of the asymmetric boundaries produced by glycogen and similar macromolecular substances The analysis of the Schlieren pattern of shell fish glycogen (D_M/D_A ratio 1 25), assuming pure Gaussian distributions, is shown in Fig 1 At

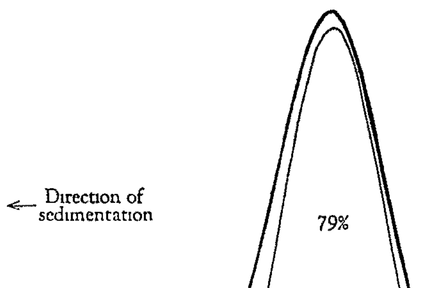

Fig 1 The broad outer line is the Schlieren profile of shell fish glycogen sedimenting at 16,000*g* The inner curves show the position and relative proportions of three components as indicated by the 310 'Curve Resolver'

least three components are present in this preparation and their relative proportions are indicated Such analysis might possibly be used to investigate metabolic disorders in liver in terms of distribution changes in the synthesized glycogen

The recent introduction of the monochromatic light scanning system has now made it possible to record ultracentrifuge data directly in a form suitable for computer analysis[17] This technique may well become the accepted way of measuring molecular weights and finally provide a general procedure for characterizing heterogeneity.

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[1] Charlwood P A, *Biochem J*, **88**, 394 (1963)
[2] Watkins, J, Tee, D E H, Wang, M, and Tarlow O, *Biochim Biophys Acta*, **127**, 66 (1966)
[3] Stacy, C J, and Foster, J F, *J Polymer Sci*, **25**, 39 (1957)
[4] Gralén, M, dissertation, Univ Uppsala (1944)
[5] Baldwin, R L, and Williams, J W, *J Amer Chem Soc*, **72**, 4325 (1950)
[6] Baldwin, R L, *Biochem J*, **65**, 490 (1957)
[7] Schachman, H K, *Ultracentrifugation in Biochemistry*, 128 (Academic Press, New York and London, 1959)
[8] James, K, Henney, C S, and Stanworth, D R, *Nature*, **202**, 563 (1964)
[9] Tee, D E H, Watkins, J, and Wang, M, *Nature*, **208**, 251 (1965)
[10] Fahey, J L, and Sell, S, *J Exp Med*, **122**, 41 (1965)
[11] Bloch, K J, *Fed Proc*, **24**, 1030 (1965)
[12] Tee, D E H, Watkins J, Wang, M, Gammack, D B, and Raper, J H *Immunology*, **9**, 85 (1965)
[13] Van Holde, K E, and Baldwin, R L, *J Phys Chem*, **62**, 734 (1958)
[14] Tee, D E H, and Watkins, J, *Brit Med J*, **4**, 210 (1967)
[15] Porter, R R, *The Plasma Proteins* (edit by Putnam, F W) 247 (Academic Press, New York and London, 1960)
[16] Pain, R H, *Biochim Biophys Acta*, **94**, 183 (1965)
[17] Spragg, S P *Anal Chim Acta* **38** 137 (1967)

MICROBIOLOGY

Photosynthetic Bacterium growing under Carbon Monoxide

OXIDATION and utilization of carbon monoxide by bacteria have often been observed[1–4] The metabolism of these bacteria was assumed to be chemolithotrophic because of the absence of any added organic energy source

The enrichment and growth of carbon monoxide oxidizing bacteria were enhanced if the culture was kept under continuous illumination, an effect which might be a result of photoreversal of the binding of carbon monoxide to a cytochrome *c* oxidase (my unpublished results)

Photosynthetic bacteria growing under carbon monoxide have been accidentally discovered in enrichment cultures for carbon monoxide oxidizing bacteria Forty-three strains of various genera of these bacteria were purified by streaking illuminated enrichment cultures (30–70 per cent carbon monoxide) on mineral salts agar contained in 'Dispo' plates (medium 337 of Hirsch and Conti[5], without carbon source, but with 0 01 per cent yeast extract added) Incubation was under incandescent light and at room temperature, in desiccators containing an atmosphere of 70 per cent carbon monoxide, 20 per cent oxygen, 1 per cent carbon dioxide and 9 per cent nitrogen Growth of the aerobic carbon monoxide bacteria ceased after several weeks probably because of lack of oxygen Red pigmented colonies then appeared on several plates Microscopic examination revealed photosynthetic bacteria strikingly similar to *Rhodopseudomonas* spp (Fig 1) The rod or club-shaped cells often had side branches and/or swellings on both ends, budding cells were observed frequently in any population Budding of different *Rhodopseudomonas* spp has been recently investigated[6]

These purple bacteria were purified easily by streaking on the same medium and incubating under the atmosphere of carbon monoxide+oxygen+carbon dioxide+nitrogen in the light Growth became abundant only after other carbon monoxide bacteria had removed the oxygen from the gas mixture, as is evident from manometric measurements as well as from tests with alkaline pyrogallol solution

The enrichment and isolation procedure was repeatedly successful with either garden soil (podsolic, *A*-1 layer, *p*H 6 8) or sewage mud (shore of New Haven, Connecticut harbour, *p*H 7 2) as the inoculum Several weekly gas changes seemed to be advantageous and helped to build up the population of carbon monoxide dependent bacteria

Pure cultures of these purple bacteria grew well anaerobically in the light in media normally used for *Rhodomicrobium vannielii*[7], with sodium acetate or sodium lactate as the hydrogen donors Growth on mineral salts agar under carbon monoxide and in the light was slow but abundant The absence of any added organic carbon or hydrogen source as well as lack of hydrogen sulphide was accomplished by employing gases and chemicals of highest available purity (Matheson Comp, Fisher's and Baker's reagent grades, Difco Noble agar, washed) All glassware was cleaned with acid after detergent had been used, and the desiccators were sealed with Dow Corning high vacuum silicone lubricant, with negligible vapour pressure Rubber tubing was excluded

In these conditions, light, carbon monoxide and ammonium ions seemed to be the only sources of energy available, either carbon monoxide or carbon dioxide or both would have to serve as the source of carbon Oxidation of ammonia could not be detected, and thus water cleavage must render an available form of hydrogen for these photolithotrophic bacteria The small increment of yeast extract could be left out with little or no effect on the total growth yield

Recently, *Rhodopseudomonas* spp have been reported to utilize ("photo-oxidaze") C1 compounds such as formate[8] and possibly methane (personal communication from W Vishniac) Utilization of carbon monoxide by a photosynthetic bacterium could be taken as another demonstration of the versatility of members of this group Further investigations will involve the use of labelled

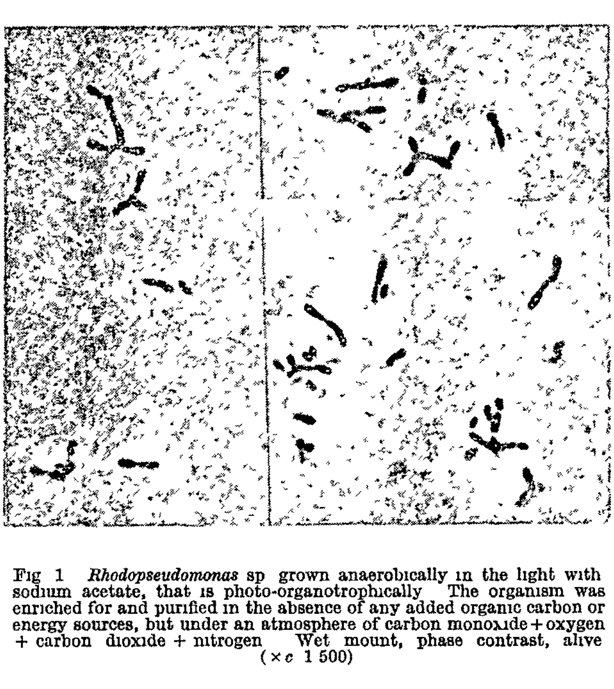

Fig 1 *Rhodopseudomonas* sp grown anaerobically in the light with sodium acetate, that is photo-organotrophically The organism was enriched for and purified in the absence of any added organic carbon or energy sources, but under an atmosphere of carbon monoxide+oxygen + carbon dioxide + nitrogen Wet mount, phase contrast, alive (×c 1 500)

carbon dioxide and examine the enzyme mechanisms involved

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[1] Hasemann, W , *Biochem Z* , 184, 147 (1927)

[2] Kistner, A , *Kon Nederl Akad v Wetensch* , Proc Series *C*, 56, No 4 (1953)

[3] Kistner, A , *Kon Nederl Akad v Wetensch* , Proc Series *C*, 57, No 2 (1954)

[4] Hirsch, P , *Bact Proc* (1965)

[5] Hirsch, P , and Conti, S F , *Arch Mikrobiol* , 48, 358 (1964)

[6] Whittenbury, R , and McLee, D A , *Arch Mikrobiol* , 59, 324 (1967)

[7] Conti, S F , and Hirsch, P , *J Bact* , 89, 503 (1965)

[8] Hoare, D S , *Bact Proc* (1967)

Competitive Binding of Streptomycin and Magnesium with *Escherichia coli* Ribosomes

SEVERAL laboratories have presented evidence that streptomycin inhibits protein synthesis of sensitive bacteria[1-4] Studies with cell free systems have clearly shown that the 30*S* sub-unit of the ribosomes derived from streptomycin-sensitive cells is the site of action of this antibiotic[5-8]. It has also been shown that one to two molecules of streptomycin bound to one ribosome particle suffice to exert an inhibitory effect on polypeptide synthesis[9,10]

Streptomycin contains the diguanido base, streptidine, and it has been shown to combine with nucleic acids to produce a polymeric compound which is dissociable in 1 molar sodium chloride in like manner to thymic nucleohistone or nucleoprotamine[11] When dihydrostreptomycin (DHSM) is converted to dideguanyldihydrostreptomycin (DGSM), the net cationic charge of DHSM is decreased from 3+ to 1+ without other detectable structural change[12]. This modified compound does not have any biological effect on streptomycin-sensitive *E coli*, suggesting that the biological activity of streptomycin is primarily related to the cationic groups[12,13] In fact, it has been suggested by some investigators that the observed effects of streptomycin in bacterial systems are the result of competition of streptomycin with Mg^{2+} (and polyamines) for a critical site or sites on the ribosome[8,14,15].

We have studied the cation exchange properties of *E. coli* ribosomes and have shown that small cations (K^+, NH_4^+, spermidine^{3+}, spermine^{4+}) can compete with Mg^{2+} for the anionic binding sites but to a variable degree[16] It has also been shown that there is no difference in the ion binding capacities between the 30*S* and 50*S* sub-units of the ribosomes In a further effort to relate the cation exchangeability of streptomycin to its biological effect, we therefore examined the possibility of a competitive effect between the antibiotic and Mg^{2+} bound to the ribosomes isolated from streptomycin-sensitive *E coli B*

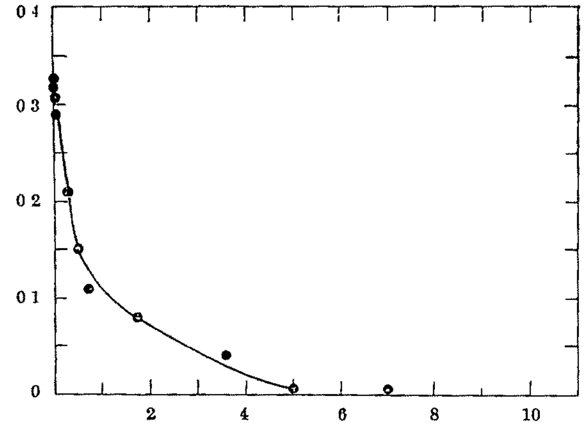

Fig 1 Effect of streptomycin on Mg^{2+} binding to *E coli* ribosomes in the presence of 10^{-2} molar *tris* buffer (*p*H 7 4)

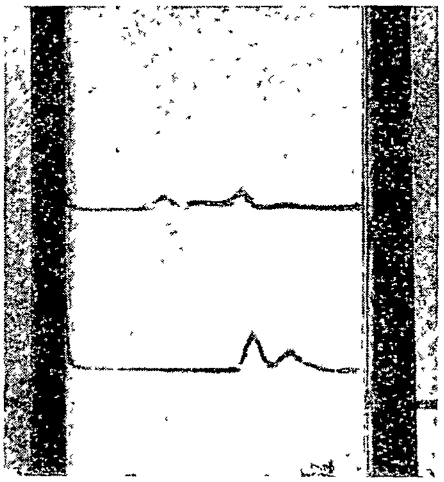

Fig 2 Analytical ultracentrifuge pattern of *E coli* ribosomes showing changes in ribosomes when one-half of the Mg^{2+} is displaced by streptomycin The lower pattern represents the 30*S* and 50*S* ribosomes in 10^{-2} molar *tris* buffer (*p*H 7 4) containing 5×10^{-5} molar Mg^{2+} The upper pattern represents the same ribosomes to which have been added $6\ 25 \times 10^{-4}$ molar streptomycin

A mixture of the 30*S* and 50*S* sub-units was prepared from *E coli B* as described by Tissieres *et al* [17], and a 0 1 per cent suspension of the ribosomes was made in 10^{-2} molar *tris* buffer, *p*H 7 4, containing 5×10^{-5} molar magnesium acetate. The change of Mg^{2+} binding to the ribosome in the presence of various concentrations of streptomycin sulphate (Nutritional Biochemicals Corporation, Cleveland, Ohio) was measured by means of equilibrium dialysis The details of our technique have been described before[16] As shown in Fig 1, the amount of Mg^{2+} bound to the ribosome decreased markedly when the concentration of streptomycin was increased, indicating that the antibiotic can displace Mg^{2+} on the binding sites of the ribosomes When the ribosomes from which the bound Mg^{2+} had been partially displaced by streptomycin were examined by the analytical ultracentrifuge, they were not the usual mixture of the 30*S* and 50*S* ribosomes (Fig 2) The ribosomes in the presence of 0 625 mmolar streptomycin showed a quite different picture of aggregation from that of the ribosomes to which streptomycin was not added This result indicates that a considerable change takes place in the ribosomal structure when half of the bound Mg^{2+} is displaced by streptomycin

Spotts and Stanier[18] proposed a hypothesis that streptomycin prevents a strand of messenger RNA from attaching to the ribosome of streptomycin-sensitive bacteria It has also been shown that messenger RNA is released from the ribosomes at a very low concentration of Mg^{2+} [19,20] The inhibitory effect of streptomycin on polyuridine-directed ^{14}C-phe-*t*RNA binding to the ribosomes is competitively relieved by increasing the concentration of Mg^{2+} in the solution[9,19] Studies with artificial polyribosomes suggest that the inhibitory effect of streptomycin is related not to the formation of polyribosomes but to the interference with the function of polyribosomes[8,9,15,21,22] This perturbation of the function of polyribosomes which is revealed by specific alteration of the recognition process of codons is also related to the concentration of Mg^{2+} and NH_4^+ in the amino-acid incorporation system Furthermore, the inhibitory effect of streptomycin is partially relieved when polyamines like spermine and spermidine are added to the cell free system containing *E coli* ribosomes[14] This antagonistic relationship between streptomycin and various cations has also been demonstrated *in vivo*, as reflected by induction of β-galactosidase[13] But the concentration of streptomycin employed in the studies of its inhibitory effect on the cell free amino-acid incorporation into protein (that is, approximately 0 1 mmolar) was considerably lower than that used in our experiment The change of sedimentation pattern of the ribosomes in the presence of 0 625 mmolar streptomycin may be an exaggerated picture of a more subtle configurational change which may occur at a lower concentration of streptomycin (that is, 0·1 mmolar) It would be very interesting to see whether the same result could be obtained in a similar experiment with the ribosomes from streptomycin-resistant *E coli*

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[1] Fitzgerald, R J , and Bernheim, F , *J Bact* , 55, 765 (1948)

[2] Erdoes, T , and Ullman, A , *Nature*, 183, 618 (1959)

[3] Hahn, F E , and Ciak, J , *Bact Proc* , 95 (1959)

[4] Kirk, J T O , *Biochim Biophys Acta*, 59, 476 (1962)

[5] Flaks, J G , Cox, E C , and White, J R , *Biochem Biophys Res Commun* , 7, 385 (1962)

[6] Flaks, J G , Cox, E C , Witting, M L , and White, J R , *Biochem Biophys Res Commun* , 7, 390 (1962)

[7] Speyer, J F , Lengyel, P , and Basilio, C , *Proc US Nat Acad Sci* , 48, 684 (1962)

[8] Davies, J E , *Proc US Nat Acad Sci* , 51, 659 (1964)

[9] Pestka, S , *J Biol Chem* , 241, 367 (1966)

[10] Kaji, H , and Kaji, A , *Proc US Nat Acad Sci* , 54, 213 (1965)

[11] Cohen S S , *J Biol Chem* , 166, 393 (1946)

[12] Polglase, W J , *Nature*, 206, 298 (1965)

[13] Bragg, P D , and Polglase, W J , *J Bact* , 85, 590 (1963)

[14] Mager, J , Benedict, M , and Artman, M , *Biochim Biophys Acta*, 62, 202 (1962)

[15] Cox, E C , White, J R , and Flaks, J G , *Proc US Nat Acad. Sci* , 51, 703 (1964)

[16] Choi, Y S , and Carr, C W , *J Mol Biol* , 25, 331 (1967)

[17] Tissieres, A , Watson, J D , Schlessinger, D , and Hollingworth, B R , *J Mol Biol* , 1, 221 (1959)

[18] Spotts, C R , and Stanier, R Y , *Nature*, 192, 633 (1961)

[19] Schlessinger D , and Gros, F , *J. Mol Biol* , 7, 350 (1963)

[20] Risebrough, R W , Tissieres, A , and Watson, J. D , *Proc US Nat Acad Sci* , 48, 430 (1962)

[21] Davies, J , Gilbert, W , and Gorini, L , *Proc. US Nat Acad Sci* , 51, 883 (1964)

[22] Pestka, S , Marshall, R and Nirenberg, M , *Proc US Nat Acad Sci* , 53, 639 (1965)

IMMUNOLOGY

Erasure of Immunological Memory with Anti-lymphocyte Serum

Levey and Medawar[1] have reported that treatment with anti-lymphocyte serum (ALS) can restore "virgin" reactivity to mice which have been previously sensitized by skin homografts across H-2 barriers Confirmation of this finding has not yet been published, and some authors[2] have failed to reproduce erasure of memory in similar experimental models

I have studied this phenomenon in three separate experiments In the first one, mice were made immune to a protein antigen In the other two experiments, skin was transplanted, the donors differing from the recipients at the H-2 locus

ALS was raised in rabbits to mouse thymocytes according to the method of Levey and Medawar[3] and was administered subcutaneously to *CBA* mice A total of 2 0 ml. of ALS was given to each experimental animal 0 5 ml followed by 0 25 ml × 6 on alternate days Controls received normal rabbit serum (NRS) in equivalent dosage and at the same time intervals *CBA* mice were made immune to bovine serum albumin (BSA) by the intraperitoneal injection of 5 mg of BSA in saline and were challenged by a second intraperitoneal injection of 100 μg of BSA Mouse sera were collected 10 days after challenge and were analysed for the presence of precipitating antibodies to BSA by a modification of the Farr technique[4]

CBA mice were sensitized to transplantation antigens by *A* strain tail skin grafts according to the method of Billingham and Medawar[5] Challenge with a second *A* strain skin graft was performed at varying intervals after immunization Skin grafts were inspected daily from the seventh day onward until rejection occurred Life tables were constructed for the various groups

In all experiments the time between initial immunization, serum treatment and subsequent challenge was systematically varied The protocols for the three experiments are shown in Tables 1, 2 and 3. The results which were obtained after immunization with BSA are summarized in Table 1 In no case had reversion to an immunologically "virgin" state been accomplished with ALS treatment All animals responded to challenge with titres above those which are characteristic of the primary response In two groups (B and H) the response of animals which had been treated with ALS was significantly lower than that of the corresponding controls which had been treated with NRS None the less, the response in all animals must be viewed as a secondary response because the challenge dose of 100 μg of BSA would not be expected to provoke a detectable response by this technique when given to *CBA* mice *de novo*[6]

In experiments 2 and 3, *CBA* mice were allowed to reject first-set *A* strain tail skin grafts before ALS was administered (Tables 2 and 3) The ALS which was administered in experiment 2 was of only moderate

Table 1 EFFECT OF ALS IN MICE SENSITIZED TO BOVINE SERUM ALBUMIN

Group	No of animals*	Interval between immunization and treatment (weeks)	Treatment†	Interval between treatment and challenge‡ (weeks)	Antigen binding capacity Mean log titre
Primary response	24	—	—	—	−0 2302
A	6	2	ALS	8	0 1137§
B	3	2	NRS	8	1 1933
C	1	2	ALS	12	0 3140
D	6	2	NRS	12	0 4027
E	6	4	ALS	8	0 2874
F	6	4	NRS	8	0 3095
G	6	4	ALS	12	0 6257§
H	5	4	NRS	12	1 1878
I	5	6	ALS	8	0 6009
J	6	6	NRS	8	0 4847
K	1	6	ALS	12	1 2011
L	4	6	NRS	12	1 0427

* *CBA* female mice immunized by intraperitoneal injection of 5 mg of fluid BSA at time zero

† Subcutaneous administration of either ALS or NRS 0 5 ml , then 0 25 ml × 6 on alternate days

‡ Challenge effected by intraperitoneal injection of 100 μg fluid BSA

§ Significant difference from control ($P < 0\ 05$ by *t* test)

Table 2 EFFECT OF ALS ON CBA MICE SENSITIZED TO *A* STRAIN SKIN

Group	No of animals	Interval between immunization* and treatment (weeks)	Treatment†	Interval between treatment and challenge	Fate of second skin graft MST (days)	Fate of second skin graft Range (days)
A	4	2	ALS	6	8 3	7 5–9 5
B	6	2	ALS	10	9 3	8 5–10 5
C	5	4	ALS	6	8 1	7 5–8 5
D	6	4	ALS	10	9 3	7 5–10 5
E	6	6	ALS	6	9 6	8 5–11 5
F	6	6	ALS	10	7 8	6 5–9 5
G	6	6	NRS	10	< 7 0	< 6 0–8 5
Primary response	41	—	—	—	10 2	7 5–15 5

* All mice immunized on day zero with an *A* strain tail skin graft

† Either ALS or NRS administered subcutaneously 0 5 ml , then 0 25 ml × 6 on alternate days

MST, mean survival time

strength The formal assay of this antiserum yielded a mean survival time of skin homografts (*A* → *CBA*) of 23 days The ALS used in experiment 3 was considerably more potent (MST 30 days) In experiment 2 (Table 2), the mean survival times of second set grafts on animals which had been treated with ALS fell between those characteristic of first and second set rejection The most rapid rejection of challenge homografts was found in the NRS control group Thus, although the secondary response would have been modified, the animals which had been treated with ALS still gave some evidence of earlier sensitization

Table 3 EFFECT OF ALS ON CBA MICE SENSITIZED TO *A* STRAIN SKIN

Group	No of animals	Interval between ALS treatment and challenge* (weeks)	Fate of second skin graft MST (days)	Fate of second skin graft Range (days)
A	9	0	39 5	23 5–55 5
B	10	2	14 5	11 5–18 5
C	9	6	11 2	9 5–12 5
D	9	10	11 5	8 5–16 5
Primary response	46	—	10 5	7 5–15 5

All animals received an *A* strain skin graft and 2 weeks later began ALS treatment by subcutaneous injection of 0 5 ml , then 0 25 ml × 6 on alternate days

MST, mean survival time

* Relative to commencement of ALS treatment

The findings in experiment 3 are quite different (Table 3) Regardless of the time which elapsed between ALS treatment and challenge by a second set graft, no animal reverted to a sensitized state (Fig 1) Challenge homografts survived longest when applied near the beginning of ALS administration, thus confirming the remarkable ability of ALS to overcome the sensitization which was aroused by homografts As the interval between serum treatment and challenge increased, the survival time of the second graft decreased By 6 weeks the rate of rejection of the second grafts equalled that of the first set Despite increase of the interval to 10 weeks no further curtailment of survival was found, and in no case did reversion to a sensitized state occur A time interval of 10 weeks should have been more than sufficient to allow complete recovery from the effects of ALS administration[7]

The results obtained in experiment 3 thus fully confirm the findings of Levey and Medawar[1] Memory of a previous sensitization to transplantation antigen was ablated, and with recovery of immunological capacity the animals reverted to a non-sensitized state, rejecting skin grafts at a rate characteristic of the primary response Failure to achieve this result in experiment 2 was probably the result of inadequate treatment with a less potent antiserum

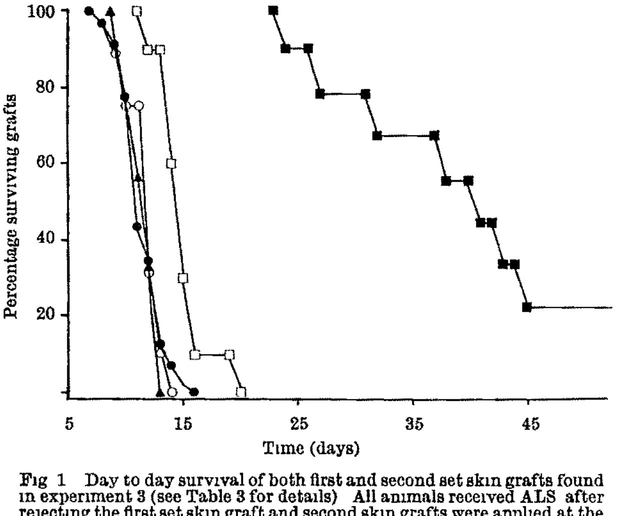

Fig 1 Day to day survival of both first and second set skin grafts found in experiment 3 (see Table 3 for details) All animals received ALS after rejecting the first set skin graft and second skin grafts were applied at the intervals shown relative to the commencement of ALS treatment First set, ● Second set ■, 0 weeks, □, 2 weeks, ▲, 6 weeks, ○, 10 weeks

The failure to erase "memory" of immunization with BSA must be viewed differently The explanation cannot be simply one of inadequate treatment because the potencies of antisera used in experiments 1 and 3 were comparable Furthermore, my experience with other systems (unpublished observations) (*S typhi* "H" antigen and sheep red blood cells) has indicated an extraordinary resistance of the secondary antibody response to the effects of ALS (ref 8) This distinction could reflect the different natural histories of the cells involved in the two types of transactions

Levey and Medawar[9] have shown that the primary action of ALS is on peripheral lymphocytes We have confirmed this view (unpublished observations) and have suggested that the immunosuppressive action of ALS results from the progressive depletion in the host of the pool of circulating small lymphocytes[7] In a skin graft system the responding cells leave central lymphoid tissue and travel to the graft site The pace of graft rejection presumably depends on the proportion of cells "programmed" to respond to the foreign antigens encountered in the graft[10,11] Previous sensitization increases the relative number of circulating cells which are capable of reacting with graft antigens Prolonged treatment with ALS eliminates the population of circulating cells and the regenerating population assumes the original (before sensitization) immunological capabilities which are characteristic of the animal In humoral immune systems the effector cell does not migrate out of the central lymphoid tissue and thereby receives a measure of protection from the effects of ALS When a second dose of antigen arrives at the lymph node or spleen, there remains an enriched population of cells capable of responding While there may be other differences between the primary and secondary response such as the interposition of the macrophage, these need not be invoked to explain the differences which have been observed

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[1] Levey, R H , and Medawar, P B , *Proc US Nat Acad Sci* , **56**, 1130 (1966)
[2] Russell, P S , and Monaco, A P , *Transplantation*, 5, 1086 (1967)
[3] Levey, R H , and Medawar, P B , *Ann NY Acad Sci* , **129**, 164 (1966)
[4] Farr, R S , *J Infect Dis* , **103**, 239 (1958)
[5] Billingham, R E , and Medawar, P B , *J Exp Biol* , **28**, 385 (1951)
[6] Mitchison, N A , *Proc Roy Soc* , B, **161**, 275 (1964)
[7] Lance, E M , in *Progress in Transplantation* (Munksgaard) (in the press)
[8] Lance, E M , in *Symposium on Cell Bound Immunity with Special Reference to Immunotherapy of Cancer, Liège* (in the press)
[9] Levey, R H , and Medawar, P B , *Proc US Nat Acad Sci* , **58**, 470 (1967)
[10] Brent, L , and Medawar, P B , *Proc Roy Soc* , B, **165**, 281 (1966)
[11] Simonsen, M , *Prog All* , **6**, 349 (1962)

Stem-cell Inactivation in Mixed Spleen Cell Cultures

A NUMBER of attempts have been made to understand the interactions possible between immunologically active cells *In vitro*, transformation of lymphocytes in response to other antigenically different lymphocytes has been noted[1] as have similar reactions involving cells explanted from spleens[2] The reactions of immune lymphocytes with their target cells have often been studied The work of Hellstrom *et al* [3] has led to consideration of the notion of allogeneic inhibition during which it is implied that the activity of a cell may be inhibited simply by the presence of a foreign cell antigen All such work is relevant to an

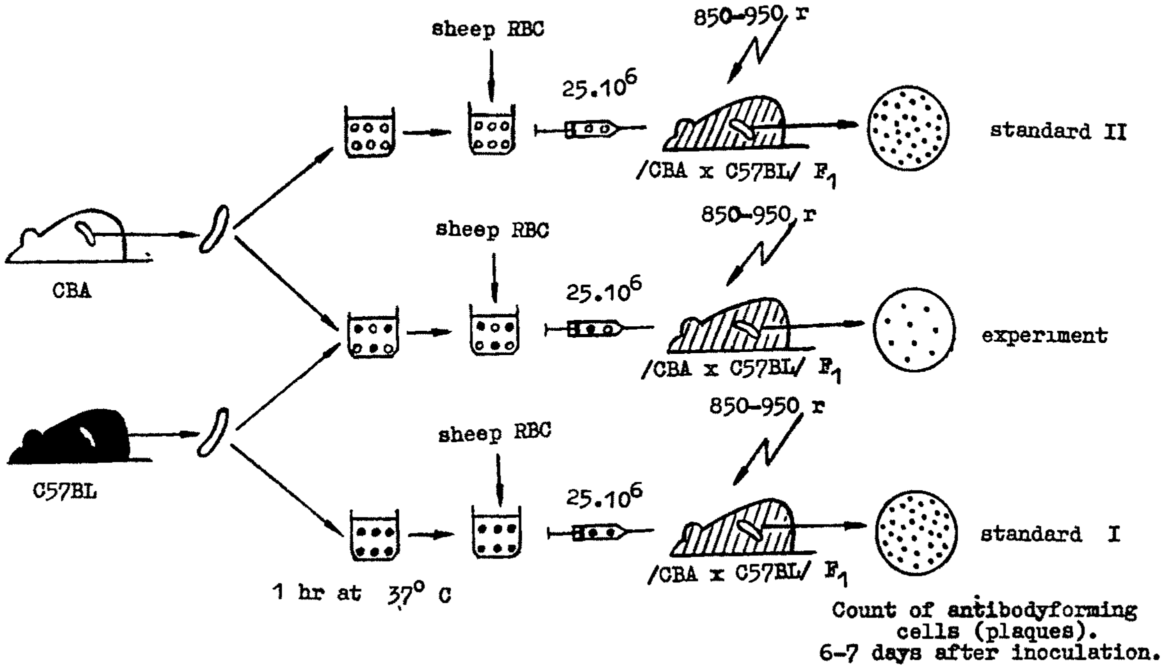

Fig 1 Experimental schedule for the demonstration of stem cell inactivation as adjudged by Jerne plaque count following *in vitro* incubation of mixtures of spleen cells before their injection into irradiated recipients

increase in our understanding of the possible importance of immunological reactions between cells *in vivo* These experiments describe the effects of the presence of foreign cells on the capacity of mouse spleen cells, first to respond to antigenic stimulation, and second to give rise to haematopoietic cell colonies The effect of irradiation on one of the components of the cell mixture is described A preliminary account of this method has already been published[4]

The experimental schedule is laid out in Fig 1 Spleen cells from either *C57Bl* or *CBA* adult mice of either sex were suspended in medium 199 and after two washings in the same medium were resuspended Samples were taken for a dye-exclusion (trypan-blue) test for cell viability The cell density was adjusted to 50×10^6 viable cells/ml The cell suspensions were then incubated separately, or mixed together (50 50 ratio), for 1 h at 37° C At the end of the period of incubation the cells were injected intravenously into irradiated $(C57Bl \times CBA)F_1$ hybrid mice. In those experiments in which we wished to study the immune response of the recipient, sheep cells were added to the spleen cell suspensions before their injection into the irradiated mice 7×10^7 sheep erythrocytes were injected into each recipient At various times after radiation, between the sixth and eighth day, the recipient mice were killed and either direct Jerne plaque assay[5] or spleen colony counts[6] were made In two experiments, *CBA* spleen cells were irradiated *in vitro* with 10,000 r before incubation and transfer All irradiations were effected with gamma-rays

The results are given in Tables 1 and 2 From Table 1 it can be seen that *CBA* and *C57Bl* cells differ in their capacities to react to sheep red cells in irradiated F_1 recipients Furthermore, incubation of the two cell types together seems to result in considerable inactivation of their immunological potential Comparison of the expected with the actual result gives an inactivation index by which different degrees of inactivation may usefully be compared The figure quoted of 75 per cent has been found to be reproducible over a number of experiments Table 2 shows that a similar result is obtained when spleen colonies are counted In this case, however, the *C57Bl* cells have a better capacity than *CBA* cells for responding to the haematopoietic depletion in the irradiated mouse Inactivation of haematopoiesis follows incubation of the two spleen cell types together This inactivation should be thought of as impairment of the activity of haematopoietic stem cells, for there is evidence that spleen colonies are clones It is noteworthy that the inactivation index is similar when measured by estimation of immunologically or haematopoietically active cells

The experiments which involved irradiation of the *CBA* cells must be regarded as preliminary because of the small numbers of mice involved They both, however, give the same result, which suggests that inactivation requires the active participation of both the component cell types in the mixture On this basis, the inactivation phenomenon may be distinguishable from allogeneic inhibition

These experiments show that the interaction between two genetically different mouse spleen cell populations *in vitro* leads to diminution in the capacity of one or other and probably both to respond to antigenic stimuli and to function as sources of haematopoietic stem cells The fact that the inactivation process is initiated and

Table 1 MEAN NUMBER OF PLAQUE-FORMING CELLS PER MILLION SPLEEN CELLS OF $(CBA \times C57Bl)F_1$ MICE 6 (EXPERIMENT 2) OR 7 (EXPERIMENT 1) DAYS AFTER 900 R AND INJECTION OF VARIOUS KINDS OF SPLEEN CELLS

Experiment No	Donor spleen cells	No of recipients	Mean No of plaque forming cells per million spleen cells ± standard error	Expected No of cells	Inactivation index $\left(100 - \frac{O}{E} \times 100\right)$
1	*C57Bl*	12	29 ± 6		
	CBA	6	62 ± 13		
	C57Bl + *CBA*	12	11 ± 2 (*O*)	46 (*E*)	75
2	*C57Bl*	5	8 ± 3		
	CBA (10,000 r)	8	0		
	C57Bl + irradiated *CBA*	12	4 ± 1	4	0

A total of 25×10^6 nucleated cells was injected into each mouse

Table 2 MEAN NUMBERS OF HAEMATOPOIETIC COLONIES PER SPLEEN OF $(CBA \times C57Bl)F_1$ MICE 8 DAYS AFTER 850 R AND INJECTION OF 2×10^6 *CBA* OR *C57Bl* SPLEEN CELLS OR 4×10^6 CELLS OF A 50 50 MIXTURE OF THE TWO

Donor spleen cells	No of recipients	Mean No of colonies/spleen	Expected No of colonies	Inactivation index
C57Bl	25	17 6 + 0 9		
CBA	22	4 5 ± 0 5		
C57Bl	25	5 3 ± 0 4	22 1	76
C57Bl	15	15 7 + 0 8		
CBA (10,000 r)	14	0 2 ± 0 1		
C57Bl + irradiated *CBA*	9	15 5 ± 1 9	15 9	3

perhaps completed *in vitro* holds hope that in future experiments its nature may be explored

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[1] Schwarz, M R , *Immunology*, 10, 281 (1965)
[2] Festenstein, H , *Ann NY Acad Sci* , 129, 567 (1966)
[3] Hellstrom, K E , Hellstrom, I , and Bergheden, C , *Nature*, 208, 458 (1965)
[4] Petrov, R V , and Seslavina, L S , *Rep Acad Sci* , *USSR*, 176, 1170 (1967)
[5] Jerne, N K , and Nordin, A A , *Science*, 140, 405 (1963)
[6] Till, J E , and MacCulloch, E A , *Rad Res* , 14, 213 (1961)

Use of Horse Anti-pig Leucocyte Serum to suppress the Homograft Reaction in Pigs

Interest in the use of heterologous anti-leucocyte serum as a means of suppressing the homograft reaction was stimulated by the work of Woodruff and Anderson[1,2], who showed that rejection of skin allografts in rats could be delayed by administering rabbit anti-rat leucocyte serum to the skin graft recipients Similar findings pertaining to allografts of skin in mice have since been reported by Monaco *et al* [3] and Levey and Medawar[4].

This communication reports preliminary observations on the value of horse anti-pig leucocyte serum in suppressing the rejection of skin allografts in pigs

Two horses were immunized with a cell suspension in Medium 199 (Glaxo) obtained by gently grinding fragments from the mesenteric lymph nodes of outbred pigs, using a hand operated glass piston blender The cell suspension was washed by centrifugation at 580*g* for 5 min and resuspended in fresh Medium 199

Table 1 TIME, IN DAYS, TO COMMENCEMENT OF SKIN ALLOGRAFT REJECTION IN UNTREATED PIGS AND PIGS RECEIVING NORMAL HORSE OR HORSE ANTI-PIG LEUCOCYTE SERUM

Category	Dose of serum	Individual values	Mean
No treatment (group 1 two pigs)		6 8 8 10 7 7 10 10	8·3
Normal horse serum (group 2 two pigs)	3 0 ml /kg body weight/day, intravenously	6 6 7 7 7 7 7 9	7 0
Anti-leucocyte serum (group 3 two pigs)	0 75 ml /kg body weight/day, intravenously	6* 8* 10* 10* 8 8 10 8	8 5
Group 4 two pigs	1 5 ml /kg body weight/day, intravenously	>21 18 18 14 17 >21 18	>18 1

* Received ALS from horse 1. The other pigs in groups 3 and 4 received ALS from horse 2

Horse 1 received three intravenous injections, each of 1×10^9 cells, at intervals of 7 days, and 4 l. of whole blood was taken from it 7 days later After a further 24 days the horse was given another injection of $0\ 44 \times 10^9$ lymph node cells and a further 3 5 l of blood was taken 7 days later

Horse 2 received three intravenous injections at intervals of 7 days, the injections being of 1×10^9, $1\ 7 \times 10^9$ and $1\ 86 \times 10^9$ cells. This horse was bled to give 5 0 l of blood after a further 7 days

The blood was allowed to stand at room temperature for approximately 2 h and was then stored overnight at 4° C The serum was separated after centrifugation at 1,800*g* for 10 min and absorbed, using one-third of its volume of packed pig erythrocytes, at 37° C for 1 h and then overnight at 4° C The serum was again separated after centrifugation at 1,800*g* for 10 min, sterilized by Seitz filtration and stored at − 20° C

Normal horse serum was obtained by bleeding a non-immunized horse and processed as before except that absorption against pig erythrocytes was omitted

Each pig studied received two skin autografts and four skin allografts Skin grafting was carried out as described by Jaffe *et al* [5] with the exception that the grafts were made larger, 1 in.^{2}, to facilitate the recognition of early thickening

The pigs were divided into four groups (Table 1). Pigs of group 1 were untreated controls in which the skin allografts were allowed to reject naturally Pigs of group 2 received 3 0 ml /kg body weight of normal horse serum by intravenous injection daily, commencing on the day of skin grafting Pigs of groups 3 and 4 received 0 75 ml or 1 5 ml /kg body weight of horse anti-pig, anti-leucocyte serum daily by intravenous injection, commencing on the day of skin grafting. In all animals of groups 2, 3 and 4, administration of serum was continued until graft rejection was judged to be complete.

All skin grafts were examined, without previous knowledge of the origin of a particular graft, at 2 day intervals, starting on day 5 after skin grafting. Skin graft rejection was considered to have commenced on the first day that a graft was palpably thickened It proved difficult to assess the time of complete rejection on account of the thickness of pig skin

The times in days to the commencement of skin allograft rejection are shown in Table 1 It may be seen that normal horse serum, group 2, or the lower dose of horse anti-pig leucocyte serum, group 3, had no influence on the time of skin allograft rejection In a dose of 1 5 ml /kg body weight/day, however, the anti-leucocyte serum produced a striking delay in the commencement of skin allograft rejection

Necropsies were performed on all the pigs between the twenty-first and twenty-eighth days. No differences were observed with the naked eye or histologically between the lymph nodes of the groups given anti-leucocyte serum and the control groups All contained a normal amount of lymphoid tissue with normal germinal centres The lymph nodes which drained the graft areas were histologically similar to the other lymph nodes The spleens were also normal, and no abnormalities were seen in the kidneys by conventional staining and light microscopy.

The results indicate that it is possible to obtain a marked prolongation of skin allograft survival without serious toxicity It would be interesting to determine whether a still greater delay in skin graft rejection could be produced by larger daily doses of anti-leucocyte serum, or by a single massive injection of the anti-serum on the day of skin grafting

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[1] Woodruff, M F A , and Anderson, N F , *Nature*, 200, 702 (1963)
[2] Woodruff, M F A , and Anderson, N F , *Ann NY Acad Sci* , 120, 119 (1964)
[3] Monaco, A P Wood, M L , Gray J G , and Russell, P S , *J Immunol* , 96, 229 (1965)
[4] Levey, R H , and Medawar, P B , *Ann NY Acad Sci* , 129, 164 (1966)
[5] Jaffe, W P , Symes, M O , and Terblanche, J , in *The Liver* (edit by Read, A E), Colston Papers, 19 (Butterworth, London, in the press)

Cross-reaction of Anti-lymphocyte Serum with Haemopoietic Stem Cells

THE risk of provoking a fatal "secondary disease syndrome" has been an obstacle to the use of bone marrow transplantation for restoring haemopoiesis in radiation- or drug-induced aplasia This syndrome results from a graft versus host attack by immunologically competent cells derived from the grafted marrow Various methods have been tried for differentially eliminating immunologically competent cells from marrow preparations[1-3], but these have not been sufficiently effective to be of practical value The possibility that anti-lymphocyte serum (ALS) could be sufficiently specific to achieve this object prompted us to examine the extent to which haemopoietic stem cells cross-react with ALS Anticipating that some degree of cross-reaction would occur, we set out to obtain quantitative data for immunologically competent cells and for haemopoietic stem cells in terms of the proportions of cells inactivated by different dilutions of ALS

Haemopoietic stem cells were assayed by their capacity to restore haemopoiesis in recipients previously treated with supralethal doses of dimethyl myleran[4] The method was an adaptation of that described by Hodgson[5] Marrow cells were obtained from the femora and tibiae of rats of a highly inbred Marshall strain The cells were flushed out with ice-cold Ringer solution, and were dispersed in a glass homogenizer with a loosely fitting 'Teflon' plunger Recipients, which were either of the Marshall or of a (Marshall × August) F_1 hybrid strain, were injected intraperitoneally, on the day before marrow transfusion, with 5 8 mg/kg of dimethyl myleran dissolved in a mixture of 1 part of propylene glycol and 16 2 parts of physiological saline (Dimethyl myleran was first dissolved in propylene glycol at 70°–80° C to give a concentration of 10 mg/ml ; the dose of diluted material was then 1 ml /100 g) Seven days after marrow transfusion each recipient was injected intravenously with 0 2 μc of iron-59 (5–11 c /g, Radiochemical Centre, Amersham) as citrate and was bled 24 h later by cardiac puncture In calculating the proportion of the injected dose of iron-59 incorporated into circulating red cells, the blood volume was assumed to be 6 6 per cent of the body weight

Immunologically competent cells were assayed by their capacity to induce a graft versus host reaction Because of their greater abundance of cells in the spleen, this tissue was used as a source in preference to the bone marrow Suspensions of Marshall spleen cells in Ringer solution were prepared by finely mincing and straining the spleens through a 100μ mesh stainless steel sieve The increase in spleen weights of (Marshall × August) F_1 hybrids induced by Marshall spleen cells and measured 9 days after their intraperitoneal injection is, in this strain combination, linearly related to the number of cells injected, within the range used in these experiments

Rabbit anti-rat lymphocyte serum was prepared by the method of Levey and Medawar[6] Eight hundred million thymocytes, obtained from five Marshall thymuses, were injected intravenously into rabbits on two occasions 2 weeks apart The rabbits were bled 1 week after the second injection The sera were inactivated by heating at 56° C for 30 min, and were absorbed twice for 1 h with equal volumes of Marshall red cells (from which leucocytes had been largely removed by centrifugation), the first time at 37° C, the second at room temperature For comparison of effects on immunologically competent and on haemopoietic stem cells the same batch of ALS was always used in any one experiment

Spleen or bone marrow cells were incubated with ALS for 45 min at 37° C All dilutions of ALS and of cells were made with Ringer solution Spleen suspensions contained 100×10^6 cells/ml , marrow suspensions contained 13×10^6 cells/ml for Marshall or 20×10^6 cells/ml for hybrid recipients respectively Spleen cells (2 ml) or marrow cells (1 ml) were injected All rats in these experiments were males, weighing 120–150 g

A preliminary experiment was carried out to determine the range of ALS titres for which inactivation of immunologically competent and of haemopoietic stem cells could be demonstrated Marshall bone marrow and spleen cells were incubated with serial 1 2 dilutions of ALS and were then injected into Marshall and F_1 hybrid recipients, respectively An almost parallel inactivation pattern was obtained for these two classes of cells Dilutions of ALS up to 1 8 gave virtually complete inactivation in both cases The degree of inactivation then appeared to diminish with increasing dilutions of ALS, to approximately 50 per cent, at dilutions of 1 . 64 to 1 128, as compared with cells incubated with Ringer solution alone

On the basis of these results a more critical comparison was made of the extent to which the respective cell types were inactivated by ALS at two selected dilutions, namely, 1 10 and 1 100 The repopulating ability of ALS-treated Marshall haemopoietic stem cells was assayed in F_1 hybrid as well as in Marshall recipients, in order to test for any effect on transplantability into the hybrid strains of cells treated with antiserum prepared against the Marshall strain There were thus three test systems (*a*) Marshall spleen cells assayed by splenomegaly induced in F_1 hybrids ; (*b*) Marshall marrow cells assayed by repopulating ability in Marshall recipients, and (*c*) Marshall marrow cells assayed by repopulating ability in F_1 hybrids Each test system consisted of six groups, containing six rats in a group The first two groups of rats received cells treated with the two dilutions, 1 10 and 1 100 respectively, of ALS A third group received cells treated with normal rabbit serum at a dilution of 1 10 The remaining three groups received either the standard number of cells or half this number, in either case without previous serum treatment, or no cells at all The results are listed in Table 1

Table 1 INACTIVATION OF IMMUNOLOGICALLY COMPETENT AND HAEMOPOIETIC STEM CELLS OF MARSHALL RATS BY RABBIT ANTI-RAT LYMPHOCYTIC SERUM

Rabbit serum	Cell system tested: Immunologically competent cells (spleen weight of F_1 hybrid, mg)	Haemopoietic stem cells (% of iron-59 administered on day 7 incorporated into red cells in 24 h)	
		in Marshalls	in F_1 hybrids
Anti-lymphocytic serum 1/10	610 (39) ‡	12 2 (5 1)	14 3 (1 3)
Anti-lymphocytic serum 1/100	786 (43)	20 3 (0 7)	20 0 (2 0)
Normal rabbit serum 1/10	1,012 (56)	29 5 (4 2)	21 8 (6 0)
None (*a*) Full dose of spleen* or marrow† cells	923 (59)	25 3 (3 6)	29 4 (5 9)
(*b*) Half dose of spleen or marrow cells	784 (49)	23 3 (5 6)	21 0 (2 5)
(*c*) No spleen or marrow cells	600 (11)	8 2 (2 3)	14 2 (1 3)

* 200×10^6 Marshall spleen cells given intraperitoneally to (Marshall × August) F_1 hybrids 9 days before killing

† Marshall rats given 13×10^6, F_1 hybrids 20×10^6, Marshall marrow cells intravenously 24 h after treatment with 5 8 mg/kg of dimethyl myleran

‡ Mean values for six rats, standard errors in parentheses

ALS in a dilution of 1 10 completely inactivated both the immunologically competent cells and the haemopoietic stem cells Both these cell types were reduced in effectiveness by ALS in a dilution of 1 100 to levels corresponding almost exactly (although confidence limits were wide) with those observed with half the standard number of untreated cells Normal rabbit serum in a dilution of 1 10 was without effect (The apparent reduction in the repopulating ability of stem cells in F_1 hybrids after treatment with normal serum was probably not real ($P > 0$ 1) In contrast to the normal rabbit serum used in the other two experimental systems, the batch used in this system had not been absorbed with rat red cells This may have contributed to this effect)

In these experiments no difference could be demonstrated in the effectiveness of ALS against splenic lymphoid cells and against bone marrow stem cells Several hypotheses can be advanced to explain the cross-reactivity of these two cell types The morphological identity of haemopoietic stem cells has never been established and it has been suggested that they may even be indistinguishable from marrow lymphocytes. This contention lends support to the monophyletic theory of the origin of lymphoid and erythroid series[7,8] and if correct would also explain their antigenic similarity. An alternative explanation may be that thymocytes, as opposed to splenic lymph node or blood lymphocytes, were the source of antigen in the preparation of the ALS used in these experiments. Thymocytes are derived from the bone marrow[9], so the similarity in antigenic composition with haemopoietic stem cells may result from a common origin. It is possible that a greater degree of antigenic disparity may exist between marrow stem cells and true lymphoid cells which might be brought to light if the latter cells were used for immunization. Recent work has shown that thymocytes possess some antigens not present in other lymphoid cells[10].

These experiments give no explanation of the mechanism of inactivation by ALS of either of these two types of cell. Our (unpublished) observation, that the addition of guinea-pig complement does not enhance the inactivation of either cell type *in vitro* by dilutions of ALS down to 1:128, supports the view that the action of ALS is predominantly cytotoxic. If a process operated *in vivo* that did not depend on the action of the recipient's own complement, a synergistic effect could be expected between this process and the complement present during *in vitro* incubation. It is possible that coating the cell surface with antibody interferes with the homing property of haemopoietic stem cells to the bone marrow and of immunocompetent cells to the sites in lymphoid tissues in which they initiate a graft versus host reaction.

There is an unexplained discrepancy. On the one hand, ALS has an immediate effect on immunocompetent cells *in vitro*, as reported in these studies and by Brent *et al.*[11], who used mortality in mice injected neonatally with allogeneic lymph node cells as their assay system. On the other hand, a delay of 3–6 days has been reported to occur between the injection of ALS *in vivo* and maximal suppression of immune function, as demonstrated by the capacity of the animal to reject allogeneic skin grafts[12], and of lymph node cells to produce haemolysins and agglutinins to foreign red cells[13], and to induce fatal graft versus host disease[11]. Although injected ALS produces an immediate fall in number of circulating lymphocytes[14], it is apparently prevented from reaching extravascular lymphoid tissues for several days.

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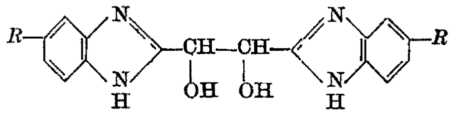
[1] Mathé, G., Amiel, J. L., Matsukura, M., and Mery, A. M., *Brit. J. Haematol.*, 10, 257 (1964).
[2] Mathé, G., Amiel, J. L., Schwartzenberg, J. F., Doré, P., Golstein, M., Sekiguchi, M., and Bechet, J. M., in *The Lymphocyte in Immunology and Haemopoiesis* (edit. by Yoffey, J. M.), 292 (Edward Arnold, Ltd., London, 1966).
[3] Van Bekkum, D. W., *Transplantation*, 2, 393 (1964).
[4] Blackett, N. M., in *Le Greffe des Cellules Hematopoietiques Allogeniques*, 55 (Centre National de la Recherche Scientifique, Paris, 1964).
[5] Hodgson, G. S., *Acta Physiol. Latino Amer.*, 12, 365 (1962).
[6] Levey, R. H., and Medawar, P. B., *Ann. NY Acad. Sci.*, 129, 164 (1966).
[7] Keiser, G., Cottier, H., Bryant, B. J., and Bond, V. P., in *The Lymphocyte in Immunology and Haemopoiesis* (edit. by Yoffey, J. M.), 149 (Edward Arnold, Ltd., London, 1966).
[8] Yoffey, J. M., Hudson, G., and Osmond, D. G., *J. Anat.*, 99, 841 (1965).
[9] Ford, C. E., and Micklem, H. S., *Lancet*, i, 359 (1963).
[10] Potworowski, E. F., and Nairn, R. C., *Nature*, 213, 1135 (1967).
[11] Brent, L., Courtenay, T., and Gowland, G., *Nature*, 215, 1461 (1967).
[12] Monaco, A. P., Wood, M. L., Gray, J. G., and Russell, P. S., *J. Immunol.*, 96, 229 (1966).
[13] Berenbaum, M. C., *Nature*, 215, 1481 (1967).
[14] Abaza, H. M., Nolan, B., Watt, J. G., and Woodruff, M. F. A., *Transplantation*, 4, 618 (1966).

PATHOLOGY

Inhibitory Effect of 1,2-*bis*(2-Benzimidazolyl) 1,2-ethanediol Derivatives on Poliovirus

THE inhibitory properties of 2-(α-hydroxybenzyl) benzimidazole (HBB) on the replication of poliovirus have been fully demonstrated[1,2]. O'Sullivan *et al.*[3] also reported that 1,2-*bis*(2-benzimidazolyl) 1,2-ethanediol (I) has a selective inhibitory action on the multiplication of poliovirus. On the basis of these findings, we have synthesized, 1,2-*bis*(2-benzimidazolyl) 1,2-ethanediol derivatives and tested them for their anti-poliovirus effect.



$R = H$ (I), $R = CH_3$ (II), $R = Cl$ (III), $R = OCH_3$ (IV)

1,2-*bis*(2-Benzimidazolyl) 1,2-ethanediol derivatives were prepared by the following procedures. *o*-Phenylenediamine or 4-methyl, 4-chloro, and 0.04 molar 4-methoxy *o*-phenylenediamine were dissolved in 50 ml. of 4 normal hydrochloric acid in a 300 ml round flask. To this solution was added 0.02 molar tartaric acid and the mixture was refluxed gently on a sand-bath for 2–3 h. The reaction mixture was cooled externally with cold water and neutralized with 20–30 per cent sodium carbonate solution while stirring. The solution was kept in an ice-box overnight to complete crystallization. The crystals were collected by filtration and then recrystallized three times from dil. ethanol. The properties and yields of the compounds are given in Table 1.

The anti-poliovirus activity of these compounds was tested by using a poliovirus type 1 (Mahoney strain) and HeLa S_3 cells in the following way. A suspension of HeLa S_3 cells (4 ml containing 2×10^5 cells/ml.) in YLE growth medium (1.0 g of yeast extract and 5.0 g of lactalbumin hydrolysate in 1,000 ml of Earle's balanced salt solution, with 100 U of streptomycin and 500 U of penicillin supplemented with 10 per cent calf serum) was put into a plaque bottle, and incubated at 37° C for 4 days. After the monolayer cell sheet has been established, the growth medium was removed and, immediately after washing with phosphate buffered saline (PBS, *p*H 7.2), 0.05 ml of different dilutions (10^{-3}–10^{-6} molar) of each tested compound and 0.45 ml of the poliovirus dilution (30–40 PFU/bottle) were added. After incubation for 1 h at 37° C, the culture fluid was removed, and the cells were washed three times with PBS. Then 4.5 ml of agar overlay medium and 0.5 ml of the diluted compound were added in turn to the bottle. The agar overlay medium consisted of equal volumes of 1.8 per cent Bacto-Difco agar and twice concentrated YLE medium supplemented with 8 per cent calf serum and 0.0025 per cent (final concentration) neutral red. After incubation for 4 days at 37° C plaques were counted. For untreated control groups, the same amount of PBS was used instead of one of the compounds being tested. The results shown in Table 2 indicate that 1,2-*bis*(2-benzimidazolyl) 1,2-ethanediol derivatives clearly inhibit the replication of poliovirus. Compound IV showed complete inhibition.

The anti-poliovirus activity of compound IV was also examined by the $TCID_{50}$ estimating dilution method, as shown in Table 3, which indicates that the minimum inhibitory concentration of this compound was about 2×10^{-7} molar. This effect is about 1,000 times greater than that of HBB or guanidine[4]. The possibility that compound IV affected the cells themselves in any way can be excluded. Compound IV showed no toxicity in a

Table 1. PROPERTIES OF THE COMPOUNDS TESTED

Compound	Melting point* (° C)	Yield obtained (%)	Formula	Analysis Calculated	Analysis Found
(I) 1,2-*bis*(2-Benzimidazolyl) 1,2-ethanediol (ref 3)	> 300	14		—	—
(II) 1,2-*bis*[5 (or 6)-Methyl-2-benzimidazolyl] 1,2-ethanediol	230–231	20	$C_{18}H_{18}N_4O_2\ H_2O$	C = 63 51 H = 5 92 N = 16 46	C = 63 53 H = 6 06 N = 16 30
(III) 1,2-*bis*[5 (or 6)-Chloro-2-benzimidazolyl] 1,2-ethanediol	219–220	13	$C_{16}H_{12}N_4O_2Cl_2\ H_2O$	C = 50 41 N = 14 70	C = 50 65 N = 14 68
(IV) 1,2-*bis*[5 (or 6)-Methoxy-2-benzimidazolyl] 1,2-ethanediol	213–214	23	$C_{18}H_{18}N_4O_4\ H_2O$	H = 5 41 N = 15 05	H = 5 64 N = 15 01

* Decomposition point is shown

Table 2 EFFECT OF 1,2-*bis*(2-BENZIMIDAZOLYL) 1,2-ETHANEDIOL DERIVATIVES ON REPLICATION OF POLIOVIRUS

Compounds No	Added* amounts (molar)	No of plaques (mean and dispersion) Untreated groups	No of plaques (mean and dispersion) Treated groups	Percentage inhibition† (mean and dispersion)
I	10^{-3}	37 0 ± 1 87 (35, 35, 38, 38, 39)	10 2 ± 1 30 (9, 9, 10, 11, 12)	72 6 (76 9–65 6)
	10^{-4}	33 6 ± 1 14 (32, 34, 34, 33, 35)	11 0 ± 3 35 (7, 8, 11, 14, 15)	67 2 (80 0–53 1)
	10^{-5}	34 4 ± 2 62 (32, 33, 34, 35, 38)	21 4 ± 3 19 (18, 18, 22, 24, 25)	37 8 (52 6–21 8)
	10^{-6}	36 0 ± 2 45 (34 34, 37, 39)	31 5 ± 2 64 (28, 31, 33, 34)	12 5 (28 2–0 0)
II	10^{-3}	35 0 ± 1 00 (34, 34, 35, 36, 36)	6 8 ± 2 77 (3, 5, 8, 8, 10)	80 6 (91 6–70 6)
	10^{-4}	30 0 ± 2 55 (28, 28, 29, 31, 34)	7 2 ± 2 58 (4, 7, 6, 8, 11)	76 0 (88 2–71 4)
	10^{-5}	31 2 ± 1 90 (28, 31, 32, 32, 33)	21 0 ± 0 70 (20, 21, 21, 21, 22)	32 7 (39 4–21 4)
	10^{-6}	27 0 ± 3 29 (24, 24, 27, 29, 31)	24 5 ± 2 44 (22, 23, 26, 27)	9 8 (24 1–0 0)
III	10^{-4}	45 6 ± 3 03 (42, 43, 46, 48, 49)	2 4 ± 1 51 (0, 2, 3, 3, 4)	92 4 (100 0–90 4)
	10^{-5}	48 0 ± 0 70 (47, 48, 48, 48, 49)	32 8 ± 3 14 (30, 31, 32, 33, 38)	31 7 (38 7–29 8)
	10^{-6}	48 0 ± 2 55 (45, 46, 48, 50, 51)	36 6 ± 2 70 (33, 35, 37, 38, 40)	23 8 (35 3–11 1)
IV	10^{-4}	31 8 ± 0 83 (31, 31, 32, 32, 33)	0 0 ± 0 00 (0, 0, 0, 0, 0)	100 0 (100–100)
	10^{-5}	34 6 ± 1 52 (33, 34, 34, 35, 37)	0 0 ± 0 00 (0, 0, 0, 0, 0)	100 0 (100–100)
	10^{-6}	36 0 ± 2 12 (34, 34, 36, 37, 39)	0 0 ± 0 00 (0, 0, 0, 0, 0)	100 0 (100–100)

* Final molar concentration is shown

$$\dagger\ \frac{\text{No of plaques of the untreated group} - \text{No of plaques of the treated group}}{\text{No of plaques of the untreated group}} \times 100$$

concentration of 10^{-4} molar in 7 days, but in a concentration of 10^{-3} molar cells were degenerated or separated from tissue culture tubes Recent results (reported at the Fifteenth General Meeting of the Society of Japanese Virologists in October 1967 at Chiba) show that (*a*) the incorporation of 2-^{14}C-uridine and ^{3}H-valine did not differ between cell cultures without compound IV and cultures treated with 10^{-4} molar compound IV, and (*b*) compound IV strongly reduced the yield of intracellular virus This compound can therefore be supposed to be non-toxic.

Table 3 ANTI-POLIOVIRUS ACTIVITY OF 1,2-BIS[5(OR 6)-METHOXY-2-BENZIMIDAZOLYL] 1,2-ETHANEDIOL

Added* amount	$-$Log *TCID*$_{50}$ Untreated groups	$-$Log *TCID*$_{50}$ Treated groups	Difference $-$log *TCID*$_{50}$
10^{-4}	9 00	0 75	8 25
10^{-5}	9 00	1 25	7 75
10^{-6}	9 00	1 50	7 50
10^{-7}	9 00	8 75	0 25
10^{-8}	9 00	9 00	0 00

* Final molar concentration is shown.

The high inhibitory effect of this compound should be valuable not only for anti-viral chemotherapy but for analysing the mechanism of viral replication

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[1] Tamm, I , and Bablanian, R , *J Exp Med* , **113**, 625 (1961)
[2] Eggers, H J , and Tamm, I , *Virology*, **18**, 426 (1962)
[3] O'Sullivan, D G , and Wallis, A K , *Nature*, **198**, 1270 (1963)
[4] Zhdanov, Yu A , and Dorofeenko, G N , *Zhur Obshckei Khim* , **29**, 2677 (1959)
[5] Rightsel, W A , and Dice, J R , *Science*, **134**, 558 (1961)
[6] Crowther, D , and Melnick, J , *Virology*, **15**, 65 (1961)
[7] Ueda, T , Toyoshima, S , and Seto, Y , *Keio J Med* ,**10**, 257 (1961)

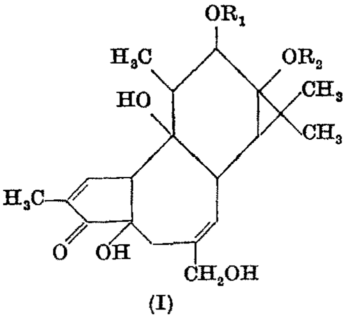
Response of an Active Component of Croton Oil to Short-term Tests of Carcinogenicity

THE cocarcinogenic properties of croton oil have been known for a long time[1], and there have been many attempts to identify its active components[2,3]

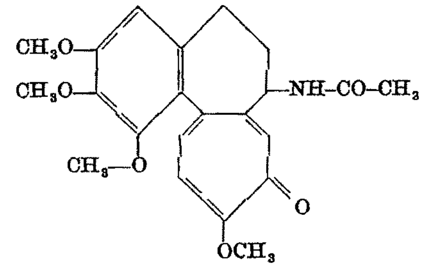
Hecker *et al* [3-8] succeeded in isolating a set of phorbol esters from croton oil According to these authors phorbol is a tetracyclic diterpene $C_{20}H_{28}O_6$ Its structural formula is now known to correspond to the formula (I) shown[8], where $R_1 = R_2 = H$ Hecker's experiments showed that these esters, apart from being promoters of cancer, also have a mild but definite tumour initiating effect[9].

Among the isolated esters of phorbol, Hecker's substance A_1 (ref 5) proved to be the most powerful This product has the same melting point (72° C) as "substance *C*-3" of Arroyo and Holcomb[10] and the same mass spectrum as "substance *C*" of Van Duuren and Orris[2] Substance A_1 is supposed to be *c*-acetyl-*b*-myristoyl-phorbol (that is, 13-O-acetyl-phorbol-12-myristate) $C_{36}H_{56}O_8$, corresponding to formula (I), where R_1 is a myristoyle, and R_2 an acetyl residue[5,8]

We have investigated the response of Hecker's substance A_1 to the short-term tests of carcinogenicity Two tests—the sebaceous gland suppression test and the epidermal hyperplasia test—were made by our usual



(I)



(II)

Table 1 RESULTS OF THE SEBACEOUS GLAND SUPPRESSION AND HYPERPLASIA TESTS

	Controls	Dose 1 10,000	Dose 1 1,000*
Mean of No of sebaceous glands ± 2 standard errors	19 1±1 2	4 4±1 3	4 9±1·9
Mean epidermal thickness ± 2 standard errors	19 6±2 2	63 3±3 6	71 6±9 5

* In some cases ulceration was present

routine[11]. Mice of the *IC* strain, 49 days old, were used in the tests The substance was dissolved in acetone and was tested in two dilutions 1 1,000 and 1 10,000 There was a strongly positive reaction in both cases (Table 1)

The phorbol ester which we have studied is one of the substances which, as well as being cancer initiators or promoters, give positive sebaceous gland suppression and hyperplasia tests These findings emphasize the correlation between carcinogenicity and positive response to short-term tests This relationship is particularly interesting because so far it has usually been described within the polynuclear hydrocarbons[12,13]; our results enlarge the field of application of such tests

It is also interesting that there is, according to Sivak, an analogy of structure and configuration between phorbol and colchicine (formula (II)) This latter substance gives a positive short-term test[12,13] without any known property of initiating or promoting cancer

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[1] Berenblum, I , *Adv Cancer Res* , 2, 151 (1954).
[2] Van Duuren, B L , and Orris, L , *Cancer Res* , 25, 1871 (1965)
[3] Hecker, E , *Z Krebsforsch* , 65, 325 (1963)
[4] Hecker, E , Jarczyk, H , Meyer, J G , Bresch, H , and Brachmann, I , *Z Krebsforsch* 66, 478 (1965)
[5] Hecker, E , and Bresch, H , *Z Naturforsch* , 20, 216 (1965)
[6] Hecker, E , and Kubinyi, H , *Z Krebsforsch* , 67, 176 (1965)
[7] Clarke, E , and Hecker, E , *Z Krebsforsch* , 67, 192 (1965)
[8] Hecker, E , and Schairer, H U , *Z Krebsforsch* , 70, 1 (1967)
[9] Hecker, E , *Colloq Gesellsch f Physiolog Chemie, Mosbach* (Baden), 105 (1966)
[10] Arroyo, E R , and Holcomb J *J Med Chem* , 8, 672 (1965)
[11] Lazar P Libermann, C , Chouroulinkov, I , and Guérin, M , *Bull. Assoc Fr Ét Cancer*, 50, 567 (1963)
[12] Bock, F G , and Mund, R , *Cancer Res* , 18, 887 (1958)
[13] Guérin M , and Cuzin, J , *Bull Assoc Fr Ét Cancer*, 48, 112 (1961)

Infectious Mononucleosis: Continuous Suspension Culture of Peripheral Blood Leucocytes

THE atypical lymphocyte in patients with infectious mononucleosis, like the leukaemic lymphocyte, is capable of spontaneous *in vivo* proliferation This is shown by dividing and binucleate forms in circulation[1], increased concentrations of cellular enzymes associated with nucleic acid synthesis[2], markedly increased premitotic activity with incorporation of tritiated nucleoside precursors[3] and early *in vitro* division of some of these labelled cells[4] Spontaneous lymphoblastoid transformation of fibroblastic elements derived from bone marrow of patients with infectious mononucleosis has been described[5] with long term culture of these cells in suspension[6] Despite the increased proliferative potential of peripheral atypical lymphocytes in this disease, little is known about the progeny of these cells No established culture of lymphoid cells derived from peripheral blood of patients with infectious mononucleosis has previously been reported

Our attempts to establish long term cultures of peripheral blood leucocytes from patients with infectious mononucleosis, evaluated at the National Institutes of Health and diagnosed with criteria suggested by Hoagland[7], include thirty-one samples taken from fifteen individuals at various stages of the disease Twenty-six samples from thirteen patients with clinical diseases without infectious mononucleosis or known malignancy and fourteen samples from ten individuals without clinical illness were studied collaterally (Table 1).

Table 1 ATTEMPTS TO ESTABLISH LONG TERM SUSPENSION CULTURE OF PERIPHERAL BLOOD LEUCOCYTES

Group	No of individuals	No of specimens	Lymphocytes/10 ml of medium
No clinical illness	10	14	$3 0\times10^{6}$–$2 0\times10^{7}$
Clinical disease other than infectious mononucleosis or malignancy	13	26	$3 4\times10^{6}$–$2 0\times10^{7}$
Infectious mononucleosis	15	31	$3 0\times10^{6}$–$2 5\times10^{7}$ (1 specimen at $5 6\times10^{7}$)

Leucocyte cultures were prepared by gravity sedimentation of heparinized peripheral blood at 37° C for 1–2 h and centrifugation of the plasma supernatant rich in leucocytes at 250*g* in the International *PR*-2 at 20° C for 30 min A sample ($3 0\times10^{6}$ to $5 0\times10^{7}$) of lymphocytes (determined by direct differential count on a haemocytometer) was cultured at 37° C in 2 ounce screw top prescription bottles containing 10 ml of RPMI 1640 medium[8], supplemented with 20 per cent heat-inactivated foetal calf serum, penicillin, streptomycin and *l*-glutamine Between 30 per cent and 50 per cent of the supernatant culture fluid was removed and replenished with fresh medium after gravity sedimentation of cells in each culture twice a week for the first 2 weeks and then weekly until a culture was terminated No effort was made to maintain the original cell concentration Cultures were observed daily with a Zeiss inverted microscope for signs of spontaneous proliferation and were discarded when intact lymphocytes were no longer visible

Eight specimens from peripheral blood of five patients with infectious mononucleosis have proliferated in long term suspension culture (Table 2) Initial signs of proliferation were evident after 21–44 days of culture with progressive *p*H (acidic) changes in the medium, clumping of cells in suspension and increase in cell number At present these established lines do not require feeder layers and have no fibroblastic component when grown in medium containing foetal calf serum Cells adherent to glass were noted, however, in the first few weeks of culture in seven of the eight lines established and either fibroblastic or epithelioid elements or both appear in all cultures grown in medium lacking foetal calf serum The cells in suspension culture have been maintained for at least 3–7 months and show no loss of vitality or signs of termination No long term suspension cultures were obtained from individuals without clinical illness or individuals with diseases other than infectious mononucleosis with the culture techniques used

Table 2 SPECIMENS OF PERIPHERAL BLOOD LEUCOCYTES FROM PATIENTS WITH INFECTIOUS MONONUCLEOSIS PROLIFERATING IN LONG TERM SUSPENSION CULTURE

Cell line	Total lymphocytes cultured/10 ml of medium	Signs of proliferation evident (days in culture)	Patient's highest guinea-pig kidney adsorbed serum heterophil titre
J P (*H*)	$1 0\times10^{7}$	36	1 896
J P (*J*)	$1 0\times10^{7}$	21	
W R (*D*)	$6 0\times10^{6}$	44	1 1,792
W R (*F*)	$1 0\times10^{7}$	41	
E C (*B*)	$5 6\times10^{7}$	35	1 7,168
L M (*B*)	$2 4\times10^{7}$	24	1 112
L M (*D*)	$1 4\times10^{7}$	40	
M K (*B*)	$2 4\times10^{7}$	34	1 448

The established lines grow essentially as free-floating pleomorphic forms with a tendency to clump despite agitation which easily reseparates them into individual cells. Giemsa staining reveals wide morphological heterogeneity with a fundamental lymphoid character with densely stained nucleus, readily visualized nucleoli, vacuoles and variable amounts of basophilic to faint staining cytoplasm. There is considerable variation in cell size and shape and quantity of uropodia. The cells look quite immature, resembling lymphoblasts and lymphocytes stimulated with phytomitogen (phytohaemagglutinin/pokeweed mitogen)[9,10]. Cells reminiscent of plasmacytoid Downey cells[11] and bizarre nuclear forms are frequently seen. Ultrastructure analysis shows a spectrum of lymphoid cells varying from small cells with close morphological similarity to atypical lymphocytes obtained from peripheral blood of patients with infectious mononucleosis, to large multinucleate forms. Varying amounts of rough surfaced endoplasmic reticulum with occasional dilated cisternae and scattered free polyribosome complexes are present.

Chromosomal analysis shows a predominantly diploid mode for all lines with a pseudodiploid population accounting for 4 per cent of metaphase plates examined in one of the lines. Doubling times range from 48–80 h in the standard culture medium. Cells actively ingesting cellular and nuclear material are present in all cultures. Following incubation with sterile polystyrene particles (0.8 mm, Dow Chemical Co.) or heat-killed *Staphylococcus albus*, 15–30 per cent of the cells showed evidence of phagocytosis. Ingestion of *Staphylococcus albus* was not associated with release of endogenous pyrogen[12]. Histochemical analysis revealed varying quantities of lysosomal enzymes but complete absence of peroxidase and leucocyte alkaline phosphatase. Most cells contain material which stains positively by the periodic acid–Schiff reaction. No morphological differences could be discerned for phagocytic and non-phagocytic cells before incubation with foreign particles.

The ability of these lymphoid cells to synthesize immunoglobulins was assayed by the radioimmunoelectrophoretic technique[13] in 18 h culture containing various cell concentrations in 2 ml of medium. Dried immunoelectrophoresis plates were exposed to standard X-ray film for 5–14 days before developing. Nascent synthesis of IgM, IgA and IgG, detectable at cell concentrations as low as 5.0×10^5/ml, was demonstrated with mono-specific anti-human immunoglobulin sera. Antibody specificity of these newly synthesized immunoglobulins is under investigation.

Interferon-like activity has been demonstrated in the supernatants from these cell lines (unpublished results). This material is not sedimented by centrifugation at 105,000*g* for 90 min. It exhibits specificity for cells of human origin, is sensitive to trypsin and cannot significantly inhibit adenovirus replication. This activity is reduced by pretreatment with actinomycin *D* of the host cell. No bacteria, virus, fungus or mycoplasma have been isolated from these lines.

Multiple continuous, long-term suspension cultures of lymphoid cells derived from peripheral blood of patients with lymphoblastic leukaemia[14], myelogenous leukaemia[15], Burkitt's lymphoma[16], Hodgkin's lymphoma, lymphosarcoma and multiple myeloma[17] have been described. Lethally irradiated Burkitt's lymphoma cells with herpes-type virus particles have been reported to induce long term culture of normal leucocytes with acquisition by these cells of herpes-type viral antigen and *C*-group chromosomal markers[18]. With special techniques established cultures of lymphoid cells from peripheral blood of "normal" individuals have been obtained as well[8]. Many of these normal lines, however, harbour herpes-type virus particles, and long-term culture may well be a result of latently infected cells circulating in individuals without clinical illness. With the techniques used, this background potential was not encountered in our control samples. Collateral specimens from patients with infectious mononucleosis have proliferated in long-term culture.

Infectious mononucleosis may simulate acute leukaemia and is thought by some observers to represent an atypical form with a self-limiting course[19]. Demonstration of the increased *in vitro* proliferation of peripheral blood leucocytes from patients with infectious mononucleosis similar to neoplastic cells in leukaemia and lymphoma supports the thesis of common cellular potential in these lymphoproliferative states and offers a tool for investigation of this relationship.

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[1] Carter, R. L., *Blood*, 26, 579 (1965).
[2] Bertino, J. R., Simmons, B. M., and Donohue, D. M., *Blood*, 19, 587 (1962).
[3] Epstein, L. B., and Brecher, G., *Blood*, 25, 197 (1965).
[4] MacKinney, A. A., *Blood*, 26, 36 (1965).
[5] Benvesh-Melnick, M., Fernbach, D. J., and Lewis, R. T., *J. Nat. Cancer Inst.*, 31, 1311 (1963).
[6] Rabin, E. R., Benvesh-Melnick, M., and Brunschwig, J. P., *Exp. Mol. Pathol.*, 7, 196 (1967).
[7] Hoagland, R. J., *Blood*, 16, 1045 (1960).
[8] Moore, G. E., Gerner, R. E., and Franklin, H. A., *J. Amer. Med. Assoc.*, 199, 519 (1967).
[9] Tanaka, Y., Epstein, L. P., Brecher, G., and Stohlman, F., *Blood*, 22, 614 (1963).
[10] Chessin, L. N., Borjeson, J., Welsh, P., Douglas, S. D., and Cooper, H. L., *J. Exp. Med.*, 124, 873 (1966).
[11] Downey, H., and McKinlay, C. A., *Arch. Intern. Med.*, 32, 82 (1923).
[12] Bodel, P., and Atkins, E., *Proc. Soc. Exp. Biol. and Med.*, 121, 943 (1966).
[13] Fahey, J. L., Feingold, I., Rabson, A. S., and Manaker, R. A., *Science*, 152, 1259 (1966).
[14] Moore, G. E., Ito, E., Ulrich, K., and Sandberg, A. A., *Cancer*, 19, 713 (1966).
[15] Iwakata, S., and Grace, J. T., *NY State Med. J.*, 64, 2279 (1964).
[16] Epstein, M. A., Barr, Y. M., and Achong, B. G., *Wistar Inst. Symp.*, 4, 69 (1965).
[17] Moore, G. E., Grace, J. T., Citron, P., Gerner, R., and Burns, A., *NY State Med. J.*, 66, 2757 (1966).
[18] Henle, W., Diehl, V., Koch, G., zur Havsen, H., and Henle, G., *Science*, 157, 1064 (1967).
[19] Dameshek, W., and Gunz, F., *Leukemia*, 403 (Grune and Stratton, London, 1964).

Seizures in Experimental Porphyria

WE report work which represents the first experimental model for neurological effects observed in acute intermittent porphyria (AIP), a genetically determined disorder, clinically characterized by gastrointestinal, neurological and psychotic signs. Increased urinary excretion of the porphyrin precursors delta-aminolaevulinic acid (ALA) and porphobilinogen (PBG) is found in overt as well as in most of the latent asymptomatic cases. Severe, often fatal, attacks may be precipitated or aggravated by certain drugs, especially barbiturates. The mechanisms responsible for the clinical signs in AIP, all of which seem to be on a neurogenic basis, are totally unknown[1,2], the risk involved by the use of drugs precludes clinical studies on the possible factors involved until more information can be derived from experimental models. Chemical manifestations, similar to those of the human disorder—

that is, increased synthesis and excretion of porphyrins and their precursors—can be produced in laboratory animals by the use of certain drugs. No clear-cut neurological abnormalities, however, have been described in experimental porphyria[2].

We have found that rats, made porphyric by allylisopropylacetamide (AIA), are very susceptible to induced convulsions. The induction of seizures in these animals is a tool for studies of abnormalities in neurological functions associated with disturbances in porphyrin metabolism. These in turn might shed some light on the factors involved in the deranged functions of the nervous system in human porphyria.

Female Sprague–Dawley rats, weighing 150–160 g, were injected subcutaneously with AIA solution in 0·15 molar sodium chloride, at a dose of 400 mg/kg, between eight and ten daily injections were given. Excessive urinary excretion of ALA and PBG was noted by established methods[3,4]. Twenty-four hours after the last injection of AIA, groups of these rats, as well as control animals, were injected intraperitoneally with solutions—made in 0·15 molar sodium chloride—of the following compounds: (*a*) isonicotinoylhydrazide (INH), (*b*) 4-methoxymethylpyridoxol hydrochloric acid, adjusted to *p*H 7, (*c*) 4-deoxypyridoxol hydrochloric acid, adjusted to *p*H 7. Some animals were injected intraperitoneally with pyridoxol hydrochloric acid solution in 0·15 molar sodium chloride, adjusted to *p*H 7, 15 min before injections of these compounds. The rats were observed for 4 h after the injections and checked again 24 h later.

A dose of 0·75 mmoles/kg of INH induced generalized clonic-tonic convulsions in 60 per cent of the porphyric rats whereas none of the control animals convulsed; at a dose of 1·12 mmoles/kg, 90 per cent of the porphyric rats convulsed as compared with 20 per cent of the control group. At this and at a higher dose level very severe convulsions were noted in the porphyric rats as compared with mild convulsions seen in the control animals. In general, the convulsions occurred at 30–60 min after the injection. None of the animals died (Fig. 1). Pyridoxol at doses of 0·5–2 mmoles/kg did not modify the pattern of convulsions induced by INH at a dose of 1·12 mmoles/kg.

A marked difference between control and AIA treated rats was found after injection of 4-methoxymethylpyridoxol (Fig. 2). Seventy per cent of the porphyric rats convulsed after injection of 0·25 mmoles/kg. All porphyric rats convulsed when given 0·75 mmoles/kg, whereas none of the control rats was affected by this dose. At a dose of 1 mmole/kg, 20 per cent of normal rats showed generalized but mild convulsions occurring at 30–60 min after the injection, as compared with very severe repeated tonic-clonic convulsions in all porphyric animals at 10–30 min after the injection. None of the animals died. Some protection was noted upon injection of pyridoxol; 2 mmoles/kg reduced the convulsions induced by 0·75 mmole/kg 4-methoxymethylpyridoxol from 100 per cent to 40 per cent. The severity of the convulsions induced by this and by higher doses of 4-methoxymethylpyridoxol was decreased in animals injected with pyridoxol, and there was a delay in the appearance of seizures from less than 30 min to about 1 h. No complete protection, however, was achieved in any of the groups by varying the doses of pyridoxol.

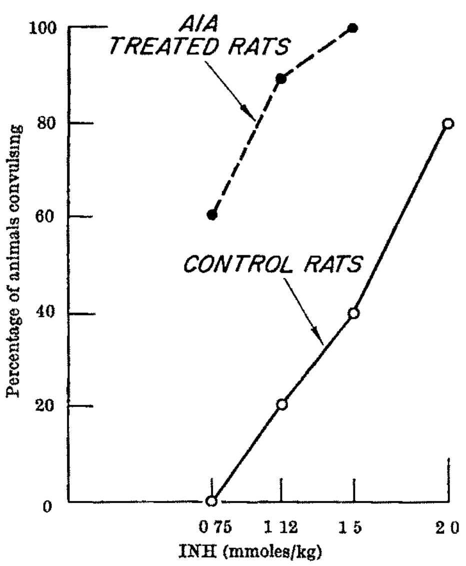

Fig. 1. Convulsions induced by INH in rats treated with AIA and in control rats. Ten to fifteen animals were used for each point.

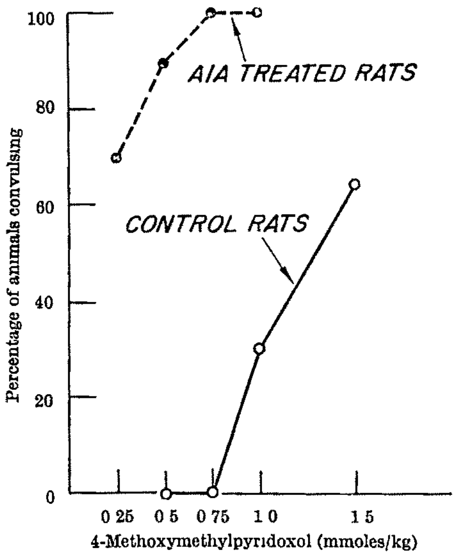

Fig. 2. Convulsions induced by 4-methoxymethylpyridoxol in rats treated with AIA and in control rats. Nine to twelve animals were used for each point.

No difference was found between rats treated with AIA and control rats after injection of 4-deoxypyridoxol (Table 1). The dose-response curve was found to be very steep for both groups. One control and one rat treated with AIA died and several other AIA treated rats showed only mild convulsions at a dose of 3·0 mmoles/kg; most animals in both groups died within 60 min after injection of 3·5 mmoles/kg of 4-deoxypyridoxol.

Table 1. EFFECT OF 4-DEOXYPYRIDOXOL ON CONTROL RATS AND ON RATS TREATED WITH AIA

Dose mmoles/kg	Rats treated with AIA		Control rats	
	Convulsions	Mortality	Convulsions	Mortality
3·0	4/12	1/12	1/8	1/8
3·5	8/10	7/10	9/10	8/10

The mechanisms of action of carbonyl reagents and of pyridoxol analogues are not clearly understood. The convulsions induced by these reagents have been attributed to interference with the metabolic pathways of the nervous system, in which pyridoxal-5-phosphate is an essential cofactor: for example, the γ-aminobutyric acid pathway. It has been assumed that INH forms hydrazones with pyridoxal and pyridoxal-5-phosphate, thereby inhibiting pyridoxal kinase and some enzyme reactions involving pyridoxal-5-phosphate. Pyridoxol analogues, such as 4-deoxypyridoxol, may, after their phosphorylation, serve as competitive inhibitors of several pyridoxal-5-phosphate dependent enzymes, but the exact modes and sites of action, as well as the possible active derivatives of these compounds, are not known[5-7]. An increased sensitivity to INH is associated with decreased brain content of pyridoxal-5-phosphate[8].

ALA synthesis, for which pyridoxal-5-phosphate serves as a cofactor, is markedly increased in the liver of porphyric animals and in the liver of patients suffering from AIP[9-12]. An increase in the liver pool of this cofactor could lead to a relative deficiency in other organs, such as the nervous system. Indeed, many of the clinical and pathological signs of porphyria are similar to those in other syndromes where pyridoxine deficiency has been postulated[13,14]. A simple deficiency of pyridoxine, however, does not explain the results reported here. Whereas the rats treated with AIA showed a marked sensitivity to 4-methoxymethylpyridoxol, no difference from normal was noted after 4-deoxypyridoxol injection. The different action of the two pyridoxol analogues and the lack of a definite protection by pyridoxol against convulsions induced by 4-methoxymethylpyridoxol and INH point to a more specific mode of action than is implied by a general deficiency. No deterioration in the clinical state of a patient suffering from AIP was noted after treatment with 4-deoxypyridoxol[15]. Treatment of patients with pyridoxol during acute attacks of porphyria either did not change the clinical course or it coincided with a worsening of the clinical state (unpublished observations). One cannot therefore imply a simple deficiency of the cofactor in any of these cases. In other situations, where abnormalities in pyridoxine metabolism have been assumed to occur, no simple deficiency is sufficient to explain the observed signs; while some of the neurological abnormalities, observed in patients who receive INH, can be prevented by therapy with pyridoxol, increase in the severity of symptoms was observed in some patients being treated with INH as well as in some patients with a variety of nervous system disorders[16,17].

Our experiments have shown that there are neurological disturbances in porphyric rats which are related to some aspects of pyridoxine action. It seems most probable that the initial site of action of the antagonists is a pyridoxal dependent enzyme. Obvious possibilities are glutamic acid decarboxylase and γ-aminobutyric acid transaminase. Glutamic acid and γ-aminobutyric acid have been implicated as transmitter substances in nervous tissue. Other possible sites include metabolic pathways of catecholamines, acetylcholine and of structural proteins in reactive membranes[5-7]. We have found that ALA rapidly forms imines with pyridoxal and pyridoxal-5-phosphate (our unpublished results). The kinetic and thermodynamic properties of the products are compatible with a role as competitive inhibitors *in vivo* at locations far from their point of generation. Such compounds, for example, imines and their derivatives, can have sufficient structural and steric specificity to serve as inhibitors to some of the key enzymes. Such an inhibition could change functional activity of the nervous tissue and lower the threshold for convulsions.

Further studies on this experimental model might help in clarifying the influence of disturbances in porphyrin metabolism on metabolic pathways of nervous tissue.

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[1] Schmid, R., in *The Metabolic Basis of Inherited Disease* (edit. by Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S.), second ed., 813 (McGraw-Hill, New York, 1966).
[2] Goldberg, A., and Rimington, C., *Diseases of Porphyrin Metabolism* (Charles C. Thomas, Springfield, Illinois, 1962).
[3] Mauserall, D., and Granick, S., *J. Biol. Chem.*, **219**, 435 (1956).
[4] Watson, C. J., Bossenmaier, I., and Cardinal, R., *J. Amer. Med. Assoc.*, **175**, 1087 (1961).
[5] Holtz, P., and Palm, D., *Pharmac. Rev.*, **16**, 113 (1964).
[6] Webb, J. L., *Enzyme and Metabolic Inhibitors*, **2** (Acad. Press, Inc., New York, 1966).
[7] Roberts, E., Wein, J., and Simonsen, D. G., in *Vitamins and Hormones*, **22**, 503 (1964).
[8] Bonasera, N., Smorto, M., and Bonavita, V., *Brain Res.*, **4**, 383 (1967).
[9] Granick, S., and Urata, G., *J. Biol. Chem.*, **238**, 821 (1963).
[10] Marver, H. S., Collins, A., Tschudy, D. P., and Rechcigl, jun., M., *J. Biol. Chem.*, **241**, 4323 (1966).
[11] Tschudy, D. P., Perlroth, M. G., Marver, H. S., Collins, A., Hunter, jun., G., and Rechcigl, jun., M., *Proc. US Nat. Acad. Sci.*, **53**, 841 (1965).
[12] Nakao, K., Wada, O., Kitamura, K., and Uono, K., *Nature*, **210**, 838 (1966).
[13] Cavanagh, J. B., and Ridley, A. R., *Lancet*, ii, 1023 (1967).
[14] Price, J. M., Brown, R. R., and Peters, H. A., *Neurology*, **9**, 456 (1959).
[15] Elder, T. D., and Mengel, C. E., *Amer. J. Med.*, **41**, 369 (1966).
[16] Lowe, C. U., *et al.* (American Academy of Pediatrics, Committee on Nutrition), *Pediatrics*, **38**, 1068 (1966).
[17] Hottinger, A., Berger, H., and Krauthammer, W., *Schweiz. Med. Wschr.*, **94**, 221 (1964).

CYTOLOGY

Heterochromatic Segments in *Vicia* revealed by Treatment with HCl–Acetic Acid

YAMASAKI[1] reported that *H* segments became visible in the mitotic metaphase chromosomes of *Cypripedium debile* following treatment with a 1 : 9 mixture of 1 normal HCl and 45 per cent acetic acid or acetic orcein for 15 min at 60° C. Sharma and Roy[2] and Sen[3], using the orcein mixture at 90° C, claimed that the treatment caused chromosome breakage at non-specific sites within a minute. The present experiments on *Vicia faba* ("Nagasaya Soramame") studied the effects of these mixtures and the separate effects of 1 normal HCl and 45 per cent acetic acid. Treatments lasting 1–5 or 10 min were given at 60° C and 90° C. Pretreatment with colchicine was necessary to facilitate observation, but fixation in acetic alcohol before treatment was not helpful.

At 90° C, treatment of up to 2 min with a 1 : 9 mixture of 1 normal HCl and 2 per cent orcein in 45 per cent acetic acid had no effect while treatment for more than 4 min gave reduced stainability. *H* segments were invariably revealed, especially in the *M* chromosomes (Fig. 1) after 3–4 min treatments, and no indications of breakage were observed in any of the experiments.

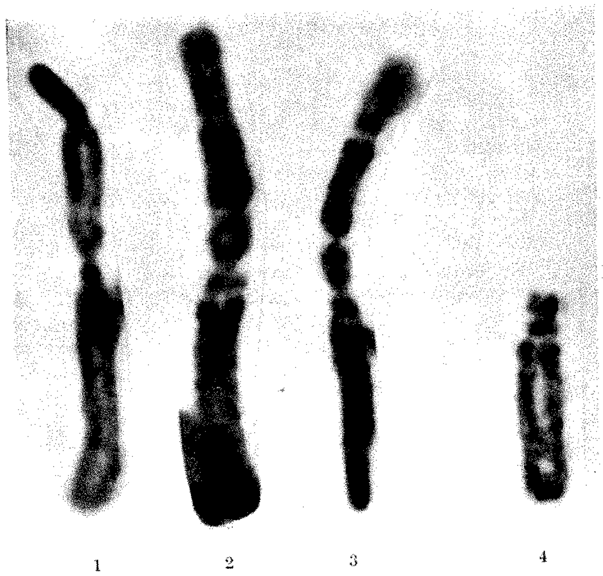

Fig. 1. 1, 2 and 3: the *M* chromosomes showing heterochromatic segments are arranged according to the order in which the *H* segments appear. 4 is an *S* chromosome showing a heterochromatic segment. (Same magnification.)

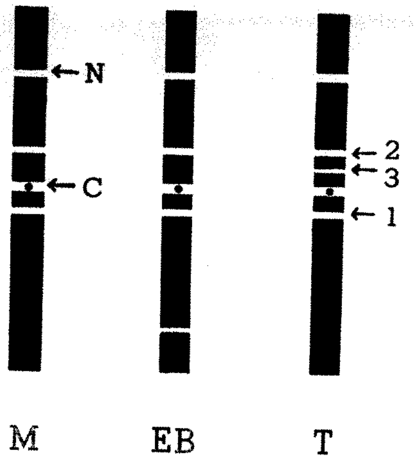

Fig. 2. The positions of the centromere (C), nucleolar organizer (N) and heterochromatic segments (other gaps) as described by McLeish[4] (M), Evans and Bigger[5] (EB) and myself (T). 1, 2 and 3 indicate the sequence in which the *H* segments become evident.

Treatment with HCl–acetic acid followed by Feulgen staining was preferable to treatment with HCl–orcein and the best results were obtained after 3 min treatment at 90° C.

The *H* segments described by McLeish[4] following cold treatment in *Vicia* are even more obvious after treatment with HCl–acetic acid. But the additional *H* segment described by Evans and Bigger[5] is not evident. HCl–acetic acid, however, reveals further segments which have not been described following cold treatment (Fig. 2).

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[1] Yamasaki, N., *Chromosoma*, 7, 620 (1956).
[2] Sharma, A. K., and Roy, M., *Chromosoma*, 7, 275 (1955).
[3] Sen, S., *Nucleus*, 8, 63 (1965).
[4] McLeish, J., *Heredity* (Suppl.), 6, 125 (1953).
[5] Evans, H. J., and Bigger, T. R. L., *Genetics*, 46, 277 (1961).

Chromosome Aberrations during Normal Epithelial Proliferation

EARLIER investigations[1] have shown that a spectacular cellular proliferation occurs in the epithelium of the mouse Müllerian vaginal anlage and in the uterine cervix during approximately the first week after birth. This proliferation is paralleled by a cellular degeneration[2]. It has been suggested that the latter phenomenon is caused, at least in part, by a degeneration of cells with abnormal mitoses which appear during the proliferation[3].

Because abnormal mitoses appear during this proliferation, we thought that it would be interesting to investigate also whether some cells have a chromosome number which is different from the normal diploid number in a mouse (40). This communication presents evidence for the existence of such cells.

Young albino mice from a closed stock were used. The animals were injected with colchicine (1 μg in 0·05 ml. of Tyrode solution) on the day of birth and were killed 6 h later. The anterior part of the vaginal anlage and the uterine cervix were dissected out. The preparations were put into a 3 per cent solution of trypsin (Difco, 1 : 250) in Tyrode solution free from calcium and magnesium at 37° C for 5 min. While still in the trypsin solution but now at room temperature, the epithelium was dissected free from mesenchyme with fine glass needles. The epithelium was then transferred to a hypotonic solution (Parker 199 and distilled water, 1 : 5) for 90 min. The preparations were fixed in a solution of acetic acid, normal hydrochloric acid and distilled water (6 : 1 : 3); they were stained in acetic orcein, and squashed. The cover glass was framed with Kroenig cement.

Because the squash technique can introduce errors when chromosomes are lost from a cell, we did not analyse any cells with less than forty chromosomes. The metaphases studied were all well circumscribed and the chromosomes in each metaphase were all contracted to the same extent.

The chromosomes of 469 cells, hypodiploid cells not being included, were analysed from about twenty-five newborn mice. Among these 469 cells, forty-eight had more than forty chromosomes. Twenty-seven cells (5·4 per cent) had 41–49 chromosomes, ten cells (2·1 per cent) had 50–69 chromosomes, and eleven cells (2·3 per cent) had 70–80 chromosomes. A cell with forty-two chromosomes is shown in Fig. 1.

Four metacentric chromosomes were seen. One cell had forty acrocentric chromosomes and one metacentric chromosome. The remaining three metacentrics (Fig. 2) were found in groups of chromosomes which could not definitely be considered to belong to a single cell.

Aneuploidy and structural differences in chromosomes are common phenomena in cancer and precancerous conditions[4–7], in cells in tissue culture[8], in short term cultures of the human endometrium[9] and after cells have been exposed to X-rays[10]; opinions differ about their occurrence in regenerating liver[4,11]. Special types of chromosomal aberrations can be seen in patients with an abnormal sex chromosome set, Down's syndrome and similar conditions.

Chromosomal aberrations can be tolerated in a cell as long as the genome for replication is intact and as long as the cell is not exposed to special demands. When this occurs—for example, during the differentiation of the epithelium in the anterior part of the vaginal anlage and the uterine cervix—it seems unlikely that the unbalanced genome is compatible with cell life, for cells with deviating genotypes are exposed to a strong negative selection[12]. We think that cells with abnormal genotypes occur by chance during proliferation. When the epithelium differentiates later, these cells die and could contribute to the degeneration in the same region[13].

A tetraploid chromosome number is usually found in some tissues such as bone marrow, liver, transitional

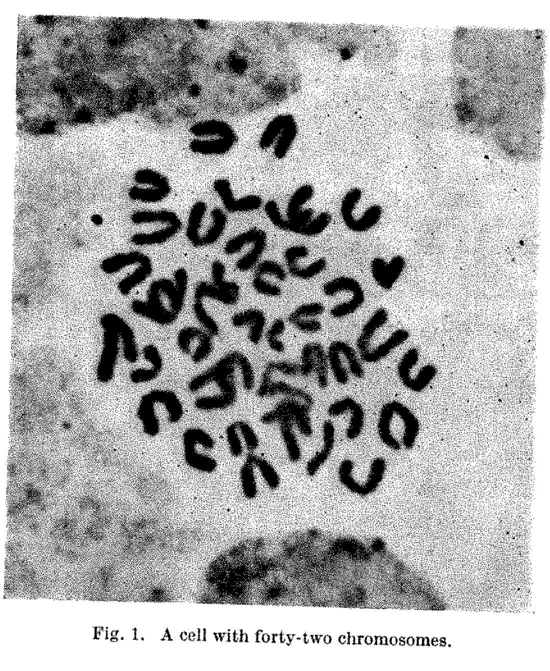

Fig. 1. A cell with forty-two chromosomes.

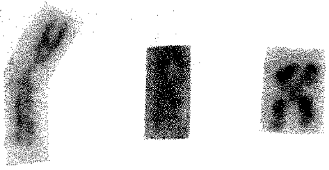

Fig. 2. Three of the four metacentric chromosomes.

epithelium and in glia cells in the cerebellum[11,14-16]. Müllerian epithelium from the anterior part of the vaginal anlage and uterine cervix in neonatal mice can also be included in this group.

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[1] Forsberg, J.-G., *Acta Anat.*, **62**, 266 (1965).
[2] Forsberg, J.-G., *J. Embryol. Exp. Morph.*, **17**, 433 (1967).
[3] Forsberg, J.-G., *Experientia*, **23**, 841 (1967).
[4] Gläss, E., *Krebsforsch*, **60**, 362 (1960).
[5] Boddington, M. M., Spriggs, A. I., and Wolfendale, M. R., *Brit. Med. J.*, i, 154 (1965).
[6] Miles, Ch. P., *Cancer*, **20**, 1274 (1967).
[7] Sandberg, A., Yamada, K., Kikuchi, Y., and Takagi, N., *Cancer*, **20**, 1099 (1967).
[8] Levan, A., and Biesele, J. J., *Ann. NY Acad. Sci.*, **71**, 1022 (1958).
[9] Hughes, C. E., and Csermely, T. V., *Amer. J. Obstet. Gynec.*, **93**, 777 (1965).
[10] Ford, C. R., in *Cytogenetics of Cells in Culture* (edit. by Harris, R. J. C.), (Academic Press, New York and London, 1964).
[11] Hsu, T. C., *Int. Rev. Cytol.*, **12**, 69 (1961).
[12] Forsberg, J.-G., and Källén, B., *Rev. Roum. d'Embryol. et de Cytol., Serie d'Embryol.* (in the press).
[13] Källén, B., *Progress in Brain Research*, **14**, 77 (1965).
[14] Epstein, C. J., and Gatens, E. A., *Nature*, **214**, 1051 (1967).
[15] Hauschka, T. S., *Cancer Res.*, **21**, 957 (1961).
[16] Lapham, L. W., and Johnstone, M. A., *Arch. Neurol.*, **9**, 194 (1963).

GENETICS

Production of Foetal Haemoglobin in Marrow Cultures of Human Adults

IN the usual conditions of marrow culture, haemoglobin synthesis is maintained for only short periods. Kraus (ref. 1 and personal communication) reported on a long-term bone marrow culture method maintained at less than 37° C. In experimenting with this technique, we observed increased foetal ($\alpha_2\gamma_2$) haemoglobin synthesis in culturing adult marrows.

Marrow from adults was obtained during routine aspirations or from ribs obtained at cardiac surgery. Haematological diagnoses included various anaemias, leukaemia, suspected metastatic disease and Gaucher's disease. None of these patients had an elevated amount of Hb-F. Marrow suspensions (5×10^5–5×10^6 nucleated cells/mm^3, using 0·5 ml. of marrow in each culture) were drawn into 1 per cent sterile heparin, stored in the dark for less than 1 h at room temperature and placed in a 25 ml. vessel fitted with a ground glass top and containing 18 ml. of Tyrode solution, phenol red as *p*H indicator, 400 U of penicillin, 400 μg of streptomycin/ml. of media and 2·5 ml. of the patient's plasma or foetal calf serum. Incubation in the dark at 27° C lasted for various times between 1 and 20 days. ^{14}C-Leucine (5 μc./culture) or citrated iron-59 in 1·5 ml. of patient's serum or foetal calf serum (10 μc./culture) was added to label any haemoglobin synthesized during incubation. At the end of incubation, the contents of the culture were washed four times with isotonic saline. Cells were lysed with 2 ml. of distilled water and all cell debris was discarded after high-speed centrifugation. The haemoglobins of the resultant supernates were separated either by starch gel electrophoresis using TEB–borate buffer[2] or by starch grain electrophoresis using a buffer which separates Hb-A from Hb-F (15·5 g of H_3BO_3, 54·5 g of *tris* 7–9 and 2·9 g of ethylenediamine tetraacetic acid diluted with water to 2,000 ml. (*p*H 8·6). Borate buffer for the vessels (*p*H 8·5)—222·7 g of H_3BO_3 and 43·8 g of NaOH diluted with water to 24 l.—was suggested by Miss J. Anderson of the King County Blood Bank, Seattle. The alkali denaturation method of Betke[3] was also used to separate foetal haemoglobin.

Haemoglobin synthesized during incubation was estimated by measuring the amount of radioactivity incorporated into various haemoglobins separated by these methods. Non-radioactive carrier foetal haemoglobin was added to the radioactive haemolysates just before separation. When ^{14}C-leucine was used, liquid scintillation counting was carried out on the various haemoglobin fractions recovered from starch grain using Bray's counting solution[4] in a Packard 'Tri-Carb' liquid scintillation spectrometer. When iron-59 was used, the samples of haemoglobin were separated by both starch gel electrophoresis and starch grain electrophoresis. The radioactivity of the starch gel separations was measured in an auto-scaler after cutting the gel into a series of separate bands.

Marrows incubated in these conditions showed a reduced number of nucleated cells: 1×10^6/mm^3 of culture media on the first day and 3×10^4/mm^3 of culture media on the eleventh day of incubation. The amount of radioactivity incorporated into haemoglobin each day also decreased with prolonged incubation (Table 1).

Table 1. INCORPORATION OF ^{59}FE INTO HAEMOGLOBIN IN CULTURES INCUBATED FOR INCREASING TIMES

Day of incubation	Total c.p.m. in Hb
2	7,000
3	10,500
5	14,000
7	15,500
9	16,000
11	18,500

Iron-59 was added to all cultures on the first day.

In contrast, the proportion as well as the total amount of radioactivity incorporated into foetal haemoglobin increased during incubation (Figs. 1 and 2). The patterns found were similar when the analyses were carried out on starch gel electrophoresis using iron-59 or on starch grain electrophoresis with either label. A typical electrophoretic analysis on starch grain is shown in Fig. 3. To confirm that the radioactivity was in fact incorporated in Hb-F, this pigment was also isolated by alkali denaturation. Recoveries using the two techniques showed good agreement (Table 2).

To determine whether the increase in production of Hb-F was dependent on newly formed RNA, actinomycin

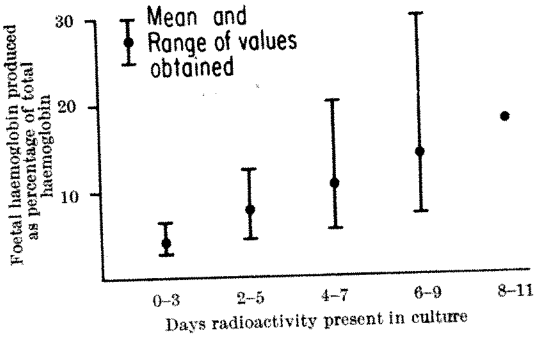


Fig. 1. Effect of length of incubation on rate of incorporation of radioactivity into foetal haemoglobin. Each point represents fifteen experiments except for days 8–11 when only one experiment was performed.

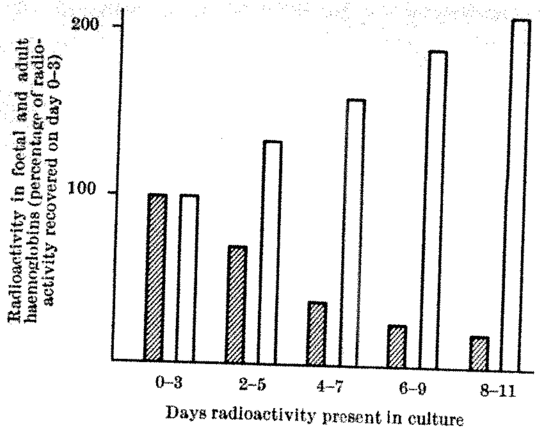


Fig. 2. Effect of length of incubation on incorporation of radioactivity into foetal (white columns) and adult haemoglobins (hatched columns). The columns for each comparison are based on the means of seven experiments. The amount of radioactivity present in foetal and adult haemoglobin on day 0–3 was taken as 100 per cent, and the amount of radioactivity in haemoglobin thereafter was expressed as the percentage of the radioactivity present on day 0–3. The ranges of foetal haemoglobin were as follows: days 2–5: 47–285 per cent; days 4–7: 47–350 per cent; days 6–9: 65–510 per cent; days 8–11: 190–265 per cent.

D was added to the cultures[5] to depress synthesis of DNA dependent RNA (0·1 ml. of actinomycin *D* dissolved in alcohol to give a final concentration of 5^{-10} molar). Two samples of a culture from the same individual, one with added actinomycin, were incubated in fourteen experiments and the counts in the various haemoglobin fractions were compared (Table 3). Addition of actinomycin *D* suppressed the production of foetal haemoglobin (mean suppression 88 per cent) more than that of adult haemoglobin (mean suppression 29 per cent). This difference was highly significant ($P < 0·01$) using the Wilcoxon rank order test.

Table 2. COMPARISON OF ALKALINE DENATURATION AND ELECTROPHORESIS IN ESTIMATION OF FOETAL Hb SYNTHESIS IN CULTURES

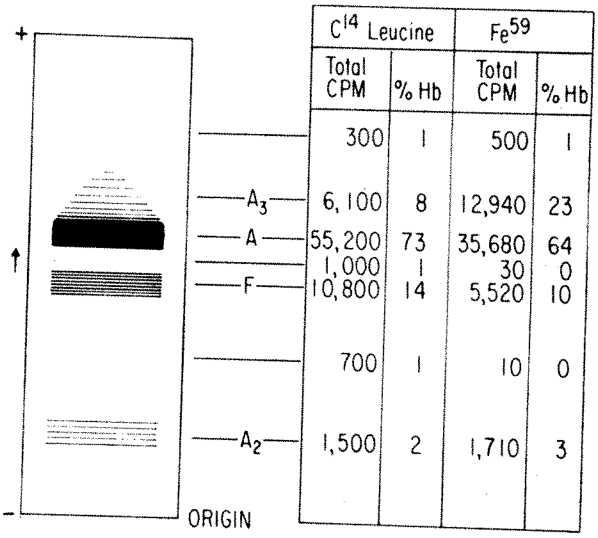
Experiment	Percentage alkali resistant haemoglobin*	Percentage F Hb* (elution from starch gel electrophoresis) Iron-59
1	18	21
2	28	33
3	1	5
4	2	3·5

* Percentages refer to the amount of radioactivity recovered from relevant fractions after culturing.

No differences in the amount of radioactivity incorporated into foetal haemoglobin in culture were noted when foetal calf serum was used rather than the patient's own serum. There was no relationship between the patient's clinical diagnosis and the amount of radioactivity incorporated into foetal haemoglobin. Essentially similar results were obtained using carbon-14 and iron-59 labelling.

Several mechanisms could account for the findings of increased Hb-F synthesis. (*a*) The culture conditions favour increased transcription of messenger RNA for the gamma chains of foetal haemoglobin. (*b*) Erythropoietic precursors which normally produce foetal haemoglobin were more resistant to cell death in culture conditions. (*c*) There was increased resistance of gamma chain messenger RNA to destruction in culture conditions. (*d*) Assuming that equal amounts of beta and gamma chain messenger RNA are made at all times and that control of haemoglobin synthesis is exerted at the ribosomal level[6], the conditions of culture favoured a shift towards translation of gamma chains by ribosomes.

The finding of an absolute increase in synthesis of Hb-F (Fig. 2) together with the more pronounced depression of Hb-F than of Hb-A synthesis with actinomycin (Table 3) suggests that increased transcription of messenger for gamma chains is the most likely explanation for our findings. Any one of the other mechanisms could also be operative. Whatever the mechanism, these studies indicate that haemoglobin synthesis may be active in culture for



	C^{14} Leucine		Fe^{59}	
	Total CPM	% Hb	Total CPM	% Hb
	300	1	500	1
A_3	6,100	8	12,940	23
A	55,200	73	35,680	64
	1,000	1	30	0
F	10,800	14	5,520	10
	700	1	10	0
A_2	1,500	2	1,710	3

Fig. 3. Schematic representation of results of separation of various haemoglobins by starch grain electrophoresis and elution. A_3, A, F and A_2 refer to the location of these haemoglobin fractions on the starch plate. The numbers refer to the results of elution. The unlabelled lines refer to elution results of starch segments between and ahead of haemoglobin fractions. The data were from two cultures from the same individual, one labelled with ^{14}C-leucine, the other with iron-59.

Table 3. EFFECT OF ACTINOMYCIN ON HAEMOGLOBIN SYNTHESIS

Experiment No.	Days radio-activity present in culture	Total haemoglobin synthesis per culture: Total c.p.m. without actinomycin	Total c.p.m. with actinomycin	Percentage suppression	Adult haemoglobin synthesis per culture: Total c.p.m. without actinomycin	Total c.p.m. with actinomycin	Percentage suppression	Foetal haemoglobin synthesis per culture: Total c.p.m. Without actinomycin	Percentage Hb F Without actinomycin	Total c.p.m. With actinomycin	Percentage Hb F With actinomycin	Percentage suppression
1	1–11	49,050	36,800	25	44,000	35,400	20	3,900	8·0*	810	2·2*	79
2	8–11	200,000	160,000	20	162,000	149,000	8	34,000	17·0†	8,600	5·0†	76
3	4–7	150,000	62,000	59	133,000	59,700	55	14,000	9·3†	1,100	1·8†	92
4	4–7	53,000	10,700	80	48,700	10,300	79	3,280	6·2†	214	2·0†	93
5	6–9	220,000	202,000	8	203,000	195,000	4	12,300	5·6†	2,828	1·4†	77
6	6–9	141,000	129,000	8	126,000	106,000	16	12,100	8·6†	0	0†	100
7	8–11	110,000	84,000	24	94,000	80,600	14	13,300	12·1†	1,848	2·2†	86
8	6–9	13,300	9,100	31	11,800	8,630	27	1,210	9·1†	291	3·2†	76
9	4–7	160,000	70,000	57	136,000	65,900	52	20,800	13·0†	2,730	3·9†	87
10	1–11	12,700	9,900	22	11,160	9,605	14	1,320	10·4*	115	1·1*	91
11	6–9	87,000	59,000	33	77,200	57,200	26	8,180	9·4†	590	1·0†	93
12	1–11	55,200	25,300	55	48,100	24,450	50	6,100	11·0*	350	1·4*	94
13	4–7	11,600	10,500	9	9,060	8,100	11	2,300	19·8†	205	2·0†	91
14	4–7	55,500	63,000	+14	48,000	61,800	+29	6,440	11·6†	0	0†	100
Mean				32			29		10·9		1·9	88
Range				80– +14			79– +29		5·6–19·8		0–5·0	76–100

* Starch grain separation.
† Separation by alkaline denaturation.

more than 10 days. The technique provides a tool for the study of mechanisms controlling foetal haemoglobin synthesis

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[1] Kraus, L M, *Nature*, 192, 1055 (1961)
[2] Huehns, E R, and Shooter, E M, *J Med Genet*, 2, 48 (1965)
[3] Betke, K, Marti, H R, and Schlicht, I, *Nature*, 184, 1877 (1959)
[4] Bray, G A, *Anal Biochem*, 1, 279 (1960)
[5] Goldberg, I H, and Rabinowitz, M, *Science*, 136, 315 (1962).
[6] Wilt, F H, *Amer Zoologist*, 6, 67 (1966)

PHYSIOLOGY

Powerful New Aggregator of Blood Platelets—2-Chloroadenosine-5′-diphosphate

ADENOSINE-5′-DIPHOSPHATE causes reversible aggregation of blood platelets in many mammalian species both *in vitro*[1,2] and *in vivo*[3,4] An optimal concentration of calcium ions[5] and the presence of one or more plasma factors[6] are required for aggregation to take place Studies by Born and Cross[2] of the aggregation of human platelets *in vitro* have shown that there is a linear relationship between the rate of aggregation and the logarithm of the concentration of ADP Aggregation *in vitro*, caused by ADP, is inhibited by adenosine, adenosine-5′-phosphate (AMP) and adenosine-5′-triphosphate (ATP) whether the platelets are human[2,7], rabbit[8] or pig[5]. It has been suggested by Clayton *et al* [9] that adenosine and AMP inhibit aggregation by occupying the ADP receptor

The nature of the ADP receptor and the mechanism by which ADP causes platelets to aggregate is not known although a number of different theories have been proposed Clayton *et al* [9] and Hellem and Owren[10] have suggested the interplatelet calcium bridge theory which envisages the binding of the purine moiety of ADP to a receptor on the surface of the platelet possibly by means of a plasma protein[10], and the bridging of the pyrophosphate moieties of adsorbed ADP molecules by calcium ions The inhibitory properties of adenosine, AMP and ATP are thought to be a result of their inability to form calcium bridges, though they still occupy the ADP receptor[9-11]

Salzman *et al* [12] have shown that platelets have an ATPase on their surface and they suggested that this "ecto-ATPase" provides the energy to maintain the platelets in the non-sticky state. ADP is thought to arrest this membrane regulatory process by product inhibition of the "ecto-ATPase", resultant changes in the membrane then permit the exposure of receptor sites which can take part in the platelet–platelet bridging by plasma protein and calcium ions

Robinson *et al* [13] and Mitchell and Sharp[14] have explained the phenomenon of the ADP-mediated platelet aggregation by postulating that the adsorption of ADP to the platelet surface alters the platelet's surface charge which normally causes platelets to repel each other

Recently another hypothesis has been put forward by Born[15], who found that dithiol compounds, such as dithiothreitol, inhibit the ADP-mediated aggregation of platelets He suggested that the interaction of ADP with its specific receptor produces a configurational change in a component of the platelet surface, and that this change is necessary for the formation between platelets and the plasma protein fibrinogen, of disulphide bonds which bring about the aggregation. Whatever the mode of action of ADP, whether it is initially simple adsorption to a specific receptor site on the surface of the platelet or the inhibition of a membrane regulatory enzyme, it seems most likely that ADP and adenosine act on the same receptor

2-Chloroadenosine has been found to be more potent than adenosine in inhibiting the ADP-mediated aggregation of human[16], rabbit[17] and sheep[18] platelets, and its action is more prolonged than that of adenosine The greater duration of action of 2-chloroadenosine can be explained, as suggested by Born[16], by the fact that it is probably not inactivated by plasma adenosine deaminase; 2-chloroadenosine is not deaminated by either rat kidney[19] or ox heart[20] adenosine deaminase The greater potency of 2-chloroadenosine compared with adenosine could be a result of a greater affinity of the analogue for the adenosine receptor If this is so, and adenosine and ADP do act at the same receptor, then the 2-chloro analogue of ADP would also be expected to have a greater affinity for the ADP receptor and to be an aggregator of greater potency than ADP

2-Chloroadenosine-5′-diphosphate (2-Cl-ADP) has been synthesized in this institute; it was isolated as the diammonium salt and was analytically pure Details of the synthesis will be reported elsewhere (G Gough and M H Maguire)

Aggregation studies with 2-Cl-ADP using sheep and human platelet-rich plasma have shown that 2-Cl-ADP is nine to ten times as potent as ADP

Sheep platelet-rich plasma was prepared as previously reported[18], and human platelet-rich plasma was prepared similarly from citrated blood collected from healthy volunteers The aggregation of platelets was measured by a modified version of Born's turbidimetric technique[5] In this method, the maximal change in optical density which could be induced by ADP was first recorded, and then the recording system was adjusted so that the change in optical density between non-aggregated and maximally aggregated platelet-rich plasma resulted in a standard reading on the recorder scale In this way results obtained from different batches of plasma could be pooled for statistical purposes To obviate further differences from one batch of platelet-rich plasma to another, comparative experiments were always carried out on the same day on the same batch of plasma

The aggregation of sheep platelet-rich plasma, brought about by varying concentrations of 2-Cl-ADP, was found to be reversible and dose dependent, just as the ADP-mediated aggregation is[18] Fig 1 shows the aggregation of sheep platelet-rich plasma by varying concentrations of 2-Cl-ADP. The log dose response curve for this aggregation was obtained by measuring the initial rate of the fall in optical density in platelet-rich plasma after the addition of 2-Cl-ADP. The log dose response curve for the ADP-mediated aggregation was obtained similarly Both log dose response curves are shown in Fig 2 and their parallel nature demonstrates that these two agonists are acting on the same receptor Both diphosphates in sufficient concentration cause complete aggregation This is indicated on the log dose response curves by the extrapolated broken line

Equimolar concentrations of 2-chloroadenosine and adenosine were found to inhibit the 2-Cl-ADP-mediated aggregation (Fig 3), and 2-chloroadenosine can be seen to be the more potent inhibitor Inhibition by both nucleosides is concentration dependent Log dose response curves were obtained from the aggregation curves for 2-Cl-ADP alone and in the presence of three concentrations of adenosine and 2-chloroadenosine In Fig 4 these log dose response curves form a family of parallel lines, indicating that the inhibition is competitive and that the agonist 2-Cl-ADP, and the antagonists 2-chloroadenosine and adenosine, are all acting on the same receptor The log dose response curves for the ADP-mediated aggregation in the absence of inhibitor and inhibited by adenosine and 2-chloroadenosine formed a similar family of parallel lines (Fig 5), showing the competitive nature of the inhibition by adenosine and 2-chloroadenosine of the ADP-mediated aggregation Both pairs of agonists and antagonists are acting on the same receptor.

Figs 2–5 show that the agonist and the antagonist in which the hydrogen of carbon 2 of the adenine moiety is replaced by chlorine have a greater affinity for the receptor than the parent compounds Values for the affinities of the antagonists were obtained from Arunlakshana and Schild plots of log $(x-1)$ versus the log of the concentration of the antagonist[21] where x is the ratio of the concentrations of inhibited agonist to uninhibited agonist which both give the same response. Data for the plots were obtained from Figs 4 and 5

The four Arunlakshana and Schild plots (Fig 6) form straight parallel lines, and in the case of the antagonist adenosine the plots obtained with both agonists, ADP and 2-Cl-ADP, are almost superimposable The plots obtained for the antagonist 2-chloroadenosine are not superimposable but not very different, and do overlap within the limits given by the standard errors. For clarity of illustration the standard errors are not shown The logarithmic values of the affinity constants, K_b, of the two antagonists are obtained from the intercepts with the line $\log (x-1)=0$, for adenosine, each agonist gives an identical value. 5 83, while for 2-chloroadenosine the intercept values are 6 43 with ADP as agonist and 6 40 with 2-Cl-ADP as agonist These values are expressed as affinity constants in Table 1 The two affinity constants obtained for 2-chloroadenosine, 0 27 × 10^7 and 0 25 × 10^7, are averaged

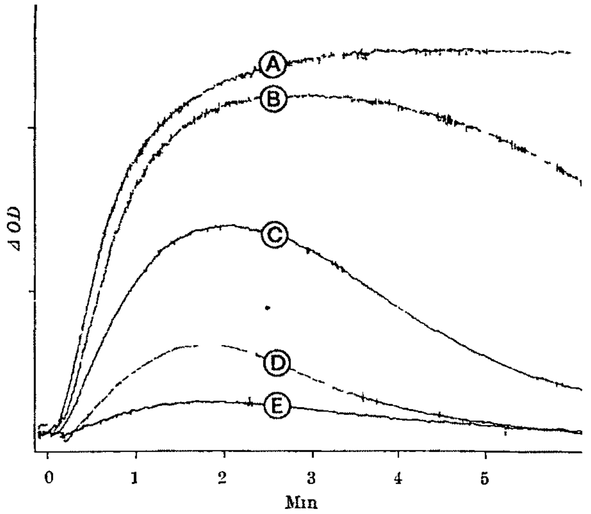

Fig 1 Changes in the optical density of sheep platelet-rich plasma caused by 2-Cl-ADP added at zero time 2-Cl-ADP concentrations × 10^{-8} molar *A*, 12 8, *B* 6 4, *C*, 3 2, *D*, 1 6, *E*, 0 8

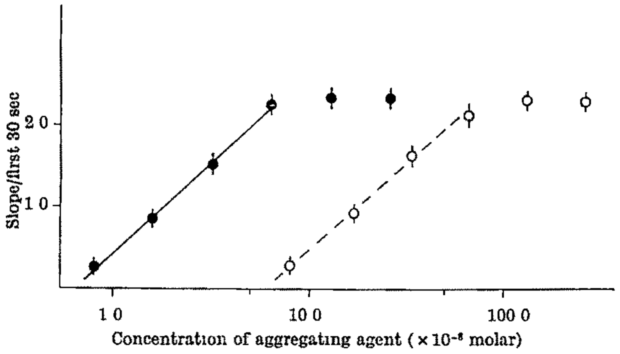

Fig 2 Log dose response curves for the aggregation of sheep platelet-rich plasma by ADP (○) and 2-Cl-ADP (●) Rates of aggregation were obtained from the initial slopes of the aggregation curves The points on each curve represent means of not less than ten experiments, the standard errors of the means are indicated by the vertical lines

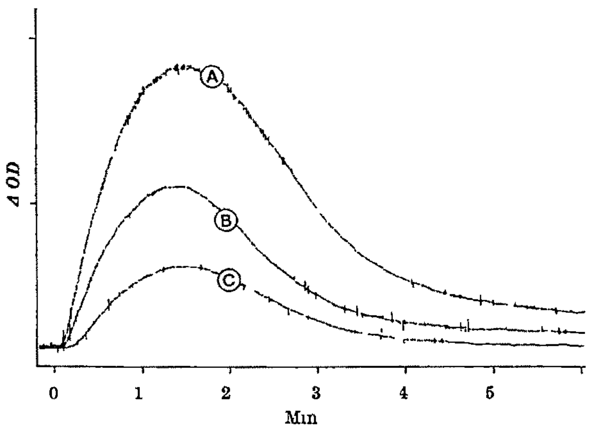

Fig 3 Changes in optical density of sheep platelet-rich plasma caused by 3 2 × 10^{-8} molar 2-Cl-ADP alone (*A*), and in the presence of 1 2 × 10^{-6} molar adenosine (*B*) and 1 2 × 10^{-6} molar 2-chloroadenosine (*C*) Adenosine and 2-chloroadenosine were added to the platelet-rich plasma 2 min before the addition of 2-Cl-ADP at zero time

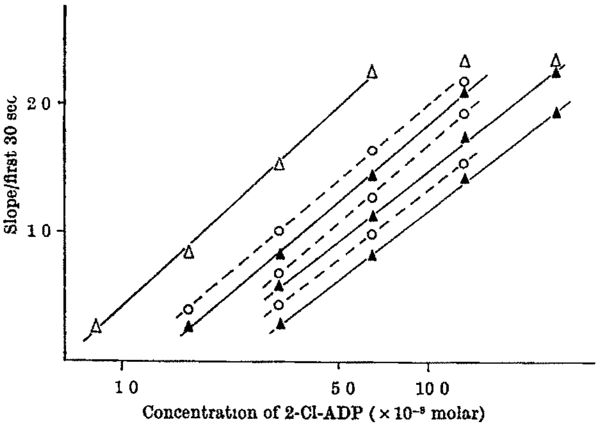

Fig 4 Log dose response curves for the aggregation of sheep platelet-rich plasma by 2-Cl-ADP alone (△), and in the presence of adenosine and 2-chloroadenosine Concentrations of adenosine (○) reading from left to right were 1 2, 2 4 and 4 8 × 10^{-6} molar, concentrations of 2-chloroadenosine (▲) reading from left to right were 0 45, 0 9 and 1 8 × 10^{-6} molar

Table 1 AFFINITY CONSTANTS K_a AND K_b, OF AGONISTS AND ANTAGONISTS FOR THE PLATELET ADP RECEPTOR

2-Cl-ADP	5 61 × 10^7
ADP	0 58 × 10^7
2-Chloroadenosine	0 26 × 10^7
Adenosine	0 07 × 10^7

Affinity constants can also be obtained for the agonist ADP and 2-Cl-ADP from the log dose response curves Because both agonists will elicit the maximum aggregation possible, and because there would seem to be negligible diffusion barriers for the agonists to pass through before having access to the platelet receptors, it may be assumed that the response, that is, the percentage of maximum aggregation, is proportional to the number of receptors occupied At 50 per cent response, half the receptors will be occupied and the affinity constant K_a will be the reciprocal of the concentration of the agonist The values of K_a obtained in this way from Figs 4 and 5 are also given in Table 1 The values show that the agonists have a greater affinity for the ADP receptor than the antagonists and that in the case of the agonists a chlorine substituent in the 2 position increases affinity 9 7-fold, while

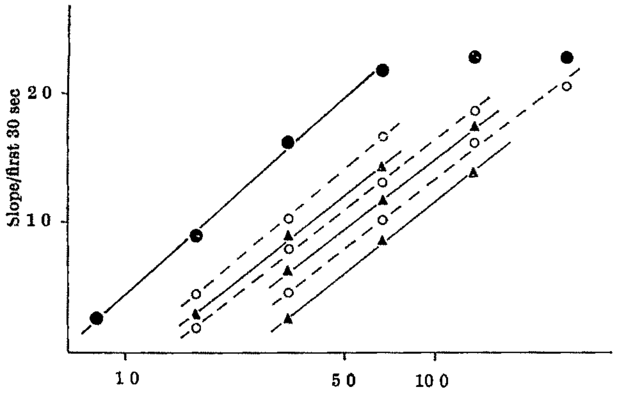

Fig 5 Log dose response curves for the aggregation of sheep platelet-rich plasma by ADP alone (●), and in the presence of adenosine (O) and 2-chloroadenosine (▲), in concentrations as described for Fig 4

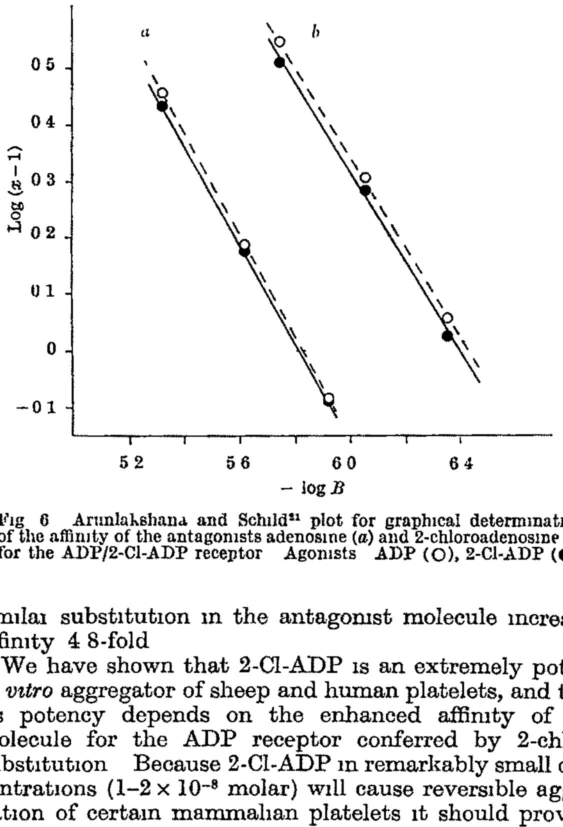

Fig 6 Arunlakshana and Schild[21] plot for graphical determination of the affinity of the antagonists adenosine (a) and 2-chloroadenosine (b) for the ADP/2-Cl-ADP receptor Agonists ADP (O), 2-Cl-ADP (●)

similar substitution in the antagonist molecule increases affinity 4 8-fold

We have shown that 2-Cl-ADP is an extremely potent *in vitro* aggregator of sheep and human platelets, and that its potency depends on the enhanced affinity of the molecule for the ADP receptor conferred by 2-chloro substitution Because 2-Cl-ADP in remarkably small concentrations ($1-2 \times 10^{-8}$ molar) will cause reversible aggregation of certain mammalian platelets it should prove a valuable tool for research into the mechanism of ADP-mediated platelet aggregation

We thank Professor R H Thorp for his continued interest in these studies

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[1] Gaarder, A , Jonsen, J , Laland, S , Hellem, A , and Owren, P A , *Nature*, 192, 531 (1961)
[2] Born, G V R , and Cross, M J , *J Physiol* , 168, 178 (1963)
[3] Davey, M G , and Lander, H , *Nature*, 201, 1037 (1964)
[4] Regoli, D and Clark, V , *Nature* 200, 546 (1963)
[5] Born G V R , and Cross, M J , *J Physiol* , 170, 397 (1964)
[6] Deykin, D , Pritzker, C R , and Scolnick, E M *Nature* 208 296 (1965)
[7] Born, G V R *Nature*, 194 927 (1962)
[8] Sinakos Z , and Caen, J P , *Thrombos Diathes Haemorrh* , 17, 99 (1967)
[9] Clayton, S , Born, G V R , and Cross, M J , *Nature*, 200, 138 (1963).
[10] Hellem, A , and Owren, P A , *Acta Haemat* , 31, 230 (1964)
[11] Gaarder, A , and Laland, S , *Nature*, 202, 909 (1964)
[12] Salzman, E W , Chambers, D A , and Neri, L L , *Nature*, 210, 167 (1966)
[13] Robinson, C W , Kress, S , Wagner, R H , and Brinkhous, K M , *Exp Mol Path* , 4, 457 (1965)
[14] Mitchell, J R A , and Sharp, A A , *Brit J Haematol* , 10, 78 (1964)
[15] Born, G V R , *Fed Proc* , 26, 115 (1967)
[16] Born, G V R , *Nature*, 202, 95 (1964)
[17] Constantine, J W , *Nature*, 205, 1075 (1965)
[18] Michal, F , and Thorp, R H , *Nature*, 209, 208 (1966)
[19] Clarke, D A Davoll, J , Philips, F S , and Brown, G B , *Brit J Pharmacol Exp Therap* , 106, 291 (1952)
[20] Rockwell, M , and Maguire, M H , *Mol Pharmac* , 2, 574 (1966)
[21] Arunlakshana, O , and Schild, H O , *Brit J Pharmacol* , 14, 48 (1959)

Sites of Action of some Partial Agonists on the Isolated Guinea-pig Ileum

It is well known that if in a homologous series of drugs a special group is changed by heavier ones, they gradually change from agonists through partial agonists into competitive antagonists[1] It has been reported that isoamyl acetate, its analogues[2] and pilocarpine[3], some of which are partial agonists, contract the isolated guinea-pig ileum through the liberation of acetylcholine on the one hand, and antagonize the action of released acetylcholine on the other hand, when they combine with the acetylcholine receptor The sites of action of partial agonists, however, have never been studied precisely, and so we have investigated the effects of cooling, cocaine, morphine and hexamethonium on the actions of two series of drugs which are esters of choline and dimethylaminoethanol

Strips of male guinea-pig ileum (3–4 cm long) were suspended in Lock–Ringer solution at 32° C and bubbled with air The responses of the ileum to test drugs were recorded isotonically on a smoked paper The results given are the means of at least ten experiments

The drugs are listed in Table 1 Pentanoylcholine, hexanoylcholine, benzoylcholine, dimethylaminoethyl butyrate and pilocarpine were partial agonists All drugs listed and also nicotine, and those drugs which affect the nerve structures, contracted the ileum through the acetylcholine receptors because their responses were antagonized by 10^{-8}–10^{-7} molar atropine The responses to the partial agonists and nicotine were greatly reduced, or abolished, by cooling the ileum to 12° C for 1–2 h, or by 5 min of treatment with cocaine (5×10^{-5} molar), while the responses to the agonists such as acetylcholine were not much affected by those treatments The responses to drugs which contracted the smooth muscle by stimulating the cholinergic nerve structures were greatly reduced by cooling the ileum, by anoxia or by cocaine[4] Furthermore, the responses to the partial agonists, with the exception of pilocarpine, were much reduced by 5×10^{-5} molar hexamethonium after 5 min, by 3×10^{-5} molar morphine after 2 min or by 10^{-6} molar tetrodotoxin after 5 min The results indicate that the site of action of the partial agonists is in the nerve structures of the ileum, the partial agonists, except with the ion of pilocarpine,

Table 1 INTRINSIC ACTIVITIES AND AFFINITIES (pD_2 AND pA_2)

C O [CNCCOC–R]$^+$X$^-$ C	R	X	At 32° C Intrinsic activity	At 32° C pD_2	At 12° C pA_2 (agonist = ACh)
ACh	CH_3	Cl	1 00	7 12	
PrCh	C_2H_5	I	1 00	5 65	
BuCh	n-C_3H_7	I	1 00	4 50	
PenCh	n-C_4H_9	I	0 66	4 18	4 08
HeCh	n-C_5H_{11}	I	0 54	4 58	4 51
BeCh	C_6H_5	I	0 52	3 39	4 45
O [^C_CNCCOC–R]HCl					
DMAEAc	CH_3		1 00	5 25	
DMAEBu	n-C_3H_7		0 70	3 90	3 80
Pilocarpine			0 87	6 44	6 27

Intrinsic activity is maximal contraction by test drug/maximal contraction by ACh seem to stimulate the ganglion cells and accelerate the liberation of acetylcholine which is depressed by morphine or strychnine In addition, pilocarpine also stimulates the nerve structures and liberates acetylcholine through a mechanism which is unaffected by morphine or strychnine[3,5]

The affinities of the agonists were expressed as the pD_2 values or the negative logarithm of the concentration which produced 50 per cent maximal contraction of muscle The competitive inhibitory activities were expressed as the pA_2 values which were calculated from the shift of a dose response curve of an agonist[6] All the partial agonists, however, displaced a dose response curve of acetylcholine towards greater concentrations in the experiments at 12° C The pA_2 values of the partial agonists were calculated from a shift of the dose response curve of acetylcholine in the experiments at 12° C The intrinsic activities and affinities are summarized in Table 1 The pA_2 values of competitive inhibitors against acetylcholine were little affected by cooling the ileum to 12° C, so the pA_2 values of the test drugs listed in Table 1 may represent their affinity for the acetylcholine receptor on the smooth muscle (ref 2 and unpublished work of Takagi, Takayanagi and Maezima) The responses to pentanoylcholine or a partial agonist were inhibited more by dibenamine (3×10^{-5} molar) than those to acetylcholine[1] This suggests that the responses to the partial agonists are antagonized by what may be a synergism of their own atropine-like action plus the anti-acetylcholine action of dibenamine, on the other hand, the responses to the pure agonists may be considered to be antagonized by an unaided anti-acetylcholine action of dibenamine As suggested, partial agonists may contract the guinea-pig ileum by liberating acetylcholine from the cholinergic nerves and antagonize an action of the acetylcholine, which they have released, by their atropine-like action

To confirm this hypothesis, the mode of action of an equivalent mixture of acetylcholine and atropine was tested The intrinsic activity or the ratio of the maximal contraction produced by the mixture to that produced by acetylcholine is about 0 7 and the concentration which produced 50 per cent of the maximal contraction was 10^{-8} g/ml Furthermore, 3×10^{-5} molar dibenamine inhibited the responses to the mixture more than the responses to acetylcholine

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[1] Ariens, E J , *Molecular Pharmacology*, 1 (Academic Press, New York, 1964)
[2] Tagaki, K , and Takayanagi, I , *J Pharm Pharmac* , 18, 795 (1966)
[3] Takagi, K , Takayanagi, I , and Shih, C K , *Chem Pharm Bull* , 15, 1747 (1967)
[4] Kosterlitz, H W , and Lees, G M , *Pharmac Rev* , 16, 301 (1964)
[5] Takagi, K , and Takayanagi, I , *Jap J Pharmac* , 16, 211 (1966)
[6] van Rossum, J M , *Arch Intern Pharmacodyn Thér* , 143, 299 (1963)

Daily Rhythm in the Noradrenaline Content of Rat Hypothalamus

The noradrenaline content of several organs of the rat has been shown to vary during each 24 h period[1,2] Almost all of the catecholamine in these organs (pineal, salivary gland and liver) is located not in the parenchymal tissue but within the nerve endings of sympathetic axons[3] Noradrenaline is also concentrated in several regions[4-6] of the brain, where it is found within both nerve endings and cell bodies It will be shown that in normal lighting conditions the noradrenaline concentrations in the anterior and posterior rat hypothalamus are not constant but, like the catecholamine in peripheral organs, depend on the time of day when the tissue is sampled.

Three separate studies were performed during two summers, each used female Sprague–Dawley rats weighing 160–180 g The animals were kept in a controlled light environment (25–50 ft.-candles of illumination, provided by cool-white fluorescent bulbs) for at least 2 weeks before they were killed The lights were turned on for 12 h starting at 6 00 a m or 7.00 a m Groups of between four and nine animals were killed by decapitation after 5 5 h of light, at the end of the light period, after 6 h of darkness or after 11 5 h of darkness The brains were removed and chilled, five regions (anterior hypothalamus, posterior hypothalamus, striatum, midbrain and thalamus-subthalamus) were dissected, using a technique similar to that described by Glowinski and Iversen[7] The lateral cuts bounding the hypothalamus were slanted toward the midline in order to avoid including subthalamic structures (such as Forel's fields) The hypothalamus was then subdivided into anterior and posterior portions by a transverse cut 2 mm caudal to the optic chiasma The weights of each region of the brain were relatively reproducible from animal to animal, their standard deviations, among the sixty-seven rats studied ranged from 10 to 21 per cent of their means

Noradrenaline was assayed in samples of individual rat brains Each region was homogenized in 5 ml. of perchloric acid (0 4 normal) After centrifugation, the supernatant was transferred to a beaker containing 200 mg of alumina which had been washed with acid, 5 ml. of 2 normal ethylenediamine tetraacetic acid and 15 ml of sodium bisulphite. The *p*H was adjusted to 8 6 with concentrated sodium hydroxide and the contents of the beaker were poured over a column containing an additional 200 mg of alumina which had been brought to *p*H 8·6 The column was washed with 0 2 normal sodium acetate and with glass-distilled water, the noradrenaline was then eluted with 3 ml of 0 2 normal acetic acid and assayed spectrophotofluorometrically[9] The recovery of added noradrenaline averaged 67 per cent

In each of the three experiments, the mean noradrenaline concentration of each brain region was calculated, and individual measurements taken at different times of day were expressed as percentages of the daily mean value for the region Data from all three experiments were pooled, and the statistical significance of temporal differences was determined using a *t* test Similar results were obtained in all three experiments The regional concentrations of noradrenaline are shown as a function of hour of the day in Table 1

Significant daily rhythms were observed in the anterior hypothalamus and the posterior hypothalamus The noradrenaline contents of both regions were greatest at the middle of the daily dark period, in the anterior hypothalamus, the catecholamine concentration was low throughout the light period, in the posterior hypothalamus, the nadir was reached at the end of the dark period In both regions, the magnitude of the daily change was 22–24 per cent Daily noradrenaline

Table 1 NORADRENALINE CONCENTRATION OF VARIOUS BRAIN REGIONS AT DIFFERENT TIMES OF DAY

Brain region		Mean noradrenaline concentration γ/g	Time brain removed 5 5 Light	12 Light	6 Dark	11 5 Dark
			Noradrenaline concentration (per cent of mean ± *SE*)			
Anterior hypothalamus	63	1 454	92 6 ±5 3	92 1* ±4 7	112 8 ±5 5	103 3 ±3 9
Posterior hypothalamus	67	1 094	103 2 ±5 7	99 6 ±7 6	110 2 ±6 0	88 7* ±4 2
Midbrain	66	0 398	95 3 ±5 6	93 8 ±5 3	102 4 ±5 1	108 3 ±8 0
Striatum	60	0 224	98 1 ±5 6	106 1 ±6 5	101 8 ±8 0	96 3 +7 2
Thalamus-subthalamus	48	0 226	101 3 ±16 0	91 9 ±10 6	84 1 ±8 4	122 5 ±21 5

* $P < 0\ 01$ Noradrenaline content differs significantly from that observed after 6 h of darkness

fluctuations of greater amplitude were found in the thalamus-subthalamus, but these changes were not statistically significant (Table 1). A daily noradrenaline rhythm has been demonstrated in the lateral hypothalamus of the cat[10]. In the present experiment, the region designated thalamus-subthalamus contained the lateral hypothalamic area, as well as the thalamus. It is possible that a daily rhythm exists in lateral hypothalamic noradrenaline and that the rhythm was obscured in the present study because the hypothalamic tissue was diluted with relatively large amounts of thalamus. The noradrenaline concentrations in the rat midbrain and striatum did not vary during the day; similar findings have been reported in the cat[10].

The neuroanatomic structures responsible for hypothalamic noradrenaline rhythms are not known, nor are the biochemical mechanisms which generate them clear. The increased concentration of the neurotransmitter during the dark period could be caused by enhanced noradrenaline synthesis, decreased release of the amine, or a decrease in its rate of intracellular metabolism. It seems likely that the amount of hypothalamic noradrenaline which can be liberated by a drug also depends on the time of day that the drug is administered. If this is true, it is possible that psychotropic drugs (such as amphetamine) which produce their effects by releasing brain noradrenaline may show time-dependent variations in potency.

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[1] Wurtman, R. J., and Axelrod, J., *Life Sci.*, **5**, 665 (1966).
[2] Hokfelt, B., *Acta Physiol. Scand.*, **25**, Suppl. 92 (1951).
[3] Wurtman, R. J., *Catecholamines* (Little Brown and Co., Boston, 1966).
[4] Bertler, A., and Rosengren, E., *Acta Physiol. Scand.*, **47**, 350 (1959).
[5] Carlsson, A., Falck, B., and Hillarp, N. A., *Acta Physiol. Scand.*, **56**, suppl. 196 (1962).
[6] Dahlstrom, A., and Fuxe, K., *Acta Physiol. Scand.*, **62**, suppl. 232 (1964).
[7] Glowinski, J., and Iversen, L. L., *J. Neurochem.*, **13**, 655 (1966).
[8] Vogt, M., *J. Physiol.*, **123**, 451 (1954).
[9] Von Euler, U. S., and Lishajko, F., *Acta Physiol. Scand.*, **51**, 348 (1961).
[10] Reis, D. J., and Wurtman, R. J., *Life Sci.* (in the press).

Thermoregulation in Tortoises

ALTHOUGH reptiles in a laboratory cage may show feeble powers of thermoregulation, it is now known that, when active in natural conditions, their body temperature is often regulated by behavioural means at a thermal level higher than that of many mammals[1]. Tortoises are no exception and sun themselves when the air is cool. Experiments on young desert tortoises, *Testudo sulcata* Miller (= *T. calcarata* Schn.), have shown that water loss by evaporation increases greatly in dry air at temperatures above 40°–41° C. At ambient temperatures of 55° C the body temperature rises rapidly to about 40.5° C, at which level it is maintained by copious salivation which wets the head, neck and front legs. Although they do not usually take water when supplied with green food, these tortoises drink deeply after such experiments.

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[1] Schmidt-Nielsen, K., and Dawson, W. R., *Adaptation to the Environment Handbook of Physiology*, Sect. 4, 467 (Amer. Physiol. Soc., 1964).

Application of Laser Interferometry to Physiological Studies of Excitable Tissues

INTERFEROMETRIC techniques offer a sensitive means of measuring small displacements, film thickness and changes in refractive index. By applying interferometry to studies of excitation processes, Kayushin and Lyudkovskaya[1,2] and Lyudkovskaya[3] reported that they had detected small movements of frog sciatic nerve in association with excitation. This communication describes a technique of applying laser interferometry to the investigation of various excitable tissues; we have tried to detect small movements accompanying the action potentials of fresh water algae (*Nitella*) and frog and lobster nerve trunks.

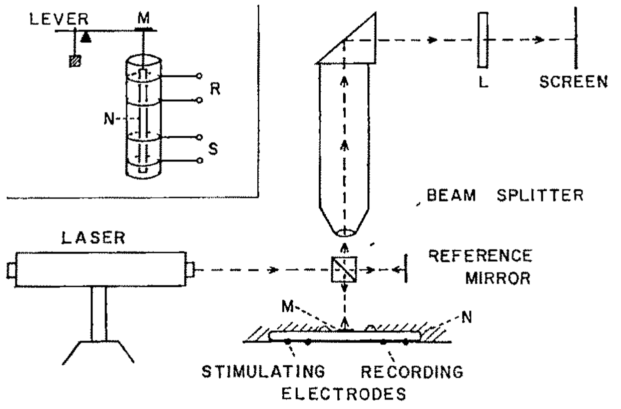

Fig. 1. Schematic diagram showing the interferometry set-up used to examine excitable tissue for the presence of small movements associated with the production of action potentials. *M* represents a small mirror placed on the surface of a *Nitella* internode or a nerve trunk (*N*). Two 'Vaseline' partitions were used to separate the middle section of the tissue from the lateral portions where the platinum wire stimulating and recording electrodes were placed. *L* represents a cylindrical lens used to focus the interference pattern on to the screen of the oscilloscope used for recording the action potential. The inset illustrates the technique used to examine changes in the length of an excitable tissue (*N*) in response to electric stimulation. Stimulating (*S*) and recording (*R*) electrodes were made of rings of platinum wire (separated by approximately 10 mm) placed near the wall of the cylindrical 'Lucite' chamber (filled with sea water diluted by a factor of 2,000).

A helium-neon gas laser (Perkin-Elmer, model 5200) served as a source of continuous, coherent, plane-polarized light (0.6328μ wavelength). An interference objective attachment (W. Watson and Sons, England) was modified by removing the condenser lens system and was fitted to a standard monocular microscope. The experimental set-up is illustrated schematically in Fig. 1. The laser beam was split in two by means of a half-silvered mirror. Interference was obtained between one portion of the beam reflected back from a reference surface mirror to the half-silvered mirror and the other part of the beam reflected from a small surface mirror attached to the biological preparation. A prism at the top of the microscope tube and a cylindrical lens were used to focus the resulting interference pattern onto the upper half of a cathode ray oscilloscope screen. In this manner, the interference pattern and the electrical response of the tissue to stimulation (displayed on the lower half of the oscilloscope screen with a standing beam) could be recorded simultaneously on moving photographic film.

In one series of experiments, the small surface mirror (0.5 mm^2) was placed directly on the (moist) surface of a *Nitella axillaris* internode, frog sciatic nerve or lobster circumoesophageal connective (Fig. 1). These excitable tissues were mounted horizontally in a 'Lucite' chamber provided with two pairs of platinum electrodes (one pair

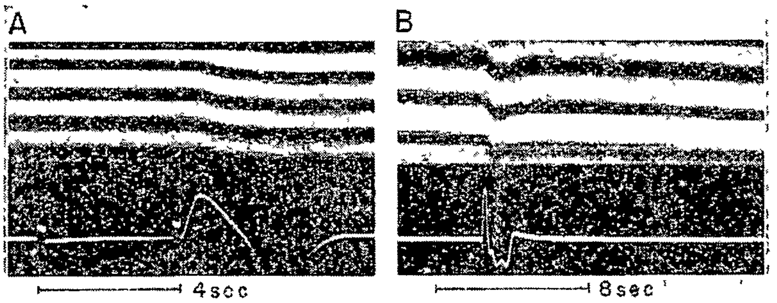

Fig 2 Shifts of interference pattern representing small movements associated with the excitation process in *Nitella* internodes Record *A* was obtained by using a surface mirror placed directly on the internode (horizontal arrangement) Note that a barely sub-threshold electric shock produced no detectable movement of the interference pattern Record *B* was taken from a *Nitella* internode mounted in the vertical chamber (see inset, Fig 1) For both experiments, the fluid medium was sea water diluted by a factor of 2,000 The tension applied to the *Nitella* cell in the vertical chamber was approximately 0 25 g A shift of the interference pattern of one full period corresponds to a 0 3164μ movement of the mirror The room temperature was 20° C

for stimulation, the other for recording action potentials) The mirror was placed on the preparation between the electrode pairs, this portion of the chamber contained only sufficient fluid to prevent the preparation drying After adjusting the two mirrors to give an interference pattern, brief pulses of current were delivered to the tissue through the stimulating electrodes In this way, a transient shift in the interference pattern, representing a small displacement of the surface of a *Nitella* internode, was detected whenever an action potential was produced (Fig 2*A*). There was no detectable shift in the interference pattern when the stimulating current failed to produce an action potential (that is, when the stimulus was sub-threshold) The displacement associated with an all-or-none action potential was generally between 0 1 and 0 3μ at the peak The direction of movement of the cell surface was always downward (compatible with a reduction in diameter of the *Nitella* cell) The internodes of the *Nitella* used were 350–450μ in diameter and 30–40 mm in length Similar experiments with frog and lobster nerve were performed, but no movement was detected even when a train of impulses (50 shocks/sec) was evoked

Reversible changes in the length of the *Nitella* cell with excitation were also observed when the internode was mounted vertically in a 'Lucite' chamber containing sea water diluted by a factor of 2,000 (inset, Fig 1) One end of the cell was secured to the bottom of the chamber and the other end was tied to a micro-lever with fine thread Interference patterns were obtained with a surface mirror attached to the lever A typical record obtained in these conditions is shown in Fig 2*B* The reversible shift in the interference pattern represents a shortening of the internode with excitation The observed shortening ranged between 0 1 and 0 3μ at the peak The recovery process (that is, the process of returning toward the initial length following shortening) was seen to start during the falling phase of the extracellularly recorded response (This phase of the extracellular response roughly coincides with the peak of an intracellularly recorded action potential) The initial, rapid phase of recovery was followed by a late, gradual recovery phase The results obtained by this method are consistent with those of Kishimoto and Ohkawa[4], who detected an average shortening of *Nitella* internodes of 0 1μ using a sensitive micro-lever technique (without interferometry).

When frog or lobster nerve was used in the vertical chamber, no detectable change in length was observed during repetitive stimulation (50 shocks/sec) At present, the reason for our failure to detect small movements during the excitation of these nerves is not clear An attempt is now being made to improve the sensitivity of our system by using a photomultiplier instead of the present photographic technique for detecting shifts of the interference pattern The relationship between the present experimental finding in *Nitella* and the mechanism of excitation in this alga (see, for example, Mullins[5]; Mailman and Mullins[6]; Kishimoto and Ohkawa[4]) remains to be clarified

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[1] Kayushin, L P, and Lyudkovskaya, R G, *Dokl Akad Nauk USSR*, 95 2 (1954)
[2] Kayushin L P, and Lyudkovskaya, R G, *Trud Inst Biol Fiz*, 1 40 (1955)
[3] Lyudkovskaya, R G, *Biofizika*, 2, 577 (1957)
[4] Kishimoto, U, and Ohkawa, T *Plant Cell Physiol*, 7, 493 (1966)
[5] Mullins, L J, *Nature*, 196, 986 (1962)
[6] Mailman, D S, and Mullins, L J, *Austral J Biol Sci* **19**, 385 (1966)

RADIOBIOLOGY

Composition of Trabecular Bone in Children and its relation to Radiation Dosimetry

Trabecular bone is a composite tissue containing both bone marrow and bony tissue, but the radiation dose to the composite tissue from a β-emitting bone seeking nuclide is, to a first approximation, proportional to the amount of bone present Children are of particular interest in this respect because of the rapid turnover of their bone mineral[1] We report here the results of chemical analyses of specimens of trabecular bone from young children, which enable the amount of bone in trabecular bone tissue to be estimated

Information about the physical and chemical composition of trabecular bone is sparse in the literature Erf[2] reports chemical analyses for the phosphorus content of compact bone and trabecular bone specimens (vertebral

Table 1. CALCIUM AND PHOSPHORUS CONTENT OF TRABECULAR AND CORTICAL BONE AND BONE MARROW

Specimen	Ratio, ash weight/wet weight	Percentage calcium by weight (in ash)	Percentage phosphorus by weight (in ash)	Calcium content, mg/g wet weight	Phosphorus content, mg/g wet weight
Vertebral trabecular bone tissue (*A*)	0.139	34.2	18.2	47.5	25.3
Vertebral trabecular bone tissue (*B*)	0.136	34.4	18.1	46.6	24.6
Trabecular bone tissue, upper end of femur (*B*)	0.132	35.9	18.3	47.4	24.1
Cortical compact bone, midshaft of femur (*B*)	0.504	38.5	17.9	194	90.3
Femur trabecular bone after washing out marrow (*B*)	—	37.9	18.7	—	—
Red marrow from femur shaft (*B*)	0.0107	(in marrow) 0.26	(in marrow) 0.22	2.1	1.8

A, 17 month old child; *B*, 20 month old child.

bodies), taken from a case of lymphatic leukaemia, in a patient who died at 65. Jones and Ellis[3] found that trabecular bone tissue, taken after death from the vertebral bodies of two adults, had a density of 1.1 g/cc. From the data given in these references, and using reasonable assumptions, it may be calculated that the proportion of bone by weight in trabecular bone tissue is 20–30 per cent. It should be remembered, however, that these experiments were made primarily for other purposes.

Calculations of the gravimetric composition of trabecular bone tissue have been made from measurements of the dimensions of the bone marrow cavities and the bony trabeculae, and lead to significantly higher estimates of the proportion of bone. For example, the trabecular bone model proposed by the International Commission on Radiological Units and Measurements (ICRU)[4] corresponds to a proportion of bone by weight of more than 65 per cent. None of this work was on child bone, and the results are not consistent.

Our measurements were made on bone specimens taken at post mortem from two children, aged 17 and 20 months. The children died in hospital, following acute respiratory illnesses. The bone specimens were considered to approximate closely to normal bony tissue and were stored at −20° C. Lumbar vertebrae were obtained from both children and a femur from one child. Trabecular bone tissue was dissected out from the vertebral bodies and from the upper end of the femur. Care was taken to avoid including any cartilage in the trabecular bone samples. The marrow cavity of the femur provided a small specimen of red bone marrow (1.3 g), and specimens of compact (cortical) bone were obtained from the femur shaft.

Specimens were weighed, dried and finally dry ashed in a muffle furnace up to a maximum temperature of 750° C. The resulting ash specimens were weighed after cooling in a desiccator. Phosphorus was determined in the ash specimens by the well known procedure using ammonium molybdate. Calcium was determined gravimetrically as the oxalate[5]. Analyses were carried out in duplicate and the coefficient of variation of the phosphorus method was 1 per cent and of the calcium method 0.2 per cent. The present results may be slightly below this standard because of the small weights of the ash samples. Results are summarized in Table 1, expressed as percentage of calcium and phosphorus, by weight, in the ash. The last two columns give the calcium and phosphorus contents of the original samples, and are obtained from the chemical analyses together with the known ash weight and wet weight of each specimen.

The calcium and phosphorus contents of trabecular bone ash are seen to be very similar to those of compact bone ash. Both approximate quite closely to a simple stoichiometric hydroxyapatite $[Ca_3(PO_4)_2]_3$, $Ca(OH)_2$, which contains 39.9 per cent calcium and 18.5 per cent phosphorus by weight[6]. Our results are also in accordance with those of Gong *et al.*[7] In work with cortical bone, and vertebral trabecular bone free of marrow, from ten adult humans, these authors found the density of cortical bone to be 1.991 g/cc and of trabecular bone 1.924 g/cc. For present purposes we may assume that the bone in trabecular bone tissue is quantitatively the same as compact bone.

We assume also that marrow in trabecular bone has the same calcium and phosphorus content as femur shaft bone marrow. A comment should be made on our bone marrow results; it is very difficult to be certain that no tiny spicules of bone remain in a sample of marrow taken from the marrow cavity in a long bone. The presence of a small percentage of bone markedly affects results for the calcium and phosphorus content of bone marrow, and possibly explains widely differing values quoted in the literature[8]. For an organ roughly comparable in cellularity with bone marrow, namely spleen, ICRP[9] gives the calcium content as 0.084 mg/g and phosphorus as 2.2 mg/g. The contribution of the bone marrow to the calcium and phosphorus content of trabecular bone tissue is therefore small.

The two assumptions discussed here allow the amount of bone in trabecular bone tissue to be obtained by proportion from the calcium measurements, or from the phosphorus measurements, or more simply from the ash weights, with the results shown in Table 2. The explanation of the small differences between these results is not clear, but for present purposes it is sufficient to consider that one-quarter the weight of trabecular bone tissue is bone.

Table 2. PROPORTION OF BONE IN TRABECULAR BONE TISSUE IN YOUNG CHILDREN

Method	Weight of bone in trabecular bone tissue (mg/g)
Calcium content	235
Phosphorus content	258
Ash weights	254

Erf's[2] analyses for phosphorus are comparable with ours, for he obtained 20.8 mg/g wet weight in vertebral bodies, and around 90 mg/g in the diaphyses of the tibia and femur, in his 65 yr old leukaemia patient. The density measurements of Jones and Ellis[3] are also compatible with our results: they found 1.1 g/cc for the density of adult vertebral trabecular bone tissue, and we would calculate 1.14 g/cc by using a density of 1.924 (ref. 7) for trabecular bone and 1.0 for marrow.

If trabecular bone tissue is formalized into a regular cubic lattice, following ICRU[4], we may calculate the thickness of the bony walls surrounding each cube of bone marrow by using the results of Table 2. If the cubes of marrow have sides of 400μ, for example (ICRU quote a range of 200–950μ), the thickness of the bony wall is only about 20μ. This is the smallest observed thickness of bone trabeculae according to ICRU, the largest thickness they report is 250μ.

In conclusion, chemical analyses of bones from children about 18 months old show that the proportion of bone by weight, in trabecular bone tissue from vertebral bodies and the upper end of the femur, is almost exactly 25 per cent. This finding supports a previous chemical analysis and a density measurement of adult trabecular bone tissues, but disagrees with estimates made from geometrical measurements of bone sections.

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[1] Fletcher, W., Loutit, J. F., and Papworth, D. G., *Brit. Med. J.*, 2, 1225 (1966).

[2] Erf, L. A., *Proc. Soc. Exp. Biol. and Med.*, 47, 287 (1941).

[3] Jones, D. E. A., and Ellis, R. E., in *MRC Special Rep.*, 295 (1957).

[4] *International Commission on Radiological Units and Measurements (ICRU)*, Nat. Bureau Standards Handbook, 78 (1961).

[5] Bryant, F. J., Morgan, A., and Spicer, G. S., *UKAEA Research Group Report AERE-R3030* (1959).

[6] Neuman, W. F., and Neuman, M. W., *The Chemical Dynamics of Bone Mineral* (Univ. Chicago Press, 1958).

[7] Gong, J. K., Arnold, J. S., and Cohn, S. J., *Anat. Rec.*, 149, 325 (1964).

[8] Spector, W. S., *Handbook of Biological Data* (Saunders, Philadelphia, 1956).

[9] *International Commission on Radiological Protection Report of Committee II* (Pergamon, London, 1959).

BIOLOGY

Regulation of Albumin Synthesis and Catabolism by Alteration of Dietary Protein

There is good evidence that catabolism of albumin is slowed in conditions of low-protein feeding and in clinical states of protein deficiency, for example, kwashiorkor[1-3]. There is less certainty about alterations in the rate of synthesis, measurement of which is usually derived indirectly from the metabolic behaviour of isotopically labelled albumin[3,4].

It has also been difficult to define the processes which regulate albumin synthesis and catabolism in states of protein deprivation. It has been particularly difficult to prove whether the adaptive changes reflect a response to altered concentration or mass of plasma albumin, or whether they are a more direct response to reduced availability of dietary protein[3,5]. In an attempt to resolve this difficulty, we have made serial measurements of the plasma albumin pool and of synthesis and catabolic rate in rats given diets containing little or no protein. Similar studies were made in rats re-fed a normal diet after being deprived of protein.

The plasma albumin pool was calculated from the product of albumin concentration and plasma volume (determined by isotope dilution using ^{131}I-albumin). Synthesis rate was measured by the method of McFarlane based on the relative rates of incorporation of carbon-14 into urea and into the guanidine carbon of albumin arginine[6,7]. Rat albumin labelled with iodine-131 was administered intravenously to measure catabolic rate which was calculated from daily urinary loss of radioactivity, derived indirectly from serial whole-body counts and expressed as a function of plasma specific activity. The method is valid even in an unsteady state which might exist in these experimental conditions[4]. ^{131}I-albumin was prepared by ammonium sulphate precipitation and labelled by the iodine monochloride method[8].

Male albino rats of Wistar strain, housed in conditions of controlled temperature and humidity, were fed one of three diets: normal protein diet consisting of 20 per cent mixed protein, low-protein diet consisting of 5 per cent mixed protein, and a protein-free diet. All were isocaloric on a weight basis. Appropriate vitamin and mineral supplements were added to the low-protein diet and to the protein-free diet. The rats were allowed free access to water. Iodide was added to the water when the catabolic rate was being measured in order to block uptake of iodine-131 by the thyroid. We set out initially to test the agreement between the synthesis rates, measured with ^{14}C-carbonate, and the catabolic rates measured with ^{131}I-albumin in animals under the same nutritional conditions. The two rates in this case should be equal. Catabolic rate was determined daily for 14 days on twenty control rats receiving the normal protein diet. At the end of this period ^{14}C-carbonate was injected to measure the synthesis rate. Five groups of four rats were studied and plasma from each group was pooled. Table 1 shows excellent agreement between synthesis rate and catabolic rate in these animals. There was also good correlation for similar figures derived from five rats studied individually after 60 days on the low-protein diet (Table 2). In this case both the synthesis rate and the catabolic rate are significantly lower than the control values ($P < 0.001$), which indicates adaptation to a lower protein intake.

Table 1. COMPARISON OF ALBUMIN SYNTHESIS AND CATABOLIC RATES DERIVED BY TWO INDEPENDENT METHODS, IN TWENTY RATS ON NORMAL PROTEIN INTAKE

Group	Synthesis rate (mg/h/300 g of rat)	Catabolic rate (mg/h/300 g of rat)
1	12.8	12.9
2	11.7	10.7
3	11.2	10.4
4	11.6	11.9
5	11.1	10.0
Mean	11.7	11.2

Each figure represents pooled values from a group of four rats.

Table 2. COMPARISON OF ALBUMIN SYNTHESIS AND CATABOLIC RATES IN FIVE RATS STUDIED INDIVIDUALLY AFTER 60 DAYS OF LOW-PROTEIN DIET

Rat	Synthesis rate (mg/h/300 g of rat)	Catabolic rate (mg/h/300 g of rat)
1	4.3	2.0
2	4.0	3.5
3	3.5	3.1
4	1.7	2.2
5	2.6	5.6
Mean	3.2	3.3

Serial measurements were made on rats on a protein-free diet, in order to study the sequence in which these changes occur. Synthesis rate was measured daily for 8 days and on days 10 and 12, each assay being based on pooled plasma from a group of four rats. Simultaneous estimations on groups of rats fed on a normal protein diet provided controls for each assay. In a repeat experiment, duplicate values for synthesis rate were obtained for days 3, 4, 6, 7 and 10. Daily catabolic rate was measured on eleven rats, previously given ^{131}I-albumin, for 4 days before and 9 days after being fed on a protein-free diet.

The timing of adaptation to protein repletion was also studied. Rats were fed on a protein-free diet for 12 days, and then allowed free access to a normal diet. On days 1, 3, 4, 5 and 7 after resumption of the normal diet, synthesis rate was determined, plasma from a group of four rats was again pooled for each assay. Catabolic rate was measured on seven rats on the last 2 days of the protein-free diet and daily for 12 days when they were on a normal protein diet.

Overall results of this depletion and repletion experiment are presented in Fig. 1. Solid lines for albumin concentration, and broken lines for synthesis rate during depletion and catabolic rate during repletion, were calculated by the least means squares method, and are included to indicate the probability of gradual change

The concentration of plasma albumin and of the intravascular pool mass decreased in a similar way The change in pool mass was all caused by a decrease in albumin concentration, for the plasma volume remained constant throughout the experiment Catabolic rate was maintained for 6–7 days (by which time synthesis rate and albumin concentration were much reduced) before falling precipitously On repletion there was an immediate increase in the synthesis rate to normal or above-normal values This change was reflected by a gradual rise in plasma albumin concentration (and pool mass) Catabolic rate rose slowly after what appeared to be a slight lag

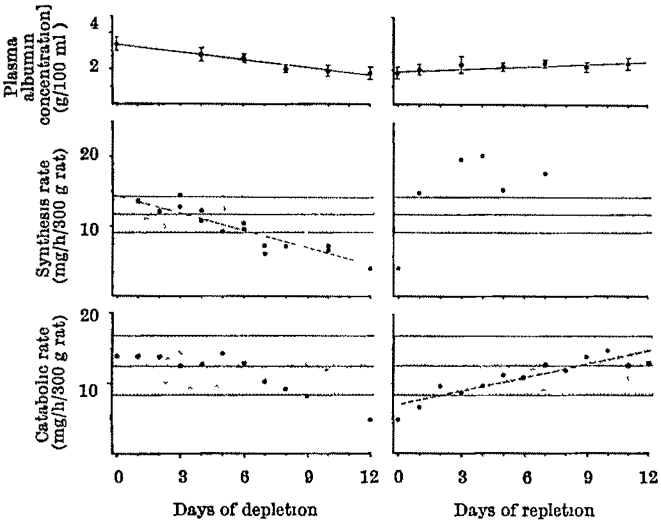

Fig 1 Serial measurements of plasma albumin concentration, synthesis and catabolic rates in rats during depletion and repletion of dietary protein Shaded areas indicate means ± 2 *S D* derived from thirty-eight normal rats

These changes seem to demonstrate conclusively the existence of separate, independent control mechanisms for albumin synthesis and catabolism, at least in our experimental conditions These results seem to support the following hypothesis Reduction or withdrawal of dietary protein causes a reduction in the synthesis rate of albumin, presumably through a reduction in the availability of amino-acids Catabolism continues at a normal rate, so that a gradual decrease in the concentration of plasma albumin and pool size occurs At some critical level, reduction in catabolic rate follows This is probably an attempt to conserve the albumin pool in the face of impending depletion If adequate dietary protein is now made available, synthesis rate increases abruptly The concentration of plasma albumin and the size of the pool gradually increase until there is no need for a compensatory decrease of the catabolic rate and normal values are re-established

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[1] Cohen, S , and Hansen, J D L , *Clin Sci* ,23, 351 (1962)
[2] Picou, D., and Waterlow, J C , *Clin Sci* ,22, 459 (1962)
[3] Hoffenberg, R , Black, E , and Brock, J F , *J Clin Invest* , 45, 143 (1966).
[4] Matthews, C M E , *J Clin Invest* , 40, 603 (1961)
[5] Waterlow, J C , *Lancet*, ii, 1279 (1962)
[6] McFarlane, A S , *Biochem J* , 89, 277 (1963)
[7] McFarlane, A S , Irons, L , Koj, A , and Regoeczi, E , *Biochem J* 95 536 (1965)
[8] McFarlane, A S , *Ann NY Acad Sci* , 70, 19 (1957)

Association between *Pandalus borealis* and Fine-grained Sediments off Northumberland

The deep-sea prawn, *Pandalus borealis*, is a north-boreal species It is found in the North Atlantic, North Pacific and Arctic Oceans It also occurs in the northern part of the North Sea, and populations off the Norwegian coast are exploited commercially[1] On the British side of the North Sea, *P borealis* occurs off northern Scotland and off Northumberland The Northumberland population is probably at the southern limit of the range of this species[2].

The first record of *P borealis* in the North Sea was by Kemp[3], who noted that several specimens had been taken in 1907 in 57 fathoms off Northumberland It has been taken on numerous occasions since then but never, apparently, in very large numbers Allen[2] published an extensive study in 1959, and reached the conclusion that although numbers of prawn were present off the Northumberland coast it was unlikely that the population could support a commercial fishery Elsewhere, *P borealis* can occur in considerable quantities on soft mud bottoms in deep-water troughs[1,4,5], but off the Northumberland coast it seemed that, even though both the bottom topography (Fig 1) and sediment distribution (Fig 2) were suitable, large concentrations of *P borealis* did not occur

In September 1966 the distribution of prawns and bottom deposits was surveyed, and in March 1967 a partial survey was made of the prawn distribution. Prawn samples were taken with a 40 mm mesh otter trawl (ground rope, 82 ft) towed for 0 5 h, and sediment

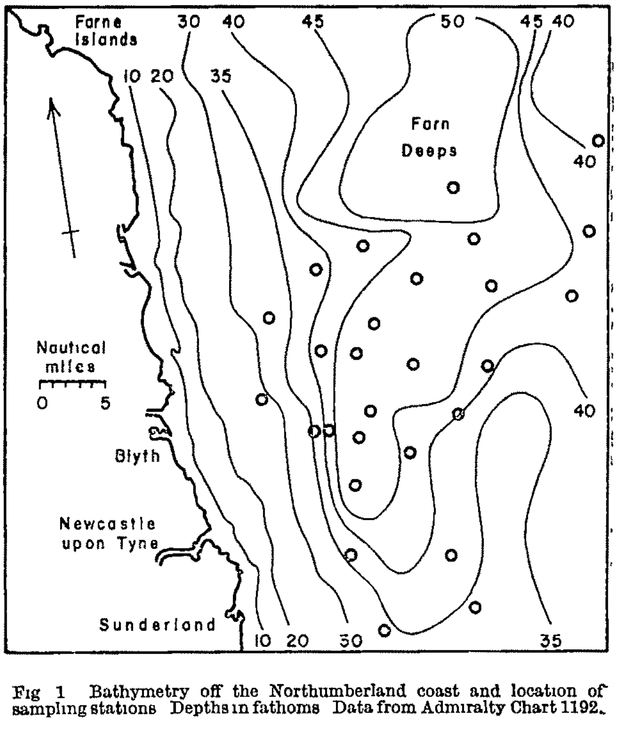

Fig 1 Bathymetry off the Northumberland coast and location of sampling stations Depths in fathoms Data from Admiralty Chart 1192.

samples were taken with a 0.2 m^2 Van Veen grab. The survey was carried out from the research vessel Platessa (Ministry of Agriculture, Fisheries and Food), a wooden side trawler of 90 ft overall length. The locations of the sampling stations (September 1966) are shown in Fig. 1.

The distribution of bottom sediments was published by Buchanan[6] in 1963. He found that near the coast, and to about 10 miles offshore, the bottom is either bare rock or sand and gravel, but further offshore there is a large area of very fine sand. This fine sediment occurs in the central part of a trough in water depths from 35 to more than 50 fathoms (64 to more than 91 m). It occupies an area about 30 miles long by 10 miles wide (65 × 18 km). The sediment samples taken by us during September 1966 were used chiefly to check that no significant changes in the distribution of sediment types had taken place since Buchanan's survey.

Large numbers of *P. borealis* were taken only from deep water in the area of very fine sand and silt; none was taken from shallower water on fine or medium sand (Fig. 3). The very fine grained sediment was similar to that associated with *P. montagui* at depths of about 5 fathoms (9 m) on the Essex coast[7]. We have found similar sediment associated with *P. jordani* at two localities off the British Columbian coast; in Georgia Strait it occurs at 45 fathoms (82 m) and in Queen Charlotte Sound in 90 fathoms (165 m). Similar sediment is also associated with *P. borealis* at 100 fathoms (183 m) off the coast of Nova Scotia.

Allen[2] reported very low population densities of *P. borealis* in 1955–58 at a point 13 miles east of Blyth. But it was in this same general area that 50 kg/h was taken in September 1966. In March 1967 the work was limited by unfavourable weather, but again in the same general area four hauls of more than 100 kg/h and one of 480 kg/h were made. Although our large otter trawl was more efficient than the small beam trawl used by Allen, there has clearly been a considerable change in the population during the 10 years or so between the two surveys. This is not particularly surprising, because considerable fluctuations can occur in populations with only a small number of year-groups.

It is difficult to estimate the rate of catch which is commercially profitable, because profitability depends on many factors not directly associated with the fishery. A rate of 20 kg/h would probably be worth while[5], and 50 kg/h should give a reasonable profit. Catches of more than 200 kg/h are probably the result of a chance combination of favourable circumstances and it is doubtful if they could be maintained for any length of time.

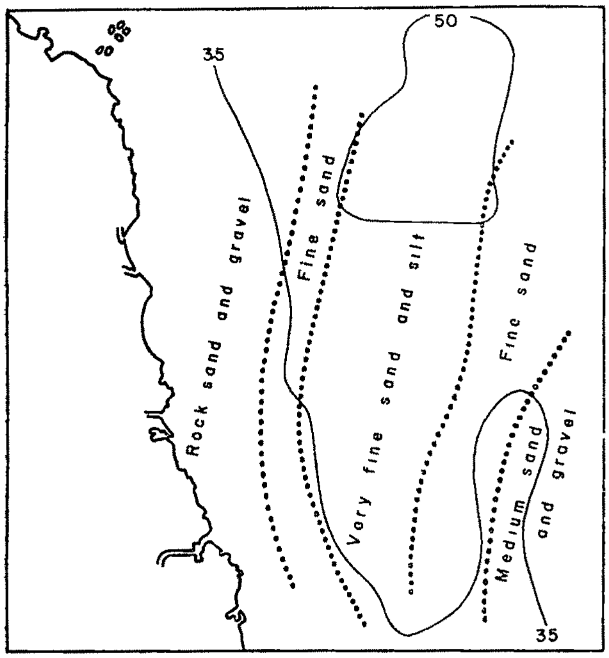

Fig. 2. Sediments off the Northumberland coast (simplified from Buchanan[6]). Depths of 35 and 50 fathoms are also shown.

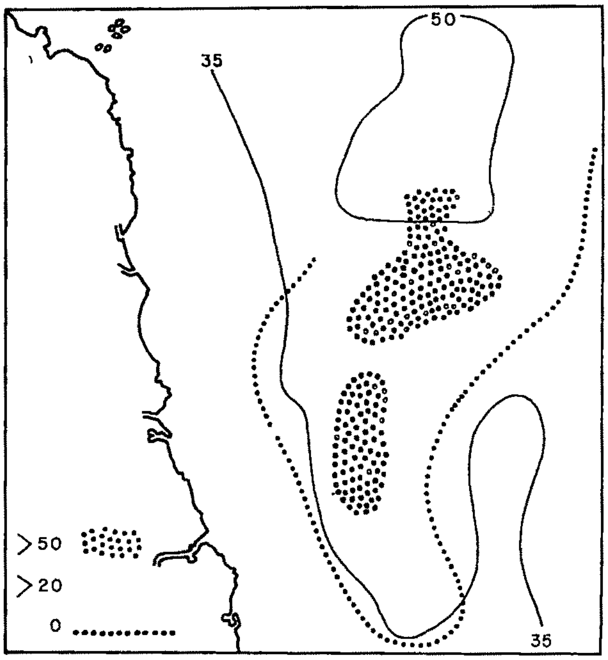

Fig. 3. Catch of *P. borealis* (kg/h). Depths as in Fig. 2.

This area should be able to support a profitable fishery in the years when *P. borealis* is abundant. Boats from various European ports took considerable quantities during the summer of 1967, but as far as we are aware no large catches were landed by British vessels.

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[1] Hjort, J., and Ruud, J. T., *Hvalrad Skr.*, 17, 1 (1938).
[2] Allen, J. A., *J. Mar. Biol. Assoc. UK*, 38, 189 (1959).
[3] Kemp, S., *Scient. Invest. Fish. Brch. Ire.*, *1908*, 1 (1910).
[4] Bigelow, H. B., and Schroeder, W. C., *Biol. Bull. Mar. Biol. Lab. Woods Hole*, 76, 305 (1939).
[5] Horsted, S. A., and Smidt, E., *Meddr. Danm. Fish.-og Havunders.*, 1, 1 (1956).
[6] Buchanan, J. B., *Oikos*, 14, 154 (1963).
[7] Warren, P. J., and Sheldon, R. W., *J. Fish. Res. Board Canad.*, 24, 569 (1967).

Red Light Induction of Gibberellin Synthesis in Leaves

GIBBERELLIC acid is known to mimic the morphogenetic effects of red light[1] on certain plants. Brian[2] has suggested that gibberellins are synthesized in plants after the absorption of red light and Kohler[3] has shown that there is a significant increase in the content of gibberellic acid in etiolated peas after 24 h of irradiation with red light

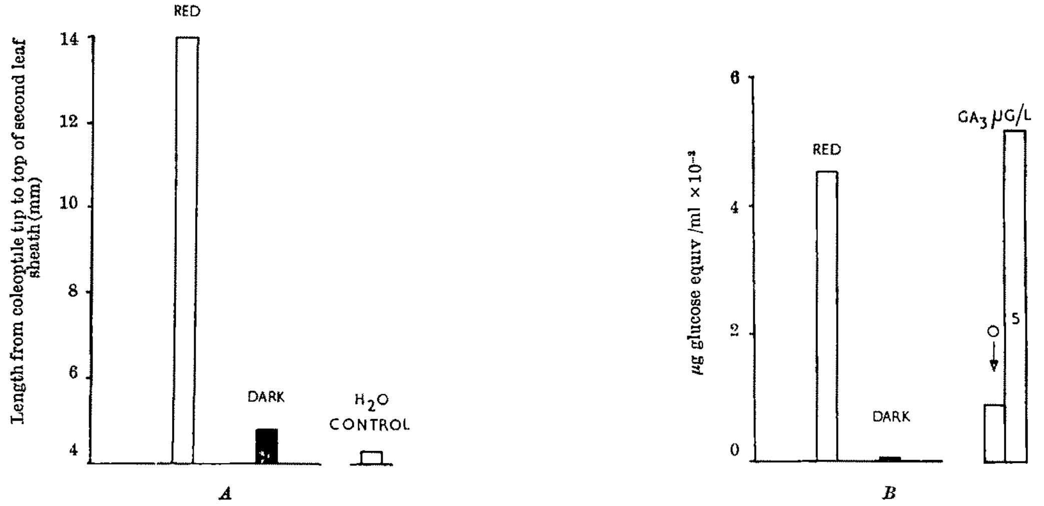

Fig. 1. The effect of red illumination on the gibberellin content of leaves of barley seedlings. *A*, Dwarf-1 maize assay; *B*, barley endosperm assay.

This and other work[4] suggests that phytochrome in the P_{fr} form (after irradiation with red light) initiates synthesis, or at least brings about increases in the content of gibberellin in plants and seeds. Many responses of phytochrome, however, can be observed a few hours (or in some cases in less than 1 h (ref. 5)) after illumination with red light. Thus to explain effects of phytochrome in terms of the action of gibberellin, it is necessary to show that gibberellins are synthesized during or very soon after the end of a period of red irradiation. This is what we have tried to do.

Barley seedlings (variety 'Pallas') were grown in moist sand, in darkness, for 1 week at 25° C. Working in a green safe light[6] a known number of 2 cm segments were cut 1 cm below the leaf tips. These were randomized and placed on moist filter paper and either exposed to red light or kept in darkness. The red source consisted of two 4 ft Atlas red fluorescent tubes and a red 'Plexiglass' filter (Cornelius No. 501). The leaf segments were 80 cm from the tubes. At 660 nm the light intensity received by the segments was 1.38 μW/cm^2.

Extracts were assayed using both the dwarf-1 maize[7] assay and the barley endosperm assay[8]. In the endosperm assay three replicates of each sample were incubated with pieces of endosperm as well as a fourth without endosperm. The blank values were subtracted from the mean sugar values for the other three replicates containing pieces of endosperm.

After receiving the appropriate light treatment leaf segments were homogenized in darkness at a high speed in an MSE top-drive macerator in 10 ml of cold 0.01 molar phosphate buffer (*p*H 7.0) for 5 min. The homogenate was centrifuged at 600*g* for a further 5 min, and the supernatant was adjusted to *p*H 2.5 and extracted three times with ethyl acetate. After separation, the organic fraction was kept at −25° C. The aqueous phase was re-adjusted to *p*H 7.5 and extracted three times with ethyl acetate. This second organic phase was separated from the aqueous layer. The two organic fractions were bulked and evaporated to dryness at 28° C in a vacuum. The solid residue was taken up in buffer (0.01 molar phosphate) with 0.01 per cent 'Tween 20'.

Three hundred leaf segments were irradiated for 40 min with red light, and then returned to darkness and extracted after 30 min. A similar batch of segments was kept in darkness. After extraction half the extract was assayed on the dwarf-maize assay and half on the barley endosperm assay.

Results are shown in Fig. 1*A* and *B*. In both assays the extract from the segments kept in darkness showed no gibberellin-like activity, while that from the segments treated with red light showed considerable activity. This increase of gibberellin activity could be brought about either by release from bound forms[9] or by *de novo* synthesis. Two inhibitors of gibberellin biosynthesis, 2-chloroethyl trimethyl ammonium chloride (CCC) and 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride (AMO 1618), were used to determine whether the gibberellins are synthesized after red light treatment. The inhibitors do not affect the endosperm assay[10]. The segments (200 per treatment) were infiltrated[6] with water, CCC (1 or 10 g/l.) or AMO 1618 (100 mg/l.) before being irradiated with red light.

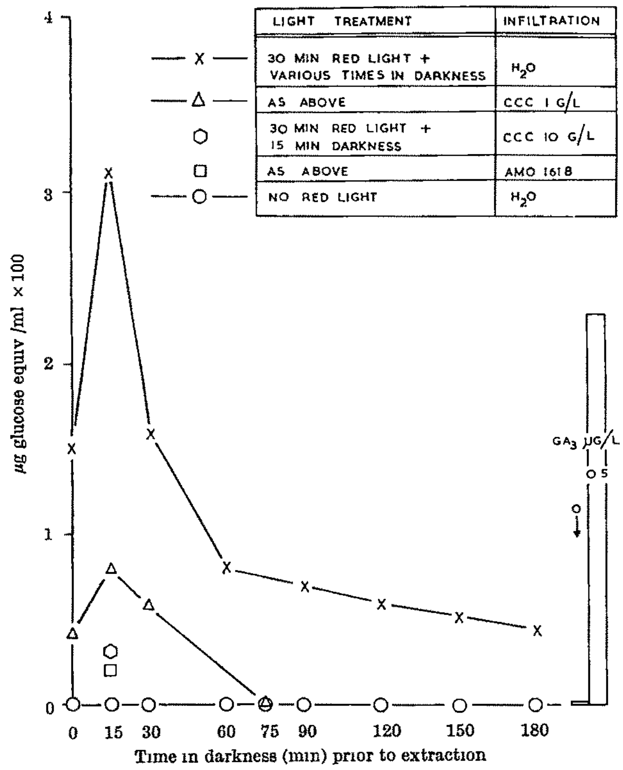

Fig. 2. The effect of CCC and AMO 1618, given before red illumination, on the gibberellin content of leaves of barley seedlings.

After 30 min in red light the leaf tissue was kept for various times in darkness, before homogenization and extraction

The results (Fig 2) show that even immediately after the end of the period of red illumination the leaf segments infiltrated with water contain appreciable amounts of gibberellin activity as compared with dark controls The gibberellin content continues to increase, reaching a maximum within 15 min The level of activity then rapidly falls to a steady low level The transient nature of this peak may explain why many workers have failed to find such an increase of gibberellins after red light treatment The lower curve of the diagram shows the considerable reduction of gibberellin activity when the segments are pre-treated with 1 g/l of CCC This compound, at 10 g/l and AMO at 100 mg/l, almost completely blocks synthesis of gibberellin in the leaf segments These results indicate that the observed rise in gibberellins must be largely a result of a red light-induced synthesis rather than a release of gibberellins from some site of storage

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[1] Nakamura, T , Yamada, T , and Takahashi, N , *Bot Mag* , Tokyo, 79, 404 (1966)
[2] Brian, P W , *Nature*, 181, 1122 (1958)
[3] Kohler, D , *Planta*, 70, 42 (1966)
[4] Bewley, J D , Black, M , and Negbi, M , *Nature*, 215, 648 (1967)
[5] Fondeville, J C , Borthwick, H A , and Hendricks, S B , *Planta*, 69, 357 (1966)
[6] Carr, D J , and Reid, D M , *Planta*, 69, 70 (1966)
[7] Phinney, B O , and West, C A , *Encyc Plant Physiol* , 14, 1185 (1961)
[8] Nicholls, P B , and Paleg, L G , *Nature*, 199, 823 (1963)
[9] McComb, A J , *Nature*, 192, 575 (1961) Jones, D F , *Nature*, 202, 1309 (1964)
[10] Paleg, L , Kende, H , Ninnemann, H , and Lang, A , *Plant Physiol*,, 40, 165 (1965)

Estimation of the Distribution of Plant Roots in Soil

The difficulty of measuring the distribution of living roots in soil is an obstacle to quantitative studies of the performance of root systems in natural conditions Even the most careful procedures for separating roots from soil are subject to limitations[1] Errors occur especially in estimates of the distribution of the finer lateral roots, which may be of major interest because they can account for a considerable fraction of the total nutrient uptake[2] It is, moreover, often impossible to distinguish living from dead roots An alternative approach to this problem is the injection into plant shoots of radioactive tracers which become reasonably uniformly distributed throughout the root systems Racz *et al* [3] and Halstead and Rennie[4] injected phosphorus-32 into plant shoots and estimated the distribution of living roots from measurements of phosphorus-32 in soil samples The low penetrating power, however, of the beta radiation from phosphorus-32 makes it possible to assay only small soil samples, and the quantities of tracer (for example, 300 μc /plant) which are convenient for measurement[4] can cause the concentration in roots to be within the range which may induce radiation injury[5] A tracer which emits gamma radiation of reasonable energy would have obvious advantages and the use of rubidium-86 was therefore investigated

Preliminary studies in water culture showed that about 10 per cent of the rubidium-86 injected into shoots of barley plants 3 weeks old reached roots in 24 h, the fraction transferred decreased with increasing age, being lower by a factor of about three for plants 8 weeks old Downward movement was similar when 2 μl droplets were injected into leaf sheaths or into the laminae of expanded leaves When plants were grown in well stirred solutions, the quantities of rubidium-86 recovered from the solutions after 3 days did not exceed 10–15 per cent of that in the roots; the loss of rubidium-86 from roots to the soil would thus be a minor source of error over short periods Autoradiographs showed that rubidium-86 was more concentrated in root apices and in the adjoining zones, where cell extension had occurred in the interval between injection and sampling, than in older tissues Because the former tissues, however, accounted for only a small fraction of the total root volume, this lack of uniformity might cause only small errors in assessments of quantities of root in appreciable volumes of soil To examine the distribution of rubidium-86 on this larger scale each tiller of plants grown in water culture was injected, and the root systems were divided into twelve approximately equal samples to simulate as nearly as possible the roots which might be present in different zones of soil The coefficient of variability of measurements of activity per unit fresh weight was 20 per cent or less In view of the errors of other methods for measuring root distribution the agreement was considered sufficient to justify experiments in the soil

Because of the variability in root distribution in the field, it was desirable to assay relatively large soil samples, a method developed for surveys of environmental radioactivity enabled samples 2 5 to 3 kg in weight to be handled[6] After thorough mixing, the soil was packed into cylindrical polythene containers of 17 cm internal diameter and 17 cm deep, re-entrant cavities 7 5 cm deep in the bases of the containers enabled them to be fitted over the stainless steel housing of a sodium iodide scintillation crystal activated with thallium (nominal dimension of crystal, 3 × 3 in) A multi-channel pulse height analyser was used to reduce the sample background When the same sample was re-packed, the reproducibility was about 3 per cent and 1 μc of rubidium-86 distributed throughout a sample gave about 2,300 c p m ; separate calibrations were made for each soil

To facilitate examination of sampling and analytical errors in the preliminary experiments, it was necessary to have a relatively uniform distribution of roots Barley plants were therefore grown in well mixed soil contained in boxes approximately 0 5 m^2 in surface area Plant rows were 15 cm apart Seven weeks after sowing, when the fifth leaf on the main axis had developed, two injections of 6 μc of rubidium-86 were made into every plant, the injected tissues were removed after 24 h Soil cores 4 5 cm in diameter were taken and divided into sections 5 cm long, and fifteen of the segments were combined to provide a sample for analysis from each depth Duplicate samples of this type were made within the plant rows in each box, alternate cores being assigned to each replicate; similar samples were taken mid-way between the rows. The mean results for four such boxes are shown in Table 1, the values for the different depths being expressed as percentages of that in the surface horizon within the rows, where root development was greatest, the counting rates of samples from this zone averaged 3 5 × 10^3 c p m above background The standard errors reflect differences between boxes and between replicates in the same box;

Table 1 ESTIMATES OF THE DISTRIBUTION IN SOIL OF THE ROOTS OF BARLEY PLANTS 7 WEEKS OLD GROWN IN ROWS 15 CM APART

Soil horizon (cm from surface)	Within plant rows	Mid-way between plant rows
0–5	100	7 4±2 1
5–10	14 1±2 6	5 8±0 8
10–15	5 4±0 4	4 0±0 6
15–20	4 3±0 7	2 8±0 5
20–25	4 7±0 8	3 1±0 4
>25	7 0±1 1	6 3±0 9

Standard errors based on eight observations are shown

Results expressed as percentages of the value for the surface horizon within the plant rows the former errors were shown to be responsible for over two-thirds of the total error Because considerably greater variability must usually be expected between field plots, it is to be expected that the procedure for subdividing and assaying soil cores will be a minor source of experimental error in field investigations

The applicability of the procedure to other types of plants will depend both on the extent to which the tracer is translocated to roots and on the uniformity of its distribution within them Perennial woody species may present special problems and the type of information sought in any study may influence the degree of variability in the distribution of the tracer throughout the root system which can be tolerated It should be possible, for example, to demonstrate whether changes in agronomic practice alter the distribution of living roots—often a question of major interest—even if variations in the activity of tracer per unit weight of roots cause uncertainty in estimates of the actual amounts of root present at different depths

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[1] Schuurman, J J , and Goedewaagen, M A J , *Methods for the Examination of Root Systems and Roots* (Centre for Agricultural Publications, Wageningen, 1965)

[2] Russell, R S , and Sanderson, J , *J Exp Bot* , 18, 491 (1967)

[3] Racz, G T , Rennie, D A , and Hutcheon, W L , *Canad J Soil Sci* , 44, 100 (1964)

[4] Halstead, E H , and Rennie, D A , *Canad J Bot* , 43, 1359 (1965)

[5] Martin, R P , and Russell, R S , *J Exp Bot* , 5, 91 (1953)

[6] Mercer, E R , in *Radioactivity in Human Diet* (edit by Russell, R S), 489 (Pergamon Press, Oxford, 1966)

APPLIED SCIENCE

Difference in Flight Capacity between Naturally Occurring Spring and Summer Forms of *Myzus persicae* (Sulz.) (Hemiptera:Aphididae)

One approach to the problem of the distances flown by aphids is to study the length of time they retain the ability to fly In many species, flight capacity is restricted to the first few days of adult life, and this has important implications for the epidemiology of plant viruses and, in particular, viruses of the "persistent" type, such as potato leaf roll virus Alate "alienicolae" of *Myzus persicae*, in common with several aphids of economic importance, lose the ability to fly about 3 days after the final moult, provided they settle down on a host plant, for settling is a prerequisite for the physiological processes leading to flight muscle autolysis[1–3].

In order to acquire sufficient potato leaf roll virus to infect a second plant, initially uninfected aphids must settle and feed for several hours on an infected source plant Smith[4] showed that the virus is not acquired by *M. persicae* until it has fed for at least 6 h on an infected potato plant, and Kassanis[5] confirmed this From Johnson's work[1–3] it might be expected that spring migrant *M. persicae* which fly to the potato crop from the primary host plants (*Prunus* species) would not take flight again after they have settled long enough to acquire the leaf roll virus In England, however, most potato leaf roll spreads usually early in the year when spring migrants of *M persicae* are entering the crops[6,7] These spring migrants include both alate "fundatrigeniae"—the first winged progeny of the parthenogenetic generations derived from the fertilized eggs laid on the primary host—and alate "alienicolae", winged parthenogenetic aphids produced in the spring by a stock of parthenogenetic aphids overwintering on secondary hosts.

The relative importance of alatae and apterae in the spread of leaf roll and other persistent viruses is still controversial[8], and Ribbands[9] attributed the early spread of leaf roll to the apterous progeny of alatae which had settled on infected potato plants These alatae might be either alate "fundatrigeniae" or alate "alienicolae", and it is possible that different forms of the same species vary in their biology, either intrinsically or because of differences in the conditions in which they were reared

Small branches of peach, bearing masses of fourth instar alate nymphs, were cut from trees growing in the garden of the Entomological Field Station, Storey's Way, Cambridge The branches were placed in jars of water in a large cage standing in a dark constant-temperature room at 21° C Many of the nymphs moulted to adult during the night, and many of these alate "fundatrigeniae" took off within a few minutes when an overhead 400 W mercury vapour lamp was switched on in the morning Alate "alienicolae" were collected at the same time in a similar way, by bringing into the constant-temperature room sprouting mangolds from a clamp in Cambridgeshire heavily infested with *M persicae*

The "fundatrigeniae" required a much longer bout of flight time than the "alienicolae" before they would settle on potato leaves Alatae which had just completed their teneral development were flown in groups in the flight chamber described by Kennedy and Booth[10] After the flight each aphid was placed on to a young detached leaf of potato, variety 'Majestic' The leaves were kept in tubes of water for 24 h in the dark in the constant-temperature room at 21° C, and then put into a "non-return box"[10] and illuminated by a 40 W "daylight" fluorescent lamp for 1 h Table 1 shows the number of aphids remaining settled on the leaves after 24 h in the dark and after a further hour in the light

The flight capacity of the "fundatrigeniae" and the "alienicolae" was compared by flying batches of newly flight-mature alatae in the flight chamber for at least 5 h to promote settling Aphids were kept on young 'Majestic' potato plants in cages in the laboratory at room temperature (20° C) They were gently removed from the plants each morning with a fine paint brush and kept in glass tubes for 1 h in a brightly illuminated flight-testing chamber To test their ability to fly they were dropped from a paint brush held about 75 cm above the floor[3] A maximum of ten tests per aphid was made Fig 1 shows only those aphids which ceased to fall and began to gain height on being dropped The "fundatrigeniae" retained the ability to fly for an average of 3 61 days, the "alienicolae" retained the ability to fly for an average of 2 36 days The difference is significant at $P < 0\ 001$

While it is evident that there were significant differences in the flight behaviour of the two forms of aphid compared in these experiments, it should be noted that the aphids came from only two samples and that equally large differences in flight behaviour might well be found within alatae of either form from different sources Nevertheless, both groups of aphids came from naturally occurring populations of *M persicae* found in the field at the normal time

Table 1 NUMBERS OF FUNDATRIGENIAE AND VIRGINOPARAE REMAINING SETTLED ON POTATO LEAVES AFTER FLIGHTS OF 1 AND 5 H

	Flight duration					
	1 h			5 h		
		No settled			No settled	
	No flown	After 24 h in dark	After 1 additional h, in light	No flown	After 24 h in dark	After 1 additional h, in light
Fundatrigeniae	40	22*	12†	12	12	8‡
Virginoparae	30	26*	20†	15	15	15‡

* Significant at 5 per cent $\chi^2 = 6\ 57$
† Significant at 1 per cent $\chi^2 = 7\ 87$
‡ Significant at 1 per cent $\chi^2 = 5\ 869$

of year Alate "fundatrigeniae" were collected from several peach trees and alate "alienicolae" were collected from many sprouting mangold roots Furthermore, a difference in the same sense was reported by Ronnebeck[11], who concluded that *M persicae* from peach trees ("fundatrigeniae") flew farther in the field than did alate "alienicolae" from stocks overwintering on savoy cabbage seed crops

It therefore seems likely that many alate "fundatrigeniae" entering the potato crop in early summer remain flight-worthy long enough to spread even a persistent virus like leaf roll if they feed for sufficient time on infected plants during the first few days after their migratory flight Moreover, their flight capacity is greater in the cool temperatures prevailing at this time of the year, for in experiments carried out in this laboratory, alate "fundatrigeniae" kept at 15° C retained the ability to fly for more than twice as long as alate "fundatrigeniae" kept at 25° C

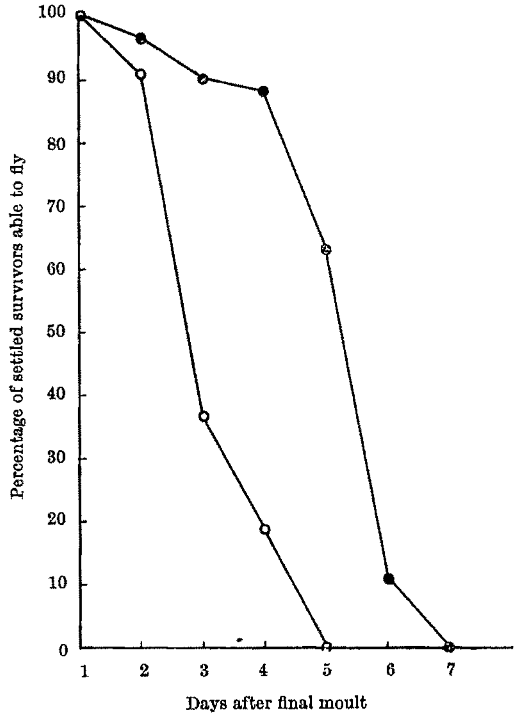

Fig 1 Flight-worthiness of alatae after an initial flight of 5 h ●, Fundatrigeniae, n = 110, ○, virginoparae, n = 22

This work was started under the supervision of the late Dr C R Ribbands, for whose encouragement I am grateful I thank Dr J S Kennedy for advice and discussion, Professor Sir Joseph Hutchinson for providing facilities, and the Agricultural Research Council for a grant

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[1] Johnson, B, *Nature*, 172, 813 (1953)
[2] Johnson, B, *Nature*, 173, 831 (1954)
[3] Johnson, B, *J Insect Physiol*, 1, 248 (1957)
[4] Smith, K M, *Ann Appl Biol*, 18, 141 (1931)
[5] Kassanis, B, *Ann Appl Biol*, 39, 157 (1952)
[6] Doncaster, J P, and Gregory, P H, *Agric Res Coun Rep*, No 7 (1948)
[7] Burt, P E, Heathcote, G D, and Broadbent, L, *Ann Appl Biol*, 54, 13 (1964)
[8] Ossiannisson, F, *Ann Rev Entomol*, 11, 213 (1966)
[9] Ribbands, C R, *Proc Twelfth Intern Cong Entomol*, London, 1964, 525 (1965)
[10] Kennedy, J S, and Booth, C O, *J Exp Biol*, 40, 67 (1963)
[11] Ronnebeck, W, *Z Pflanzenkrankh*, 59, 13 (1952)

GENERAL

Holographic Model of Temporal Recall

Professor Longuet-Higgins[1] has called attention to the remarkable property of human memory of recognizing and recalling long sequences of which at first only a small fraction is consciously remembered, and he has devised a most interesting, physically realizable mathematical model which achieves just this He has rightly called this a temporal analogue of holography I wish to show that there is at least one other mathematical model, which I consider to be a closer analogue of holography, because it operates with triple products of the temporal function to be recognized or recalled

Let the signal $A(\tau)$ start at $\tau = 0$ At the time t_1 we record the convolution integral

$$\varphi(t_1) = \int_0^{t_1} A(\tau)A(t_1 - \tau)\mathrm{d}\tau \quad 0 < t_1 < T$$

where T is the total duration of the signal In order to recognize or to recall the signal we take $\varphi(t_1)$ from the store and form a convolution integral with a signal A' which need be only a fraction of the longer sequence A The recalled function is

$$R(t) = \int_t^T A'(t_1 - t)\varphi(t_1)\mathrm{d}t_1 = \int_t^T A'(t_1 - t)\mathrm{d}t_1 \int_0^{t_1} A(\tau)A(t_1 - \tau)\mathrm{d}\tau$$

Reversing the order of integration in the trapezoidal region $t < t_1 < T$, $0 < \tau < t_1$, we obtain

$$R(t) = \int_0^t A(\tau)\mathrm{d}\tau \int_t^T A'(t_1 - t)A(t_1 - \tau)\mathrm{d}t_1 + \int_t^T A(\tau)\mathrm{d}\tau \int_\tau^T A'(t_1 - t)A(t_1 - \tau)\mathrm{d}t_1$$

If now the function A is one which correlates sharply with itself, that is, if it is as often positive as negative and not regular-periodic, the two factors of $A(\tau)$ under the integral signs will be of the nature of delta-factors, $\delta(\tau - t)$, which become noticeably different from zero only for $\tau = t$, so that

$$R(t) \propto A(t)$$

with more or less "noise" if A' is only a fraction of A

This is therefore a possible mechanism for recall It is also a mechanism for association It is easy to see that, if we replace $A(\tau)$ by some other function $B(\tau)$ and record its convolution with $A(\tau)$, we shall be able to recall B by means of A or a part A' of it

The process can also be digitalized The functions A, B can be discrete sequences of $+1$ and -1, or of "yesses or noes", defined only for certain points of time, and the integrals are then replaced by sums over the lattice points in the trapezoidal area This makes the model realizable with "McCulloch–Pitts neurons" The best procedure would be probably to record first $A(\tau)$ in a first store, and then transfer it to a second store, where it is multiplied with itself, back-to-front, one multiplicand starting at $\tau = 0$ and the other, backwards, at $\tau = t_1$ The signal A could then be recalled, by a similar operation in a third, "associative" organ, by a fraction A' of itself, or by any other sequence which has a part (such as a word, or several words) in common with it

I do not wish to suggest, of course, that this model corresponds any more closely to the reality of the nervous system than Longuet-Higgins's system of resonators

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[1] Longuet-Higgins, H C, *Nature*, 217, 104 (1968)

BOOK REVIEWS

TOP BIOLOGISTS

Nobel Lectures

Physiology and Medicine 1901–1921, including Presentation Speeches and Laureates' Biographies. Pp. xii + 561. (Amsterdam, London and New York: Elsevier Publishing Company, 1967. Published for the Nobel Foundation.) 180*s*.

THE Nobel Foundation has arranged for the publication, in English, of the Nobel Lectures in the various subjects (physics, chemistry and so on). This volume contains the lectures in physiology and medicine for 1901–21. In trying to make overall judgments about this series of statements one is handicapped by their small number, which gives any generalizations small statistical reliability, especially because the authors and their subjects are very varied. It would perhaps have been better to review all the lectures in one subject when they have been published. This would involve reading three times more of them and the strain of following the great thoughts of so many great men might lead to disillusionment.

As it is, however, the volume is a pleasure and a stimulus. Many thoughts occur as one reads, mainly thoughts of admiration. The staff of professors of the Royal Caroline Institute have done their work well. There is nothing here that is silly, very little that is wrong and an enormous amount that was highly original in its day and is still interesting now. Only one prize was awarded for work that turned out to be of quite minor scientific and clinical importance (that of Finsen in 1903 for phototherapy of *Lupus*, and he gave no address). But what a galaxy of success there is, Ross and Laveran, Koch and Pavlov, Golgi and Cajal, Metchnikoff, Ehrlich and Krogh, Kossel and Carrel. Of course, such a selection cannot always be exactly right. The electors have had their quirks, such as choosing Ross five years before Laveran, and pairing off Golgi and Cajal together, in spite of their disagreements and dislikes. But such differences of personal and scientific style are with us still. To see the lucky ones ill-matched together at least gives sardonic amusement to the less fortunate.

It would not be easy to make a convincing analysis of the success of these biologists and medical scientists. But some general points emerge. The unity of biological and medical science stands out on almost every page. Some assert it openly as Metchnikoff: "To solve medical problems comparative pathology had to be brought in". In his case it involved the study of the larvae of starfishes and it was with these that the phenomena of inflammation and phagocytosis were revealed, to be confirmed later by work on water fleas!

Nearly all of these prize-winners used and refer to animal experiments at the very centre of their work, some without being theoretical about it, others like Pavlov, meditating on the necessity to "ignore the mental state of the animal". Having revealed how "four simultaneously acting lethal causes were deliberately eliminated" he comments: "A striking proof of the power of science that regards the organism as a machine", a power we still need as much as ever today, but are often too mealy-mouthed and frightened of conventional views to admit it.

Of course, the awarders of the prizes have not been unheedful of the importance of the practical clinical results of medical research. We cannot, therefore, use the lectures to estimate how far investigators work with practical ends in view. But the awarders carefully avoided over-attention to this aspect and it is interesting that those of their choices who gave least attention to immediate practice were conspicuously the more influential and also in the end produced the greatest effects. The surgeon Kocher no doubt did a great deal for our understanding of the pathology of the thyroid and his address is an interesting account of it, but it is incomparably less important than the entirely academic presentations such as that by Cajal on neurones or by Kossel on the nucleic acids, protein and histones of the nucleus. Again both of these two chose material from all over the animal kingdom. Kossel took histones from the red cells of birds and the sperm of sea urchins and cephalopods and protamines from the milt of fishes. What a lot he knew about the nucleus even in 1910.

Indeed it is inspiring, if humbling, to see the width of knowledge, experience and indeed wisdom shown by most of these men. Of course they wrote their addresses carefully, but also in something of a hurry. The products must reflect their natural capacities and tastes, and these are mostly admirable. Many of the Nobel Prize-winners write well, often personally, but with respect for their predecessors and contemporaries, and seldom rancour over the opposition which many of them encountered. Final reflections: no single address in twenty years contains a mathematical formula, and only Ross, Cajal and Golgi felt the need for any great amount of illustration. Even considering the formal nature of the occasions of presentation these are curious features of the addresses delivered by the greatest ones of physiology and medicine (1901–21). J. Z. YOUNG

BRAIN–HORMONE INTERACTIONS

Neuroendocrinology

Vol. 2. Edited by Lucian Martini and William F. Ganong. Pp. xix + 777. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 256*s*.

THE first of the two volumes of this treatise on neuroendocrinology dealt mainly with the anatomy of the hypothalamo-pituitary system in mammals, methodology, and the classic problem of the neural control of the secretion of the anterior and posterior pituitary hormones. It was reviewed (*Nature*, 215, 448, 1967) as an addition to the literature of a rather specialized area of research that was already extensively covered by other reviews, monographs and reports of symposia. Considerable duplication was inevitable and, although some excellent chapters were noted, the book as a whole was not considered to offer a great deal of material that was not already fairly easily available elsewhere to a reader reasonably familiar with the field. The same cannot be said of the second volume, for the editors, Ganong and Martini, have now made available a more extensive and complete survey of neuroendocrine topics than has been attempted previously in a single work.

The overall plan seems to have been to assign a general field, rather than a limited topic, to contributors actively engaged in a wide range of biological research. On the one hand this approach has resulted in an expert compilation and sifting of scattered results from many sources that are relevant to neuroendocrine investigations, while on the other hand those workers concerned with varied aspects of a particular problem have been able to present their specialized knowledge in a coherent form for the general reader. Contributions of the first type include a chapter by Gold and Ganong on the effects of drugs on neuroendocrine processes, a critical review by Ganong and Lorenzen on brain neurohumours and endocrine function, and discussions of invertebrate and lower vertebrate neuroendocrinology by Hagadorn and by

Jørgensen and Larsen Specific problems discussed in depth include the control of the onset of puberty (Critchlow and Bar-Sela), the effects of light on endocrine function (Wurtman), and control of the pars intermedia (Etkin)

The action of hormones on the central nervous system is also discussed in detail in this volume Barraclough reviews the long-term effects of pre-natal and post-natal exposure of the brain to abnormal gonadal steroid levels on reproductive function, one of the most exciting areas of neuroendocrine research in the last decade The specific effects of gonadal steroids in eliciting sexual behaviour and the influence of these and other hormones on brain excitability in general receive comprehensive treatment from other contributors The "feedback" action of the target organ hormones on the hypothalamus or pituitary to modify trophic hormone secretion was dealt with in the earlier volume Indeed, there are few topics that could reasonably be included under the heading of "Neuroendocrinology" that have escaped notice in one or other volume although the higher mental functions are perhaps one No discussion of the studies of the acute and chronic effects of hormones on conditioned aversive behaviour in animals was noted, nor was there any attempt to review the extensive, though often contradictory, literature on the possible role of endocrine factors in psychiatric disorders in man

Although this second volume covers such a diversity of topics, the overall impression is not one of discontinuity On the contrary, the juxtaposition of findings from so many branches of biology serves to emphasize the relevance of data from other fields to the specialist's understanding of the significance of brain–hormone interactions in his own area of interest The high standards of editing and production achieved in the first volume have been maintained in this second volume, together they provide a valuable source of factual information for the endocrinologist in search of details and a very complete presentation of current views and interests in the field of brain–hormone interrelations for the biologist who is not himself involved in such studies but needs an overall view of the activities of his contemporaries in this area of research

K BROWN-GRANT

FROM MIND TO MIND

The Psychology of Human Communication

By John Parry Pp 248 (London University of London Press, Ltd, 1967) 30*s*.

"COMMUNICATION is all", a twentieth century Keats might say, surveying the triumphs of communication theory in the sciences and the arts, for all of which it makes bold to offer a new Esperanto Psychologists too have profited from this general enrichment of the world of learning by the acquisition of novel tools of conceptual analysis, but they have been apt to regard the idea of communication they that of information) as too sacred to tamper with In the result, communication theory has sometimes turned out to be a Procrustean bed for psychological phenomena, since a blind eye has been turned to the purely human facts of communication to which models must be fitted

Hence the task Dr Parry has set himself He has endeavoured to convey to the reader the distinctive and sometimes imponderable character of the psychological phenomena in the domain of communication, and to consider the types of message one individual wishes to transmit to another, the barriers that prevent or distort transmission, and the lines of research relevant to these problems.

In the first part of this book he deals with various types of information among which he distinguishes human from artificial systems. He then proceeds to discuss the role of language as a medium and of feeling as a message, compares scientific with artistic expression, and differentiates between "sign" and "content" information, categories which are perhaps more familiarly known as "selective" and "semantic", respectively

The second part is devoted to an exposition of barriers to communication Seven are described, they are limitation of the receiver's channel capacity, distraction, unstated assumptions, incompatible schemas, unconscious pressures, poor presentation, and absence of channels The third part moves on to examine problems of communication in complex activities such as personnel selection and the administration of the law, and finally, in the fourth part, Dr Parry presents his theme as a branch of research

Dr Parry is clearly well versed in the literature Nevertheless, there are significant omissions which, if reference had been made to them, would have strengthened his thesis There is no mention, for example, of the epidemiological model which L S Penrose and others have used in a variety of contexts of human communication, in particular, where ideas are disseminated throughout a group.

While the treatment on the whole is reasonably critical, there are occasional lapses Thus a chapter is given to ten instances of failure in communication at the end of which we are told the "likely reasons" for the failure in each case. But these reasons are not very illuminating Thus case 4 is "A father and son are at odds. Each says that the other refuses to consider his point of view" The "likely reason" is "schema incompatibility" Are we now much wiser ? Dr Parry defines a "schema" as "an enduring pattern of brain activity", but unless we know something about this pattern what do we achieve by giving it a name ? Dr Parry might have heeded a warning given by Professor R C Oldfield that the word "schema" has an important role in contemporary psychology because "it stands for something of which we are ignorant"

It is also surprising not to find any mention of communication in committees and conferences, or to the technique of "role reversal" which several investigators have fruitfully employed

Nor is there any mention of recent experiments on subjective scaling of selective or sign information which would have been germane to his theme, and, while he realizes that selective information is not the whole story, there remains much more to be said about the scope and nature of subjective (semantic, content) information.

It is doubtful whether, in the fourth part of the book, Dr Parry has succeeded in conveying the gist of relevant research to readers unfamiliar with the original sources And the abstracts are presented in a sequence which does not bring out any coherence, if any they possess.

Nevertheless, in spite of these shortcomings, the volume as a whole is a timely and salutary corrective to the dehumanization of communication theory in contemporary psychology

JOHN COHEN

LAYMAN'S ACADEMIC PSYCHOLOGY

Psychology and Social Problems

By Michael Argyle Pp 232 (London Social Science Paperbacks in association with Methuen and Co, Ltd, 1967) 16*s* net

IT is difficult to project a book primarily at the layman while maintaining interest and precision and academic respectability Such a book may well end as a mass of fascinating but unwarranted generalizations or, at the other extreme, as an uninteresting list of experimental findings which are thought to be of particular everyday relevance In his *Psychology and Social Problems*, Mr Argyle neatly avoids either of these poles He has produced an easily readable book which deals with topics of social importance in an acceptable academic manner The chapter headings read like a list of problem areas, on which (often to his embarrassment) the professional psychologist is frequently asked to pontificate by chance acquaintances. Argyle deals with these topics in an authoritative manner, making few statements which he cannot support with sound scientific evidence.

Consequently, I feel that this book is useful at two levels. First, as a direct fund of information to the layman, and second, as a source book for the psychologist who wishes to answer the questions of the layman but whose specialized interests lie in other areas of behaviour.

At a more particular level, Argyle is especially lucid in his discussions of interpersonal relationships, crime, mental health and racial prejudice. In my estimation, however, he does not fare as well when considering child rearing and the motivation of social behaviour. Here, it can be argued, he places too little emphasis on that body of research and thought which offers the most promising approach, that is, that stemming from learning theory. Further, I would criticize the book with respect to the arrangement of its references. It is frustrating to have to turn continually to the final section of the book in order to check these. These criticisms, however, are somewhat trivial and detract very little from what is basically a well-written and useful book.

Finally, it is interesting to note Argyle's observations on measures which would help to reform various undesirable social conditions. They are a mixture of enlightened common sense and suggestions springing directly from research. One has the impression that they would satisfy the final analysis demanded by society—they would work.

K. T. STRONGMAN

DATA TO THEORY IN SOCIAL SCIENCE

Theory and Methods of Social Research

By Johan Galtung. Pp. 534. (Oslo: Universitetsforlaget; London: George Allen and Unwin, Ltd., New York: Columbia University Press, 1967.) 70*s* net.

THIS thought-provoking treatise is, as its author says, "neither an introduction nor a handbook" to social research methodology. "Rather," he explains, "the intention has been to try to integrate things usually kept apart: techniques of data collection, techniques of analysis, statistical methods, and some philosophy of science." The result is a unique and stimulating work, in the tradition of Lazarsfeld and Hyman, but with an added breadth and comprehensiveness. For all but a few British sociologists at the present time, the mathematical treatment is likely to prove difficult, but no one seriously interested in social research method can fail to derive benefit from the book as a whole.

The unifying concept is the data matrix, the tripartite structure into which all social science data can be fitted, consisting firstly of elements or units of analysis, secondly of dimensions or variables and thirdly of the values of the units on the variables studied. The first part of the book deals with data collection, the second part with data analysis. Developing the logic of the data matrix, there are chapters on the units (and problems of sampling), on the variables (and levels of measurement) and on the values (with the arguments for and against rating or ranking them). There follow sections on the main forms of data collection and on surveys. This second part subdivides into three, beginning with data processing, statistical distributions, patterns and indices, continuing with a discussion of hypotheses and their "dimensions" and methods of multi-variate analysis, and concluding with a chapter on the logic of theory formation.

So bare a summary cannot convey the book's rich content. While some tools of analysis discussed are "close to or beyond the limit of what contemporary social science can digest", no method, however humble, is taken for granted. We are made to think through each mental operation afresh and emerge with our logical muscles considerably and no doubt beneficially stretched. The author's combination of reasoning power and pragmatic awareness of the social science research context is unusual and impressive.

Classical statistics from Galton to Fisher largely assumed interval scale variables and normally distributed universes. The publication of Siegel's *Non-parametric Statistics for the Behavioral Sciences* in 1956 opened up ampler perspectives. But much is still missing. "The field of ordinal scale statistics is in wild disorder" and this is by far the most important field for the social scientist, who typically can develop ordinal measures but rarely interval scale measures. "What social science analysis lacks most at the moment is a language, in which *degrees* of correlations can be expressed; a language which is stronger than ordinary prose, yet weaker than interval scale mathematics of functional relationships."

Looking perhaps yet further ahead, he calls for a development of the language of process. There is no simple relation between this language and that of correlation, nor can one in general infer from synchronic to diachronic correlation or vice versa. Therefore there should be much more emphasis on longitudinal data in social science, and much less on the synchronic type of study. Social theory should be presented in the process language as a set of variables related by one-way or two-way, negative or positive processes. The pragmatic concept of process is preferable to the dogmatic concept of cause and effect. Processes are difficult to study because of the time they take to unfold, but a variety of methods are suggested, including a combination of historical source data with modern sampling methods and the analytic machinery of content analysis.

The book is well produced, with an exceptionally beautiful jacket, on the back of which we read that our author was born in 1930, holds degrees in both mathematics and sociology (Oslo, 1956, 1957), taught at Columbia University, 1958–1960, and was Unesco Professor of Sociology at Santiago, 1962–63 and 1965. Since 1959, he has been the Director of the International Peace Research Institute, Oslo.

C. MADGE

PHYSICAL CHEMISTRY OF PIGMENTS

Pigments

An Introduction to their Physical Chemistry. Edited by David Patterson. Pp. xii+210. (Amsterdam, London and New York: Elsevier Publishing Company, Ltd., 1967.) 65*s*.

THE scarcity of reliable and really informative scientific books on the subject of inorganic and organic pigments is the cause of great difficulty to those who are students or teach in this field. The organic or inorganic chemistry of pigments is in general fairly straightforward even though it is often cloaked by industrial secrecy. The difference between a coloured insoluble compound and a pigment, however, is as great as that between carbon black and a diamond, and sometimes more costly to achieve. It is the study of the properties of pigments which make them useful as distinct from simple coloured matter which constitutes the subject of the physical chemistry of pigments. In this field we must be concerned with molecular phenomena such as electromagnetic absorption, optical dispersion and photoexcitation, and with particular phenomena including crystal structure, nucleation, particle size and surface properties. Additionally important techniques must be considered, notably colour measurement and computation, and particle size analysis.

In July 1964 a summer school was organized under the auspices of the department of colour chemistry at the

University of Leeds on the topic "Progress in the Chemistry and Technology of Pigments" which covered the physical chemistry of pigments Dr Patterson has taken the proceedings of the school as a basis and with additional material has compiled the first available book on the topic. Normally summer school proceedings do not provide a sufficiently coherent body of information to make a readable book, but the policy of combining a chapter on some aspect of general theory with another on the application of the theory to pigment problems has avoided the dangers most effectively The text still betrays its spoken origins For example, there is the occasional "verbless sentence" This tends, however, to maintain a brisk informal style which carries the reader forward

Five of the ten chapters are concerned with colour Theories of colour of inorganic and organic molecules are considered in the first two chapters In dealing with organic molecules very great emphasis is placed on the free electron method of calculating light absorption with perhaps too little attention paid to molecular orbital and valence bond methods One chapter is concerned with the colour of organic crystals with interesting information about orientation effects on light absorption mechanisms Two further chapters on colour are concerned with instrumental methods of colour measurement and colour match prediction, respectively The particular aspects of pigments are considered in four chapters concerned with nucleation and growth of particles, particle size measurement, surface activity and theories of dispersion, flocculation and flotation Photoexcitation in pigment crystals is considered in relation to the question of photoconduction and the degradation of organic molecules This chapter is accommodated less easily than the others, but the editor's contention that photoconduction studies in this field are of special relevance to the question of the light fastness of pigments is well supported

This book succeeds in what it sets out to do, namely, to indicate in some detail the scope of a far ranging subject It will prove of great value to students or research workers coming fresh to pigment problems, providing a guide to their further education. I D RATTEE

REFERENCE TO PLUTONIUM

Plutonium Handbook

A Guide to the Technology Vol 1. Edited by O J Wick Pp x+520 (New York and London· Gordon and Breach, 1967) $26

THE aims of the two volumes of this work are stated as being the provision of information on a wide range of topics concerned in the utilization of plutonium and of a first reference on its technology In view of the progressive relaxation of classification restrictions since the first limited release of information on the properties of plutonium in the United States and Britain in 1954, these objectives constitute a very considerable task The editor is therefore to be congratulated on producing a first volume of manageable proportions which covers the physics, metallurgy and chemistry of the metal and its compounds The sub-title of the work, "A Guide to the Technology", seems inappropriate for the first volume, although a preview of the chapter titles for the second volume suggests that it may be more applicable to the latter

The individual chapters have been written by acknowledged experts in the appropriate fields from a number of American atomic energy laboratories Not unexpectedly, major contributions have been made by the Los Alamos and former Hanford, now Pacific North West, Laboratories Clearly it has taken a considerable time to bring together all the individual contributions into a single volume and the most recent references quoted are dated 1965 In particular, no reference is made to any of the papers presented at the Third International Conference on Plutonium held in London in November 1965 Some qualification must be made, therefore, about using the work as a first reference Within individual chapters the authors have attempted to incorporate references to work throughout the world, though naturally there is a preponderance of American references in view of the large proportion of the work which has been done in that country The chapter lengths are very uneven and a more balanced result might have been achieved if subjects of equal weight had been chosen for each chapter Minor chapters such as "Metallography" and "Laboratory Techniques" might well have been combined or even omitted There is also some overlap between different chapters which could have been cut out without great loss, for example, data on the Pu–O and Pu–C systems The author index and subject index are only to be published with the second volume which provides a slight limitation on the value of the first volume alone Having made these criticisms, however, it must be said that the principal chapters are in general excellent, both in technical content and in readability

The book will undoubtedly find use in all plutonium laboratories as a compilation of the principal features of the chemistry and metallurgy of plutonium and its compounds, although the combined price of the two volumes is likely to be rather prohibitive for individual purchasers.

P G MARDON

TWENTY YEARS ON

Dynamic Aspects of Biochemistry

By Ernest Baldwin Fifth edition Pp xiv+466 (London Cambridge University Press, 1967) 55s net, $9 50

IT says much for the original dynamism of Professor Baldwin's book that it reappears now in its fifth edition some twenty years after it first burst on the biochemical scene The two previous editions had given the impression that the book was beginning to lose ground a little in the rapid advance of the subject It was to be hoped that this would be redressed in a new edition, which has been reset in a larger format and for which, it is claimed a major revision has been made It is a great disappointment to have to report that these hopes are largely in vain. Two chapters, those on protein biosynthesis and fat metabolism, have indeed been completely rewritten and another, on special aspects of nitrogen metabolism, has been dismantled, but much remains which is now outdated For example, it detracts much from the value of the book to read speculations concerning the position of the extra phosphate group in NADP$^+$, or again those about the origin of deoxyribose, which take no account of Reicherd's work on reduction at nucleotide level Even worse is the misstatement concerning the localization in the cell of the iso-citrate dehydrogenases and there are erroneous formulae such as those for active methionine and ubiquinone which have been transferred uncorrected from the fourth edition

The chief criticism of the book, however, must be its failure to deal with important new developments in biochemistry One could list such topics as isoenzymes, allosterism and the relationship between structure and function in enzymes as exemplified by ribonuclease and lysozyme

The opportunity has been missed to revitalize *Dynamic Aspects* to meet the demanding requirements of the honours student who has, nowadays, a number of excellent modern texts available To those, however, approaching biochemistry for the first time and for whom a complete and balanced up to date coverage is not a requirement, then *Dynamic Aspects* may well be recommended C A ROSS

IUWDS International Geophysical Calendar for 1968

1968 JANUARY

S	M	T	W	T	F	S
	1	2	△3	4	5	6
7	8	9	△10	11	12	13
14	15	⑯	◇17	⑱	19	20
21	22	23	△24	25	26	27
28	29	30	△31			

1968 FEBRUARY

S	M	T	W	T	F	S
				1	2	3
4	5	6	△7	8	9	10
11	12	⑬	△⑭	⑮	16	17
18	19	20	△21	22	23	24
25	26	27	△28	29		

1968 MARCH

S	M	T	W	T	F	S
					1	2
3	4	5	△6	7	8	9
10	11	⑫	△⑬	⑭	15	16
17	18	19	△20	21	22	23
24	25	26	△27	28	29	30
31						

1968 APRIL

S	M	T	W	T	F	S
	1	2	△3	4	5	6
7	8	9	△10	11	12	13
14	15	⑯	◇17	⑱	19	20
21	22	23	△24	25	26	27
28	29	30				

1968 MAY

S	M	T	W	T	F	S
			△1	2	3	4
5	6	7	△8	9	10	11
12	13	⑭	△⑮	⑯	17	18
19	20	21	△22	23	24	25
26	27	28	△29	30	31	

1968 JUNE

S	M	T	W	T	F	S
						1
2	3	4	△5	6	7	8
9	10	⑪	△⑫	⑬	14	15
16	17	18	△19	20	21	22
23	24	25	△26	27	28	29
30						

1968 JULY

S	M	T	W	T	F	S
	1	2	△3	4	5	6
7	8	9	△10	11	12	13
14	15	⑯	◇17	⑱	19	20
21	22	23	△24	25	26	27
28	29	30	△31			

1968 AUGUST

S	M	T	W	T	F	S
				1	2	3
4	5	6	△7	8	9	10
11	12	⑬	△⑭	⑮	16	17
18	19	20	△21	22	23	24
25	26	27	△28	29	30	31

1968 SEPTEMBER

S	M	T	W	T	F	S
1	2	3	△4	5	6	7
8	9	10	△11	12	13	14
15	16	⑰	△⑱	⑲	20	21
22	23	24	△25	26	27	28
29	30					

1968 OCTOBER

S	M	T	W	T	F	S
		1	△2	3	4	5
6	7	8	△9	10	11	12
13	14	⑮	◇16	⑰	18	19
20	21	22	△23	24	25	26
27	28	29	△30	31		

1968 NOVEMBER

S	M	T	W	T	F	S
					1	2
3	4	5	△6	7	8	9
10	11	⑫	△⑬	⑭	15	16
17	18	19	△20	21	22	23
24	25	26	△27	28	29	30

1968 DECEMBER

S	M	T	W	T	F	S
1	2	3	△4	5	6	7
8	9	10	△11	12	13	14
15	16	⑰	△⑱	⑲	20	21
22	23	24	△25	26	27	28
29	30	31				

1969 JANUARY

S	M	T	W	T	F	S
			△1	2	3	4
5	6	7	△8	9	10	11
12	13	⑭	△⑮	⑯	17	18
19	20	21	△22	23	24	25
26	27	28	△29	30	31	

⑯ Regular World Day (RWD)

22 Day of Solar Eclipse

3 Day with Unusual Meteor Shower Activity

8 9 World Geophysical Interval (WGI)

◇17 Quarterly World Day (QWD) also a PRWD and RGD

⑭ Priority Regular World Day (PRWD)

△10 Regular Geophysical Day (RGD)

TABLE OF WORLD DAYS MARKED ON THE CALENDAR

1968	RWD	PRWD	QWD	RGD	WGI	ECL	METEORS
Jan	16, 17, 18	17	17	3, 10, 17, 24, 31	8-21	—	4
Feb.	13, 14, 15	14	—	7, 14, 21, 28	—	—	—
Mar	12, 13, 14	13	—	6, 13, 20, 27	—	28-29	—
Apr	16, 17, 18	17	17	3, 10, 17, 24	8-21	—	21-22
May	14, 15, 16	15	—	1, 8, 15, 22, 29	—	—	4-5
June	11, 12, 13	12	—	5, 12, 19, 26	—	—	6-10
July	16, 17, 18	17	17	3, 10, 17, 24, 31	8-21	—	28-30
Aug	13, 14, 15	14	—	7, 14, 21, 28	—	—	10-13
Sep	17, 18, 19	18	—	4, 11, 18, 25	—	22	—
Oct	15, 16, 17	16	16	2, 9, 16, 23, 30	7-20	—	20
Nov	12, 13, 14	13	—	6, 13, 20, 27	—	—	16
Dec	17, 18, 19	18	—	4, 11, 18, 25	—	—	12-14 22

University News :

Cornell

Dr Carl Sagan, assistant professor of astronomy at Harvard University, has been appointed an associate professor in the Department of Astronomy

London

Mr R N Pryor, general manager of the Rio Tinto Patino SA, has been appointed to the chair of mining tenable at the Imperial College of Science and Technology

Manchester

Mr Peter Dovell, at present head of the Birmingham School of Planning and Landscape, has been appointed to a newly established chair of urban design.

Announcements

The Council of the Geological Society of London has made the following awards for 1968 Wollaston Medal to Professor R C Moore, Lyell Medal to Mr M Black, Murchison Medal to Dr G Wilson, Wollaston Fund to Dr S Moorbath; Murchison Fund to Mr R K Harrison, a moiety of the Lyell Fund to Dr R G C Bathurst, another moiety of the Lyell Fund to Mr R E Elliott

The Institute of Metals has made the following awards Institute of Metals (Platinum) Medal for 1968 to Dr W J Kroll in recognition of his services to the non-ferrous metal industries, Rosenhain Medal for 1968 to Dr D W Pashley in recognition of his contributions in the field of physical metallurgy; W H. A. Robertson Medal for 1967 to Mr T Z Blazynski for his paper, "An Assessment of a Combined Rotary Piercing and Elongating Process for Tube Making", published in the *Journal of the Institute of Metals*, April 1967

Meetings

Tenth Anniversary Meeting of the Phytochemical Society, April 1–3, University of Cambridge (Dr J B Harborne, Hartley Botanical Laboratories, The University, Liverpool 3)

Properties and Metrology of Surfaces, April 1–4, Oxford (Mr R S Glynn, Conference Department, The Institution of Mechanical Engineers, 1 Birdcage Walk, London, SW1)

Structure of Tracks of Charged Particles in Condensed Media, Second L H Gray Memorial Conference, April 8–10, 1969, Trinity College, Cambridge (Professor J. W Boag, Second L H Gray Memorial Conference, Department of Physics, Institute of Cancer Research, Clifton Avenue, Belmont, Sutton, Surrey).

Structure, Solid Mechanics and Engineering Design in Civil Engineering Materials, April 21–25, 1969, University of Southampton (Secretariat, M Te'eni, Department of Civil Engineering, University of Southampton, Southampton)

NATO Advanced Summer Study Institute on Superconductivity, June 17–29, McGill University (P R Wallace, Institute of Theoretical Physics, McGill University, Montreal, Quebec, Canada)

Technological Forecasting, June 23–25, University of Strathclyde (Mr R V Arnfield, University of Strathclyde, Livingstone Tower, Richmond Street, Glasgow)

Precision Electromagnetic Measurements, June 25–28, Boulder Laboratories of the National Bureau of Standards (Donald D King, Aerospace Corporation, PO Box 95085, Los Angeles, California 90045)

Natural Rubber Conference, August 28–September 5, Kuala Lumpur (Natural Rubber Conference 1968, RRIM, PO Box 150, Kuala Lumpur, Malaysia)

Physical Aspects of Noise in Electronic Devices, September 11–13, University of Nottingham (The Meetings Officer, 47 Belgrave Square, London, SW1)

Theoretical Chemistry Summer School, September 16–28, Somerville College and Mathematical Institute, Oxford (Professor C A. Coulson, Mathematical Institute, 24–29 St Giles, Oxford)

Erratum In the communication "Effect on Brain Enzyme and Behaviour in the Rat of Visual Pattern Restriction in Early Life" by Devendra Singh *et al* (*Nature*, 216, 1337, 1967), Table 2 should read

Table 2

Rearing condition	ChE activity*	Anterior cortex	Posterior cortex	Total cortex
VP	Mean	55 00	55 80	53 09
	σ_M	7 90	4 55	4 45
RVP	Mean	38 67	27 33	33 00
	σ_M	5 11	6 06	4 02
Difference		16 33†	28 47‡	20 09‡

* All ChE values are in moles ACh × 10^{10} hydrolysed/min/mg of tissue
† Significant at 0 05 level
‡ Significant at 0 01 level

The ChE activity for restricted visual pattern group (RVP) and visual pattern group (VP)

Erratum In the communication "Sites of Inhibition of DNA Synthesis by Kethoxal *bis*(Thiosemicarbazone)" by Barbara A Booth *et al* (*Nature*, 217, 250, 1968), dCTP in the legend to Fig. 1 should represent deoxycytidine not deoxycytosine

Erratum In the communication "Sialotonin Vasopressor Substance in Cat Submaxillary Saliva" by C Moriwaki *et al* (*Nature*, 217, 270, 1968), the second line of the penultimate paragraph refers to indolealkylamines. these should be indoleamines

Erratum In the article "Radio Emitting Electrons in the Galaxy and the Solar Modulation of Cosmic Rays" by K C Anand *et al* (*Nature*, 217, 25, 1968), the scale of the legend to Fig 1 should be in BeV and not GeV as shown The abscissa of Fig 2 should be Frequency (MHz) (Anticentre), and the third sentence of the third paragraph on page 27 should read "It may also be pertinent to stress here that the fitting in the high frequency end of Fig. 2*A* depends primarily on the assumed magnetic field because in this frequency region $I_\nu \propto L < H_\perp >^{y}$, where $y > 1$"

Erratum In the communication "Ultraviolet Photolysis of Sodium Nitrate Solutions in the Laboratory and by Sunlight" by E G Gori *et al* (*Nature*, 217, 248, 1968), columns 2 and 3 of Table 2 should read

α'	β'
Mole l^{-1} cal $^{-1}$	Cal $^{-1}$
2 70 × 10^{-3}	6 27 × 10
4 10 × 10^{-3}	3 47 × 10

Corrigendum In the communication "Proposed Procedure for the Allocation of Trivial Names to the Gibberellins" by J MacMillan and N Takahashi (*Nature*, 217, 170, 1968) the structure of Canavalia-II was incorrectly drawn, the OH group should not have been present

Corrigendum *Textbook of Fluid Dynamics*, by Frank Chorlton, which was reviewed in *Nature* (217, 199; 1968), is available as a library edition in cloth boards for 70*s*, as well as the paperback version at 35*s*, which was reviewed

FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, February 12

University of London (in the Botany Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Dr R J Benthem (Utrecht) "Conservation of Land Use Development I Reconstruction of Landscape" *

Institution of Mechanical Engineers, Manipulation and Mechanical Handling Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Passenger Handling"

Tuesday, February 13

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, NW1), at 5 p m —Scientific Papers

Institution of Electrical Engineers, Control and Automation Division (joint meeting with the Automatic Control Group of the I Mech E, at Savoy Place, London, WC2), at 5 30 p m —Discussion meeting on "Control in Respiration" opened by Professor J M Nightingale

Royal Institution (at 21 Albemarle Street, London, W1), at 5 30 p m — Professor R J Harrison "Reproductive Patterns" (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties To be repeated on February 14, 20 and 21)

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Dr D A J Tyrrell "The Possible Chemotherapy of Respiratory Virus Diseases" (Tenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Institution of Electronic and Radio Engineers (at 8–9 Bedford Square, London, WC1), at 6 p m —Mr P Cooke "The M O S T Integrated Circuit"

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Mr C G O'Neill "Mixture Conditioning for Reduced Exhaust Emission"

Institution of the Rubber Industry (at the Eccleston Hotel, Victoria, London, SW1), at 7 p m —Mr M Williams "Value Analyses—a Management Technique"

Wednesday, February 14

Royal Institution, History of Science Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Professor James Greig "Engineering and Scientific Technique—The Contribution of John Hopkinson" *

Institute of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, SW1), at 2 15 p m —Mr L R Colquhoun "The Operation of Hovercraft"

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street London, WC1), at 5 p m —Professor A P Dempster "A Generalization of Bayesian Inference"

Institute of Petroleum and the Institute of Fuel (at 61 New Cavendish Street, London, W1), at 5 30 p m —Mr W R Dorrensteijn, Mr H Verkoren and Mr G P Reman "Causes and Remedies of Combustion Noise in Refinery Plant"

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, WC2), at 5 30 p m —Mr F J M Laver "Stimulus or Constraint? The Interplay of Computer Design and Use"

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Professor B S Platt "Some Features of Protein-Calorie Deficiency in Man and Animals" *

University of London (in the Botany Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Dr R J Benthem (Utrecht) "Conservation of Land Use Development II Landscape Planning in New Areas" *

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 6 p m —Professor Harry Harris, FRS "Human Genetics and Biochemical Individuality" (Sir William Jackson Pope Memorial Lecture)

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College, London, SW7), at 6 p m —Dr P Grootenhuis "The Use of Visco-Elastic Materials to Control Vibrations"

University of London (at the Institute of Archaeology, 31–34 Gordon Square, London, WC1), at 6 p m —Lt Comdr D W Waters, RN "Sources of Maritime Archaeology—History of Navigational Instruments" *

Society of Chemical Industry, Food Group—Food Engineering Panel (at 14 Belgrave Square, London, SW1), at 6 15 p m —Mr N G Ross "The Importance of Residence Time in Continuous Food Processing"

Institute of Information Scientists (at Knightway House, 20 Soho Square, London, W1), at 6 30 p m —Mr M Goldsmith "The Science of Science Foundation"

Pharmaceutical Society of Great Britain, and the British Society for the History of Pharmacy (at 17 Bloomsbury Square, London, WC1), at 6 30 p m —Mr F W Adams "The Pharmacy Act 1868"

Thursday, February 15

London Mathematical Society (at Burlington House, Piccadilly, London, W1)—Dr P Dembowski "Designs and Permutation Groups"

Institution of Electrical Engineers, Control and Automation Division (joint meeting with the Automatic Control Group of the I Mech E, and the Institute of Measurement and Control, at Savoy Place, London, WC2), at 2 30 p m —Colloquium on "Direct Digital Control of Systems and Processes"

Oil and Colour Chemists' Association, London Section (in the New Engineering Block, University College, Gower Street, London, WC1), at 2 30 p m —Paint and Printing Ink Conversazione Papers by Mr T R Bullett, Mr C C Mill, Mr C J H Monk, Dr Rohrer, and Dr Wagener

Society for Analytical Chemistry, Biological Methods Group, an Radiochemical Methods Group (at the Pharmaceutical Society, 17 Bloomsbury Square, London, WC1), at 2 30 p m —Meeting on "Radiochemical Methods Applied to Biological Investigations"

Royal Society (at 6 Carlton House Terrace, London, SW1), at 4 30 p m —Professor W S Fyfe "Experiment and the Crust of the Earth"

Institution of Mining and Metallurgy (at the Geological Society Burlington House, Piccadilly, London, W1), at 5 p m —Dr E Cohen, Dr J Mizrahi, Mr C H J Beaven and Mr N Fern "The Residence Time of Mineral Particles in Hydrocyclones", Dr J Mizrahi and Dr E Cohen "Studies of Some Factors Influencing the Action of Hydrocyclones", Mr M P Jones, Dr J Gavrilovic and Mr C H J Beaven "Colour Synthesis as an Aid to Electron-Probe X-ray Microanalysis"

Linnean Society of London (at Burlington House, Piccadilly, London, W1), at 5 p m —Dr G L C Bertram and Dr C K R Bertram "The Bionomics of Dugongs and Manatees", Professor A J E Cave "The Cetacean Reniculus"

Institute of Petroleum, Economics and Operations Group (at 61 New Cavendish Street, London, W1), at 5 30 p m —Mr G W Sears "Distribution Models"

Institution of Civil Engineers, Transportation Engineering Group (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "Transportation Systems in New Towns" introduced by Mr J M Stevens and Mr P B Green

Institution of Electrical Engineers, Power Division (at Savoy Place, London, WC2), at 5 30 p m —Discussion meeting on "Voltage Measurements of High Voltage Transmission" opened by Mr N Ashton

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Dr R J W Rees "Recent Advances in the Study of the Leprosy Bacillus" (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, SW1), at 6 p m —Mr J R Ryder and Dr A J Majumdar "Glass Fibre Reinforced Cement and Plaster"

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W1), at 7 30 p m —Dr J R Audy "Implications for Trematode Control of Inter-Specific Larval Antagonisms within the Snail Host", Dr C A Wright "Some Views of Biological Control of Trematode Diseases"

Thursday, February 15—Friday, February 16

Biochemical Society (at the Middlesex Hospital Medical School, Cleveland Street, London, W1)—479th Meeting Joint Colloquium with the Society for Endocrinology on "Characterization and Identification of Small Molecules of Biological Importance", and an ordinary meeting followed by a lecture on "New Methods for Finding Scientific Information"

Friday, February 16

British Computer Society (at the Connaught Rooms, Great Queen Street, London, WC2)—Symposium on "The Basis of Business Information"

Royal Institution, Photochemistry Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Dr C M Trumbore "Excited States in Radiation and Photochemistry" *

Society of Chemical Industry, Oils and Fats Group (at 14 Belgrave Square, London, SW1), at 2 30 p m —Mr H B S Conacher "Rearrangement of Methyl Epoxyoleate and Related Epoxy Esters", Mr R W Williams "The Effect of Cholesterol on the Lecithin/Water System", Mr B K Bullimore "Evaluation of a Cheap NMR Apparatus for Oils and Fats Analysis", Mr D Illingworth "Zone Refining of Fatty Acids"

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, WC2), at 5 30 p m —Mr J K V Lee "A Position Fixing Aid to Marine Surveying"

Royal Institution (at 21 Albemarle Street, London, W1), at 9 p m — Dr Roger Bannister, CBE "Science and the Athlete"

Saturday, February 17

Inner London Education Authority (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3 30 p m —Mr Nicholas Guppy "Forest Indians of Southern Guiana—the Wai-Wais and Mawayans" *

Saturday, February 17—Monday, February 19

Field Studies Council (at the Orielton Field Centre, Pembroke)— Symposium on "The Biological Effects of Oil Pollution on Littoral Communities"

Monday, February 19—Wednesday, February 21

Materials Science Club (at the City University, St John Street London, EC1)—Informal Meeting on "Materials for Fuel Cells and High Energy Density Rechargeable Batteries"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Senior Demonstrator (with an honours degree in geology with particular interests in mineralogy, petrology or economic geology, and preferably experience in geochemistry or in X-ray techniques) in Geology at the University College of Townsville, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (February 16)

Reader/Senior Lecturer (with substantial research experience in building acoustics and noise) in Building Science—The Staff Officer (606/6), The University of Aston in Birmingham, Gosta Green, Birmingham, 4 (February 17)

Assistant Lecturer in Organic Chemistry—The Registrar, University of Leicester, Leicester (February 24)

Agricultural Science Graduate (with an honours degree or equivalent) in the Soil Fertility/Chemistry Department—The Director, An Foras Taluntais (The Agricultural Institute), 33 Merrion Road, Dublin 4, Republic of Ireland, quoting Ref N3 (February 28)

Chair of Veterinary Pathology—The Secretary of the University Court, The University, Glasgow (February 29)

Chemist (with HNC, LRIC or BSc degree) to work on soil organic matter/trace elements, and a Chemist/Physicist (with HNC, LRIC or BSc degree) to study soil minerals by X-ray diffraction—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref 1052/105 (February 29)

Laboratory Steward (with HNC or higher qualification and considerable experience in a university laboratory)—The Secretary, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford (February 29)

Temporary Lecturer or Assistant Lecturer in the Department of Psychology to lecture on animal behaviour and the biological foundations of psychology—The Registrar, University Senate House, Bristol 2 (February 29)

Lecturer or Assistant Lecturer (preferably with postgraduate experience and interests in the field of metamorphic petrology) in Geology at Fourah Bay College, University of Sierra Leone—The Inter-University Council, 33 Bedford Square, London, WC1 (March 14)

Lecturer or Assistant Lecturer (with a degree in agriculture or tropical agriculture plus graduate experience in the tropics or sub-tropics, and preferably experience in the field of agricultural education) in Crop Production at the University of Botswana, Lesotho and Swaziland, for duties in Swaziland at the Agricultural College and University Centre—The Inter-University Council, 33 Bedford Place, London WC1 (March 20)

Chair of Physics at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, April 8)

Assistant Professor (with experience in bacterial chemistry and/or metabolism) of Microbiology—Dr A J Rhodes, Head, Department of Microbiology, School of Hygiene, University of Toronto, Toronto 5, Ontario, Canada

Biochemist (preferably with experience with liver perfusion techniques and enzymes) to assist in research on bile pigment metabolism—Dr B H Billing, Medical Unit, Royal Free Hospital, Gray's Inn Road, London, WC1

Botanist/Plant Physiologist (with a good honours degree, sound experience in physiological and biochemical techniques and adequate research achievement) for studies on the biological changes accompanying maturation and senescence of fruits under selected storage regimes, with particular reference to the physiological disorders arising during long storage and to the application of the results of basic research—The Secretary, East Malling Research Station, Maidstone, Kent

Graduate Biochemist (preferably with research experience in separative biochemical methods) in the Department of Pharmacology to assist in a research programme on the metabolism of cerebral tissues—The Clerical Officer, Unit for Brain Metabolism, Department of Pharmacology, University Medical School, Teviot Place, Edinburgh, 8, Scotland

Research Assistant in the Department of Chemistry for research on proteins—The Administrator, Department of Chemistry, University College London, Gower Street, London, WC1

Research Assistants (qualified students wishing to work towards the MSc or PhD degrees) in Physiology—Dr D H Copp, Head, Department of Physiology, University of British Columbia, Vancouver, 8, B C, Canada

Research Technician/Junior Technician in the Pathology Department to work full-time on projects involving tissue culture, fluorescent microscopy, histology immunological techniques—The Secretary, Guy's Hospital Medical School, London Bridge, London, SE1

Senior Technical Assistant (with previous experience of work in chemical laboratories and ability to undertake responsibility for experimental investigations without detailed supervision) in the Chemistry Department of the Chester Beatty Research Institute Fulham Road, SW3—The Secretary, Institute of Cancer Research, Royal Cancer Hospital, 34 Sumner Place, London, SW7, quoting Ref 301/B/309

Technical Officer in Chemistry (with a university degree or equivalent) in the Metabolic Unit—The Secretary, Royal Postgraduate Medical School, Ducane Road, London, W12

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Directory of British Associations Edition 2, 1967–8 Pp x+294 (Beckenham, Kent CBD Research, Ltd, 1967) 80*s*, $15 [2811

National Lending Library for Science and Technology List of Current Serials Received from Asia Pp 42 (Boston Spa National Lending Library for Science and Technology, 1967) [2911

Imperial College of Science and Technology (Royal College of Science, Royal School of Mines, City and Guilds College) Calendar 1967–68 Pp xi+569 (London Imperial College of Science and Technology, 1967) [412

Northern Ireland Ministry of Agriculture Leaflet No 25 Sheep—Management of Upland Flocks Pp 14 Leaflet No 26 Sheep Diseases Pp 16 (Belfast Ministry of Agriculture 1967) [412

University of Hull Annual Report 1966–67 Pp 146 (Hull The University, 1967)

Department of Education and Science The Sophistication Factor in Science Expenditure By A V Cohen and L N Ivins (Science Policy Studies, No 1) Pp 53 (London H M Stationery Office, 1967) 6*s* net [512

Marine Biological Station, University of Liverpool Annual Report No 79 for 1966 Pp 45 (Liverpool Liverpool University Press 1967) [512

National Institute of Agricultural Engineering Report for the period 1st October, 1964—31st March, 1967 Pp 68 (Silsoe, Bedfordshire National Institute of Agricultural Engineering, 1967) 20*s* [512

Glasshouse Crops Research Institute Annual Report 1966 Pp 170 (Rustington, Littlehampton Glasshouse Crops Research Institute, 1967) 13*s* 6*d* [612

Other Countries

Canada Department of Energy, Mines and Resources Bulletin 155 Middle and Upper Triassic Spiriferinid Brachiopods from the Canadian Arctic Archipelago By A Logan Pp 37+plates 1–5 $2 Paper 67–4 Abstracts of Publications in Scientific Journals by Officers of the Geological Survey of Canada, January, 1966 to March, 1967 Compiled by D Snowden Pp 61 $0 75 Paper 66–54 Proceedings, Symposium on Geochemical Prospecting, Ottawa, April, 1966 Edited by E M Cameron Pp v+282 $2 Paper 67–2 Geological Survey of Canada—Radiocarbon Dates VI By J A Lowdon, J G Fyles, and W Blake Jr Pp 42 $0 75 Paper 67–43 Vendom Fiord Formation—a New Red-Bed Unit of Probable Early Middle Devonian (Eifelian) Age, Ellesmere Island, Arctic Canada By J W Kerr Pp v+8 $1 50 (Ottawa Queen's Printer, 1967) [412

National Science Foundation NSF 67–12 Basic Research, Applied Research, and Development in Industry, 1965—A Final Report on a Survey of R & D Funds, 1965, and R & D Scientists and Engineers, Jan 1966 (Surveys of Science Resources Series) Pp v+128 (Washington, D C National Science Foundation, 1967 Available from U S Government Printing Office) $0 65 [412

United States Department of the Interior Fish and Wildlife Service Technical Papers of the Bureau of Sport Fisheries and Wildlife No 11 Surface Temperature Gradients Observed in Marine Areas Receiving Warm Water Discharges By James L. Squire, Jr Pp 8 $0 10 No 14 Interspecific Hybridization of Esocids Hatching Success, Pattern Development, and Fertility of Some F_1 Hybrids By K Buss and Jack Miller Pp 30 $0 25 (Washington, D C Government Printing Office, 1967) [412

Bulletin of the American Museum of Natural History Vol 138, Article 2 Annotated List of Birds of the Adelbert Mountains, New Guinea—Results of the 1959 Gilliard Expedition By E Thomas Gilliard and Mary Lecroy Pp 51–82+plates 9–14 (New York American Museum of Natural History, 1967) $2 50 [412

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FEBRUARY 17, 1968

Is Technology Here to Stay?

THE Ministry of Technology has enlivened and sometimes illumined the character of government in Britain for three years now, and after a shaky start it has won for itself a degree of permanence or at least the appearance of permanence. It is, for example, a long time since even the people in charge were not quite sure how to translate into administrative action the profound conviction of their government that the deficiencies of the British economy were largely attributable to defects in the structure of British industry. It is also a long time—or at least it seems like it—since there was a hankering after the belief that a handful of wise men around a committee table could solve problems which were beyond the wits of rapacious industrialists. Paradoxically, the succession of economic crises in the past three years has helped to give Mintech, as the Ministry of Technology likes to call itself, a better sense of proportion. After all, governments which are forced against their will and to their own surprise to devalue their currencies are not in the strongest position to offer unsolicited advice to industrial companies which, for all their faults, have at least managed to stay in business. Inevitably, and sensibly, the Ministry of Technology has become in the brief spell of its existence more like a midwife of change than an aspiring magician.

In the circumstances, however, it is not impertinent to ask precisely what the Ministry of Technology is for, and in looking for an answer it is probably more charitable to go by what the ministry does than by what it says. The trouble is that public statements on the subject are almost inevitably filled either with platitudes or even with fatuities. In a recent lecture at Imperial College, for example, the Minister of Technology, Mr Wedgwood Benn, cheerfully announced that "for the first time it is possible to compare projects across the whole field of engineering and to begin the difficult task of evolving criteria which allow intelligent choices to be made between alternative developments in different fields". Most people will agree that this is a sensible goal at which to aim, although some will raise the question of whether governments can allow themselves to be so involved in the process of planning industrial developments without at the same time finding themselves uncomfortably identified with the failures as well as the successes. The trouble is that it is also an ambitious goal, and there is at present no reason for believing that it is within the grasp of the Ministry of Technology or even the Government as a whole. In practice, indeed, the ministry's successes so far have not been steps towards this grand design but, rather, sensible and exceedingly empirical attempts to deal with industrial problems which have become so acute that they cry out for attention. It is easy to see how a sense of progress towards distant objectives helps to sustain the ministry's courage in adversity, but it is bound to be distracting for those outside.

This is a pity, for a good many of the things which the ministry has done are likely to be most valuable. There is, for example, a scheme to encourage the development of new kinds of machine tools by making it possible to spend public money on the first few production models. This will help to secure in Britain some of the benefits which are obtained in the United States (with less fuss) by the scale and the manner of spending on defence research. There is also a great deal of good sense in the ministry's plan to do something for the chemical engineering plant manufacturing industry, partly by advice, partly by research, development and consultancy at the ministry's research establishments and partly, no doubt, by an attempt to group some of the small companies in the industry into larger units. In time, no doubt, the ministry will be able to do valuable work in this field, not least of all because the men responsible for these developments are patient creatures and knowledgeable as well. In the same way, there now seems to be some evidence that the attempt to use the efforts of the 9,000 technically qualified people under the ministry's umbrella for industrial research and development is beginning to bring rewards (see page 607), although it would probably be foolish to imagine that what has worked with some success at Harwell can become a means of avoiding altogether the problem of how to deal with defence research establishments which, for one reason or another, have outgrown their original tasks. Whether the ministry's efforts to produce "a flourishing computer industry" will eventually be successful is another matter, although it is only proper to acknowledge that luck and IBM between them will probably have a greater influence on the outcome than anything the ministry can do. In these and many other ways, the ministry has functioned well when it has set out as a kind of industrial entrepreneur, willing to seize on whatever opportunities present themselves. It has also, however, won some more general victories. The ministry has, for example, provided a good deal of the drive behind the Industrial Reorganization Corporation, which has itself done much to set in train what amounts almost to an epidemic of mergers in British industry. The ministry has also been able to establish the principle that the Government's intervention in industry should ideally consist of encouragement for the successful enterprises, not support for the unsuccessful. In the long run, this inegalitarian doctrine should bring important benefits.

In other ways, however, the Ministry of Technology has been less successful. On many occasions, for example, it has spread itself too thinly. Sometimes plans which have been broached to the people most directly affected have then been put into cold storage for lack of people or money to follow them through. On other occasions the ministry has been downright inconsistent or ambiguous. What, for example, is its policy towards the research associations? What precisely does it mean by the European Technological Community? Then, with the most human frailty, it tends to grasp the thorny nettles last of all—there is, for example, little sign of movement on the reorganization of the nuclear power industry. Elsewhere the ministry's influence, no doubt unwittingly, has been mischievous. Its joyful espousal of the cause of tribology has been indecently innocent. By going out of its way to make technology respectable (which it is), the ministry has cast what it calls pure science in a villain's role and it will be a serious matter if that becomes a permanence.

At this stage nobody can know precisely what the future holds. The Ministry of Technology may have been able to prove the point that governments can usefully play a creative part in industry, but it does not follow that the patterns of administration now being created will last indefinitely. And in many ways, of course, success implies that the ministry will do itself out of a job. Now, for example that its efforts to weld the British companies in the British motor industry into a single unit have been successful, it is much easier for the ministry to wash its hands of the motor industry's anxieties. By the same test, if and when it rationalizes the computer industry, it will be able to retire gracefully to those sidelines as well. To say this, of course, does not mean that there will be nothing for the ministry to do, but rather that its industrial interests are bound to change frequently with the passing of time. In the circumstances, it is hard to see just what part the ministry's laboratories will play in the continuing future—is there really a need for them? But if it were not for the laboratories, what distinction would there be between the Ministry of Technology and several other agencies of government—the Board of Trade, the Ministry of Power and the Department of Economic Affairs, for example? In other words, although the Ministry of Technology has made a useful start, and although the foreseeable future is crammed with work, it will not be surprising if ten years from now the pattern of administration is radically different from what it is at present. And the test by which it will eventually be judged is whether, like the midwife it should aim to be, it will be prepared to retire gracefully once it has done its obvious job in some sector of British industry. The signs are not always encouraging.

Expensive Nuclear Power

The controversy over the way in which British nuclear power plants are built is not being allowed to die a quiet death. Mr Norman Atkinson, Labour Member of Parliament for Tottenham, has tabled a motion in the House of Commons urging the Government to implement the findings of the Select Committee on Science and Technology, of which he was a member. The committee, which produced its report in December 1967, said that nuclear power stations in Britain should be designed by one large group, taking in all the experience of the existing three consortia and of the Atomic Energy Authority. Mr Atkinson has since resigned from the committee, because, he says, he did not agree that an all party body was the right way for a Labour Government to determine its policy on nuclear power. Despite this, his motion that the recommendations of the select committee be now implemented has been tabled, and has the support of over one hundred Labour Members of Parliament.

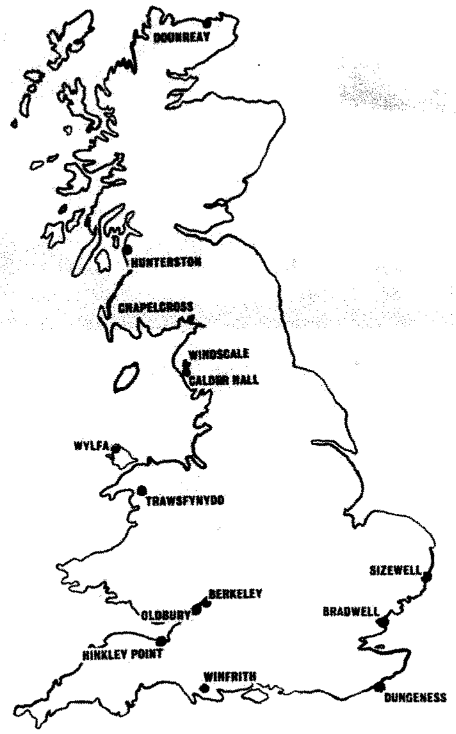
Mr Atkinson is convinced that the way in which the Central Electricity Generating Board has organized the building of power stations has cost Britain £40 million. This suspicion arose originally in the proceedings of the select committee, when it emerged that the CEGB had approved a change in the design of the channel diameter between Dungeness *B* and Hinkley Point *B*, the first two advanced gas cooled reactors to be built. It was claimed that, if this change had not been made, some £11–13 million could have been saved on Hinkley *B*. Both the CEGB and the Nuclear Power Group, makers of Hinkley *B*, indignantly dismiss the charge, and it is probably fair to say that other members of the select committee were doubtful about it too. Very little was made of it in the report. Mr Atkinson has now extended the same argument, claiming that, if all the first four AGRs had been identical, £40 million could have been saved on the capital cost of £320 million. "The CEGB commissioned virtual replicas, but did not get the economic saving of replication," Mr Atkinson says. The civil engineering work on all the power stations was also far too expensive—companies from the United States could have done it for half as much.

All this is likely to be taken as just another example of Mr Atkinson's enthusiastic approach to politics. There may well have been mistakes in the arrangements of the first few AGRs, but it is very unlikely that they will cost as much as Mr Atkinson claims. The difference between United States capital costs and British ones is at least as much a function of the design of the reactor system as it is of civil engineering inefficiency. But it is entirely sensible of Mr Atkinson to feel that it is about time the Government made up its mind about the future pattern of nuclear power in Britain. Until the select committee report was published, there was almost an excuse for inaction, but by now the Government can quite properly be accused of indecision. Mr Atkinson claims that the Government is under pressure from the consortia to retain the existing system—which is doubtless true—and that the CEGB also opposes the single design authority. Even if the Government were not on the point of announcing new nuclear power stations to provide power for aluminium smelters, there would still be no excuse for further delay.

Sites for Nuclear Power

The British Government clearly intends to build nuclear power stations much nearer to centres of population. In the House of Commons last week, Mr Richard Marsh, Minister of Power, said that some modification of the policy of building nuclear stations in remote places was now possible. The minister was acting on advice given him by the Nuclear Safety Advisory Committee, which had said that the principal safeguard to public health came from the high standards of design, construction and operation of nuclear stations. For gas cooled reactors built in pre-stressed concrete vessels, it was clear that it was safe to build them much nearer to cities than had been customary. Mr Marsh said that this meant any area with a population density sufficiently light to evacuate the people within a range of 1·5 to 2 miles in the unlikely event of an accident. He agreed that this included the areas around Hartlepools and Heysham, both possible sites for nuclear stations.

The decision raises some interesting issues. Although most people agree that a relaxation of the restrictions is timely, particularly in Britain where isolated sites are hard to find, the decision of the committee refers only to British gas cooled reactors. What would happen if the generating boards decided to build a water reactor in the United States style (as they have sometimes threatened to do) is not made clear. A direct comparison of the safety of the two reactor types has not been made—so far both have a completely clean record, although it has been suggested that gas cooled reactors are inherently safer. The decision also clears the way for the generating boards to replace some of their older coal fired stations with nuclear stations, making better use of sites nearer to towns. As the map shows, all British nuclear stations so far built are in fairly remote areas, which has tended to increase the costs of building and of transmitting the power to the point of use.



In other parts of the world which lack the sophisticated grid system in operation in Britain, the advantages of building near towns may be even greater. This, it is hoped, may help the export of British power stations, perhaps to Germany. As Mr Eric Lubbock mentioned in the short debate in the Commons, a power station is to be built near Ludwigshafen in West Germany; Mr Marsh pointed out that another is to be built within 20 miles of Hamburg. The fact that Britain is now willing to build as near as this to towns will lend some weight to the tenders made by British consortia. In the United States, Consolidated Edison did once propose to build a station in New York, but were dissuaded by the Atomic Energy Commission. Despite this, the same tendency for generating stations to get ever nearer to towns has been observed in the United States. One of the problems which dictates a cautious advance, however, is the way in which the size of nuclear stations has increased. In this sense, Mr Marsh is disingenuous to claim that Britain has 132 nuclear years of experience. Until Dungeness *B* starts operating, Britain has no actual experience of a commercial advanced gas cooled reactor. The experience is on the less sophisticated—and generally smaller—magnox reactors.

Kidney Transplants

THE ethical problems of transplanting organs are to be discussed at a conference announced on February 14 by Mr Kenneth Robinson, Minister of Health. The conference will be chaired by Sir Hector MacLennon, President of the Royal Society of Medicine, and will include lawyers, leading churchmen and laymen as well as doctors. Although the conference will be private, there seems some chance at least of a joint communiqué being issued after it is over. The ministry has emphasized that the purpose of the conference is to discuss the problems that have arisen in the provision of kidneys for transplant operations in Britain, and not to embark on lengthy discussions of the more controversial subject of heart transplants. So far, because there have been no heart transplant operations in Britain, the discussion of the subject has been less acrimonious than elsewhere, but there have already been signs of sharp disagreement. The minister will be hoping to steer the discussion away from such potentially dangerous topics.

Kidney transplantation is a much less heated subject; almost everybody agrees that in principle it is a very good thing. But technical difficulties over the provision of kidneys from donors have at times made the surgeon's task harder than it might have been. Some surgeons feel that the law should be amended so that kidneys could be taken from fatally injured people without consent from relatives. The difficulty here is that the kidney must be removed from the patient very soon after death if it is to be of any use to the patient.

Guidance for Universities

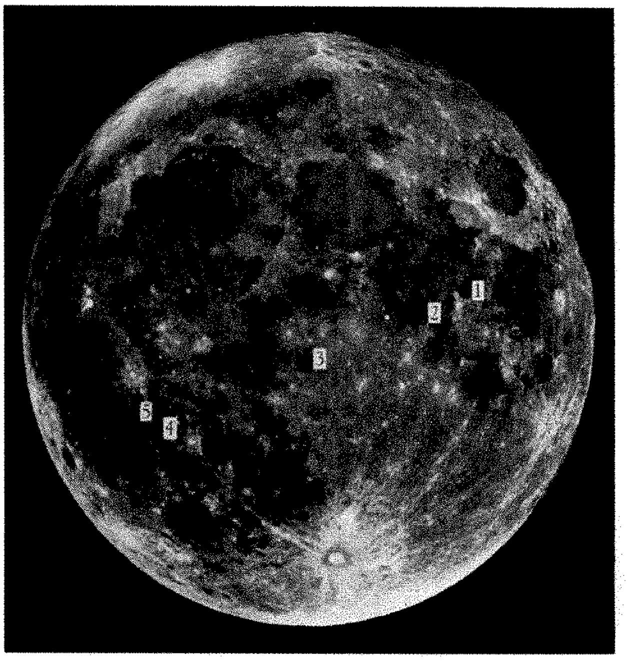
BRITISH universities have now been provided with some cautious guidance on the degree to which lecture rooms and laboratories are being used. This information is the first product of the work of the sub-committee under Mr C. F. Carter, Vice-Chancellor of the University of Lancaster, which has been brooding on the utilization of university facilities for the best part of a year. The report now being sent to universities is based on a survey in sixteen institutions which has been carried out by Mr K. S. Davies, the research officer attached to the project by the vice-chancellors' committee. At this stage it is clear that everybody is anxious not to let the universities feel that they are being coerced into a uniform pattern of teaching, and the report is offered as a means of providing "universities with a means of comparing their use of capacity with others". It remains to be seen whether the project will have more to say, in the years ahead, about the ways in which universities should manage their affairs, but the report says that the next step is to estimate what savings and qualitative improvements would be possible "if optimal patterns of use are adopted". Eventually, the argument runs, the universities would then be more able to formulate accurate plans for capital expenditure. The report also looks forward to a kind of advisory service equipped to keep a continuously watchful eye on university practices and able to offer advice about the ways in which universities might get better value for money.

Mr Davies explained, earlier this week, that there is not yet a tangible plan for setting up a continuing organization to superintend the ways in which universities make use of their facilities. The progress report which has now been issued says very little about British university practice in present circumstances, but there is a brief statement that, in the universities already surveyed, the "occupancy factor" of teaching rooms works out at 75 per cent, which is "no mean performance" compared with the average occupancy of 30 hours a week out of 45 in the universities of Washington and California. This brief statement is, of course, quite unconvincing without supporting evidence and has on this account provoked some sarcasm in the daily newspapers. Mr. Davies explained during the week that more detailed information had been circulated to the committee of vice-chancellors, though it is not yet clear whether this is already being used for purposes of comparison by British universities. He emphasized that his enquiries were only at a preliminary stage and explained that in any case the details of his work would not be understandable "to people outside the universities".

Landing Sites on the Moon

The National Aeronautics and Space Administration (NASA) has completed its selection of landing sites on the Moon for the Apollo astronauts. Five areas, each three by five miles in size, have been chosen by the Apollo Site Selection Board, it was announced last week. Site selection has been achieved through the photographic survey carried out by the Lunar Orbiter series of spacecraft reinforced by the spot checks of the Surveyor landers. At the beginning of the survey programme thirty tentative sites ranged along the Moon's earthward face were under consideration. The observations of the survey spacecraft reduced these to eight "possibles". From these, the board has selected the five target sites and the Apollo men will leave for their mission not knowing which of three they will use.

All five sites lie within a quite narrow band along the lunar equator—within 45° east and west of the visible Moon's centre and 5° of the equator, the so-called Apollo Zone of Interest, where optimum conditions for communications and surveillance from Earth of the astronauts' progress exist. Two are in the Sea of Tranquillity, one in the Central Bay, and two in the Ocean of Storms (Mare Tranquillitatis, Sinus Medii, Oceanus Procellarum). Apart from this, each site has had to satisfy seven other criteria necessary for the safety of astronauts. These are relative freedom from craters and boulders; a smooth approach path without large hills, cliffs or deep craters likely to cause inaccurate altitude signals on the landing radar; a slope of less than 2° over the landing site and approach path; central grouping enabling the least expenditure of propellant by the lunar module; optimum visibility for the approaching astronauts in guiding their craft down which requires that the Sun angle should lie between 7° and 20° and come from behind the craft at landing. This last criterion gives a 1-day launch window each month. The intervals between the sites have had to correspond with the time needed to re-cycle the Apollo/Saturn V if there is a check during initial countdown; and, finally, site location must be within reach of the Apollo "mothership" on a free-return trajectory, that is, where the spacecraft can coast round the Moon and return to Earth without employing propulsion. Altogether this will make a 3-day period each month available for launching the first Apollo flight.



The five sites chosen for the Apollo landing are in the Mare Tranquillitatis (1) and (2), the Sinus Medii (3), and the Oceanus Procellarum (4) and (5).

Contraception in France

General de Gaulle's dream of a French population of one hundred million by the end of the century is not likely to be greatly thwarted by a new French law on contraception, passed on December 19, 1967. Although this law, the Neuwirth law, may well serve to make contraception more respectable, it is in many ways unsatisfactory and has been widely criticized.

The history of events leading up to the formulation of the law reflects the absurdity of the situation. After the First World War, possibly because many Frenchmen had been killed, a notorious law was passed which made contraception illegal. Since then the only method of contraception to which the authorities have turned a blind eye has been the condom, which has come to be regarded as a prophylactic against venereal disease rather than as a contraceptive.

Various forms of oral contraceptives have been available in France for several years because of their initial therapeutic role, but not everyone can afford them.

The family planning association called the *Mouvement Français pour le Planning Familial* (MFPF) has, however, been operating "underground" and has until recently got around the law by running the organization on the lines of a club with paying members. The organization now has a membership of 100,000 and has more than three hundred centres.

According to the new law, decisions about the manufacture and importation of contraceptives will be subject to a ruling by the public authority, and the sale of contraceptive products will be subject to an authorization of the Minister of Social Affairs. The sale of such articles will be confined to chemists' shops, while those oral contraceptives placed on the so-called "special list" will only be sold under prescription; and the doctors concerned will be required to consider the patients' medical history. Minors up to 18 years old under parental control requiring contraceptives, and similar young people up to 21 years old requiring oral contraceptives on the special list, will have to have a prescription as well as the written consent of a parent or legal guardian.

Under the new law, family planning associations will not be allowed to supply contraceptives as they have done in the past. Another blow to such organizations is that all "antinatalist propaganda" will be forbidden, which means that people seeking advice from an association will have no means of finding it, either from advertisements, or, presumably, from telephone directories. The government intends to back up the law with fines and imprisonment for offenders.

Because the Social Security will not have to reimburse the costs of progesterones, as it does the cost of other medicines, some doctors contend that it is the poor who will suffer most. There is also a feeling that the law places too much responsibility on the doctors, allowing them to interpret it according to their moral and religious principles. Others feel that it is intolerable that patients should be so closely controlled in so personal a matter. One objector on medical grounds, Professor Jacques Huguier, has suggested that, if the law is not repealed before it comes into action on March 1, wholesale resignation of the medical profession might be called for. An extremist's view, perhaps, but it is evidence of the kind of opposition the law is meeting.

More European Physics

The plan for forming a European Physical Society has been modified somewhat in the light of the discussions of the steering committee which met on January 30 at the Battelle Institute in Geneva. For one thing, it has been decided to postpone the large conference which had first been hoped to inaugurate the society at Florence in September this year. This event will not now take place until 1969. Instead there will be an inaugural ceremony in Geneva in September this year. At the same time it appears that serious discussions have been opened with a number of countries from Eastern Europe—Yugoslavia, Czechoslovakia, Hungary and Rumania. Although the representatives of the physical societies in these countries have been given clearly to understand that the formation of a European Physical Society will not be obliged to allow them to determine their own relationship to it, there does now seem hope that they will eventually be associated with the work at some stage. In the meantime, it appears that there have emerged a number of difficulties about the financial provisions in the constitution although these will most probably be resolved at the next meeting of the steering committee due to be held in the spring this year. With luck this will be held in Prague, but otherwise in Vienna.

It has been agreed that the headquarters of the society will be in Geneva at the physics department of the university, 32 Boulevard d'Yvoy, 1211 Geneva 4. It has also been agreed that the working language of the society will be English. At the meeting on January 30 it was agreed that the European Chemical Society should publish a bulletin, principally to keep European physicists informed about the discussions now going on, but there seems some confusion about the way in which this will be accomplished. In the circumstances, it is not surprising that the steering committee has not yet had a chance to take up the more complicated question of how to organize the learned journals in physics which cater for European scientists. The first inclination seems to be to divide physics as a whole into fields which could be parcelled out to the journals which already publish original articles in physics, and in this spirit existing publishers are likely to be approached in the next two weeks. It would not, however, be surprising if in the end the European Physical Society decided to follow a more informal and more empirical course, if only for the sake of the flexibility it would thereby gain.

New Hope for Planning

The first rumblings of a new assault on national planning were heard last week when Mr Peter Shore, Secretary of State for Economic Affairs, presented a Government paper to a meeting of the National Economic Development Council. It appears that few details were given at the meeting, but it was stated that another paper would be presented after the budget on March 17, with broad outlines of the way the economy should develop. The economic development councils for the different sectors of industry will then be able to discuss the projections and work out what the effects will be on their own sections of industry. There are twenty-one councils, known affec-

Mr Peter Shore.

tionately and sometimes derisively as little Neddies, covering about two-thirds of private industry, with representatives from management and unions. When the results of their deliberations are known the Government will add them to results from its own studies and produce a more detailed planning document in the autumn. It is acknowledged that prices and incomes will be important considerations in any plan of this kind. The whole subject of national planning has been regarded rather sceptically since the National Plan was abandoned in July 1966, but it is thought that the present state of the economy calls for another attempt, provided that it is more flexible than the last.

New Look for Heathrow

The British Airports Authority, which runs commercial airfields in Britain, last week unveiled plans for the development of Heathrow, London's major airport. With the advent of the Boeing 747, which will be arriving on commercial flights to London by the summer of 1970, development of Heathrow to handle the increase in passengers has become urgent. Although the new plan is to cost £10 million, it represents a face-lift for only a small part of the airport.

The new section of the airport will be a T-shaped pier, with an upright 900 ft. long and a crosspiece 1,000 ft. long. The aircraft stands will be positioned along the crosspiece, seven each side, and passengers will be carried along the upright section on a travelling pavement. For those who prefer to make the journey on foot, ample space will be provided on either side of the conveyor. Telescopic air jetties will link the aircraft with waiting rooms. There will also be changes in the terminal buildings; a new passenger terminal building, prosaically called Terminal 1, will be built, and the existing Europa and Britannic buildings will be merged and called Terminal 2. The Oceanic building will be renamed Terminal 3, and a new arrivals terminal will also be built. By the time the 747 service across the Atlantic is in full swing, it will be necessary to separate arriving and departing passengers into two separate terminals. Thus by 1970, Terminal 3 (the Oceanic building to today's travellers) will be devoted to departing passengers, while the new arrival terminal looks after those coming into Britain. By this time, the authority says, the number of departing and arriving passengers on long haul services is likely to reach 2,700 an hour in each direction.

The main difficulty will arise when several 747s arrive at once, or within a few minutes of each other. Each will be carrying up to 490 passengers. Unless the capacity of the airport bus services is to be severely overstretched, a rail link between central London and the airport must be built.

Marine Science on Record

The first comprehensive directory of marine science in the United Kingdom has just been published (The Royal Society, £2). It has been prepared on behalf of the Royal Society's British National Committee for Oceanographic Research with the co-operation of the Natural Environment Research Council, and it should prove to be of great help to everyone working in this rapidly growing subject.

For the most part, the book consists of the names of scientists listed by establishment and arranged in four sections. Sections 1 and 2 include the marine science laboratories, both government and university, with details of the scientists employed in them and of the laboratories themselves. Information given under each institution includes facilities for visiting research workers, training courses, and a selected bibliography of research done there. There are as many as 57 university departments in the United Kingdom which have continuing interests in marine science.

Sections 3 and 4 cover university departments and government-supported laboratories in which marine science has only a minor role—but where there are staff working on some aspect of the subject. A further 42 academic and 13 government-supported departments are listed in these sections.

There is often confusion over the organization of research in interdisciplinary fields such as oceanography. It is helpful, therefore, that the directory includes a summary of the overall organization of oceanographic and fisheries research in the United Kingdom and the part played by the national programme in international activities.

The research ships available to British scientists and the libraries offering facilities to them are listed in two appendices.

Scientist on the Move

Mr B. T. Price, at present director of the Defence Operational Analysis Establishment of the Ministry of Defence at Byfleet in Surrey, is to become Chief Scientific Adviser at the Ministry of Transport on April 1. Mr Price has worked in turn for the Atomic Energy Authority and for the scientific group at the headquarters of the Ministry of Defence. He has been largely responsible for setting up the Byfleet establishment in the past few years. He is a physicist by trade.

Mr B. T. Price.

This new appointment comes at a time when it seems that the Ministry of Transport would like to see more of what the British Government spends on research and development devoted to the art of moving about.

Make or Break

During the past year or so, the Electrical Research Association has done its share of breast-beating in public. The decision by the Central Electricity Generating Board to withdraw its habitually automatic support was a shock from which ERA has taken some time to recover. Now, although workers at ERA are hoping the worst is over, there is still a certain amount of belt tightening to be done.

Under a scheme to be introduced next year, each item of research carried out at ERA will have to be individually sponsored. This is a system which will doubtless help to raise sums of money for jobs for individual companies, but is unlikely immediately to help ERA to continue work which is of general interest to the entire industry. The more general a problem, the less likely a company is to feel responsible for it. This difficulty is going to hit hardest at the work which is of greatest public concern—the safety of electrical devices, as ensured by the correct design of fuses. ERA performs a continuing service for fuse manufacturers, not least by ensuring that British companies keep up with changes in international standards, now recognized as a major barrier to successful exporting. The same section at ERA which is responsible for fuses also works on contractors, and has recently been able to show that British manufacturers were producing a product which was uneconomic because the standards were too stringent.

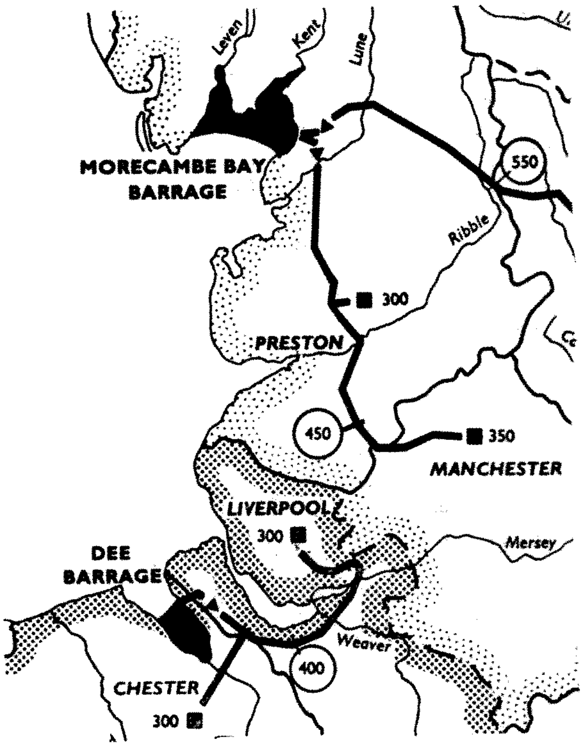
ERA has now published a summary of the literature in fuses between 1950 and 1965 (*Advances in Electrical Fuses*, by H. W. and C. Turner, ERA, 54*s*.). This, it is hoped, will stimulate British manufacturers to increase support for this important part of ERA's activities.

Biology of Barrages

Work is now in progress on the biological effects of the proposed barrage across Morecambe Bay. The full feasibility study announced early last year by the Water Resources Board is to cost about £500,000 and is expected to last another 2 to 3 years. By then the board, with reports in hand representing all sides of the project, should be able to advise the government whether the barrage is a workable proposition.

An important aspect of any barrage scheme is the effect the works will have on the ecology and fisheries of the area. The biologists must try to predict what will happen to the different animals and plants, bearing in mind the usefulness of the impounded water, the amenities of the area, the fisheries, and so on. One of the biological techniques used for a study of this kind is to find the nearest parallel project. Unfortunately, there are few examples of similar estuarine barrages to which the biologists can turn. The conversion of the Zuider Zee into the Ijsselmeer in Holland, however, may have some relevance, and Loch Neagh in Northern Ireland shows some similarities although it is an inland lake. Two biologists who are to work on the Morecambe Bay barrage have now been appointed by the Natural Environment Research Council. The co-ordinator of the ecological work is Mr John Corlett, who has been seconded to the Windermere Laboratory of the Freshwater Biological Association from the Fisheries Laboratory, Lowestoft. His assistant is based at the University of Lancaster.

The Nature Conservancy is also assisting in the study in several ways. A small department to study the overall conservation of large scale proposals has been recently established at the Nature Conservancy station at Shrewsbury under the direction of Dr R. J. Elliott. The proposed barrage is one of the projects with which it will be concerned, and it will keep in touch with the regional conservation staff and other interested parties. Meanwhile, a small ornithological project on waders is in operation in co-operation with the Royal Society for the Protection of Birds and the Ministry of Agriculture. It is necessary to know how many of these birds there are in the estuary and what they feed on. The Nature Conservancy has therefore issued a licence for a few waders to be shot so that their stomach contents can be analysed. Work on the coastal ecology of the estuary will be done from the Nature Conservancy's station at Merlewood, Grange-over-Sands.



The Morecambe Bay and Dee Barrages, with a suggested distribution system.

It will be some time before all the data collected can be assembled into a meaningful whole, but there is no doubt that there will be some important biological factors for the engineers to bear in mind when or if they come to actually designing the barrage.

Astronomers at Brighton

For the first time since 1925, the General Assembly of the International Astronomical Union is to be held in Britain. The next Assembly—the fourteenth—will meet in 1970 at the University of Sussex, near Brighton, from August 18 to 27. Judging by the numbers at previous Assemblies—the thirteenth was held in Prague last year—about 2,000 astronomers are expected to attend.

The chairman of the Preparatory Organizing Committee, Dr D. H. Sadler of the Royal Greenwich Observatory, is hoping that observatories and astronomy institutes in Britain will arrange visits for the astronomers during the Assembly. Many institutes in the United Kingdom may also wish to take advantage of the presence of visiting astronomers to organize meetings before or after the assembly. Dr Sadler, like conference organizers the world over, is hoping that his plans will not be marred by duplication.

Better Machines

The Ministry of Technology has devised a way of supporting the development of numerically controlled machine tools. It involves collaboration between a government establishment (RAE, Farnborough), a research association (the Production Engineering Research Association) and industry (Airmech AEI). These three are establishing a numerical control advisory and demonstration service, which will set out to tell industry what is possible and how it can be done.

Some industries in Britain are already aware of the importance of numerical control. Having a machine tool controlled by tape not only releases a man for other activities, but also improves precision and makes much better use of machines. A machine controlled by tape can be used for 60 per cent of the time, against 20 per cent for one controlled by hand. Numerically controlled machines need fewer jigs, and may also reduce lead times—the gap between design and production. Most of Britain's 1,400 numerically controlled machines are in the aviation industry, and other industries have tended to fight shy. The production engineers estimate that eventually 10 per cent of the machine tools in Britain will have to be controlled in this way, a total of perhaps 125,000 machines, so there is plenty of room for expansion.

One of the difficulties has been to get an unbiased assessment of just how valuable numerical control is. The new service should be able to provide this, although by identifying itself with the scheme, PERA may have sacrificed its objectivity as far as industrialists are concerned. PERA says that it has been able to show a 20–25 per cent return on the investment for most companies contemplating the investment, and that each survey costs in the region of £1,200. The new service provides for the ministry to pay between 50 and 90 per cent of the cost of such surveys, as well as paying for the training courses. At the same time, the ministry is supporting the establishment of a numerical control centre at High Wycombe by Airmech AEI Ltd. This will contain a workshop with a range of machine tools equipped with Airmech AEI control systems, and will undertake contract work for industry on a commercial basis, in order to prove how cheap and effective numerical control is. The total cost of the scheme will be £685,000, spread over three years, half of which will cover the cost of buying numerically controlled machine tools.

Infrared Geology

Fig. 1 shows an example of the infrared images (taken in the 8 to 13 micron band) which are providing information on the San Andreas fault system in the Carrizo Plain area of California. An aerial photograph is shown for comparison (Fig. 2). R. E. Wallace and R. M. Moxham have been taking pictures of 200 miles of the fault system as part of a joint project of the US Geological Survey and the National Aeronautics and Space Administration. The infrared image was taken before dawn, when the properties of the rocks and soil are best indicated. The strong white line on the left is a hard packed road. It is thought that the particularly dark patches in the fault line itself are due to accumulations of tumbleweed which have exceptionally low thermal inertia and thus produce relatively low radiation temperatures before dawn.

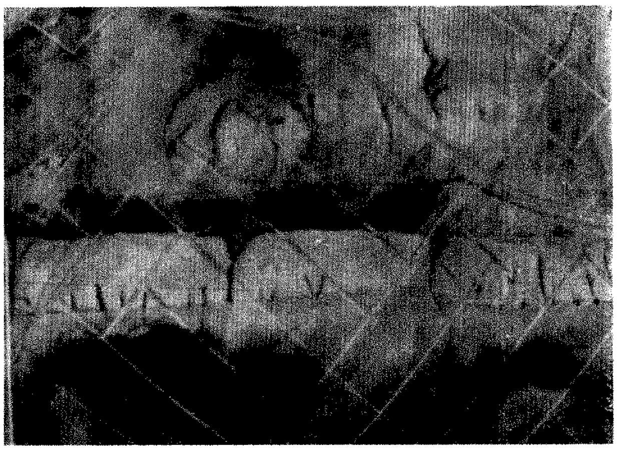


Fig. 1. Infrared image of part of San Andreas fault.

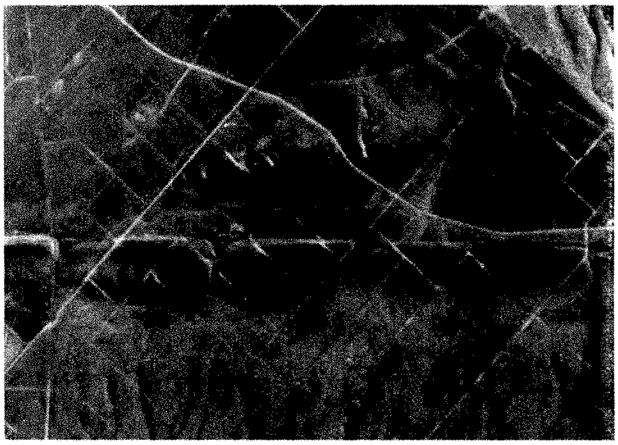


Fig. 2. Aerial photograph of the same area.

Building High

Much modern building, particularly of high rise blocks, would be impossible without the detailed knowledge of soil technology which has been developed, principally in this century. Buildings, of course, have always depended on the security of their foundations, but until comparatively recently empiricism ruled supreme. Mr R. Glossop, giving the eighth Rankine lecture to the Institution of Civil Engineers on February 14, gave some intriguing examples of this approach. When Tower Bridge was built over the Thames in the late nineteenth century, there seems to have been no serious examination of the geological conditions below the foundations of the bridge. There was little excuse for this, as the techniques were known—for Saltash Bridge, I. K. Brunel sank 175 boreholes for the central pier alone. For Tower Bridge it was decided, evidently on scanty scientific evidence, that the clay would bear a load of 4 tons/sq. ft; but contemporary engineers, who criticized the design, said that higher loadings would have been perfectly acceptable, and would have reduced the obstruction to the waterway. The consulting engineer, Mr John Wolfe Barry, fortunately stuck to his guns; Mr Glossop said that recent boreholes near the bridge had shown that greater pressures would have been unwise. Indeed, the whole design of Tower Bridge seems to have been blessed with good fortune; the depth of the clay is only some 10 feet and, had this been penetrated, there might have been serious difficulties.

This approach is happily now of historical interest only. Settlement is frequently a source of damage to old buildings, and Mr Glossop quoted the example of Westminster Cathedral, where the massive tower on the north face of the cathedral has settled more than the rest of the building. Much of the progress in soil technology is due to the classical work of Terzaghi, which has been developed since 1925 into a most important branch of engineering science. The turning point, at least as far as the loading capacity of soils is concerned, seems to have come in 1942, when the foundations of a single storey building in the Firth of Forth collapsed. The design failed because it had not taken into account all the geological factors; but it provided such a mass of data for the Building Research Station to analyse that it might have been designed as a laboratory experiment. The agreement between theory and experience was so good, Mr Glossop said, that it finally brought conviction to many unbelievers.

The most marked effect of the development of soil mechanics had come in the design and construction of earth dams. In 1866, Mr Glossop said, Jacob had confidently stated that it was "idle to pursue the investigation (of earth dams) by a mathematical mode of reasoning". He suggested a three to one slope on the upstream side, and a two to one slope on the downstream side, recommendations which were accepted without question until about 1940. In 1898, two engineers attempted to justify dams on which the slope flattened progressively from top to bottom on the grounds that Mount Fujiyama was that shape. Since then, Mr Glossop hastened to point out, the construction of earth dams had become a much more scientific activity, a claim which he proved by reference to the Derwent Dam, built on the borders of Northumberland and County Durham, and completed in 1967.

All Change at the Museums

SIR TERENCE MORRISON-SCOTT, Director of the British Museum (Natural History) since 1960, is to retire at the end of the year. The most important event in Sir Terence's term of office must have been the severing of the Natural History Museum from the British Museum on the passing of the British Museum Act, 1963. This meant that the Natural History Museum for the first time in its life was able to establish its own governing body of twelve trustees.

At the latest meeting of the trustees, two new appointments were also confirmed. Dr J. G. Sheals, Head of the Arachnida and Myriapoda section, is to be a deputy keeper in the Department of Zoology. Dr Sheals is particularly interested in the application of data processing equipment to the taxonomic catalogues in the museum. Another new deputy keeper—this time in the Department of Palaeontology—is to be Dr C. G. Adams, who works on the larger species of Tertiary Foraminifera. Dr Adams will transfer from the Zoology Department to the Palaeontology Department on taking up his appointment. Both appointments are effective from April 1.

A further announcement is that Lord Radcliffe has resigned as chairman of the Board of Trustees of the British Museum because of ill-health. His place has been taken over by Lord Eccles, who will now lead in the British Museum's efforts to persuade the Government to agree to the building of the new national library next door to the present museum. Lord Eccles appears to be as vehement as Lord Radcliffe in his condemnation of the Government's action over the museum. It is understood that Lord Radcliffe will remain a trustee, at least for the time being.

Science of Science

THE Science of Science Foundation is now in the fourth year of its existence. To mark the occasion, Dr O. M. Solandt, President of the Science Council of Canada, is to give the foundation's fourth annual lecture. The foundation has continued to mount a wide ranging programme of lectures and seminars, and is now publishing a bimonthly newsletter. It has also opened a library at the University of Sussex. These developments apart, there is as yet little sign of the activities proposed in March last year when the foundation launched an appeal for £282,000 (*Nature*, **214**, 1 and 3). It must be assumed that the appeal has not so far met with conspicuous success.

The foundation's newsletter (obtainable at the Ciba Foundation, 41 Portland Place, London, W1; annual subscription 2 guineas ($6)) is concerned with "topics of interest in the field of the science of science". With twelve pages an issue, the newsletter contains reports of the foundation's activities and other meetings, and reviews of relevant books and pamphlets. Science policy and other news from Europe, the USA and USSR are also covered. The newsletter began as a document for internal information, but outside demand for its services has already created a small but rising circulation.

Research on a Shoestring

THE Medical Research Council of Ireland has just published its annual report for 1966 (5*s*.). The impoverishment of medical research in Ireland is made blatantly obvious—the council, for example, has to do without a research director or a central research institute. The reason for this appears to be to ensure that as much of the council's grant as possible goes directly to the support of research. Most of the money is used to encourage research in medical school departments, but there is also one special university unit, the Unit for Cell Metabolism in the Department of Biochemistry, University College, Dublin, and one independent unit for studying chemotherapy specially set up by the council in Trinity College. Summaries of the research carried out by these units and research in other departments supported by the council are given in the report. This research is co-ordinated by the special committees set up recently by the council to advise it on the development of particular research fields. There are five committees in all, covering cancer, cardiovascular diseases, gastroenterology and haematology, metabolism and endocrinology, and pregnancy and congenital deformities.

In November 1965, the government increased the council's grant for general research by £10,000 a year, bringing it to £72,000 a year. The increase was, however, more than absorbed by salary increases, so that the council reports that the range of its activities is more or less at a standstill. Other grants from unspecified sources and a grant from the government for specific projects brought the total amount of money administered by the council in 1966 to £106,650.

Parliament in Britain

Foot and Mouth

LORD BESWICK said that the setting up of a committee of inquiry into control policy for foot and mouth disease was being considered as a matter of urgency. The appointment of the chairman might be announced in the fairly near future. Although the precise terms of reference had not yet been decided, they were likely to be similar to those given to the Gowers Committee: "To review the policy arrangements for dealing with foot and mouth in Great Britain and to advise whether any changes should be made in the light of present scientific knowledge and the technical and administrative experience gained in recent years in this and other countries". The experience of those employed in the present outbreak would best be made available as witnesses rather than as members of the committee. (Debate, House of Lords, February 5.)

Dungeness B

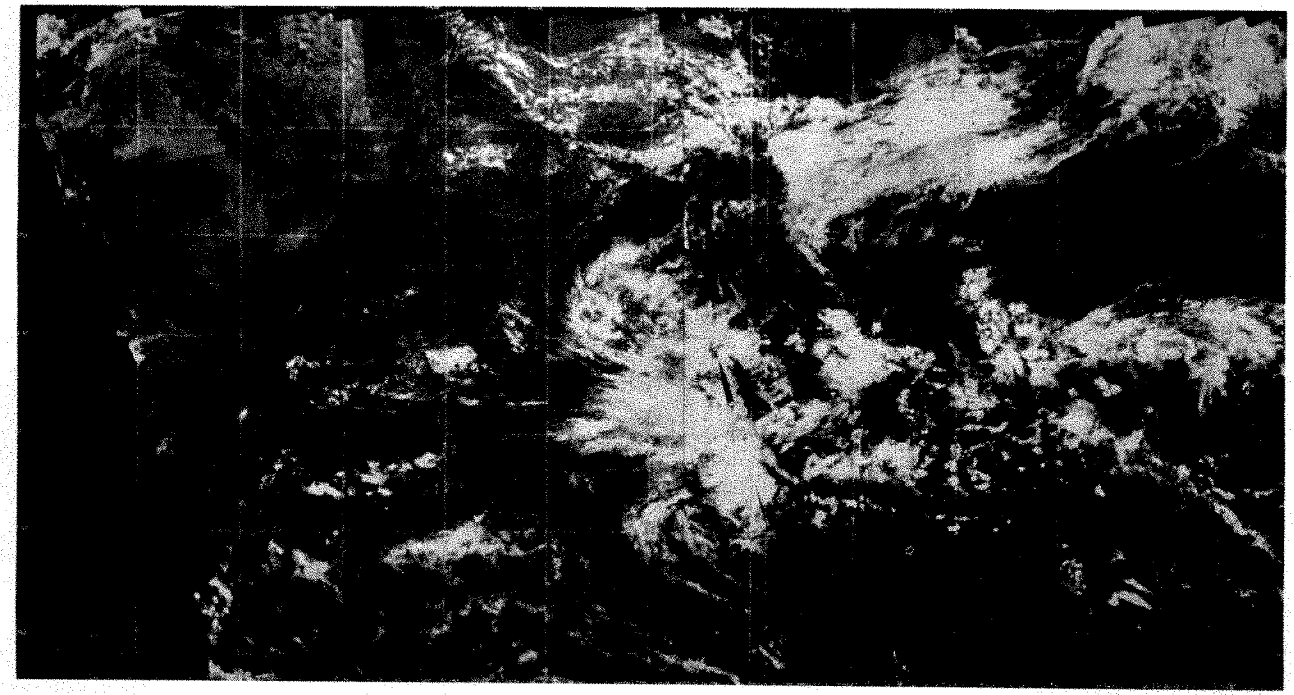
THE parliamentary secretary to the Ministry of Power, Mr R. Freeson, stated that the estimated base-load generating cost (including capital charges and operating costs) of the Dungeness B nuclear power station had increased by about 25 per cent since July 1965. The Central Electricity Generating Board advised that 8 per cent of this was due to increase in price levels, including the effects of devaluation, changes in ground rules and use of the same rate for interest during construction, 3 per cent to the inclusion of royalty payable to the Atomic Energy Authority, and 7 per cent to other causes. The station was expected to begin operating in 1970. (Written answer, February 6.)

Satellite Motors

IN answers in the House of Commons on February 5, the Minister of State, Ministry of Technology, Mr J. Stonehouse, stated that the first experimental model of an electrical thruster had been made at the Royal Aircraft Establishment and preliminary testing would start shortly. Initial research was being carried out on very low thrust electrical units suitable for maintaining the orientation of a satellite in orbit. A development programme on this initial research work came within a wider programme costing £3 million a year. (Written answer, February 5.)

Insects

A LIST of current research projects into the biological control of insect pests to which the Department of Education and Science was giving assistance was given by Mr P. Gordon Walker, Secretary of State for Education and Science. The institutions receiving assistance are: Glasshouse Crops Research Institute; Pest Infestation Laboratory; National Vegetable Research Station; Rothamsted Experimental Station; Virus Research Unit; Agricultural Research Council, Monkswood Experimental Station; Department of Zoology, Cambridge University; Department of Zoology and Applied Entomology, Imperial College of Science and Technology; Department of Agriculture, Leeds University; Haughley Research Farm; and Insect Pathology Unit. (Written answer, February 6.)



A mosaic of cloud pictures on Mercator's projection, produced by computer from ESSA 3 satellite photographs.

NEWS AND VIEWS

How Useful are Weather Satellites?

THE recent publication of a technical note on "The Use of Satellite Pictures in Weather Analysis and Forecasting" is an opportune moment to ask how useful weather satellites are proving to be. The purpose of the note, published by the World Meteorological Organization, is to help with the interpretation of pictures of cloud formations.

TIROS 1, launched in 1960, was the first satellite designed specifically for meteorological observations. It was followed by a series of TIROS and NIMBUS satellites which tested different camera systems and developed the techniques needed for an operational weather watching system. Once the development work was completed, the running of the project in the United States was transferred from the National Aeronautics and Space Administration to the Environmental Science Services Administration. The first satellite in the operational system was ESSA 1, launched early in 1966.

Weather satellites are used extensively in Britain as an aid in the plotting of weather charts. At present between 20 and 25 pictures a day are received by the Meteorological Office at Bracknell from NIMBUS 2—one of the research and development satellites still functioning—and from ESSA 6. The ESSA satellite has an automatic picture transmission facility, which means that any station in the line of sight can in principle receive pictures of conditions in the area. The forecasters at Bracknell thus have available pictures of cloud formations over large areas of the North Atlantic and Europe, sometimes only 20 minutes after they were taken.

The satellite pictures are used to correct the surface charts—the familiar maps showing the positions of fronts and isobars delineating regions of high and low pressure. The procedure is to compare the cloud distribution implied by the surface chart with that revealed by the satellite pictures. Where the two differ, a correction is made to the surface chart. Pictures of the cloud cover can also be taken during the night with infrared sensors. Such an instrument is fitted to ESSA 6 but has not worked for some time.

It is clear that weather satellites do not eliminate the need for ground observing stations. This is particularly so because there seems to be no immediate prospect of developing satellite-borne instruments for measuring pressures and wind speeds in the lower atmosphere—two parameters of great importance to forecasters. As far as meteorologists are concerned, the full benefits of earth satellites have yet to be realized. Before any really revolutionary improvements in weather forecasting are made, there will have to be many more weather stations, particularly in remote polar and oceanic areas and in the underdeveloped nations. These regions still account for enormous areas of uncertainty on the surface charts.

In addition to stations on land and fixed to buoys at sea, there are plans for the introduction of free-floating balloons to carry instruments over remote regions. Experiments with balloons of long lifetime have been made by the French space agency and by the National Centre for Atmospheric Research, Colorado. The Americans have released a series of 'Mylar' plastic balloons from New Zealand and from McMurdo Sound in the Antarctic. The balloons float at a height where the atmospheric pressure is equal to the pressure inside the balloon. Most of the American balloons were inflated so as to drift at levels between 18,000 feet and 40,000 feet. Some of them have lasted for more than two hundred days, circumnavigating the earth several times until lost because of icing. To test the satellite data collection aspects of this project, the satellite NIMBUS B is to be launched in April. This will be able to locate and interrogate free-floating balloons and relay the information to ground stations. In the first instance tests will be made with a balloon drifting over the United States. It therefore seems probable that in the 1970s, communication satellites will be the chief contribution of space technology to meteorology.

Cerebral Impasse

from our Neurophysiology Correspondent

UNDER the title "The Logical Analysis of Cerebral Functions" a meeting was held at the Royal Society on February 8 under the organization of Professors J. Z. Young and H. C. Longuet-Higgins.

Professor Weiskrantz began his paper with a plea for illogicality in research. This at first seemed at variance with the spirit of the meeting, but he proceeded to give a logical analysis of the effects of temporal lobe lesions on the classification of visual information by monkeys and man. His thesis that engineering analogies often die while the real nervous system survives carries some weight, although a distinction should be made between a logical function and the device which performs it. It is fashionable to compare retrieval by the cerebral cortex with holography and information storage. This is a useful analogy because it presents in tangible terms a system capable of performing some of the more mysterious operations of the brain it is not, of course, suggested that the brain is a hologram but merely that transformation of input into some kind of storable interference pattern is a possible mechanism of memory.

Professor Longuet-Higgins presented his theory of the temporal storage of patterns by "holophony" (*Nature*, **217**, 104). He showed that a bank of resonators could store information about the component frequencies of an auditory input and then reproduce the whole input when stimulated again by only a fragment of it, provided that the coupling constant of each resonator were altered proportionally to the power of the input signal in its frequency band. After his presentation the inventor of holography, who was sitting in the front row, declared that he had been so impressed with Professor Longuet-Higgins's idea that he had been forced to devise alternative models (Gabor, *Nature*, **217**, 585) One of these has the further property that its input and output signals can be expressed in digital terms, the model therefore corresponds to the celebrated "McCulloch–Pitts" neurones Both models are interesting attempts to supply mechanisms for the recall of extended temporal patterns such as the muscular movements made in playing a piece of music

What constitutes information storage in the brain ? Most of those interested in cerebral function favour a change in the conductance of cortical synapses, but this has proved extraordinarily difficult to demonstrate Professor Burns described the results obtained by himself and his colleagues Bliss and Uttley from isolated slabs of cat's cerebral cortex Two stimulating electrodes, one in the white matter and one on the cortical surface, were used to provide two "independent" pathways of excitation to a cell whose activity was recorded by an extracellular microelectrode The effects of stimulation by one path (x) were tested by stimulation via the other (y), measured as the probability that the cell would fire in response to each stimulus pulse A somewhat cabbalistic equation

$$(\mathrm{k}\Delta c = -0\ 50\frac{\Delta x}{x} + 0\ 12\frac{\Delta y}{y} - 0\ 38\ \frac{xy}{y}$$

where c is conductance of the cell to information and the other parameters are as defined) was said to describe the changes observed in 19 of the 26 neurones observed The equation became somewhat less mystical when we were told that the various coefficients in fact had at least 50 per cent uncertainties in either direction Notwithstanding, the main conclusion—that cortical pathways in the cat are not usually facilitated by frequent usage but are in fact de-facilitated—is indeed of great importance, if confirmed, it will be the first direct measurement of changes, apparently at the synaptic level, related to usage in the cerebral cortex One important gap in this paper was the apparent failure to consider factors such as accommodation and fatigue which might have affected the results in a systematic way

Dr Cragg, in collaboration with Dr Rose, has been investigating by light deprivation the anatomy of the rat's visual cortex in the hope of finding anatomical correlates of disuse His results, which have been presented in detail already (*Nature*, **215**, 251), were impressively ordered and carefully analysed, they showed that light deprivation led to an increase in size but a decrease in number of synaptic terminals A first period of exposure to light had the opposite effect

No meeting of this sort would be complete without a computer simulation Dr Thorne, from the Department of Machine Intelligence at Edinburgh, acted as an entertaining representative of the computer in question, describing a programme (10,000 characters in ATLAS autocode) which simulates the way in which a human undertakes the syntactical analysis of English sentences Its novel features are the lack of a comprehensive dictionary, and the relative speed with which sentences are analysed at only one reading Psycholinguistics has given a great impetus to the partitioning of brain mechanisms into those which are inborn and those which are learnt One of its most interesting conclusions is that there are syntactic universals which must be genetically determined—the "deep structure" of language—and which make it possible for a human to select from the infinite possibilities of his language

The meeting in sum was rather disappointing and little new information was produced But all present must have been impressed with the fascination which the problems of cerebral function exert on many scientists from a wide range of disciplines There is a widespread feeling that brain mechanisms will be for the next few decades what molecular biology has been for the last few

Lipid Peroxides

In suitable conditions, unsaturated fatty acids will react with oxygen to form lipid peroxides These compounds have been found in animals in states of vitamin E deficiency, and after exposure of animals to carbon tetrachloride, ultraviolet light and ionizing radiation They are thought to be responsible for at least some of the toxic effects produced by these agents

Lipid peroxides can act as oxidizing agents, probably through a free radical intermediate They readily oxidize small molecules containing the thiol (SH) group, and it was thought that the effects of lipid peroxides might partly be explained by the oxidation of protein SH groups Little and O'Brien have recently examined the oxidation of protein SH groups by linoleic acid hydroperoxide, and have shown that the presence of haem groups affects the reaction (*Biochem J*, **106**, 419, 1968) Haem groups catalysed the reaction so that haem proteins were oxidized more quickly than small thiols, which reacted more rapidly than non-haem proteins The rate of reaction was always greater with the fatty acid peroxide than with hydrogen peroxide, but fewer thiol groups were oxidized per mole of fatty acid peroxide in haem proteins than in small thiols In non-haem proteins there was relatively little thiol oxidation of the native protein by the lipid peroxide, but the denatured protein was oxidized by the lipid peroxide to the same extent as by hydrogen peroxide The addition of haematin to solutions of glutathione (GSH) decreased the amount of SH oxidation It therefore seems that the group most susceptible to lipid peroxide is the haem group, and the presence of haem groups can protect protein thiols from oxidation, but increase the rate of oxidation

Thiols can also affect the formation of lipid peroxides Oxidized glutathione (GSSG) and mixtures of GSSG and GSH will stimulate lipid peroxidation, but Christopherson (*Biochem J*, **106**, 515, 1968) has now shown that GSH actually inhibits ascorbate-stimulated peroxidation in fresh preparations but not in boiled ones Cysteamine and diethyldithiocarbamate inhibited both fresh and boiled preparations, but thioglycollate and 2-mercaptoethanol had no inhibitory effect Cysteine and homocysteine stimulated peroxidation even in the absence of ascorbate, probably because they always contained some oxidized thiol The GSH inhibition does not seem to involve the protection of protein thiols, as the same effects were observed when the preparations had been pre-treated with N-ethyl maleimide, which blocks SH groups

Slater (*Biochem J*, **106**, 155, 1968) has found that the peroxidation produced by carbon tetrachloride can be inhibited by concentrations of promethazine (a tranquillizing drug) which are likely to be found during treatment The same drug strongly inhibited a peroxidation system requiring reduced nicotinamide adenine dinucleotide phosphate, adenine diphosphate and ferrous ions both *in vitro* and *in vivo* Other phenothiazine drugs inhibited this system *in vitro*, but not all compounds which protect against carbon tetrachloride necrosis were inhibitory The author suggests that the inhibition of this system involving free radicals may mean that the phenothiazines can act as general free radical scavengers

Dismantling the Ribosome

from our Molecular Biology Correspondent

Remarkably little is known about the manner in which the ribosome carries out its catalytic function Recently, however, attempts at partial disruption of the structure and reassembly have met with success and it has been found possible to identify certain proteins or groups of proteins with particular aspects of the activity This represents a chink of light in what was until then almost total darkness The same approach has now been taken several steps further by Nomura and his colleagues (Traub *et al*, *Proc US Nat Acad Sci*, **58**, 2430, 1967)

It had already been established that 50*S* and 30*S* particles from *E coli* can be stripped of part of their protein at high concentrations of caesium chloride to yield "cores" 40*S* and 23*S* These cores were inactive, but could be restored to full viability by the addition of the abstracted proteins It was further found that these proteins could be fractionated and the different fractions returned to the cores with varying results Thus the proteins taken from the 30*S* particles in caesium chloride were separated into two groups—one acid (*A*) and one basic (*B*) When proteins of the first group were added to the cores, these remained inactive, but proteins of the second group were sufficient in themselves to restore much of the activity Some or all of these proteins are therefore indispensable

The complete fractionation of the *B* proteins has now been achieved by the use of a new system of column chromatography on phosphocellulose to give five apparently homogeneous components The five proteins have been restored to the 23*S* cores, individually and in combination, and all facets of the activity have been assayed—poly-U-stimulated phenylalanine and viral RNA-stimulated valine incorporation, binding of phenylalanyl-*t*RNA, binding formylmethionyl-*t*RNA in the presence of viral (*f*2) RNA and binding of poly-U When all five β proteins were added to the cores, all activities were restored in full, but omission of either B_3 or B_5 resulted in essentially complete inactivity, except in regard to poly-U binding (which, as the authors point out, could be at least partly non-specific) The lack of B_1 leads to low levels of activity, that of B_2 to moderately high levels, especially in terms of *t*RNA binding Here, however, the authors add the caveat that the cores contain proteins of the same electrophoretic mobility as B_1 and B_2, and if these should in fact be residual B_1 and B_2, these last results are not quantitatively significant The absence of B_4 led to unimpaired, indeed enhanced, activity in all respects except the binding of formylmethionyl-*t*RNA which was reduced by some 60 per cent

Thus at least two essential factors for protein synthesis, B_3 and B_5, have been identified, as well as another, B_4, which is not involved in incorporation, but may well be specifically concerned in chain initiation Studies on the interaction of these proteins with the various elements of protein synthesis hold the promise of momentous results

Further interesting experiments concerning chain initiation come from Ghosh and Khorana (*ibid*, 2455) These are among the dividends of the synthesis of polynucleotides of repeating sequences in the same laboratory, and they provide a pleasing proof of the mechanism previously outlined by Nomura, whereby the 30*S* particle is self-sufficient for chain initiation, whereas the 50*S* component is required for propagation In the presence of the polymers poly-*r*AUG or poly-*r*UG, which contain repeating codons for methionine, the chain initiator formylmethionyl-*t*RNA will bind to the 30*S* sub-unit, while methionyl-*t*RNA and other *t*RNAs will not do so This then defines the chain initiation step If now 50*S* particles are added, the methionyl-*t*RNA is taken up, and a complex containing the two different *t*RNAs can be isolated On addition of the cell supernatant, the dipeptide formyl-methionylmethione is produced There are thus two *t*RNA binding sites on the 70*S* ribosome, the second of which is generated when the two sub-units associate, when this occurs the process of polypeptide synthesis is set in train This adds particular attraction to the notion that the B_4 protein may be identified as part of the initiation site on the 30*S* particle

Viruses and Cancer

Professor M G P Stoker and his colleagues at the University of Glasgow have been concerned with the early events in the transformation of cells into tumour cells, using in particular the polyoma and *SV*40 viruses These viruses have not been found to cause tumours under natural conditions, but are useful tools in laboratory systems using tissue cultures On February 6, Professor Stoker gave a fascinating lecture on the subject to a large and attentive audience at the Royal Veterinary College, Camden Town

When cells are infected with transforming virus two things may happen either the virus multiplies within the cells which then die, or else the normal cells are transformed into tumour cells—Professor Stoker described the several differences that exist between the normal and transformed cells The most exciting part of his lecture, however, concerned the work that several laboratories have undertaken to discover exactly what the transforming virus does inside the cell The great problem is that once transformation has occurred, the causative virus usually disappears without a trace Two antigens—the transplantation and T antigens—have, however, been recognized in these cells, and viral *m*RNA has also been demonstrated It is also expected that techniques will be improved sufficiently to allow viral DNA to be detected—technically a difficult task

But if viral genetic material is in the cell, where precisely is it? As Professor Stoker explained, the fact that transformed cells perpetuate themselves suggests that the division of the virus is in some way associated with the division of the cell Recent work has suggested that loss of the cell chromosome results in loss of transformation, indicating that the viral nucleic acid is possibly integrated with the host cell chromosome.

It seems that the events occurring in a transformed cell are similar to the early stages of vegetative growth of viruses, but that the late functions such as viral replication and synthesis of viral protein do not take place. Why should this be so, and why are the transformed cells not killed? Two alternative explanations have been offered: either the virus is defective when it enters the cell, or else it may be that the transformed cell is unable to synthesize the late products of the virus. By fusing a cell transformed with *SV40* with a susceptible cell, research workers at the Salk Institute at La Jolla and at the Wistar Institute in Philadelphia have shown that virus synthesis begins and the cycle is completed. This suggests that there is no defect, at least not in the *SV40* virus.

Certain requirements for transformation can now be defined. These are a functional virus particle, the absence of cell death, integration of the viral DNA so that it is perpetuated, and absence of the late gene functions of the virus. But do the physiological changes in the transformed cell depend on viral DNA being permanently integrated into the host chromosome, thereby creating the switch that alters regulation, or is the change caused by a viral product which functions irrespective of the position in the cell of the virus DNA? To determine which of these is correct, Professor Stoker has made use of a phenomenon called anchorage dependence in *BHK*21 cells infected with polyoma virus. When these cells were put into agar with transforming virus, only 1–5 per cent were transformed. When, however, a viscous solution of 'Methacel' was used, a third to a half of the cells appeared to have been transformed—that is, these cells could now form colonies in the 'Methacel'. When removed and cultured further, it was found that the majority reverted to normal.

From this Professor Stoker concludes that transformation can either be temporary (in which case the cells become normal again when the virus is lost) or else permanent transformation can occur, in which the viral DNA is integrated. He suggests that although the physiology of the transformed cell does not depend on integration, transformation can only be perpetuated if the viral DNA is integrated. When polyoma virus enters any cell in which it functions, the cell is probably transformed into a tumour cell. Subsequently, the cell may die accompanied by growth of the virus, or else it may continue for a short time as a transformed cell until the virus is lost and is not therefore tagged on to cell replication. Finally, the virus DNA may be integrated, and thereby the transformed characteristics are perpetuated indefinitely, resulting in cancer.

5′ Terminus of R17 RNA

from our Cell Biology Correspondent

MESSENGER RNA is translated in the 5′ to 3′ direction, and the nucleotide sequence of the 5′ terminal region of *m*RNA molecules probably specifies both the binding site for ribosomes and the initiation of in-phase translation. The simplest expectation would be that an initiator codon, either AUG or GUG, is 5′ terminal and fulfils both these functions. Unfortunately, determination of the 5′ sequence of *m*RNA, or of any other RNA molecule, is in practice extremely difficult. First, the only *m*RNA molecules which can readily be isolated in sufficient purity and quantity to warrant such analysis are RNA phage genomes. Second, and more generally important, the ideal method for determining 5′ termini is by specifically labelling the 5′ terminal nucleotide of non-radioactive RNA and then isolating the labelled nucleotide. But, as Takanami (1966, 1967) has discovered, this is difficult without at the same time causing the degradation of the RNA molecules—and every breakage generates a spurious 5′ terminus.

Takanami found that *f*2 phage RNA had to be treated with alkaline phosphatase before it could be labelled with ^{32}P phosphate by the agency of the enzyme polynucleotide kinase, and it appears to be impossible completely to free these enzymes of ribonuclease so that some molecules are always degraded. Takanami's results have, however, one important implication—the 5′—OH group of the terminal nucleoside of *f*2 RNA made *in vivo* is phosphorylated and probably triphosphorylated, *pppXp*. Such termini might have been expected, because RNA chains grow 5′ to 3′ by the nucleophilic attack of the 3′—OH group on the growing polynucleotide on the 5′ α phosphate of the incoming nucleoside triphosphate. Not all RNA molecules possess 5′ triphosphate termini, however; Takanami claims that *E. coli* ribosomal RNA has 5′ phosphoryl termini, *pXpXp*.

The occurrence of the 5′ triphosphate group suggests an alternative approach to the determination of the 5′ nucleotide of RNA. If ^{32}P-labelled phage RNA grown *in vivo* has a 5′ triphosphate, alkaline hydrolysis of such RNA should uniquely liberate the 5′ terminal base as the tetraphosphate *pppXp*. Roblin (*J. Mol. Biol.*, **31**, 51, 1968) in some very carefully executed experiments has shown that alkaline hydrolysis of R17 RNA in fact yields *pppGp*, so that the 5′ terminus must be *pppGpX pY*. He has also shown (doctoral thesis, Harvard University) that the second base is a purine and the third a pyrimidine. This means that neither AUG nor GUG initiating codons are 5′ terminal, and so at least the first three bases must have some function other than initiating polypeptide synthesis. Likewise the last eleven bases at the 3′ terminus of phage RNA do not include a chain-terminating codon (see *Nature*, **217**, 311, 1968). In collaboration with J. T. August, Roblin (doctoral thesis, Harvard University) has also shown that the 5′ terminus of *Qβ* RNA made *in vitro* is *pppGpGpXpYp* where *X* is a purine and *Y* is a pyrimidine.

It now seems likely that all the RNA phage genomes have *pppGp* at the 5′ terminus. Dahlberg (quoted in Roblin's thesis) has apparently found this for *f*2, and both *Qβ* minus and plus strands begin *pppGp* (Banerjee *et al.*, 1967; Bishop, Pace and Spiegelman, 1967). Moreover, Maitra and Hurwitz (1965) showed the predominant 5′ termini of RNA made *in vitro* by *E. coli* RNA polymerase to begin either with *pppAp* or *pppGp*.

Why do purines predominate at the 5′ terminus of *m*RNA, and what is the effect of the 5′ triphosphate group on ribosome binding and the coding properties of these molecules? It should be possible to answer these questions as more and longer 5′ terminal sequences are determined.

Diversification at Harwell

by
JOHN MADDOX

It is now three years since the programme of diversification began at Harwell. How well is it working?

THE Atomic Energy Research Establishment at Harwell is now just over two decades old, and one of the most surprising of the signs of its maturity is that buildings which were within living memory freshly painted and a little stark are now softened by vegetation—a great many trees, ivy on some buildings and Berkshire grass everywhere Indeed, there is occasionally a suspicion that the research establishment may sink back into the landscape on which it was imposed and this, of course, chimes in well with the trough in the morale of the laboratory in the past few years when it has been hard to see just how the laboratory could justify its continued existence It is true, of course that even before the passage of the Science and Technology Act in March 1965 the laboratory had taken the lead among government establishments in planning to engage in research and development of wider interest to civilian industry than the mere development of nuclear power stations Now, looking back, the people at Harwell say that many of them were always frustrated by the need to stick to their narrow brief In any case, Section 4 of the Science and Technology Act gave the Atomic Energy Authority a licence to carry out other kinds of research and development by agreement with the Minister of Technology

Since then, diversification has been the watchword at Harwell For one thing, it seems to be the best way of giving the laboratory a continuing programme of work The fact that Dr Walter Marshall, who will succeed Dr Robert Spence as Director on April 1, has been the principal architect of the diversification programme is one sign of the importance attached to diversification But the programme being followed at Harwell is clearly regarded as a model by other government establishments anxious to find ways of cushioning the effects of financial stringency within the defence budget and even, sometimes, as the Minister of Technology would wish, to make a contribution to the health of British industry It is therefore of some importance to know how well diversification at Harwell is proceeding even though it is necessarily far too soon for anybody to make a final judgment 1970 will be the time for that

It is only sensible to acknowledge that opinions about the potential merits of the attempt to diversify the interests of a government laboratory are varied and often in conflict with those of the research establishment itself There are some, for example, who say that civil servants and quasi civil servants are necessarily less efficient than scientists and engineers who work in industry, but this is not a very serious argument, Harwell indeed has a splendid reputation for getting things done

A more cogent objection is that there must be doubt about the efficacy of programmes of research and development which are pursued at locations different from those at which good ideas are turned into manufactured products. If the people responsible for research and development are separate from the manufacturing organization, so the argument goes, there is a danger that unrealistic programmes will be designed and followed in the research laboratories. Another view is that the separation between research and production loses what may be the most valuable function of industrial research—that of providing a means by which the manufacturing process can be leavened by the spirit of the research laboratories To some people, these potential snags far outweigh the potential advantages of the admittedly magnificent resources which are available at Harwell and, to a lesser extent, at other government laboratories. Even in a preliminary assessment of the diversification programme, these are the most conspicuous questions

Projects now under way, which account for roughly 18 per cent of the research effort at the establishment, include several about which there can be no argument. Harwell is able to use its resources for providing some services to industry which could not easily be accommodated within the manufacturing companies most directly concerned Thus the Non-Destructive Testing Centre under Mr R S Sharpe seems to have a flourishing business in the design and construction of devices for special tasks in engineering industries In the nature of things, the company which commissions the design of such an instrument (paying a fee calculated on the basis of the time and materials consumed) is likely to find that its needs are satisfied for several years. In this sense, the establishment at Harwell functions exactly as if it were an independent profit-making research laboratory. Its advantages are that it can call on the services of a large and skilled engineering organization so that the design of a new instrument can benefit from what may seem to be an ideal matching of need and skill

In much the same way the diversification group at Harwell which is known as the Heat Transfer Service is carrying out work for chemical engineering companies on a fee-paying basis This same group of people, which hopes to exploit the ease with which it has access to computers and the other facilities of Harwell, plans, for example, to offer to chemical engineering companies what it calls a design information service as part of which, for a fee of £1,000 a year, a company will receive half a dozen or more detailed reports on particular pieces of design work which have been carried out The Heat Transfer Service confidently hopes to find between 30 and 50 customers It expects that something like seven people will support a turnover of about £100,000 a year and will have time and energy to spare for a programme of supporting research.

A part of the philosophy which has so far been developed at Harwell is that a research programme of some kind is an indispensable accompaniment of *ad hoc* help to industry Thus a good deal of the effort of the Non-Destructive Testing Centre is concerned with more long-term problems —the development of means of testing the strength of bonds made with adhesives between different materials and the development of means for understanding and then predicting the strength of composite materials such as those made from carbon fibres now coming into fashion

The programmes of research and development in which the establishment has a formal agreement on collaboration with some industrial companies are somewhat different in flavour even if the difference is merely one of degree By now the establishment has accumulated a number of schemes like this Probably the oldest of them are those which form a part of the programme of desalination research on which £1 3 million has been spent in the past three years and to which a further £4 million has just been allocated by the Ministry of Technology In its work on flash distillation, for example, the establishment at Harwell is linked with the Weir Westgarth Company, and there seems to have grown up a tidy division of labour between the two partners Harwell claims, and the company confirms, that the AEA provides a valuable service in carrying out rapid and accurate assessments (by computer) of designs which the company would like to submit for tender One product of this work has been that the company has taken seriously to experimenting with arrangements other than horizontal evaporator tubes in its distillation plants—a potentially important source of economy in commercial operation In practice, the desalination programme is based at Risley in Lancashire, and Harwell tends to contribute the basic research but only part of the large-scale chemical engineering design

The establishment is also deeply engaged in a programme of collaborative research which already involves the Royal Aircraft Establishment at Farnborough, the National Research Development Corporation and commercial companies of various kinds on the development of composite materials based on carbon fibres. The object here is somehow to exploit the high specific modulus of thin filaments of carbon The establishment at Harwell has experience in the development of graphite for nuclear reactors and it so happens that the same equipment can be used for the development on a small scale of methods for manufacturing carbon fibres from long lengths of a synthetic polymer Carbon fibres some hundreds of feet long have been manufactured in this way and bonded together with various resins Heat treatment is known to be a crucial part of the process of developing the full strength of the material as it is in the preparation of graphite for nuclear reactors, and a brief sight of the equipment in use is a sufficient justification of the establishment's claim that in this respect its facilities are unique

In the industrial development of materials based on carbon fibres, funds are provided partly by the National Research Development Corporation In other agreements it has made, the Harwell establishment has found itself in partnership with a straightforward commercial enterprise Thus, for example, there is a collaborative programme of research on the development of means of using ion beams in the construction of semi-conducting electronic devices In this and other agreements, the commitment of resources is said by both partners to be the only real means of knowing that the other is prepared to take the work seriously

The management at Harwell considers that the proportion of the laboratory's effort devoted to work of an industrial character may eventually rise to 25 per cent The schemes now being considered for approval may indeed mean that the target of about a quarter of the establishment's effort on diversification is reached within the coming year The establishment seems to welcome the prospect that it will then have to pick and choose rather carefully the industrial schemes on which it embarks This is entirely in line with the laboratory's view that competitiveness should determine the way in which it functions in industrial concerns Thus the laboratory takes the view that it is no part of its job to help to support unsuccessful companies or declining industries In this sense, the argument goes, its industrial interests are markedly different from those of the research associations It will do its best to reinforce success

A number of comparatively minor criticisms of the diversification programme are comparatively easy to answer in this context Thus to the complaint that the charges made at Harwell for research or consultancy are too high, the laboratory answers that industry is not in any case sufficiently aware of the real costs of professional research and that, in spite of the cost, the demand for the establishment's services has grown rapidly to the point at which it is now almost an embarrassment

The same line of argument is at least a partial response to the suspicion that a laboratory with the prestige of Harwell might be able to foist impractical ideas on a gullible industry Companies which have played a part in collaborative programmes at Harwell say that some of the ideas put up to them under the terms of the collaboration are indeed more innocent of industrial experience than would be welcome At the same time, the companies concerned seem to find no difficulty in imposing a sense of commercial reality on the joint enterprise Evidently what matters is that both partners are committed in money terms The long-term safeguard is that the partnership between the laboratory and the industry should never be one in which the industrial partner is altogether lacking in capacity for research and development The ideal is that the laboratory and its industrial partner should be complementary in some sense or another

That said, it is important that the laboratory may yet find that its new activities raise problems of an unfamiliar kind Some at Harwell point to the irony of how it has been possible in the past few years to dismantle much of the apparatus necessary to safeguard national secrets only, now, to discover that the problems of administering commercial secrecy are every bit as intricate In practice this means that the laboratory would not, for example, be able to tackle the same problem for two different companies Making sure that one company cannot use Harwell as a means of finding out what its competitors are up to is obviously a crucial matter, and years will no doubt go by before the establishment has been able to establish for itself in this respect the kind of reputation which the great independent research laboratories have built up over the years The problem is specially difficult to handle in circumstances in which the establishment collaborates with several different companies on different aspects of the same problem—water desalination, for example Then it is necessary formally to agree in advance on the exchange of information between the companies about the status of separate parts of the development programme without disclosing detailed design information

Problems affecting the staff of Harwell are likely to assume increasing importance in the years ahead At present, the people engaged on the diversification of work are dealt with as are the other employees of the Atomic Energy Authority and this, of course, is inevitable because it is often hard to predict just who is going to be caught up in some industrial programme and for what proportion of his time The result, however, is that it is comparatively easy for industrial companies to tempt away from Harwell people whom they wish to recruit and on one view, of course, this is nothing to weep about—if the laboratory can inject people from Harwell into British industry as a whole, some of its objectives in diversification will have been achieved. But in the long run it is hard to imagine that the laboratory will be able to keep its present salary structure, even with the degree of flexibility which the autonomy of the Atomic Energy Authority provides, and yet allow its diversification teams to function efficiently That, however, is a problem yet to be faced

It goes without saying that a proof of the success of diversification must also lie in the future These are early days and it is bound to be some time before the establishment at Harwell will be able to demonstrate that a substantial proportion of its diversification projects has been successful (Nobody, of course, would expect them all to be successes) So far, however, the signs are encouraging It remains to be determined how freely the establishment will be able to function in British industry—understandably, its interests so far have been largely concerned with problems more or less related to chemical engineering It is also conceivable that success will take the form of persuading industrial companies to increase their efforts in research and development, so that in the long run Harwell's function will be seen as that of a pump primer But this is a long way in the future For the time being, what seems to matter most is that the venture has begun well and has served already to revive sagging spirits at the laboratory It is perhaps worth remembering that things may have begun so well because of the great prestige and undoubted competence of what is, after all, one of the best institutions of its kind In other words, it does not follow that if diversification works at Harwell, it will also work at other government establishments

Engineers and Scientists in Industry

by

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The Royal Society arranged two meetings in Oxford last year to discuss the difficulties of persuading scientists and engineers to go into industry In this article the organizers of the meetings make some recommendations for action to help the situation

1 Industry's need for more "qualified scientists and engineers" has been widely discussed and documented during the past few years There are several aspects (*a*) What sort of engineers and scientists does industry want? (*b*) Do the universities turn out people with the right type of training and outlook for industry? (*c*) Why is industry apparently unable to attract good quality people in sufficient numbers? (*d*) What aspects of collaboration between industry and university should be further developed to improve the situation?

2. The Royal Society organized two one-day meetings to discuss these questions Each was attended by a representative group of thirty people from industry, universities and government departments The first discussion, held on March 17, 1967, covered all four topics, while the second, held on July 6, 1967, concentrated on collaboration between industry and university Although no definite conclusions or recommendations have emerged, it was felt that a summary of the views expressed at these two meetings should be given some publicity

3 Having been responsible for the arrangements of the meetings, we decided to produce such a summary It would be impossible to mention in detail all the aspects in a brief article, and so we have picked out those points which seemed to us important or novel, and drew from these our own conclusions This is a personal, idiosyncratic report and so are the recommendations, to ensure that none of the participants should feel in any way bound by what we say we do not attribute remarks and views to named individuals

Industry's Needs

4 No clear pattern emerged from the discussion on the relative numbers of B Scs (or equivalent), M Scs (or equivalent) and Ph Ds wanted by industry This is not surprising, because the participants were drawn from many diverse fields including the machine tool, electrical, motor car, aircraft, chemical and pharmaceutical industries All one can say is that, taken as a whole, British industry, as at present constituted, will want at the most 1 per cent of its intake of qualified engineers and scientists at Ph D level, although in some industries, such as electronics and chemical, the figure is much higher

Training Scientists and Engineers for Industry

5 This was probably the most controversial subject in the whole discussion and a gulf was seen to exist between the engineering professors and the industrialists Industry, with its well developed training schemes, has a vested interest in education and this makes the job of the universities difficult Industrialists on the whole regard the three year university courses as sufficient and are in favour of engineers learning their job in industry apart from periodical releases for further training This is in sharp contrast with the practice in many European countries, where universities provide a really full training of engineers lasting five to six years so that further training is not required in industry, except in the firm's specialized techniques

6 Training in industry has the drawback that it often results in narrow specialization and this militates against mobility of manpower Fully trained engineers on the continent settle down quickly in new jobs

It is said, on the other hand, that the "sheltered life" and the type of training in universities tend to make people unwilling or unable to cope with the stresses and strains of industry and to assume responsibilities

Recruitment

7 Industry does not get high quality people in sufficient numbers, and in the view of industrialists too high a proportion of the good graduates remain in the universities after their first degree, either to take advanced courses or to read for a Ph D It was pointed out, however, that one year advanced courses are in many cases a continuation of the undergraduate courses and could be regarded as a tacit acknowledgment of the fact that it is not possible to train a competent scientist or engineer in three years

8 The high proportion of those reading for a Ph D is quite another matter Too many Ph Ds, especially in chemistry and physics, stay on in universities, most of them in the so-called "penumbra" without permanent positions The longer they stay on, the less likely becomes their ultimate assimilation in industry even if industry could offer sufficient numbers of attractive openings for all Ph Ds Many felt that either the number of research studentships should be reduced, or at least more of them should be channelled into research of ultimate benefit to industry This is already being done through changes in the grants policy of the Science Research Council (SRC), for example, the institution of science and industry awards, industrial studentships, industrial fellowships and the earmarking of a proportion of research studentships for work with an industrial content

9 Why was it that graduates preferred to stay in universities rather than go into industry? One reason put forward was that in their undergraduate years students were "brain-washed" in favour of pure academic work and were left in ignorance of the needs of industry and the intellectual challenge of industrial work Equally important appeared to be the poor image of industry Many recruits felt that their abilities were not fully used, they were not "stretched" and they were not given responsibilities sufficiently early in their career It was mentioned in this connexion that industry in the United States is more compartmentalized than in the United Kingdom and as a result they have more high and well paid posts with responsibilities in only a limited field

10 Higher salaries and better working conditions (for example, longer holidays) would help in recruiting At present salaries in industry up to the age of 30–35 are about the same as in universities At least half of the industrial salaries level out at about £2,500 per annum and less than 10 per cent rise markedly above professorial salaries But even if economic considerations permitted a general raising of individual salaries the advantages would be short-lived, for, because of the "Priestley doctrine" government and university salaries would

follow suit This difficulty does not seem to exist in other countries, for example, in France where industrial salaries are markedly higher than university salaries which in turn are fully at par with those in the United Kingdom

11 In several industries insistence on parity within a grade seems to make salary differentials based on merit or qualification difficult to achieve On the other hand, there seems to be a lack of parity between the "science" and the "arts" men in industry If people on the managerial, advertising, commercial and legal side could expect higher salaries than their "technical" colleagues who prefer to continue creating wealth rather than manipulating it, this tends to discourage engineers and scientists from entering industry

Collaboration between Industry and University

12 *The doctor's degree.* One of the reasons for graduates wanting to stay on in universities is the importance attached to the possession of a doctor's degree This was strongly felt by industrialists, to find out the reasons might be the subject for a sociological study Because, however, this attitude cannot be changed quickly, some other remedies have to be found The obvious solution is for universities to award doctor's degrees for work carried out in industry A survey of most British universities has shown that although purely external degrees are offered only by one or two universities, most others do award Ph Ds provided some residential qualifications (one year in most cases) are satisfied The attitude of universities may be summarized by saying that they do not want to be degree-giving bodies for work carried out entirely outside the university

13. It was generally agreed that the work carried out in several industrial research and development laboratories was fully of the type and standard required for the Ph D degree as at present constituted But it was pointed out very forcibly that the Ph D is basically a preparation for pure research, while industry needed people of the same intellectual calibre but with a mind more attuned to industrial life The question was raised whether it would be possible to award Ph D degrees not only for work leading to new discoveries but also as a recognition of high standards of critical ability and of organizational talent required by industry In this connexion a recent scheme started at the University of Aston is worth mentioning This will have three novel features (i) it will be on applied research carried out in industry and directly related to the operation of the firm; (ii) the research project will be more broadly based, that is in addition to the basic discipline the knowledge of a complementary discipline will be required, for example, an engineering project would have an economics content, a physics project would involve operational research and psychology and so on, and (iii) wherever possible the work will be done by teams and the thesis will be written jointly

14 If more graduates of Ph D calibre were to be drawn into industry the output of research in universities would correspondingly be reduced While it is thought that at least in some disciplines a reduction in the number of Ph D students would not damage university research, it appeared that a reduction in the number of post-doctoral people might be serious

15 *Consulting work by university staff* A survey has shown that all British universities allow their staff to do consulting work in industry and that there are on the whole no serious restrictions on the amount of consulting done or on the earnings It was felt, however, that the attitude was more one of toleration than of encouragement and it was generally agreed that universities should positively encourage their staff to get involved through consultancy, in industrial life For example, successful consulting work should be taken into account together with, or in replacement of, publications when considering promotion

16 It was pointed out that there is still too much "prestige" consulting going on, consultants paying occasional visits to the firm concerned without getting involved in its work It was agreed that for consulting to become an effective bridge between university and industry it must be done properly and the consultant should as far as possible take an active part in certain aspects of the firm's work and even assume responsibility for it

17 There was also general agreement that it was undesirable for a firm to insist on the consultant not to work for any other firm in the same field or a cognate field Insistence on "sole consultancy" is to some extent a reflexion on the scientist's or engineer's professional integrity and is unknown in most other professions, for example, in the legal or financial field Although it would be difficult for universities to forbid their staff to enter into sole consultancy agreements, they should strongly discourage it

18 The small firms with no research and development sections were particularly in need of university consultants and it was pointed out that the British Council—Department of Education and Science publication *Scientific Research in British Universities* provided a good guide for finding them

19 *Industrial staff in universities* The importance of industrialists getting more involved in university life was emphasized This practice is becoming fairly widespread, but at present most visiting professors or readers from industry are only concerned with research and postgraduate training. Industrialists feel that an understanding of the aims and methods of industry should be cultivated early in the student's academic life, and so it seems important that visiting professors should get involved in undergraduate teaching as well This obviously would mean some sacrifice by industry, because the benefits of such involvement would be of a long-term nature On the other hand, universities should pay more than a nominal honorarium to visiting professors from industry, a figure in the range of £500–£1,000 per annum being regarded as reasonable The possibility of using industrial visiting professors as temporary replacements for university staff on sabbatical leave might be explored.

Conclusions and Recommendations

We want to make it clear that the following remarks are based on just two one-day discussions and on some additional bits of information we have picked up, and that we did not have the help of a powerful committee or secretariat to provide detailed information It may well be that some of the actions we are suggesting have already been taken or that some of our premises are no longer valid If this is the case, we apologize

(*A*) Not enough is known about either the actual or the desired composition (B Scs, M Scs and Ph Ds) of industry's present intake We recommend that these data be ascertained and the future policy be examined (paragraph 4) This requires action by the Confederation of British Industries (CBI) and by the Committee on Manpower Resources of the Ministry of Technology and the Department of Education and Science

(*B*) There is a great variety in the ways of training engineers and a divergence of opinion about the relative advantages We recommend that this whole question be examined in depth and that methods in the United Kingdom be compared with continental and American practices (paragraphs 5, 6). This requires action by the Council of Engineering Institutions (CEI), the CBI and the Committee of Vice-Chancellors

The following three conclusions and recommendations are related to industry's recruitment difficulties

(*C*) As a result of the "Priestley doctrine", that is, the insistence on parity between the three sectors, salaries in industry are much the same as those in universities and the Civil Service Within industry salary differentials to recognize higher academic qualifications are small There is also a strong feeling that prospects of promotion for "arts" graduates, most of whom are in the administrative or managerial sections, are better than for engineers or scientists. We recommend that these questions be examined (paragraphs 10, 11). This requires action by HM Treasury, the CBI and the professional institutions (for example, Association of Professional Civil Servants, the Association of University Teachers, and the appropriate trade unions).

(*D*) Given more attractive conditions in industry, recruitment of graduates could be improved by reducing the number of SRC and other studentships and of postdoctoral awards. We recommend that the short-term and long-term effects of such measures on university research be examined (paragraphs 7, 8, 14). This requires action by the research councils, especially the SRC, the Council for Scientific Policy, the Committee of Vice-Chancellors and the Royal Society.

(*E*) The possibility of gaining a doctor's degree on the basis of work (not necessarily of research) done in industry would help recruitment. We recommend that the whole basis of the Ph D degree, which at present is geared to a university career, should be examined (paragraph 12). This requires action by the Committee of Vice-Chancellors, the SRC and the Royal Society.

While the measures mentioned under *C*, *D* and *E* would go a long way towards improving recruitment by industry, they are not sufficient in themselves. It is important that universities, that is, both staff and students, should become more aware of the needs of industry, and that industry should make a more effective and intelligent use of high grade manpower. The following points deal with this question.

(*F*) We recommend that consultancy by university staff both at professor and lecturer level should be positively encouraged by the university authorities (paragraphs 15, 16, 17). This requires action by the Committee of Vice-Chancellors and the CBI.

(*G*) We recommend that part-time university appointments (visiting professorships, fellowships, readerships, lectureships) of industrial staff should be encouraged and that the need for involvement in undergraduate teaching be emphasized (paragraph 19). This requires action by the Committee of Vice-Chancellors and the CBI.

(*H*) We recommend that the dissemination of information already available about university activities of interest to industry (for example, *Scientific Research in British Universities*) should be made more effective (paragraph 18). This requires action by the Office of Scientific and Technical Information, the Ministry of Technology, the CBI and the Committee of Vice-Chancellors.

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Multiphase Development of the Lunar Crater Tycho

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Data collected by Orbiter V when it landed on the Moon in 1967 provide information about the history of the crater Tycho. Volcanic eruptions seem to have been involved in the crater's development

TYCHO, the rayed lunar crater 85 km in diameter, has long been regarded as the classic example of a large impact crater. Its anomalously high radar reflectivity[1] indicates that the floor of Tycho is rougher or denser than the surroundings. Its anomalous thermal behaviour[2] during a lunation, or during eclipse, indicates that the materials of the crater are better thermal conductors than the terrain around Tycho. Taken together with the facts that the rays of Tycho are stratigraphically more recent than most objects which they cross, and that the walls of the crater are sharply sculptured and nearly circular, the observations have been interpreted widely to demonstrate that Tycho is a recent impact structure.

In 1967, Tycho was photographed in medium resolution (Fig 1) by Orbiter V, and much of the floor, wall and surrounding terrain was, in addition, covered by high-resolution photography. Surveyor VII soft-landed and chemically analysed the material in the "continental" domain covered by the high-resolution shots. In this preliminary report we extract information from the Orbiter V coverage of Tycho. We find clear evidence for extensive volcanism in and around Tycho.

Geological Units

The geology of the Tycho region will be described fully when the Surveyor VII analyses are available. On the basis of marked morphological and topographical differences of the terrain immediately north of Tycho, as represented on the Orbiter V photograph *H*–128, we mapped the approximate boundaries (Figs 1 and 2) of the principal geological units in the region. Units *A* and *B* have all the characteristics of lava flows and they cover the units *C* and *D* and so must be later than these units. A high density of fine fractures, shown as short hair lines in Fig. 2, was found principally in units *C* and *D* and was used to define the boundaries of these units, which appear to overlie unit *E*. Unit *E* itself is largely devoid of similar fractures and we believe that it is the oldest geological unit mapped. Fine fractures could, of course, be filled by detritus on the older units, so the paucity of fractures there is in accord with our proposed age sequence.

We believe that units *C* and *D* are volcanic flows: in addition to some parts being fractured in a manner characteristic of lavas, they are topographically higher and are less cratered than unit *E*, also, they conform, largely, to the "deltoids" described[3] as special fracture zones from which lavas were extruded to build up complex plateaux.

Several flow structures have been mapped in addition to those shown in Fig 2. Six well-defined flows occur W. and N W. of Tycho and are shown in Fig 1. They are 3–20 km long and about 2–6 km wide. The direction of flow is indicated by arrows in Fig 1 and conforms to the slope of the terrain on which they have flowed. Several have flowed towards Tycho and others have flowed away from Tycho.

Five of these six flows have apparently issued from craters which have been breached by the flows. Their surface texture is coarse with a wrinkled or ropey appearance and they seem to have been considerably more viscous than the flows in the maria. The general appearance of the flows and the fact that they are associated with craters indicate that they are volcanic flows—probably lava rather than ash or debris flows.

The most distant flow is just over 100 km from the rim of Tycho which is near the limit of the medium resolution coverage of Orbiter V in this area. It is quite possible

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* On leave from the University of London.

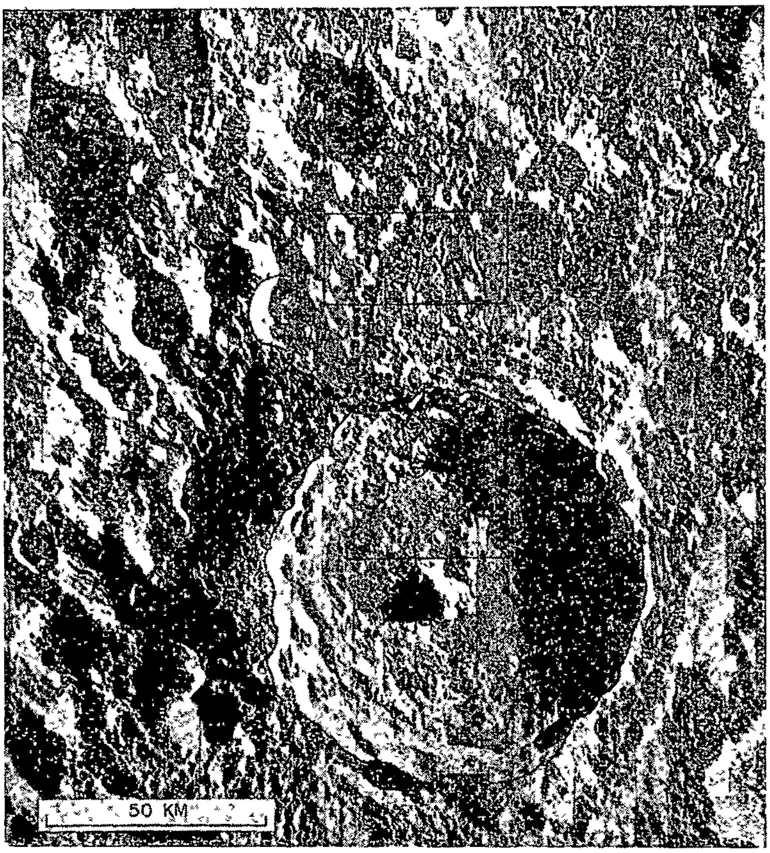

Fig 1 Medium resolution mosaic of three Orbiter V photographs of Tycho and region The rectangle north of Tycho outlines the area mapped in Fig 2 from a high resolution photograph That portion of the floor of Tycho mapped in Fig 3, from another high resolution photograph, is also indicated here The black spots give the approximate locations of those "lakes" on which craters were counted Larger "lakes" are shown in outline, chiefly east of Tycho Finally, the principal lava flows are sketched, and the direction of flow is indicated by arrows (Based on NASA photographs *M* 125, 126, 127)

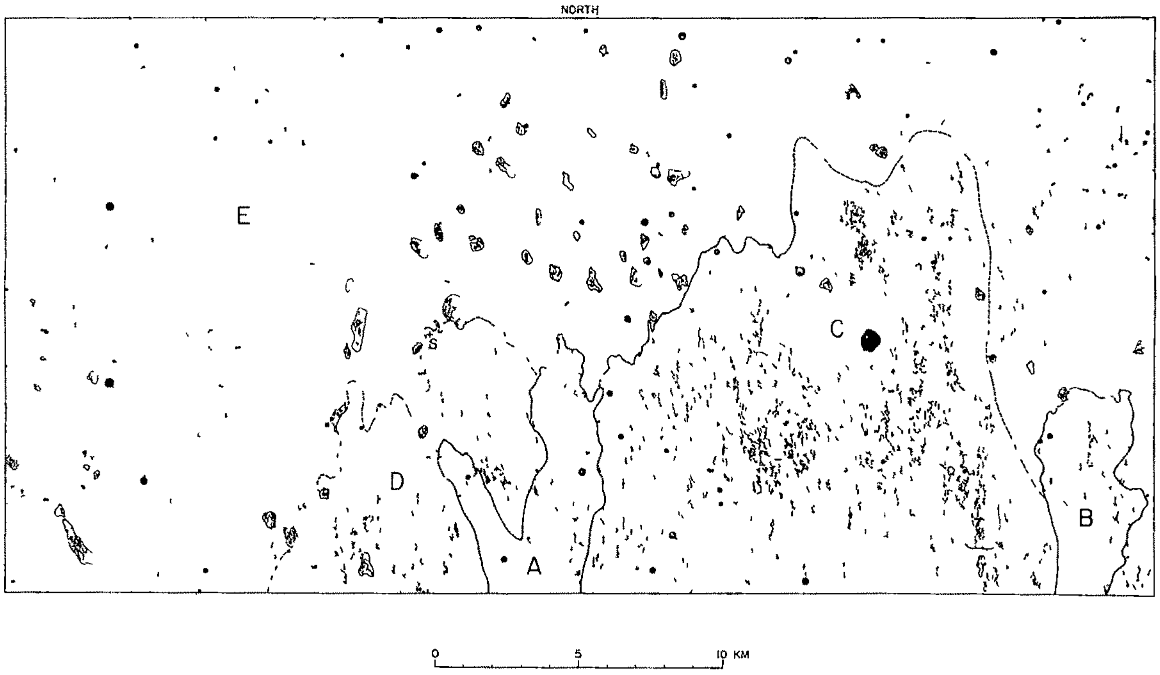

Fig 2 Principal geological units *A* to *F*, discussed in the text Broken lines are used to define boundaries in places where they are only approximately located Short hair lines define fractures The smallest, irregularly shaped units mapped are the lava "lakes" discussed in the text Craters are blacked in The position of Surveyor VII is shown as a cross labelled *S* at the northern extremity of unit *D* (Based on NASA photograph *V*–*H*–128)

that other flows occur at even greater distances. The fresh appearance of these flows, the relatively close proximity to Tycho and their similarity to flows on the rim and on the interior wall of Tycho suggest that they are associated with the formation of Tycho.

The interior slopes of Tycho also show signs of the flow of material (Fig. 1). These are primarily expressed as channels with raised rims, although there are several flows with well-defined fronts. One of these flows has issued on the floor and merged with it so that the floor and flow appear as one textural unit. Peripheral fissures on the floor continue across the flow. Thus the flow reached the floor when it was still in a fluid or plastic condition and before the formation of the peripheral fissures, and the flow must be essentially the same age as the floor. The flow channels have raised rims and several appear to begin at craters on the wall. The channels are almost identical to lava drainage channels with natural levées built up by the overflow of lava. The similarity is strengthened by the fact that several of the channels start at craters and at least one ends in a small "lake" which exhibits a flow front.

Numerous small "lakes" of relatively dark material and exhibiting fine fractures are situated in circular to irregular depressions. They are depicted in Fig. 2 and interrupt the other units (*C*, *D* and *E*), therefore, they must be later than these units. The "lakes" are concentrated within 20 km of the rim and several of the larger ones have distinct fronts where they contact the sides of the depressions in which they lie. The approximate locations of the "lakes" studied on the high resolution frames are shown as filled circles in Fig. 1. The larger "lakes" may be seen only on the medium resolution coverage and their well-defined elevated-fronts are also mapped in Fig. 1. The distribution of these lakes near the rim of Tycho indicates they are intimately associated with its formation. We believe the lakes consist of lava which has found egress to the surface through vents or fractures primarily associated with the depressions in which they lie and that, because of the paucity of craters and abundance of well-defined fractures found on them, they are relatively recent features. Finally, that part of the floor of Tycho recorded on the Orbiter V photograph *H*–125 forms a geological unit which is morphologically quite different from the other units. It is shown in Fig. 3 and is characterized by steep and gentle-sided domes (not mapped) interspersed with terrain which displays a highly fractured and ropy pattern similar to that of terrestrial lava. Several gentle domes exhibit the same texture as the floor and have fissures along their crests. They are probably updomings of the floor or tumuli produced by the hydrostatic pressure of fluid lava underneath the solid crust. Numerous crater chains and craters of highly irregular shape occur on the floor. Some of these features are shown in Fig. 3 and attest to the intense volcanic action to which the floor has been subjected.

A set of peripheral fractures is found on an inward sloping bench at the margins of the floor and indicates that the floor has subsided leaving a higher bench broken by tension fractures in the zone of maximum downbending. These features are very similar to the fractured benches found on terrestrial lava lakes—for example, in Hawaii—caused by the drain-back of lava into the vent. If the Tycho bench formed in a similar manner, then it is probable that Tycho is underlain by a magma chamber into which the lava drained.

Eumorphic (sharp-rimmed) circular craters as large as, or in excess of, 50 m in diameter have been mapped on all the geological units. These craters may be used in an attempt to date the different units.

Crater Counts

To check the stratigraphic sequence suggested by the morphological and topographical relations, we counted probable impact craters on each geological unit mapped. This was done using only eumorphic, circular craters, and corrections for the many shadowed and over-exposed areas were applied for all regions using planimetric and square-counting methods. Although the counts of craters

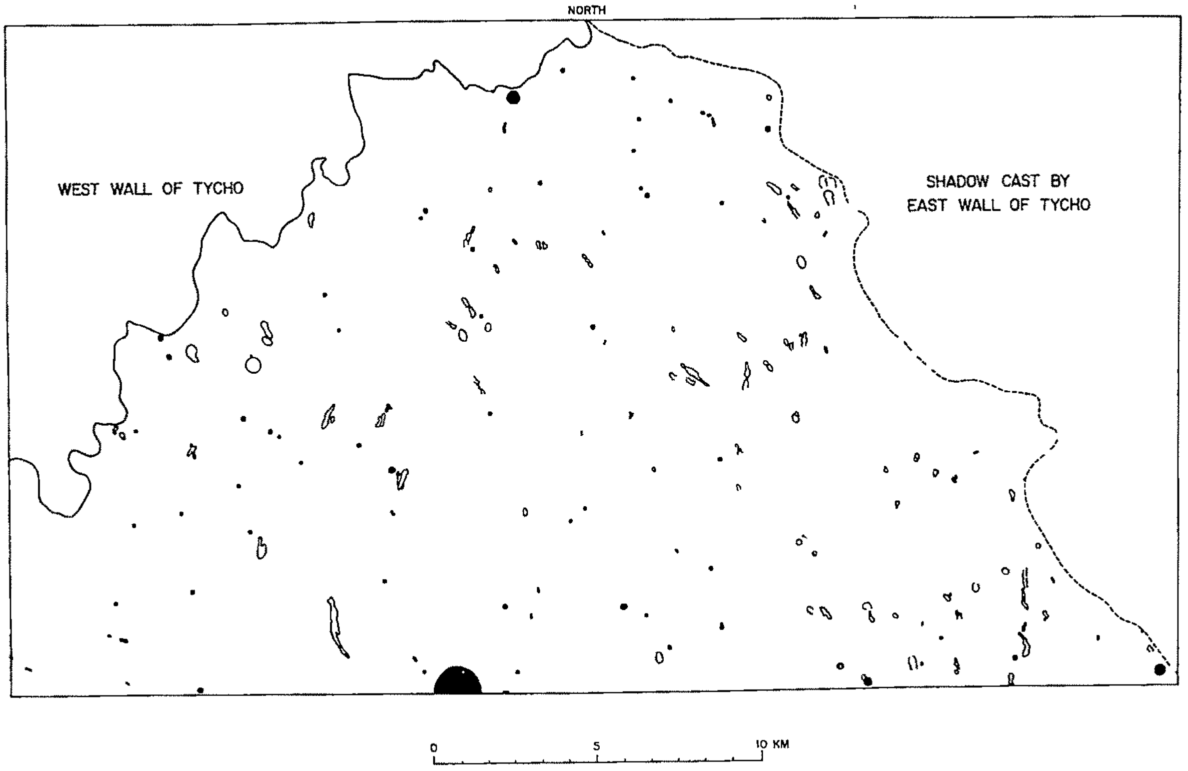

Fig. 3. Sketch of the floor unit discussed in the text. Eumorphic craters are blacked in, whereas probable volcanic craters are shown only in outline. (Based on NASA photograph *V–H*–125.)

on the floor of Tycho are restricted to eumorphic craters, the position of many at the apex of hills and on or at the end of fissures suggests that they may be volcanic rather than impact in origin The count therefore represents a maximum on this unit The measured areas and counts for the different units are given in Table 1

The geological units *A* and *B* are alike and, indeed, are found to have the same crater density, within the limits to be expected from the method Again, the apparently similar units *C* and *D* have similar number-densities of craters A less obvious result of the crater counts is that the "lakes" and the "floor" units are cratered to a similar number-density (Table 1) Unit *E* has 4·5 times as many craters per unit area as the floor of Tycho, 2·6 times as many as unit $(A+B)$ and twice as many as unit $(C+D)$

It would be natural to interpret these differences in crater density as age differences between the various geological units Random fluctuations (such as might have resulted from "secondary" cratering) in crater density as a possible cause of the observed differences can be ruled out because of (*a*) the extent of the areas mapped and (*b*) the similarity in the cratering on superficially similar geological units With the assumption that cratering is related to age, *E* would be the oldest unit mapped while the floor of Tycho and the lava lakes would be the most recently formed units The counts completely confirm the observed stratigraphic relations of the various geological units

A further analysis of the number data will now be given In Fig 4, the data are represented logarithmically as cumulative numbers per 1,000 km² of surface against minimum crater diameter The most significant points are joined with continuous lines, the less significant points with broken lines. Because the difference in crater density of units $(A+B)$ and $(C+D)$ was only 1·3 at the 50 m size (Table 1), we took sample counts in these regions for craters down to half that size (Fig 4) to see if the same difference persisted The result shows a similar difference in crater density, although we suspect that there is observational incompleteness in the counts of the smallest craters because the terrains are rough and the graphs bend inward relative to the graph for the "lakes", where equally small craters (25 m) were seen easily.

The fact that $(A+B)$ is actually younger than $(C+D)$ comes from stratigraphic evidence By themselves, the crater counts are insufficient to establish the age difference beyond doubt, although they do not conflict with the geological information Random fluctuations in crater density may be expected to render the counting method of dating invalid for small areas. Even when areas *A*, *B*, *C* and *D* are taken separately, however, either of the areas *A* and *B* has fewer craters per unit area than either of the areas *C* and *D* (Table 2); and *A* is adjacent to *C*, *D* and *E*, whereas *B* is adjacent to other parts of *C* and *E* (Fig 2). Thus it is not unlikely that the observed differences in crater density on units $(A+B)$ and $(C+D)$ are a true measure of age

Combining the results of the crater counts with the geological information on the relative age of the different units, we conclude that (*a*) materials of unit $(C+D)$ are appreciably younger than those of unit *E*, (*b*) materials of unit $(A+B)$ are somewhat younger than those of unit $(C+D)$; (*c*) the materials forming the floor of Tycho, and those forming the lakes outside Tycho's walls, are appreciably younger than those of units $(C+D)$ and $(A+B)$, and (*d*) the flow on the wall of Tycho which merges with the floor is the same age as the floor.

Table 1 TOTAL CRATER COUNTS IN DIFFERENT GEOLOGICAL UNITS

Geological unit	Area examined (km²)	Corrected area of count (km²)	Total No of craters greater than 50 m in diameter	Craters per unit area (floor = 1)
Lakes	22	22	17	0·9
Floor	460	400	347	1·0
A	24	19	30	1·8
B	19	15	22	1·7
C	185	136	278	2·4
D	51	42	81	2·2
E	463	342	1,324	4·5

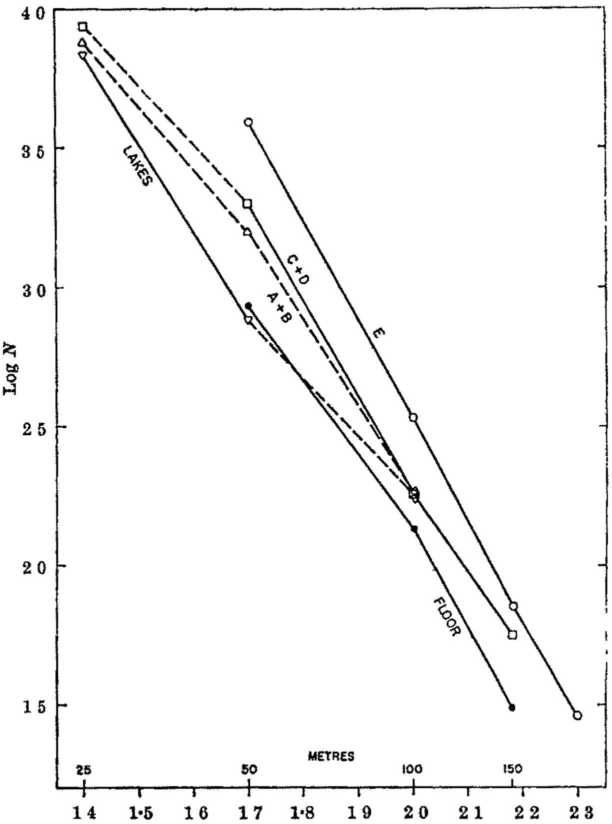

Fig 4 Numbers *N* of craters ≥ Δ metres in diameter/1,000 km² of surface, represented logarithmically for the geological units indicated

Further Implications

If these age differences are accepted, the inevitable conclusion is that at least the terminal phases in the formation of the floor and outer rim of Tycho were protracted Certainly, the rim materials that we now see cannot be ejecta. first, the ejecta blanket of an impact crater would have been formed within minutes and would tend to be uniformly cratered or to show systematic losses of craters as the rim crest was approached; second, it would not display the range and disposition of geological units, or the sharp discontinuities between them, that are observed It seems that the extrusion and flow of lava at very different times is the only way to account for the observations If, however, an impact produced Tycho, lavas might be generated at the time of the impact, as Urey[4] suggested, or later as Hartmann[5] suggested. We now show that neither hypothesis seems to be adequate to account for the present observations

The time interval between the formation of the various lava units is important in this discussion and, given the rate of cratering, the appropriate intervals of time can be calculated. An attempt to date the individual units may be made using Hawkins's[6] influx law $\log N = \log m_\infty - 7·5$, where *N* is the cumulative number of meteorites received by 1 km² of Earth per hour and m_∞ is the mass in kg of a meteorite at infinity. Assuming that the diameter of the meteorite, density 3 g cm^{-3}, required to form a crater of diameter Δ is Δ/15 (see ref 7), it follows that the mass required to form a 50 m crater is $5·8 \times 10^4$ kg. If (as we expect) the meteoroid flux is the same in the vicinity of the Moon, and if it is constant in time, then one would expect one primary lunar impact crater 50 m or more in diameter to be formed per 1,000 km² every

Table 2. INCREMENTAL AND CUMULATIVE CRATER COUNTS IN DIFFERENT GEOLOGICAL UNITS

Geological unit	n* (50–100 m)	N† ($\geq$50 m)
Lakes	580	759
Floor	733	869
A	1,421	1,579
B	1,267	1,467
$A+B$	1,394	1,575
C	1,816	2,044
D	1,881	1,929
$C+D$	1,821	2,005
E	3,529	3,871

* n Number of crater/1,000 km² between 50 m and 100 m in diameter
† N, Total number of craters/1,000 km² $\geq$ 50 m in diameter

Table 3 ESTIMATED AGES OF GEOLOGICAL UNITS

Geological unit	Age in units of 10^8 yr	
Lakes and floor	1 6	0 4
$(A+B)$	3 2	0 8
$(C+D)$	4 0	1 0
E	7 7	2 0

2×10^5 yr Using the cumulative number-data of Table 2, it follows that the various geological units may be expected to have the ages listed in the second column of Table 3

A very crude but independent method of dating the geological units is to consider the volume of lunar material eroded per unit surface per year and to assume that the 50 m craters would no longer be regarded as eumorphic after 4 m of material had been eroded from their rims and deposited in and around them Data, including microphone data, relating to the particle flux in space have been used[8] to arrive at a lunar erosion rate of $<10^{-6}$ cm³ cm⁻² yr⁻¹ Nilsson[9] showed that the microphone counts of particles in space were too high, and certainly unreliable He suggested (private communication) that a better estimate of erosion rate could be deduced from the observed decrease in reflectivity of the Earth-satellite Echo I Assuming that <5 per cent of the surface of the 100 ft balloon was etched by micrometeorites during a 4 yr period, and using the skin thickness of the balloon, $1\ 3 \times 10^{-3}$ cm, to obtain an upper limit for the volume of material eroded, it follows that $<1\ 6 \times 10^{-5}$ cm³ cm⁻² yr⁻¹ was eroded away The 10^{-6} figure indicates that all the 50 m craters counted would be $\lesssim 4\ 0 \times 10^8$ yr old If $2\ 0 \times 10^8$ yr were the absolute age of the average eumorphic craters on unit E, the incremental crater counts in Table 2 would yield the ages listed in column 3 of Table 3 These differ by a factor of 4 from the age estimates based on Hawkins's equation If the estimates are taken at face value, the time span between the formation of the lavas $(A+B)$ and $(C+D)$ is of the order of 10^7 or 10^8 yr The floor of Tycho and the lava lakes solidified $\sim 10^8$ yr later than the lava flows $(A+B)$ The figures quoted are illustrative only, but we stress that, even if the ages are in error by an order of magnitude, there must still be wide intervals of time between the formation of the various lava fields We therefore believe that polycyclic eruptions characterized at least part of the formation of Tycho

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[1] Pettengill, G H , and Henry, J C , *J Geophys Res* , **67**, 4881 (1962)
[2] Shorthill R W , Borough, H C , and Conley, J M , *Pub Astro Soc Pacific* **72**, 481 (1960)
[3] Fielder, G , *Observatory*, **82**, 196 (1962)
[4] Urey, H C , *Sky and Tel* , **15**, 161 (1956)
[5] Hartmann, W K , *Commun Lunar and Planet Lab Univ Ariz* , **2**, 184 (1964)
[6] Hawkins, G S , *Astron J* , **65**, 318 (1960)
[7] Öpik, E J , in *Progress in the Astronautical Sciences*, **1**, Chap 5, 221, 234 (North Holland Publ Co , Amsterdam, 1962)
[8] Fielder, G , *Planet and Space Sci* , **11**, 1335 (1963)
[9] Nilsson, C , *Science*, **153**, 1242 (1966)

Size Changes in Isolated Nuclei of *Amoeba proteus* on Treatment with Polyionic Substances

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Swelling of nuclei in *Amoeba proteus* is quite independent of the ionic strength of the medium or the osmolarity Rather, it seems that surface charge is important in determining the configuration of a biological membrane which could give rise to large and specific changes in its permeability

SEVERAL authors[1,2] have suggested mechanisms for amoeboid movement which locate the source of locomotory energy in contracting membranes While considering which molecular mechanisms are involved in such contractions, the studies of Kuhn and his collaborators[3] on the interconversion of chemical and mechanical energy in charged polymers led us to wonder whether the cell membrane was behaving in a manner analogous to Kuhn s polyelectrolytes, its degree of extension or contraction being determined by the effective net surface charge density over its surface Experiments performed on intact, moving *Amoeba proteus* and *Naegleria gruberii* will be reported elsewhere We wish to focus attention here on a simpler membrane system, namely, the isolated nucleus of *A proteus* and its response to various polyionic substances

Changes in the volume and size of various isolated organelles—for example, mitochondria[4-6], chloroplasts and microsomes[7] and ribosomes[8]—have often been described, and generally attributed to swelling and shrinking consequent on passage of water across the bounding membrane Experiments with isolated nuclei[9,10], however, suggest that the volume changes that have been observed are induced not by the osmolarity of the suspending medium but by more specific stimuli such as the ionic strength and the presence or absence of certain macromolecules in the solution The nuclear envelope certainly does not constitute an osmotic barrier[9,11], and we considered that in isolation it would provide a useful model for the study of contractile processes in an isolated biological membrane

Amoebae were grown in rice grain cultures in Petri dishes They were removed from such cultures and placed on a glass slide and when they had adhered to it were washed several times in Chalkley's medium Isolated nuclei of *Amoeba* are very sensitive to mechanical injury[12], and puncture of the nuclear membrane with a microneedle leads to a collapse of the nucleus into a shrivelled mass. To avoid such injury, cells were disrupted by the application of an electric field. If two electrodes are dipped into opposite sides of a drop of Chalkley's medium on a slide containing some amoebae and the applied potential is gradually increased by means of a potentiometer, then at a certain value the outer membrane of the cell will be disrupted at several points and the cell contents will rush out into the surrounding medium under the influence of the electrophoretic field. When a nucleus isolated in this manner has been allowed to stick to the slide it can be washed free of other debris by gentle irrigation. Changes in appearance of the nuclei when irrigated with different media were observed by phase contrast at × 400 magnification and changes in size were measured with an ocular micrometer. To avoid mechanical damage no coverslips were used with these preparations.

The nucleus of *A. proteus* has a discoid shape with a rather irregular border when observed in the cell by phase contrast microscopy. During movement, it undergoes some changes in shape. After isolation and washing in Chalkley's medium or in simple salt solutions at *p*H 7·2 (0·14 M and 0·01 M sodium chloride, 0·2 M, 0·01 M and 0·001 M phosphate buffer) nuclei immediately expand and take up a more regular shape. Such nuclei are quite transparent to light and their surfaces seem to be quite smooth and uniform. This swelling phenomenon is quite independent of the ionic strength of the medium or osmolarity, proceeding equally well in distilled water, 0·2 M sodium chloride or 10 per cent sorbitol. Such an expanded nucleus is shown in Fig. 1*A*.

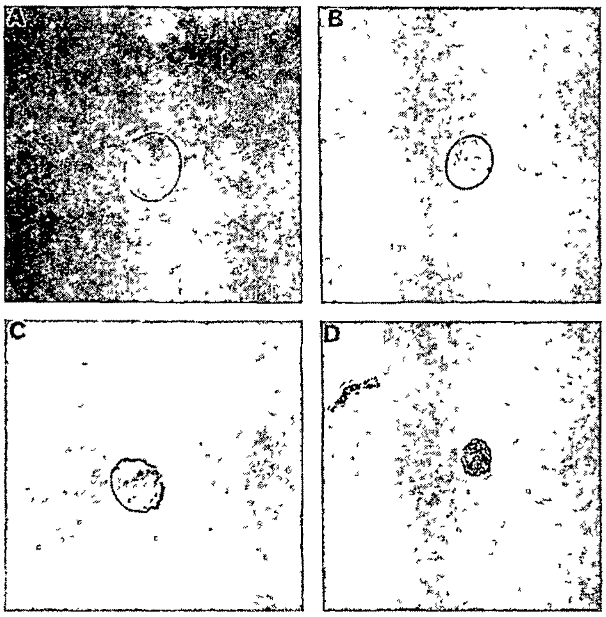

Fig. 1. *A*, Nucleus isolated in 0·14 M sodium chloride, *B*, nucleus in presence of 0·005 per cent poly-L-lysine, *C*, nucleus in presence of 0·1 per cent poly-L-lysine, *D*, nucleus in presence of 0·05 per cent poly-L-lysine.

Poly-L-lysine adsorbs very readily from very dilute solutions (3–5 μg/ml.) onto negatively charged biological membranes as our observations on *N. gruberi* had shown. Irrigation of an isolated nucleus with a 0·05 per cent solution of poly-L-lysine (molecular weight about 50,000) brought about an immediate strong contraction accompanied by changes in appearance of the nucleus. First, the normal intracellular appearance of the nucleus was restored and then further contraction took place within less than a second and a similar effect could be brought about by applying 0·2 per cent protamine, another positively charged polymer. The numerical values observed are given in Table 1, and stages in the contraction are illustrated in Fig. 1*B*, *C* and *D*.

ATP is a tetra-anion and, as might have been predicted on mechanochemical grounds, a 1 mM solution did not stimulate an isolated nucleus to contract. With 1 mM ATP + 1 mM magnesium chloride, however, a very strong contraction was obtained. The surface of the nucleus remained smooth, but the diameter was always much less than inside the cell. This dramatic contraction of the nucleus was specifically dependent on the presence of magnesium ions; substitution of calcium ions gave rise to no measurable contraction.

Nuclei were irrigated with solutions containing 1 mM EDTA, 0·1 per cent heparin or 0·2 per cent sodium polyglutamate, all containing 1 mM magnesium chloride. In all cases a contraction of the nucleus was observed although this was never so strong as in the ATP–magnesium solution. The values which were obtained are recorded in Table 1. All these polyanions in solution without magnesium ions are without effect on isolated, swollen nuclei.

Table 1. EFFECT OF VARIOUS POLYELECTROLYTES ON THE DIAMETER OF ISOLATED NUCLEI OF *A. proteus*

Diameter of nuclei as a percentage of diameter in solution of 0·14 M sodium chloride at *p*H 7·2

Protoplasm	84 ± 5
Medium alone, 0·14 M sodium chloride	100 ± 3
0·2 per cent sodium polyglutamate	101 ± 2
0·1 per cent heparin	104 ± 3
0·1 per cent heparin + 1 mM magnesium chloride	81 ± 5
0·2 per cent protamine	82 ± 4
0·05 per cent poly-L-lysine	77 ± 2
1 mM EDTA + 1 mM magnesium chloride	65 ± 3
1 mM ATP + 1 mM magnesium chloride	58 ± 3

Single nuclei were irrigated with a sequence of the reagents described here applied in various orders. It was found that the appearance and size of nuclei were always determined by the medium bathing the nuclei at the time of observation and bore no relation to their past history. Exceptions to this rule were nuclei which had contracted under the influence of ATP–magnesium or poly-L-lysine which did not recover completely on washing with Chalkley's but which required treatment with polyglutamate or heparin. By treatment of a single isolated nucleus with various media it was possible to follow it through an apparently indefinite number of cycles of expansion and contraction. An example of such an experiment is recorded in Table 2. In Table 3, a similar experiment in the presence of 10 per cent sorbitol shows the independence of the phenomena of osmolarity.

If our analogy with Kuhn's mechanochemical models is correct, change in *p*H and alteration of the degree of ionization of surface groups should also change the configuration of the membrane. Accordingly, nuclei were treated with 0·2 M acetate and phosphate buffers from *p*H 3·6 to 8·0. Fig. 2 represents the mean of results obtained from measurements on ten separate nuclei, the results in each case being expressed as a percentage of the value for the given nucleus at *p*H 8·0. The results show that depression of ionization of acidic groups at low *p*H indeed induced a strong contraction of the nucleus which proved to be quite reversible. There is also the suggestion of a point of inflexion in the curve near to *p*H 5·0 which would suggest that we are not here dealing with the ionization of a single species.

The most commonly accepted molecular theory of contraction is the well known "sliding filament" theory derived from the analysis of the contraction of muscle fibres by light and electron microscopy. It would seem, however, to be only applicable in detail within the relatively complex system of the muscle proteins. Contractile processes in living organisms are not limited to

Table 2 EFFECT OF DIFFERENT MEDIA ON THE SIZE OF AN ISOLATED NUCLEUS OF *Amoeba proteus*

Medium	Protoplasm	Medium alone	0 1 per cent heparin	0 1 per cent heparin +1 mM magnesium chloride	1 mM ATP +1 mM magnesium chloride	0 2 per cent sodium polyglutamate	0 2 per cent protamine	0 1 per cent heparin	0 05 per cent poly-L-lysine	0 2 per cent sodium polyglutamate	1 mM EDTA +1 mM magnesium chloride
Diameter of nucleus (μ)	42	50	53	45	30	49	41	51	38	48	32

All reagents were dissolved in 0 14 M sodium chloride solution at *p*H 7 2

Table 3 EFFECT OF DIFFERENT MEDIA ON THE SIZE OF AN ISOLATED NUCLEUS OF *Amoeba proteus*

Medium	Protoplasm	Medium alone	1 mM ATP +1 mM magnesium chloride	0 1 per cent heparin	0 05 per cent poly-L-lysine	0 1 per cent heparin	0 1 per cent heparin +1 mM magnesium chloride	Medium alone	1 mM ATP +1 mM magnesium chloride	0 2 per cent sodium polyglutamate
Diameter of nucleus (μ)	37	55	34	53	39	52	44	50	30	49

All reagents were dissolved in 10^{-3} M phosphate buffered saline, *p*H 7 2, containing 10 per cent sorbitol

those of muscles but on the contrary are shown by a variety of structures, so it seems to us that the explanation of these processes should stem from a rather more general theory of molecular behaviour than one designed to cope with one specialized molecular array Such a basis is provided by the mechanochemical principles laid down by Kuhn and Katchalsky and their co-workers[3,13] and we wish to discuss our results in these terms

Molecules of flexible polyelectrolytes change their shape in solution as the mean electrical charge density along their length is altered The change in configuration is brought about by a readjustment in the relation between the forces stemming from the Brownian motion of the molecule tending to bring it to a roughly spherical configuration, a force of entropic origin, and the Coulombic repulsive forces between the charges along its length which tend to extend it The higher the net charge on the molecule the greater will be its extension and asymmetry

In their reaction with hydrogen ions at low *p*H and with polylysine, nuclei of amoebae behave exactly analogously to polyanions in solution, collapsing as their overall surface charge density is reduced It is of considerable interest that three polyanionic materials will induce contraction of the nucleus when in the presence of magnesium ions although they have, as we would have predicted, no effect on their own This result suggests strongly that a mechanism similar to that proposed by Morales[14] for the interaction of ATP and magnesium with myosin is in operation He has suggested that the quadrivalent anion could be bound by ion-triplet bridges, with magnesium being the linking ion to the anionic sites on the myosin molecule, thus reducing its overall charge and causing a contraction as indicated here It is evident from these experiments that anions other than ATP are capable of fulfilling this role

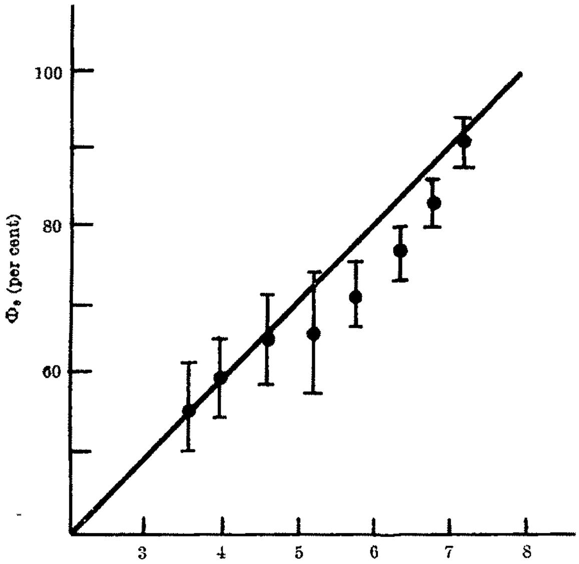

Fig 2 Effect of *p*H on the size of isolated nuclei of *A proteus* Ordinate, diameter expressed as a percentage of the average diameter at *p*H 8, abscissa *p*H of surrounding medium

Davies and Spencer[15] have ascribed the swelling of nuclei in various media containing heparin to competition between heparin and DNA for binding to histone and consequent expansion of negatively charged DNA molecules The experiments described here make this suggestion unlikely, especially because heparin can induce a nuclear contraction in the presence of magnesium ions and they draw attention to the importance of the ionic milieu in which fixation is performed when interpreting light or electron micrographs

Bryn Jones[16] has shown that addition of ATP to tissue culture cells in the presence of magnesium leads to a reduction of adhesiveness which he ascribes to a contraction of the membrane surface as a result of the presence of actomyosin proteins at the surface

Experiments with intact *A proteus* indicate that contractions can be brought about at the periphery of the cell by local application of polylysine to an advancing pseudopodium, which are similar in appearance to the natural tail of the amoeba (Gingle, Korohoda and Wolpert, unpublished) We feel that these experiments show the importance of surface charge in determining the configuration of a biological membrane which could give rise to large and specific changes in its permeability Whether these changes are themselves capable of providing the conversion from chemical to mechanical work or whether they are the initiators of the contraction of actomyosin molecules at or adjacent to the cell surface requires further experimental work to decide

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[1] Holtfreter, J , *Ann NY Acad Sci* , 49, 709 (1948)
[2] Goldacre, R J , *Exp Cell Res* , suppl 8, 1 (1961)
[3] Kuhn, W , Ramel, A , and Walters, D H , in *Size and Shape Changes of Contractile Polymers* (edit by Wassermann) (Pergamon, 1960)
[4] Lehninger, A L , *Physiol Rev* , 42, 467 (1962)
[5] Wojtczak, L , Wlodawer, P , and Zborowski, J , *Biochim Biophys Acta*, 70, 290 (1963)
[6] Suranyi, E M , and Avi-Dor, Y , *Biochim Biophys Acta*, 118, 445 (1966)
[7] Robinson, J D , *Arch Biochem Biophys* , 113, 526 (1966)
[8] Gesteland, R F , *J Mol Biol* , 18, 445 (1962)
[9] Merriam, R W , *J Biophys Biochem Cytol* , 6, 353 (1959)
[10] Anderson, N G , and Wilbur, K M , *J Gen Physiol* , 35, 781 (1952)
[11] Wiener, J , Spiro, D , and Loevenstein, W R , *J Cell Biol* , 27, 107 (1965)
[12] Kopac, M J , in *The Cell* (edit by Brachet and Mirsky), 1, 161 (1959)
[13] Katchalsky, A , *Biophys J* , 4/1 Pt 2, 9 (1964)
[14] Morales, M F , *Rev Modern Physics*, 31, 426 (1959)
[15] Davies, H G , and Spencer, M , *J Cell Biol* , 14, 445 (1962)
[16] Jones, Bryn W , *Nature*, 212, 362 (1966)

Distribution of Plasma Fatty Acid before and after Canine Hepatic Homograft

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The effect of liver transplantation on the fatty acids in the plasma of dogs suggests that fat is mobilized and dehydrogenated after the operation

ELUCIDATION of lipid metabolism in the liver transplant recipient has been hampered by the fact that survival rarely exceeded 15 days As surgical techniques improve and prolonged liver transplantation survival becomes imminent, it is increasingly important to look at the physiochemical role of the transplanted liver to determine its capacity for normal metabolic function The liver is primarily involved in lipid metabolism, and so its function may well be reflected in quantitative or qualitative changes in plasma lipid and fatty acid values

The livers of three healthy unrelated mongrel dogs were completely removed and replaced by an orthotopic hepatic homograft obtained from unrelated dogs[1] Azathioprine and antibiotics were administered daily after the operation The diet consisted of commercial dog food freely given Blood from the peripheral venous system of a fasting dog was collected in ethylenediaminetetraacetate (EDTA) pre and post-operatively twice a week for clinical chemistry and lipid analysis Clinical chemistry and liver function tests were performed routinely and are reported elsewhere[1,3]

The "lipid profile", consisting of total, free and esterified cholesterol (CE), phospholipid (PL), non-esterified fatty acid (NEFA) and triglyceride (TG) were analysed by chemical procedures[4-7]

Isolation of the lipid classes was accomplished by extracting the lipids from 1 ml of plasma with chloroform methanol (3 2 v/v) and then spotting the extract on a

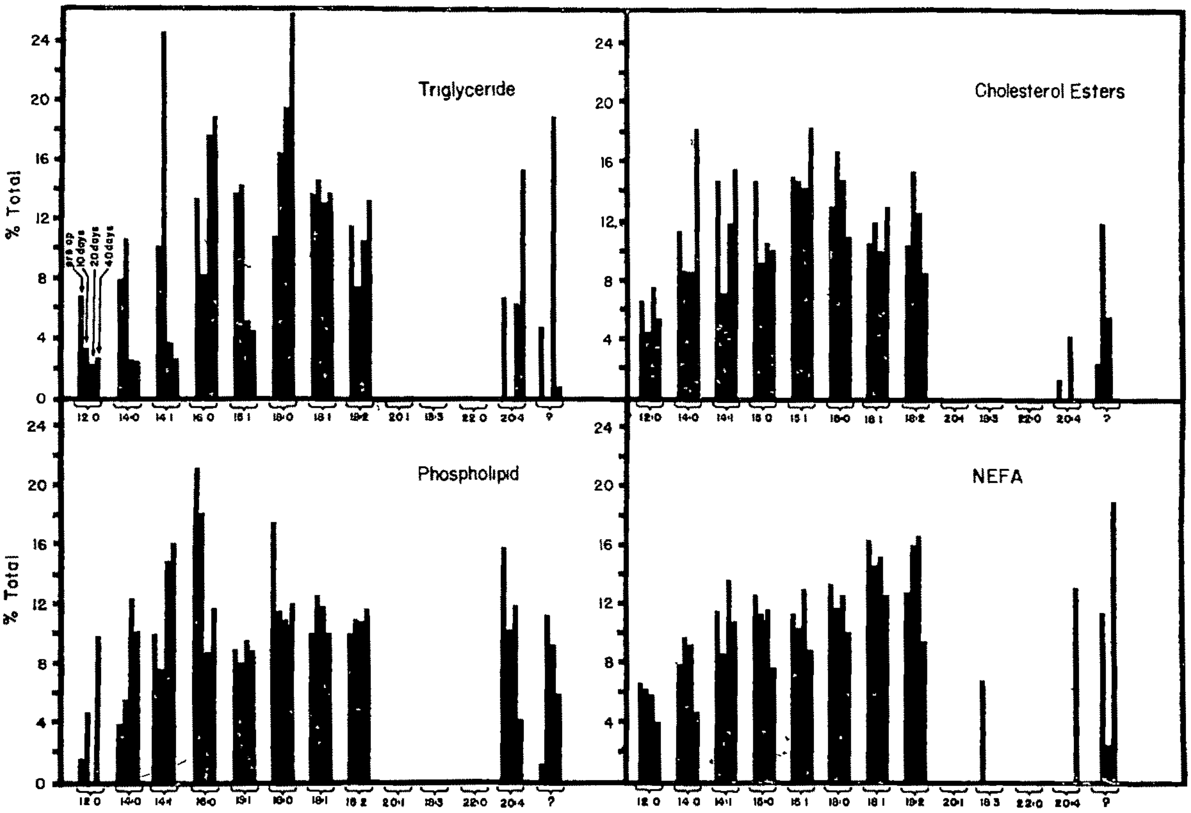

Fig 1 Total percentage of plasma fatty acids (GLC) in a canine hepatic homograft (HHM-12) The bar graph shows the fatty acids, by carbon chain length, for each of the four periods

thin-layer chromatograph (TLC) plate coated with 1 mm of silica gel *G* The solvent used consisted of redistilled Skelly Solve *B* ethyl ether glacial acetic acid (70 30 1 v/v) A standard was applied to each TLC plate to locate the four lipid classes (1) phospholipid, (2) NEFA and cholesterol, (3) triglycerides and (4) cholesterol ester[8] The plates were run, standard developed and sample fractions were immediately scraped into a test tube and extracted from the silica gel with chloroform methanol (3 2 v/v) These four fractions were individually saponified, esterified and metholated by a modification of Metcalfe's[9] methanol-boron-trifluoride fatty acid metholation procedure

The fatty acids were analysed in a Barber Colman Model-20 gas chromatograph (GLC) which was equipped with an argon–tritium ionization detector The column specifications are ethylene glycol succinate (15 per cent) on 'Chromsorb' (100–110 mesh) support, inlet pressure of 30 p s i. argon and column temperature of 185° C Hormel Institute standards were used before and after each day's run The peaks were calculated by the "absolute-peak-area" method (height × width at 1/2 height) using pure fatty acids as qualitative standards The results are expressed as percentage total for each fatty acid Each fatty acid is represented by a pre-operation, 10, 20 and 40 day post-operation value for each dog[2]

Early liver injury was observed in each of the three dogs as determined by standard liver function tests Only one of the animals (HHM-5) developed hyperbilirubinaemia and died after 100 days There was in each animal an increase in alkaline phosphatase serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) More detailed data have been presented before[1]

The lipid profile showed that total and esterified cholesterol and phospholipid dropped to about 50 per cent of the pre-operation value in the first 10 days but returned to the pre-operation range in 30–40 days Triglycerides remained in the pre-operation range[10] The NEFA fluctuated but exhibited no obvious trend

The fatty acid data are reported in two ways (1) by a graph showing a representative fatty acid pattern in one animal (HHM-12) for each lipid class (Fig 1), and (2) by a bar graph (Fig 2) showing the unit changes in short chain versus long chain and saturated versus unsaturated fatty acids in each dog as related to the individual animal's own pre-operation value The lipid class is reported during each of the four periods of the study

For convenience in this study, the fatty acids were divided into two groups Fatty acids from C 12 0 lauric to C 16 1 palmitoleic are designated as shorter chain fatty acids Long chain fatty acids are designated C 18 0 stearic to the unidentified peak which is present after C 20 4, arachidonic This unidentified peak (retention time t_R 60 min) appears after C 20 4 (t_R 50 min) but before C 24 0 (t_R 66 min) The peak is not present after hydrogenation, indicating that it is an unsaturated fatty acid

Changes occurring in the individual fatty acids of dog HHM-12 during the four time periods are illustrated in Fig 1 This dog's fatty acid pattern appears to be the median of the three dogs A typical prevalence of long chain fatty acids in the phospholipid fraction is present It is also interesting to note that in the NEFA fraction there is a general quantitative increase as the chain length increases

The data in Fig 2 are an expression of the unit variation between the pre-operation value and each of the three post-operation periods for each animal Each animal acts as its own control There is a noticeable increase in the long chain fatty acids in two of the dogs but not in dog HHM-5 Here the shorter chain fatty acids increase After transplantation the unsaturated fatty acids increase in all classes except HHM-12's triglyceride and HHM-5's phospholipid fraction, as seen in Fig 2 It is interesting to note that there is a fatty acid ratio of 40 per cent

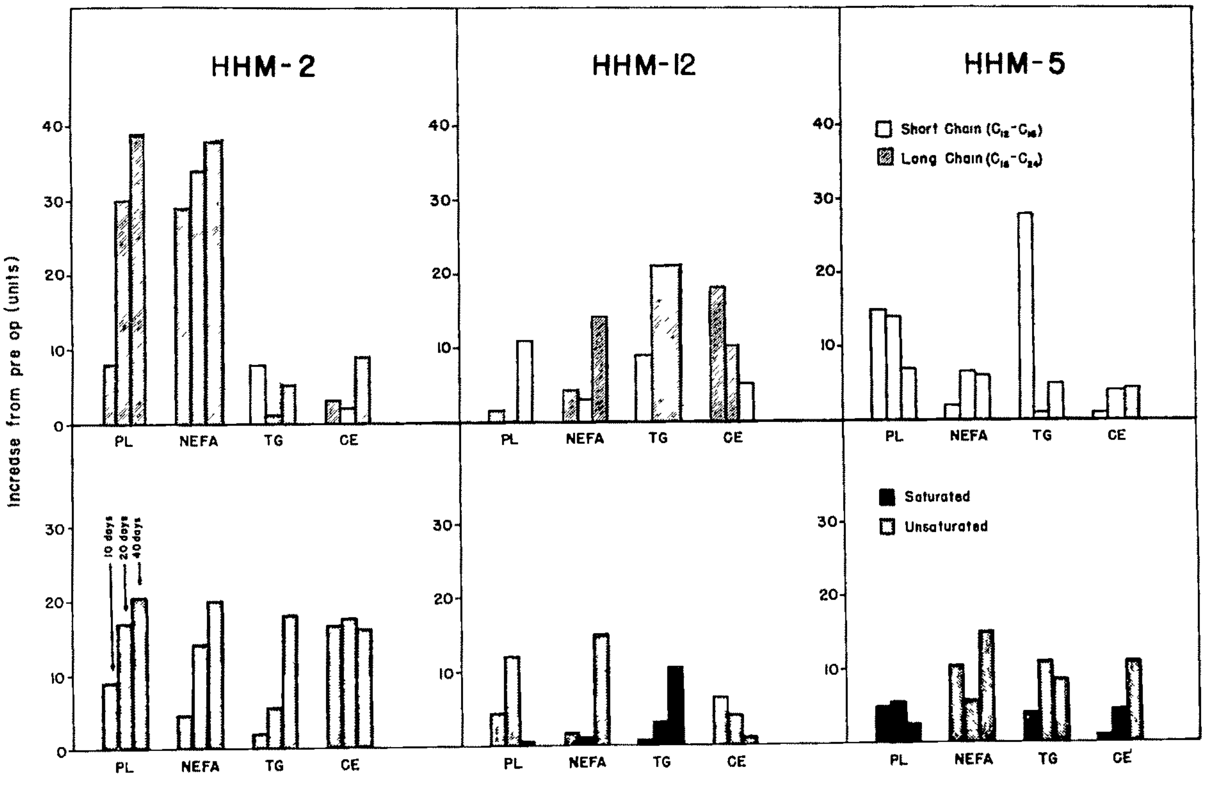

Fig 2 The data on this graph were derived from the difference between plasma fatty acids (GLC) pre-operative and post-operative for each of the three periods Each animal is its own control The results express the unit change after transplantation

saturated to 60 per cent unsaturated which remains constant for all dogs pre and post-operatively

The fatty acids most prevalent in the dog food were oleic, palmitic, linoleic, stearic and linolenic, in that order Neither the unknown peak nor the C 20 4, arachidonic, peaks were present

Dog HHM-2 differs from the other two dogs in that there is a larger percentage (53–67 per cent against 39–47 per cent) of saturated fatty acids in all classes in the pre-operation sample This is because of a high proportion of C 12 0 and C 14 0 in the phospholipid, NEFA and cholesterol ester fractions as well as C 16 0 and C 18 0 in the triglyceride fraction By the fortieth day the saturated fatty acids of dog HHM-2 had dropped to the range (36–51 per cent) of the other two dogs

As may be expected, there is more continuity between fatty acid changes within each dog rather than between the three dogs Two of the dogs (HHM-2, HHM-12) show a general increase in long chain fatty acids, while one dog (HHM-5) exhibits an increase in shorter chain fatty acids There is a general increase in the unsaturated fatty acids for all dogs, the exceptions being in dog HHM-5 phospholipid and cholesterol ester fractions and dog HHM-12 triglyceride fraction

The plasma of two dogs (HHM-2 and HHM-12) and the control dogs contains an increased amount of long chain unsaturated fatty acids This may be a result of a mobilization of the adipose tissue, for long chain fatty acids are found primarily in fat stores Carter[11] states that fatty acids with less than twelve carbon atoms cannot be stored in the animal's body and so must be oxidized as they are absorbed

Another factor which could cause an increase in long chain unsaturated fatty acids is that, theoretically, animal tissue cannot synthesize linoleic acid (C 18 1) The animal therefore depends entirely on an external source of supply, namely the diet The third largest fatty acid content in the dog food was linoleic acid Once present in the cell it undergoes a sequence of desaturation and elongation to form arachidonic acid and other long chain polyunsaturated fatty acids[12]

It is obvious that the enzyme systems of the transplanted liver are affected, for alkaline phosphatase, SGOT and SGPT are altered, as already noted[1,3] Hexadecanoyl dehydrogenase, the enzyme which acts on the acyl Co *A* derivatives of the long chain fatty acids, may therefore also be altered

Dog HHM-5 was the one which exhibited a rise in the shorter chain unsaturates, notably in the phospholipid fraction This dog died of acute pneumonia a little more than 100 days after the operation Further research will be necessary to clarify this interesting observation, for pneumonia is one of the major causes of death in liver transplants

Temporary plasma lipid changes in liver transplant dogs seem to be caused by immunogenic and operative trauma Plasma lipids of control animals (without transplant livers) were not obviously affected by the immunologic therapy but there may be subtle changes in fatty acid metabolism

In the hepatic homograft, mobilization of long chain fatty acids from the adipose tissue may be a natural defence mechanism to supply the liver with "essential fatty acids" for phospholipid synthesis Phospholipids in turn play an important part in the reduction of excess fat in the liver and alveoli function in the lung

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[1] Starzl, T E , Marchioro, T L , Porter, K A , Taylor, P D , Faris, T D , Hermann, T J , Hlad, C J , and Waddell, W R , *Surgery*, **58**, 131 (1965)

[2] Worth, W S , and Miller, N L , *Fed Proc* , **24**, 2401 (1965)

[3] Starzl, T E , Marchioro, T L , Huntley, R T , Rifkind, D , Rowlands, jun , D T , Dickinson, T C , and Waddell, W R , *Ann NY Acad Sci* , **120**, 739 (1964)

[4] Dole, V P , and Meinerty, H , *J Biol Chem* , **235**, 2595 (1960)

[5] Fiske, C H , and Subbarow, Y , *J Biol Chem* , **66**, 375 (1925)

[6] Searcy, R L , and Bergquist, L , *Clin Chim Acta*, **5**, 192 (1960)

[7] Van Handel, E , and Zilversmit, D E , *J Lab Clin Med* , **50**, 152 (1957)

[8] Randerath, K , *Thin-Layer Chromatography* (Academic Press, 1963)

[9] Metcalfe, L D , and Schmitz, A A , *J Biol Chem* , **33**, 363 (1961)

[10] Worth, W S , Miller, N L , and Taylor, P T , *Nature*, **211**, 78 (1966)

[11] Carter, H E , *Biol Symp* , **5**, 47 (1941)

[12] Dawson, R M C , and Rhodes, D N , *Conference on Metabolism and Physiological Significance of Lipids*, 24 (J Wiley and Sons, Ltd 1964)

Numerical Taxonomy and Influenza *B* Virus

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Analysis of the reactions of influenza *B* virus with various antisera has clarified the relationships among virus strains

It may not be easy to see immunological relationships within several virus strains from experimental data, such as those in Table 1, which shows titres from a cross-haemagglutination inhibition (HAI) test involving strains of influenza *B* For clarification, these data have been subjected to numerical taxonomic analysis The methods, which may be valuable in predicting pathogenicity of newly isolated strains and in determining polyvalence of vaccines required, should be useful for summarizing information about a variety of other organisms

The values given in Table 1 were "normalized" by a logarithmic transformation Virus strains were then compared two at a time, the reactions of a virus strain with each of the antisera being considered as multivariate observations on that strain Thus row 1 of Table 1 contains eleven observations on virus V_1, row 2 contains eleven observations on V_2, and so on From each replicate of the experiment there would be eleven additional observations on each strain As a measure of the similarity between V_1 and V_2, the sample product-moment correlation coefficient r was computed for the eleven pairs of (logarithmic) observations For V_1 and V_2, $r = 0\ 82$ Correlations calculated from data in Table 1 (after logarithmic transformation) are given in Table 2 V_{10} and V_{11} are most closely related because their correlation coefficient is 0 98, V_1 and V_4 have a correlation of 0 00 and are not related V_1 and V_6 have the strongest negative relationship ($r = -0\ 59$)

Table 1 CROSS-HAEMAGGLUTINATION INHIBITION TEST WITH INFLUENZA *B* STRAINS FROM 1940–61

Test strain and passage history	Immunizing strain S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}
V_1 *B*/Lee/40 *F*8–*M*137–*E*179	768	512	<8	<8	<8	<8	<8	<8	<8	24	8
V_2 *B*/Lee/40 *F*13*A*–*M*17–*E*3	256	1,536	32	24	48	12	64	16	48	24	24
V_3 *B*/*TM*/40 *F*2–*M*24–*E*5	48	<8	384	12	16	<8	12	<8	<8	<8	48
V_4 *B*/Mel/Bon/43 *E*8	48	48	<8	96	64	256	192	16	48	32	24
V_5 *B*/*AA*/Chaddick/45 *E*17	<8	<8	<8	48	64	128	48	<8	<8	8	<8
V_6 *B*/*AA*/Allen/45 *E*31	<8	<8	<8	48	32	256	192	16	16	24	32
V_7 *B*/*AA*/6/52 (Lwy) *E*8	16	<8	<8	24	96	64	128	<8	24	8	16
V_8 *B*/*GL*/1739/54 *E*4–*M*10–*E*15	12	64	24	48	48	96	256	96	256	192	384
V_9 *B*/*AA*/1/55 *E*4	16	32	16	24	24	96	256	64	256	192	512
V_{10} *B*/*AA*/1/59 *E*4	16	32	32	24	32	24	64	64	96	1,024	1,024
V_{11} *B*/Tokyo/*M*27/61 *MK*1–*E*3	<8	<8	<8	<8	<8	8	16	12	48	512	512

S_1 to S_{11} represent antisera from ferrets immunized with virus strains V_1 to V_{11}, respectively Homologous titres are underlined The value <8 was interpreted as 4

This matrix of correlations was analysed for "clusters" or "families" of virus strains One method used was Sokal and Michener's clustering by average linkage (weighted pair-group method, WPGM)[1], by which admission of a strain into a cluster is based on an average of the correlations of that strain with the existing members of the cluster Results of this analysis are represented by a dendrogram (Fig 1) The abscissa is used only to separate virus strains, whereas the ordinate shows the similarity coefficient (correlation) scale from +1 00 to −0 40 For example, V_5 and V_6 are linked at 0 82 and their average correlation with V_7 is 0 78 When correlation is 0 90 there are two clusters and seven individual strains; and when correlation is 0 75 four clusters of viruses are seen (V_1, V_2), (V_4, V_5, V_6, V_7), (V_8, V_9, V_{10}, V_{11}) and (V_3) An investigator wishing to prepare a vaccine against these strains might determine a reasonable level of correlation and include one strain from each of the clusters seen at that level

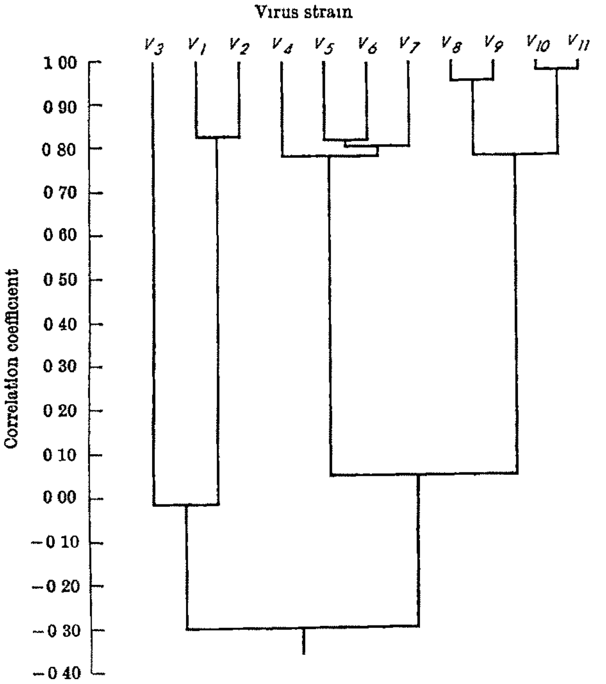

Fig 1 Dendrogram showing relationships between strains of influenza *B* virus from analysis of correlations in Table 2 by Sokal and Michener's clustering by average linkage procedure (WPGM using simple averages)

Table 2 CORRELATIONS BETWEEN VIRUS STRAINS

	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}
V_1	0 82	−0 01	−0 00	−0 41	−0 59	−0 37	−0 45	−0 36	−0 18	−0 17
V_2		−0 02	0 04	−0 35	−0 57	−0 22	−0 33	−0 34	−0 31	−0 35
V_3			−0 56	−0 24	−0 37	−0 14	−0 46	−0 36	−0 11	−0 17
V_4				0 75	0 73	0 78	0 23	0 23	−0 23	−0 12
V_5					0 82	0 80	0 04	−0 03	−0 32	−0 28
V_6						0 77	0 54	0 51	0 12	0 19
V_7							0 23	0 25	−0 16	−0 07
V_8								0 96	0 74	0 77
V_9									0 77	0 84
V_{10}										0 98

Principal components analysis[2] provided a second means of identifying clusters of virus strains From data in Table 1 each virus strain was considered as a point in eleven dimensional space the co-ordinates of which correspond to the eleven observations (titres) for the virus Analysis of correlations in Table 2 revealed that most of the variation (87 per cent) in the original data occurs in a three dimensional space, which corresponds to the first three principal components of the correlation matrix Fig 2 depicts the eleven virus strains with respect to these principal component axes Four clusters of virus strains are seen (V_1, V_2), (V_4, V_5, V_6, V_7), (V_8, V_9, V_{10}, V_{11}) and (V_3) These clusters are essentially the same as those appearing in the dendrogram, which is reassuring because principal components analysis is mathematically far more sophisticated than the average-linkage procedure

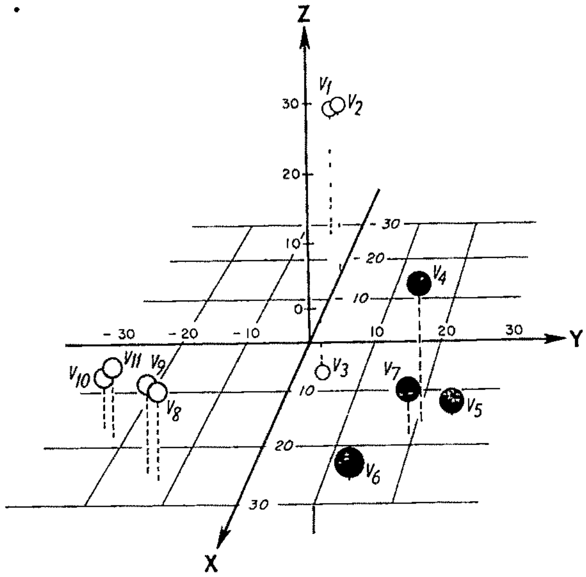

Fig 2 Graph of first (*X*), second (*Y*) and third (*Z*) principal components for eleven strains of influenza *B* virus The *Z* scale has been elevated 0 05 units above the *X*–*Y* plane to simplify presentation of "virus" points the *Z* co-ordinate of which is less than zero

The results indicate that these strains of influenza virus fall into groups according to the date of their isolation This is not a new observation, of course, but the relationships are more clearly visualized by numerical taxonomy

Computer-simulated experiments were conducted to evaluate these two methods of cluster analysis and two further methods (*a*) McQuitty's elementary linkage analysis[3] and (*b*) Sneath's clustering by single linkage[4] Artificial data, resembling real data from HAI experiments involving seven or ten virus strains, were obtained by sampling from twenty-one different multivariate normal populations The computer experiments were planned so that the "virus strains" could be expected to cluster in given patterns, correlations between strains within the same cluster ranged from about 0 60 to 0 95 in normal populations from which samples were drawn The four methods of cluster analysis were compared on the basis of the proportion of samples from each population for which the "virus strains" were "correctly" clustered.

Ease of computation was another basis for comparison of methods, and both two-replicate and unreplicated computer experiments were examined

Several conclusions were drawn from the analysis of computer experiments (*a*) When the correlation coefficient is used to measure similarity between virus strains, classifications based on two-replicate experiments are more reliable, as might be expected, than those based on unreplicated experiments regardless of the method of cluster analysis employed (*b*) Sokal and Michener's average-linkage analysis and principal components analysis are comparably "accurate" in classifying the number of virus strains used in this study (up to ten) when the correlation coefficient is the measure of similarity (The methods of average-linkage analysis and principal components analysis seem to complement each other, although the end results of the two differ somewhat in appearance, while the former method is simpler computationally, one might perform both analyses if possible) (*c*) McQuitty's elementary linkage analysis tends to yield too many clusters and is the least satisfactory of the four procedures investigated

Sneath s clustering method, which bases admission of a virus strain to a cluster on a single linkage of the virus with any individual member of the cluster, is logically less appealing than Sokal and Michener's technique, which admits a virus strain to a cluster on the basis of an average linkage with members of the cluster

Numerical taxonomic methods other than those presented here seem to be useful in the study of influenza Of the many types of cluster analysis, only four of the more widely known were included in the present study Various measures of similarity between virus strains, among them a normalized Euclidean distance function, have been considered instead of the correlation coefficient The mean titre ratio[5], a commonly used measure of serological similarity between two strains of influenza virus, is not recommended for numerical taxonomic studies, because it utilizes little of the available information in making a comparison

Numerical taxonomy affords a means for simplifying visualization not only of the results of serological tests, as presented here, but also of other biological entities on which a number of quantitative measurements have been made

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[1] Sokal, R , and Michener, C , *Univ Kansas Sci Bull* , 38, 1409 (1958)
[2] Anderson, T , *An Introduction to Multivariate Statistical Analysis*, 272 (Wiley, New York, 1958)
[3] McQuitty, L , *Educ Psychol Meas* ,17, 207 (1957)
[4] Sneath, P , *J Gen Microbiol* ,17, 201 (1957)
[5] Archetti, I , and Horsfall, F , *J Exp Med* , 92, 441 (1950)

Biological Phase Relationships

by

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By treating hydra as a multi-phase system with each cell type constituting a separate biological phase the minimum number of components necessary to control cell differentiation can be determined

BURNETT[1] has suggested that growth and cell differentiation in hydra are regulated by two factors which he called stimulator and inhibitor He further suggested that the relative concentrations of these two factors account for the morphological changes which occur along the body column of the hydra Webster[2] has proposed a slightly different model in which he suggested that the interrelation of two factors—"level of inhibition" and "threshold for inhibition"—controls hypostome formation in hydra Others (for example, ref 3) also suggest that polarity in hydra is controlled by the interrelation of two factors or materials

Recent experiments[4-6], however, have suggested that inorganic ions control differentiation in some situations The authors have suggested that cellular control can be effected by the modification of membrane properties by hormonal substances or growth factors. A change in membrane selectivity caused by growth factors would cause intercellular ionic compositions to change A change in ionic composition is then assumed to cause a change in cell properties, that is, differentiation or growth I do not intend to argue this hypothesis, but rather to indicate a possible method for determining the minimum number of controlling factors for cell differentiation The argument to be presented relates specifically to hydra, but it is applicable to other biological systems as well, and the reference to hydra was selected primarily for convenience

Biological Phases

Biological systems can be characterized as heterogeneous systems composed of separate and distinct elements called cells, spaces and fluids If we take all of the elements of a particular type (say, epithelial cells) they might be said to constitute a single biological phase Each biological phase must be distinct and must be separated from the other phases, but a given phase may itself be broken into parts as long as each portion of a phase is the same as every other portion of that phase This is analogous, for example, to an ice–water system in which ice constitutes a single phase whether it is a single piece of ice or many pieces It will be assumed that the state of a biological system is determined by the phases and the controlling components which determine the phases The controlling components are the constituents of one or more of the phases which are responsible (that is, causal factors) for the presence of the various phases That is, they are the minimum set of constituents the relative concentrations of which determine the relative amount of each phase The constituents, including the special set called components, constitute the entire biological system Each biological phase is recognized as being distinct morphologically because of the presence of distinct constituents For a biological system in steady state the mole fractions of the components in the phases can be assumed to determine completely the state of the system For the co-existence of two biological phases I assume that one independent component is required to describe the state of the system Water is assumed to have a constant concentration throughout the biological system Two solutes, with the mole fraction of one dependent on the mole fraction of the other, are therefore required to establish the relationship between two phases Implicit in this argument is the assumption that by changing the mole fractions of the components the relative amounts of the two phases will correspondingly change just as ice becomes water when the temperature or pressure changes

If three phases co-exist, then the number of independent components required is two, or a system of co-existing biological phases is specified by a minimum of one less independent component than the number of phases Furthermore, it should be noted that the same components could be responsible for the regulation of more than one phase interaction in the same system Before discussing how the phase relations apply to hydra a more precise derivation of the deductions presented here will be given

Derivation of Steady State Phase Relationship

The phase rule was derived by Gibbs[7] to apply to equilibrium systems, and other authors[8-10] in extensive discussions of the derivation or the application of the phase rule consider only equilibrium situations Biological systems, however, are not in thermodynamic equilibrium and therefore, to describe cell differentiation or other biological phase relationships, it is necessary to derive a relationship in which equilibrium is not assumed This is now done

The Gibbs function for constant temperature and pressure is given by

$$dG = \sum_{i=1}^{q} \mu_i dn_i \qquad (1)$$

where G is the Gibbs free energy, μ_i is the chemical potential of the ith component and n_i is the number of moles of the ith component If time derivatives are used, then

$$dG/dt = \sum_{i=1}^{q} \mu_i dn_i/dt \qquad (2)$$

Because G is an additive property, if the system is considered to be composed of r distinct phases with q components then

$$G = \sum_{j=1}^{r} G_j, \text{ and } dG/dt = \sum_{j=1}^{r} \sum_{i=1}^{q} \mu_{ij}\, dn_{ij}/dt \qquad (3)$$

where the double subscript refers to the ith component in the jth phase

A biological system, such as a single hydra polyp, which has a constant shape and a relatively constant composition with time is said to be in steady state That is, any accretion of material is exactly balanced by a corresponding loss of material New cell divisions occur at an approximately uniform rate and are balanced by a loss of cells at the body extremities and food input is balanced by waste product outflow The steady state situation is considered to occur when the average flow of materials, including cell division and cell death, is constant with time Analytically, this is described by saying that the change in the flow or the change in the flux of a material with respect to time is zero, that is, flows are constant At steady state therefore the time derivatives become equal to zero and

$$dG/dt = \sum_{j=1}^{r} dG_j/dt = 0, \text{ or } dn_j/dt = \sum_{j=1}^{i} dn_{ij}/dt = 0 \qquad (4)$$

Thus the flow of the ith component is assumed to be constant throughout the animal and flow between any two phases j and $j+1$ is also constant For constant flow to exist the driving force for the flow or the difference in chemical potential between two phases $(\Delta\mu^{i}_{j\rightarrow j+1})$ for the ith component must be constant There are r phases, and so this gives $r-1$ restricting conditions for each of the q components Or there is a total of $q(r-1)$ internal restricting conditions on the system relating to the distribution of the components which control the biological state

If instead of moles (n) mole percentage composition is used to describe the composition of each phase, then there are $q-1$ independent composition variables required to describe each of the r phases or $r(q-1)$ composition variables for the systems We define the number of degrees of freedom (DF) for the system as the difference between the number of restrictive conditions and the number of composition variables or

$$DF = q(r-1) - r(q-1) = r - q \qquad (5)$$

The degrees of freedom give the number of composition variables that must be arbitrarily selected so that the state of the system may be defined

Phase Relations in Hydra

For hydra the r phases consist of cell types with each cell type being a distinct phase, but all cells of the same type constitute only a single phase The q components are the factors controlling cell differentiation and growth, that is, growth factors, hormones or inorganic ions In hydra there are eleven principal cell types which are contained in two epithelial layers The two layers called the epidermis and gastrodermis are separated by a cell-free mesogloea All the cell types seem to derive from two stem cells, the interstitial or neoblast cell and the epithelial cell The two cell lines have never given evidence of interchanging, that is, a cell in one line differentiating into a cell of the other line The two cell lines seem therefore to be essentially independent, and we are probably dealing with two distinct systems This independence is related to differentiation capabilities and is not meant to suggest that certain positional or gradient effects do not act simultaneously on both cell lines

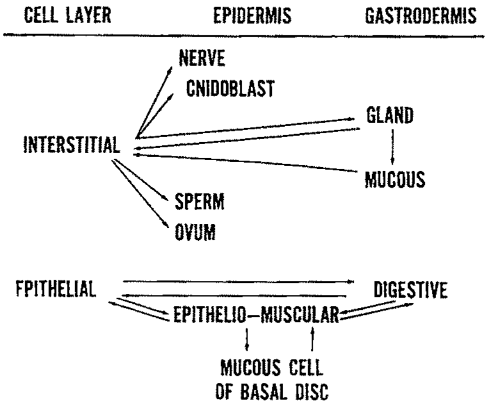

Fig 1 Pathways of cellular differentiation and dedifferentiation in hydra showing location of event by cell layer Cell divisions for both stem cells occur in both cell layers

The stem cells divide and provide a continuing supply of cells which differentiate into all of the other cell types along the pathways shown in Fig 1 The particular cell layer in which each cell type is found is also indicated in Fig 1 We have assumed that there are two distinct cell lines, and so Fig 1 could be redrawn into two figures with triangular co-ordinates (Fig 2), one for each cell line Fig 2 is drawn so that cell types found adjacent in the animal are adjacent in the phase diagram, correspondingly, cell types which do not occur in juxtaposition on the phase diagram also do not usually occur in immediately adjacent areas in the animal Cell division is included as a distinct phase because it can be considered to be a state of differentiation[1] Each cell type (phase) is represented by an area, and cell (or phase) transformations occur across boundaries An intersection of boundaries in Fig 2 represents an invariant association of cell types (phases) Moving away from an intersection along a boundary

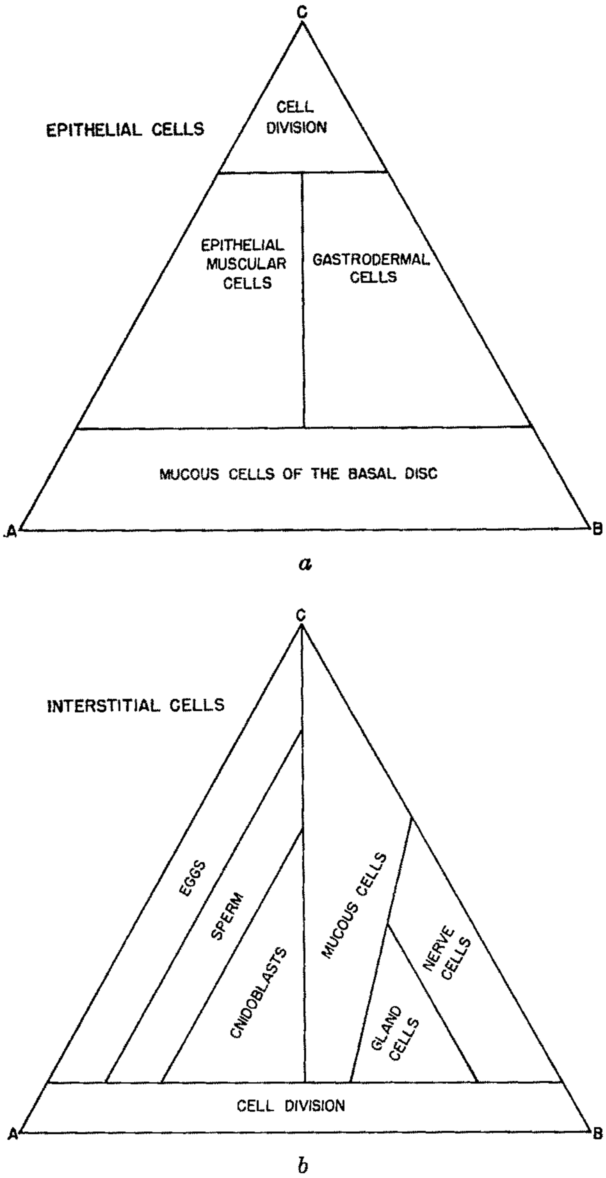

Fig 2 Phase diagrams for epithelial cell and interstitial cell lines An area on the phase diagram represents a single cell type and transformations occur across boundaries The factors *A*, *B*, *C* are shown to indicate how three components may interact to control the phase behaviour (differentiation and division) of the two cell lines The composition at any point on the diagram is found by the intersection of lines drawn perpendicular to the sides of the diagram, the proportion of *B* being read from the side opposite apex *B*, and so on

represents a state with one degree of freedom, that is, one composition variable can be arbitrarily chosen An area represents a state with two degrees of freedom The three variable factors or components, which is the minimal set for zero degrees of freedom, are represented along the three sides of the phase diagram Fig 2 represents the actual situation in hydra, in which three cell types of a given cell line jointly exist only within a very restricted area but two cells from the same cell line co-exist in a much larger area. For each cell line, for DF $=0$, we therefore have $r=3$ for the three co-existing phases at an intersection on the phase diagram; therefore $q=r=3$, or, to explain the observed phase behaviour, at least three components are necessary There is no way of predicting whether or not the same components will be active in controlling both cell lines If the interstitium between cells or the mesogloea is considered as another phase, then a four-dimensional phase diagram would be required and $q=r=4$ at an intersection on the phase diagram Thus three or four components are required to describe the biological phase behaviour in hydra depending on the part played by the interstitium in cell regulation

An alternative way of analysing the phase behaviour of the hydra cells is to ascribe properties to the stimulator (x) and inhibitor (I) substances[1] similar to those usually given in thermodynamics to temperature and pressure Now equation (5) becomes

$$\mathrm{DF} = r-q+2 \tag{6}$$

where 2 includes the variables x and I, and q includes the composition variables This system cannot be easily represented graphically, but we can imagine the x and I substances exerting a thermodynamic stress on a cell, thereby causing a change in $\Delta\mu_i$ between two phases The change in chemical potential causes $dn_i/dt \neq 0$ until a steady state is re-established This effect can be likened to the effect of temperature and pressure on phases under Le Chatlier's principle The two factor system of Burnett or Webster is therefore clearly inadequate to explain the phase behaviour of hydra cells We are therefore fully justified in searching for a three or four component system (which may be mediated by two growth factors) which exerts control over cell differentiation and division in hydra

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[1] Burnett, A L, *Amer Naturalist*, **100**, 165 (1966)
[2] Webster, G, *J Embryol Exp Morph*, **16**, 123 (1966)
[3] Lenicque, P M, and Lundblad, M, *Acta Zool*, **47**, 277 (1966)
[4] Macklin, M, and Burnett, A L, *Exp Cell Res*, **44**, 665 (1966)
[5] Barth, L G, *Biol Bull*, **129**, 471 (1965)
[6] Kroeger, H, *J Cell Comp Physiol*, **62**, Suppl 45 (1963)
[7] Gibbs, J W, *Collected Works*, **1**, 55 (Longmans, 1876)
[8] Bowden, S T, *Phase Rule and Phase Reactions* (Macmillan, 1938)
[9] Campbell, A M, and Smith, N O, *The Phase Rule* (Dover, 1951)
[10] Ricci, J E, *The Phase Rule* (Van Nostrand, 1951)

Evolutionary Rate at the Molecular Level

by
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Calculating the rate of evolution in terms of nucleotide substitutions seems to give a value so high that many of the mutations involved must be neutral ones

Comparative studies of haemoglobin molecules among different groups of animals suggest that, during the evolutionary history of mammals, amino-acid substitution has taken place roughly at the rate of one amino-acid change in 10^7 yr for a chain consisting of some 140 amino-acids For example, by comparing the α and β chains of man with those of horse, pig, cattle and rabbit, the figure of one amino-acid change in 7×10^6 yr was obtained[1].

This is roughly equivalent to the rate of one amino-acid substitution in 10^7 yr for a chain consisting of 100 amino-acids.

A comparable value has been derived from the study of the haemoglobin of primates[2]. The rate of amino-acid substitution calculated by comparing mammalian and avian cytochrome *c* (consisting of about 100 amino-acids) turned out to be one replacement in 45×10^6 yr (ref. 3). Also by comparing the amino-acid composition of human triosephosphate dehydrogenase with that of rabbit and cattle[4], a figure of at least one amino-acid substitution for every $2{\cdot}7 \times 10^6$ yr can be obtained for the chain consisting of about 1,110 amino-acids. This figure is roughly equivalent to the rate of one amino-acid substitution in 30×10^6 yr for a chain consisting of 100 amino-acids. Averaging those figures for haemoglobin, cytochrome *c* and triosephosphate dehydrogenase gives an evolutionary rate of approximately one substitution in 28×10^6 yr for a polypeptide chain consisting of 100 amino-acids.

I intend to show that this evolutionary rate, although appearing to be very low for each polypeptide chain of a size of cytochrome *c*, actually amounts to a very high rate for the entire genome.

First, the DNA content in each nucleus is roughly the same among different species of mammals such as man, cattle and rat (see, for example, ref. 5). Furthermore, we note that the G–C content of DNA is fairly uniform among mammals, lying roughly within the range of 40–44 per cent[6]. These two facts suggest that nucleotide substitution played a principal part in mammalian evolution.

In the following calculation, I shall assume that the haploid chromosome complement comprises about 4×10^9 nucleotide pairs, which is the number estimated by Muller[7] from the DNA content of human sperm. Each amino-acid is coded by a nucleotide triplet (codon), and so a polypeptide chain of 100 amino-acids corresponds to 300 nucleotide pairs in a genome. Also, amino-acid replacement is the result of nucleotide replacement within a codon. Because roughly 20 per cent of nucleotide replacement caused by mutation is estimated to be synonymous[8], that is, it codes for the same amino-acid, one amino-acid replacement may correspond to about 1·2 base pair replacements in the genome. The average time taken for one base pair replacement within a genome is therefore

$$28 \times 10^6 \text{ yr} \div \left(\frac{4 \times 10^9}{300}\right) \div 1{\cdot}2 \doteqdot 1{\cdot}8 \text{ yr}$$

This means that in the evolutionary history of mammals, nucleotide substitution has been so fast that, on average, one nucleotide pair has been substituted in the population roughly every 2 yr.

This figure is in sharp contrast to Haldane's well known estimate[9] that, in horotelic evolution (standard rate evolution), a new allele may be substituted in a population roughly every 300 generations. He arrived at this figure by assuming that the cost of natural selection per generation (the substitutional load in my terminology[10]) is roughly 0·1, while the total cost for one allelic substitution is about 30. Actually, the calculation of the cost based on Haldane's formula shows that if new alleles produced by nucleotide replacement are substituted in a population at the rate of one substitution every 2 yr, then the substitutional load becomes so large that no mammalian species could tolerate it.

Thus the very high rate of nucleotide substitution which I have calculated can only be reconciled with the limit set by the substitutional load by assuming that most mutations produced by nucleotide replacement are almost neutral in natural selection. It can be shown that in a population of effective size N_e, if the selective advantage of the new allele over the pre-existing alleles is s, then, assuming no dominance, the total load for one gene substitution is

$$L(p) = 2\left\{\frac{1}{u(p)} - 1\right\} \int_0^{4Sp} \frac{e^y - 1}{y}\,\mathrm{d}y - 2e^{-4S}\int_{4Sp}^{4S} \frac{e^y}{y}\,\mathrm{d}y + 2\log_e\left(\frac{1}{p}\right) \qquad (1)$$

where $S = N_e s$ and p is the frequency of the new allele at the start. The derivation of the foregoing formula will be published elsewhere. In the expression given here $u(p)$ is the probability of fixation given by[11]

$$u(p) = (1 - e^{-4Sp})/(1 - e^{-4S}) \qquad (2)$$

Now, in the special case of $|2N_e s| \ll 1$, formulae (1) and (2) reduce to

$$L(p) = 4N_e s \log_e(1/p) \qquad (1')$$

$$u(p) = p + 2N_e sp(1-p) \qquad (2')$$

Formula (1′) shows that for a nearly neutral mutation the substitutional load can be very low and there will be no limit to the rate of gene substitution in evolution. Furthermore, for such a mutant gene, the probability of fixation (that is, the probability by which it will be established in the population) is roughly equal to its initial frequency as shown by equation (2′). This means that new alleles may be produced at the same rate per individual as they are substituted in the population in evolution.

This brings the rather surprising conclusion that in mammals neutral (or nearly neutral) mutations are occurring at the rate of roughly 0·5 per yr per gamete. Thus, if we take the average length of one generation in the history of mammalian evolution as 4 yr, the mutation rate per generation for neutral mutations amounts to roughly two per gamete and four per zygote (5×10^{-10} per nucleotide site per generation).

Such a high rate of neutral mutations is perhaps not surprising, for Mukai[12] has demonstrated that in *Drosophila* the total mutation rate for "viability polygenes" which on the average depress the fitness by about 2 per cent reaches at least some 35 per cent per gamete. This is a much higher rate than previously considered. The fact that neutral or nearly neutral mutations are occurring at a rather high rate is compatible with the high frequency of heterozygous loci that has been observed recently by studying protein polymorphism in human and *Drosophila* populations[13–15].

Lewontin and Hubby[15] estimated that in natural populations of *Drosophila pseudoobscura* an average of about 12 per cent of loci in each individual is heterozygous. The corresponding heterozygosity with respect to nucleotide sequence should be much higher. The chemical structure of enzymes used in this study does not seem to be known at present, but in the typical case of esterase-5 the molecular weight was estimated to be about 10^5 by Narise and Hubby[16]. In higher organisms, enzymes with molecular weight of this magnitude seem to be common and usually they are "multimers"[17]. So, if we assume that each of those enzymes comprises on the average some 1,000 amino-acids (corresponding to molecular weight of some 120,000), the mutation rate for the corresponding genetic site (consisting of about 3,000 nucleotide pairs) is

$$u = 3 \times 10^3 \times 5 \times 10^{-10} = 1{\cdot}5 \times 10^{-6}$$

per generation. The entire genome could produce more than a million of such enzymes.

In applying this value of u to *Drosophila* it must be noted that the mutation rate per nucleotide pair per generation can differ in man and *Drosophila*. There is some evidence that with respect to the definitely deleterious effects of gene mutation, the rate of mutation per nucleotide pair per generation is roughly ten times as high in *Drosophila* as in man[18,19]. This means that the corresponding mutation rate for *Drosophila* should be $u = 1{\cdot}5 \times 10^{-5}$ rather than $u = 1{\cdot}5 \times 10^{-6}$. Another consideration allows us to suppose that $u = 1{\cdot}5 \times 10^{-5}$ is probably appropriate for the neutral mutation rate of a cistron in *Drosophila*. If we assume that the frequency of occurrence of neutral mutations is about one per genome per generation (that is, they are roughly two to three times more frequent than the mutation of the viability polygenes), the mutation rate per nucleotide pair per generation is $1/(2 \times 10^8)$, because the DNA content per genome in *Drosophila* is about one-twentieth of that of man[20]. For a cistron consisting of 3,000 nucleotide pairs, this amounts to $u = 1{\cdot}5 \times 10^{-5}$.

Kimura and Crow[21] have shown that for neutral mutations the probability that an individual is homozygous is $1/(4N_e u + 1)$, where N_e is the effective population number, so that the probability that an individual is heterozygous is $H_e = 4N_e u/(4N_e u + 1)$. In order to attain at least $H_e = 0{\cdot}12$, it is necessary that at least $N_e = 2{,}300$. For a higher heterozygosity such as $H = 0{\cdot}35$, N_e has to be about 9,000. This might be a little too large for the effective number in *Drosophila*, but with migration between subgroups, heterozygosity of 35 per cent may be attained even if N_e is much smaller for each subgroup.

We return to the problem of total mutation rate. From a consideration of the average energy of hydrogen bonds and also from the information on mutation of *rIIA* gene in phage T_4, Watson[22] obtained $10^{-8} \sim 10^{-9}$ as the average probability of error in the insertion of a new nucleotide during DNA replication. Because in man the number of cell divisions along the germ line from the fertilized egg to a gamete is roughly 50, the rate of mutation resulting from base replacement according to these figures may be $50 \times 10^{-8} \sim 50 \times 10^{-9}$ per nucleotide pair per generation. Thus, with 4×10^9 nucleotide pairs, the total number of mutations resulting from base replacement may amount to $200 \sim 2{,}000$. This is 100–1,000 times larger than the estimate of 2 per generation and suggests that the mutation rate per nucleotide pair is reduced during evolution by natural selection[18,19].

Finally, if my chief conclusion is correct, and if the neutral or nearly neutral mutation is being produced in each generation at a much higher rate than has been considered before, then we must recognize the great importance of random genetic drift due to finite population number[23] in forming the genetic structure of biological populations. The significance of random genetic drift has been deprecated during the past decade. This attitude has been influenced by the opinion that almost no mutations are neutral, and also that the number of individuals forming a species is usually so large that random sampling of gametes should be negligible in determining the course of evolution, except possibly through the "founder principle"[24]. To emphasize the founder principle but deny the importance of random genetic drift due to finite population number is, in my opinion, rather similar to assuming a great flood to explain the formation of deep valleys but rejecting a gradual but long lasting process of erosion by water as insufficient to produce such a result.

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[1] Zuckerkandl, E., and Pauling, L., in *Evolving Genes and Proteins* (edit. by Bryson, V., and Vogel, H. J.), 97 (Academic Press, New York, 1965).

[2] Buettner-Janusch, J., and Hill, R. L., in *Evolving Genes and Proteins* (edit. by Bryson, V., and Vogel, H. J.), 167 (Academic Press, New York, 1965).

[3] Margoliash, E., and Smith, E. L., in *Evolving Genes and Proteins* (edit. by Bryson, V., and Vogel, H. J.), 221 (Academic Press, New York, 1965).

[4] Kaplan, N. O., in *Evolving Genes and Proteins* (edit. by Bryson, V., and Vogel, H. J.), 243 (Academic Press, New York, 1965).

[5] Sager, R., and Ryan, F. J., *Cell Heredity* (John Wiley and Sons, New York, 1961).

[6] Sueoka, N., *J. Mol. Biol.*, **3**, 31 (1961).

[7] Muller, H. J., *Bull. Amer. Math. Soc.*, **64**, 137 (1958).

[8] Kimura, M., *Genet. Res.* (in the press).

[9] Haldane, J. B. S., *J. Genet.*, **55**, 511 (1957).

[10] Kimura, M., *J. Genet.*, **57**, 21 (1960).

[11] Kimura, M., *Ann. Math. Stat.*, **28**, 882 (1957).

[12] Mukai, T., *Genetics*, **50**, 1 (1964).

[13] Harris, H., *Proc. Roy. Soc.*, B, **164**, 298 (1966).

[14] Hubby, J. L., and Lewontin, R. C., *Genetics*, **54**, 577 (1966).

[15] Lewontin, R. C., and Hubby, J. L., *Genetics*, **54**, 595 (1966).

[16] Narise, S., and Hubby, J. L., *Biochim. Biophys. Acta*, **122**, 281 (1966).

[17] Fincham, J. R. S., *Genetic Complementation* (Benjamin, New York, 1966).

[18] Muller, H. J., in *Heritage from Mendel* (edit. by Brink, R. A.), 419 (University of Wisconsin Press, Madison, 1967).

[19] Kimura, M., *Genet. Res.*, **9**, 23 (1967).

[20] *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation* (New York, 1958).

[21] Kimura, M., and Crow, J. F., *Genetics*, **49**, 725 (1964).

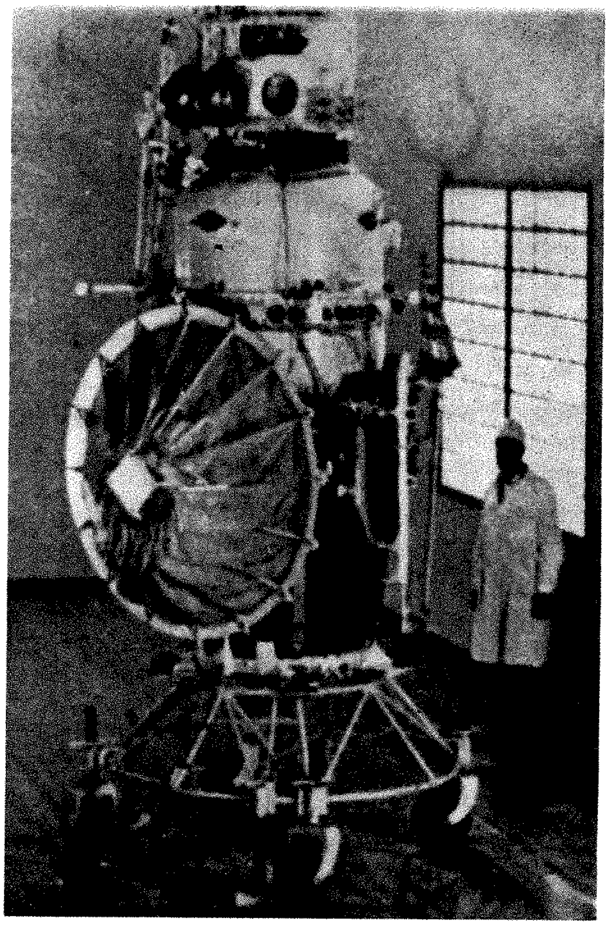
[22] Watson, J. D., *Molecular Biology of the Gene* (Benjamin, New York, 1965).

[23] Wright, S., *Genetics*, **16**, 97 (1931).

[24] Mayr, E., *Animal Species and Evolution* (Harvard University Press, Cambridge, 1965).

The USSR Venera-4 Probe photographed during Preparation for Flight (TASS)

(see opposite)



The Venera-4 probe, weighing nearly 2,500 lb., was launched from the USSR on June 12, 1967. After 128 days in flight, it was parachuted from the space rocket to make a soft landing on the surface of Venus. Information sent back to Earth from the probe was picked up by the radio telescope at Jodrell Bank and was passed to the Russians for decoding. Some of the results are discussed opposite.

LETTERS TO THE EDITOR

PLANETARY SCIENCE

Superadiabatic Atmospheric Layer on Venus, as inferred from the Venera-4 Probe Measurements

THE USSR Venera-4 probe entered the atmosphere of Venus on October 18, 1967, made the first direct measurements of the physico-chemical characteristics of the lower atmosphere and landed smoothly on the planet's surface[1,2]. The probe's whole descent took place on the night side of the planet, close to the equator and about 1,500 km from the terminator. The probe contained two resistance thermometers, an anaeroid barometer transducer, a densitometer and eleven core gas analysers. Immediately after the probe's parachutes opened at an altitude of 26 km, an atmospheric sample was inserted in five cores. The remaining six cores received samples at an altitude of 23 km. All the analysers operated. The carbon dioxide content turned out to be 90 per cent, with less than 10 per cent error. The water or moisture content turned out to be greater than 0·1 per cent and less than 0·7 per cent. In one analyser the oxygen content was greater than 0·4 per cent. In another analyser the oxygen and water content were less than 1·5 per cent, that is, the oxygen content is somewhere between 0·4–1·4 per cent. Finally, the nitrogen content is less than 7 per cent, for an analyser with this response showed no presence of nitrogen. Table 1, derived from these measurements, presents five atmospheres of differing composition for Venus, depending on the relative amounts of carbon dioxide, between the possible limits 80–99·5 per cent. Probably small quantities of argon and other inert gases are present in the atmosphere of Venus as may be deduced from the composition.

Table 2 presents the temperature and pressure for altitudes from 0 to 25 km. The temperatures are mean values determined from the measurements of the two thermometers; the deviation from the mean in the readings of the two instruments was about ± 7°. From 26 km to the surface, the temperature varied from 313° K to 543° K. The behaviour of temperature with altitude turned out to be almost linear, with a mean temperature gradient of 8·9° km^{-1}. The pressure at the same altitudes varied from 0·7 to 20 atmospheres. The average behaviour of pressure with altitude is shown in Table 2; also, possible errors in its measurement are indicated by minimum and maximum values.

From the data presented in Tables 1 and 2 it is possible to estimate the degree of convective activity in the lower atmosphere of Venus. If the actual temperature lapse rate is represented by γ and the dry-adiabatic rate by γ_d, then there are three possible atmospheric stability conditions

$$\begin{aligned} &\gamma < \gamma_d \text{ stable} \\ &\gamma = \gamma_d \text{ neutral} \\ &\gamma > \gamma_d \text{ unstable} \end{aligned} \tag{1}$$

The necessary condition for the support of vertical motions, up or down, in dry air is a lapse rate greater than the adiabatic rate or a superadiabatic lapse rate ($\gamma > \gamma_d$). The dry adiabatic process operates up to the saturation point of the water vapour. If the greatest possible moisture content of 0·7 per cent obtained by direct measurement of Venera-4 at 23 km is taken, then the condensation level turns out to be about 29 km. If the minimum amount of water vapour (0·1 per cent) is assumed, then the cloud level is about 32 km.

For any gaseous atmosphere,

$$\gamma_d = -\partial T/\partial h = g/C_P \tag{2}$$

where T is the temperature, h the altitude, g the acceleration of gravity and C_P the specific heat at constant pressure of the gas composing the atmosphere. The variation of g with h above any planet can be expressed by

$$g = g_0 R^2/(R+h)^2 \tag{3}$$

where g_0 is the acceleration of gravity at the planet's surface and R is the planet's radius. For Venus[3], $g_0 = 877 \pm 5{\cdot}0$ cm s^{-2} and $R = 6{,}085 \pm 20$ km. The variation of g with altitude is slight in the lower atmosphere of Venus (Table 3). From 1–20 atm, the variation of C_P with pressure is slight for carbon dioxide, molecular nitrogen and argon[4]. The main variation of C_P in equation (2) is therefore caused by its direct variation with temperature. The temperature decreases with altitude (Table 2), and so C_P also decreases with altitude.

Using values of C_P for individual gases accurate to three decimal places[4], calculations were made of the specific heats of the following four mixtures of gases at the temperatures and pressures listed in Table 2: (*a*) 80 per cent carbon dioxide, 7 per cent nitrogen, 13 per cent argon; (*b*) 85 per cent carbon dioxide, 7 per cent nitrogen, 8 per cent argon; (*c*) 90 per cent carbon dioxide, 7 per cent nitrogen, 3 per cent argon; (*d*) 95 per cent carbon dioxide, 5 per cent nitrogen. Calculations were made also for 100 per cent carbon dioxide. These five cases correspond closely to the five Venus atmospheres presented in Table 2. It should be noted that C_P for carbon dioxide and nitrogen are approximately the same for the assumed conditions. The slight amount of water vapour and molecular oxygen was neglected in the calculations.

The value of the specific heat at constant pressure for a gas mixture, consisting of n different gases, C_P, is[5]

$$\bar{C}_P = \frac{1}{\bar{M}} \sum_i^n C_i M_i C_{P_i} \tag{4}$$

where $\bar{M}$ is the average molecular weight of the gas mixture ($\sum_i^n C_i M_i$); C_i is the proportion of constituent gas i; M_i is the molecular weight of constituent gas i and C_{P_i} is the specific heat at constant pressure for constituent gas i.

Table 1. RELATIVE CONCENTRATION (PER CENT) OF CONSTITUENTS IN LOWER ATMOSPHERE OF VENUS (0–26 KM) FOR VARIOUS PERCENTAGES OF CARBON DIOXIDE[2]

Constituent	Carbon dioxide (per cent) 80	85	90	95	99·5
Water vapour	0·1–0·7	0·1–0·7	0·1–0·7	0·1–0·7	0·1
Molecular oxygen	0·4–1·4	0·4–1·4	0·4–1·4	0·4–1·4	0·4
Molecular nitrogen	0·0–7·0	0·0–7·0	0·0–7·0	0·0–4·5	0
Other inert gases, such as argon	10·9–19·5	5·9–14·5	0·9–9·5	0·0–4·5	0

Table 2. TEMPERATURE AND PRESSURE VERSUS ALTITUDE IN THE VENUS LOWER ATMOSPHERE[1,2]

Altitude (km)	Temperature (° K)	Pressure (atm) Minimum	Average	Maximum
0	543	18·0	20·0	22·0
5	499	12·0	13·1	14·2
10	455	7·9	8·6	9·2
15	410	4·9	5·2	5·6
20	366	2·7	2·9	3·1
25	321	0·9	1·0	1·1

Table 3. ADIABATIC LAPSE RATE (DEG/KM) VERSUS ALTITUDE IN VENUS LOWER ATMOSPHERE FOR FIVE DIFFERENT COMPOSITIONS

Altitude (km)	Temperature (° K)	Pressure (atm)	g (cm s^{-2})	Atmospheric composition 80% CO_2 7% N_2 13% Ar	85% CO_2 7% N_2 8% Ar	90% CO_2 7% N_2 3% Ar	95% CO_2 5% N_2	100% CO_2
0	543	20·0	877·0	8·8	8·6	8·4	8·2	8·2
5	499	13·1	875·2	9·0	8·8	8·6	8·5	8·5
10	455	8·6	874·4	9·3	9·1	8·8	8·8	8·8
15	410	5·2	872·6	9·6	9·4	9·2	9·1	9·1
20	366	2·9	871·7	10·0	9·7	9·5	9·4	9·5
25	321	1·0	870·0	10·4	10·2	10·0	9·9	10·0

Next, using the specific heats calculated from (4) and g from (3), adiabatic lapse rates were computed from (2). The results are given in Table 3, which shows the adiabatic lapse rate in degrees per kilometre in the lower atmosphere of Venus from 0 to 25 km for the five different possible compositions (Table 1).

The actual lapse rate of 8·9° km^{-1} is greater than the adiabatic lapse rates for mixtures in Table 3 containing 90 per cent or greater amounts of carbon dioxide at altitudes up to 10 km. For the 85 per cent carbon dioxide mixture the altitude of the superadiabatic layer is about 5 km, while for the 80 per cent carbon dioxide mixture it is at the surface (0 km). No appreciable changes are made in the values in Table 3 if they are calculated using either the minimum or maximum pressures displayed in Table 2. Likewise, no significant changes result if the calculations are made using temperatures either 10° less or 10° greater at all altitudes.

These detailed calculations therefore indicate that if the lower atmosphere of Venus consists of 85 per cent or more carbon dioxide, as seems probable, then it contains a fairly thick (5–10 km) superadiabatic atmospheric layer at its surface. According to the criterion in (1), the lower atmosphere is unstable and favours large scale vertical motions. These conclusions, based on measurements made on the night side of Venus, should hold even more strongly for the day side. The presence of such a marked convective layer and its influence on dust particles can have profound effects on the dynamics of the lower atmosphere of Venus[6].

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[1] *Pravda*, No. 295 (17977), 6 (October 22, 1967).

[2] Vinogradov, A., *Izvestiia*, No. 257 (15651, October 31, 1967).

[3] Koenig, L., Murray, F., Michaux, C., and Hyatt, H., *Handbook of the Physical Properties of the Planet Venus*, *NASA SP*-3029 (1967).

[4] Hilsenrath, J., *Tables of the Thermodynamic and Transport Properties of Air, Argon, Carbon Dioxide, Carbon Monoxide, Nitrogen, Oxygen, and Steam* (Permagon Press, New York, 1960).

[5] Kennard, E. H., *Kinetic Theory of Gases*, 265 (McGraw-Hill Book Co., New York, 1938).

[6] Davidson, G. T., and Anderson, A. D., *Science*, **156**, 1729 (1967).

Monsoon over the Indus Valley during the Harappan Period

THERE is adequate archaeological evidence[1] that the Harappans, who flourished in the Indus Valley between 2500 and 1700 ± 100 BC, lived in very much moister

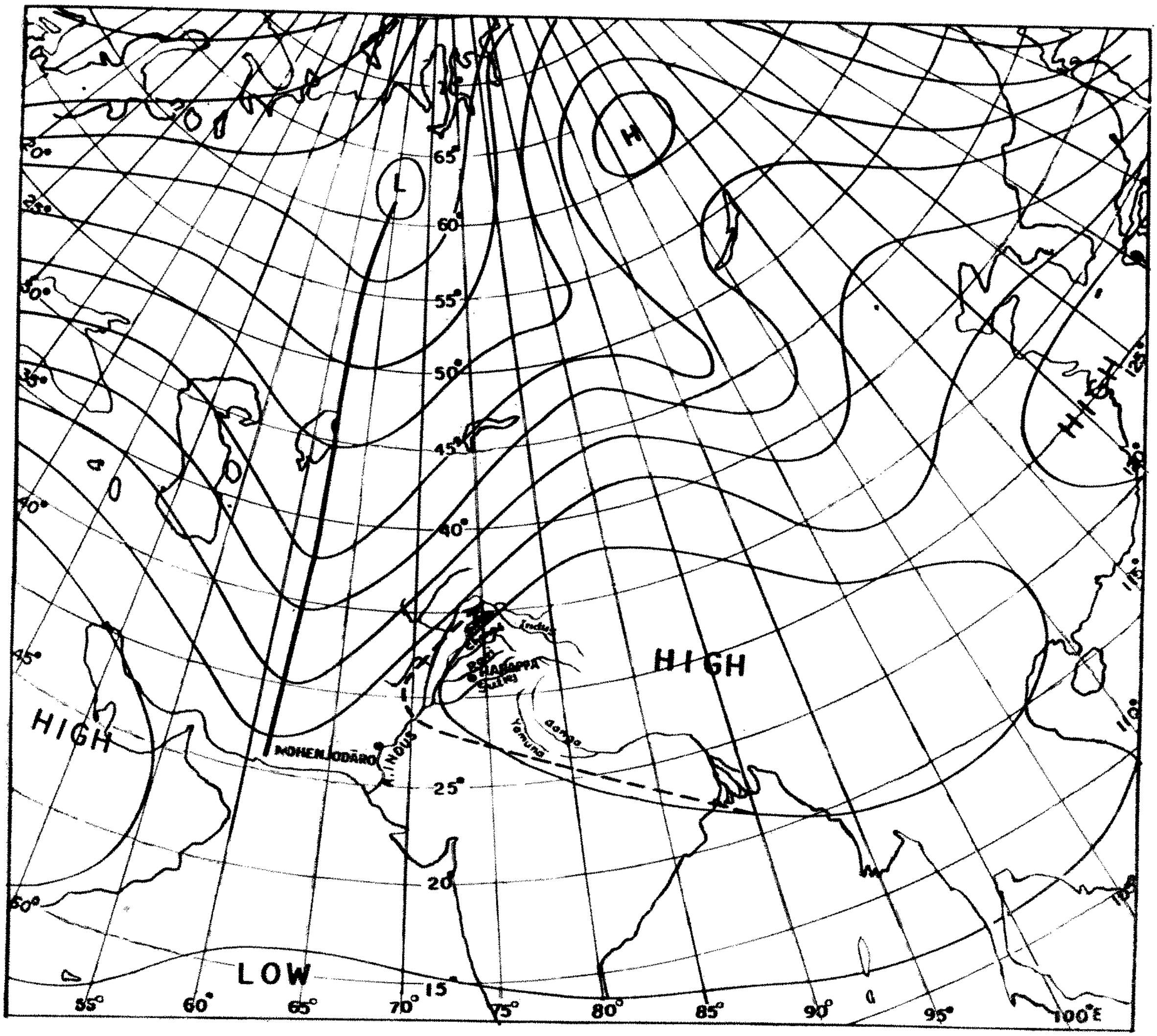

Fig. 1. Schematic 300 mbar flow patterns (approximately 9·0 km above sea level) on a day when a middle-latitude quasi-stationary trough causes a change in the track of a monsoon depression from west-north-west to north or north-east. Note the positions of the famous cities of Mohenjodāro and Harappa and the catchments of the Indus and its tributaries in relation to the middle latitude trough and the track of the monsoon depression before and after it curves to the north. ———, Contours at 300 mbar level; - - - -, track of monsoon depression at sea level.

climatic conditions than those which exist today. Archaeologists[2,3] have also emphasized the occurrence of intermittent floods in the Indus valley during the Harappan period. These conclusions, which are based on archaeological evidence, are also supported by the following meteorological considerations.

The patterns of 1,000–500 mbar thicknesses recently published by Lamb, Lewis and Woodroffe[4] clearly indicate that in 2000 and 500 BC, there was a mean trough in the upper westerlies with its axis along 70° E. and extending from high latitudes to 45° N. in the month of July. These thickness charts do not indicate the existence of such a mean trough anywhere between 0° E. and 160° E. in modern times.

I have made a detailed study of the daily northern hemisphere charts for 1962–66, with good coverage of data to the north of the Indo–Pakistan sub-continent, and have found that quasi-stationary troughs in the westerlies at the 500, 300 and 200 mbar levels occasionally extend into north-west Pakistan and induce monsoon activity ahead of such troughs[5]. They are deep enough to alter the configuration[6] of the sub-tropical high to their east and are thus indirectly responsible for causing any monsoon depressions moving in a west-north-westerly direction towards longitude 78° E., to curve to the north or north-east towards the Punjab (Pakistan), Punjab (India) and Kashmir (Fig. 1). Before the depression curves to the north, the area of heavy rainfall lies in the left front quadrant[7] of the depression. After the depression has curved to the north, however, this area of heavy rainfall shifts to the northern sector of the depression, with the active monsoon area chiefly determined by the area of high level divergence ahead of the trough in the upper westerlies[5,8]. In the later stages of its north-eastward movement, the monsoon depression causes floods in the upper reaches of the Himalayan rivers to the west of 78° E. An example of this can be seen in the monsoon depression of September 2–9, 1966. Such depressions curving to the north or north-east were, however, only very occasional during 1891–1960 (ref. 9).

It follows that during the south-west monsoon seasons around 2000–500 BC deep troughs in the upper westerlies must have extended into west Pakistan far more frequently than they do now, inducing monsoon activity and also causing any monsoon depressions from the Bay of Bengal moving towards longitude 78° E., to curve to the north or north-east. These developments would have caused frequent active monsoon conditions over the entire Indus Valley, which is basically a fertile region[10]. Thick vegetation and marsh jungles inhabited by fauna as described by archaeologists[1], and intermittent floods in the Indus and its tributaries, would therefore have characterized the period of the Harappan civilization.

These conclusions are further supported by the recent discovery of considerable reserves of ground water[11] in the arid region of extreme west Rajasthan close to the Indus Valley. Carbon-14 tests carried out[12] by staff of the Tata Institute of Fundamental Research in Bombay, at a place called Palana, 14 miles south of Bikaner (28° 00′ N., 73° 18′ E.) indicate that the ground water there is about 5,000 yr old, this being the upper limit of the true age of the water.

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[1] Wheeler, M., *The Indus Civilisation*, Cambridge History of India, 5 (Camb. Univ. Press, 1956).

[2] Wheeler, M., *Civilisation of the Indus Valley and Beyond*, 75 (Thames and Hudson, 1966).

[3] Kosambi, D. D., *Culture and Civilisation of Ancient India in Historical Outline*, 62 (Routledge and Kegan Paul, London, 1966).

[4] Lamb, H. H., Lewis, R. P. W., and Woodroffe, A., *World Climate from 8000 to 0 B.C., Proc. Intern. Symp.*, 199 (Royal Meteorol. Soc., 1966).

[5] Ramaswamy, C., *Indian J. Meteorol. Geophys.*, **16**, 2, 177 (1965).

[6] Ananthakrishnan, R., and Bhatia, K. L., *Monsoons of the World*, 157 (India Meteorol. Dept., 1958).

[7] Pisharoty, P. R., and Asnani, G. C., *Indian J. Meteorol. Geophys.*, **8**, 15 (1957).

[8] Ramaswamy, C., *Tellus*, **8**, 26 (1956).

[9] *Tracks of Storms and Depressions in the Bay of Bengal and the Arabian Sea* (India Meteorol. Dept., 1964).

[10] Stamp, D. L., *The World*, 119 (Orient Longmans, 1959).

[11] Rao, K. L., *Indian Geohydrology*, **1**, 2 (Indian Assoc. Geohydrol., 1965).

[12] *Report to the Indian National Committee for International Hydrological Decade* (Tata Institute of Fundamental Research, Bombay, 1966).

Ultra-low Levels of Terrestrial Ionization

Neher has suggested[1] that the approximate lower limit of the exposure rate resulting from soil radioactivity be taken as 1·4 μr./h, an unpublished value measured by Millikan in Manitoba. Subsequent surveys in Europe and the United States[2,3] reported minimum values for the intensity of terrestrial ionization which averaged approximately 4·5 μr./h. Millikan's value was also less than that found in the exhaustive study by Hultqvist[4], in which the lowest indoor gamma level, as measured in wooden houses, was about 5·5 μr./h.

In 1962, however, during another survey organized by the Health and Safety Laboratory of the US Atomic Energy Commission, Beck *et al.*[5] measured 1·6 μr./h at Copperopolis, California. At a nearby asbestos mine (Pacific Asbestos, Inc.) a value of 0·5 μr./h was measured. The credit for pointing out the diminished radioactivity in this region must go to Wollenberg and Smith[6], who during a search for low-activity materials for counting room enclosures "discovered" not only the Copperopolis site but also an underground facility, the abandoned Red Mountain magnesite mine in the Diablo Mountains in Santa Clara County, California.

In the late summer of 1965 we visited the Red Mountain mine with a thin-walled (0·5 g/cm^2 'Plexiglas') air-filled ionization chamber. This type of chamber has been described elsewhere[7]. After subtracting the cosmic ray contribution, estimated at 0·01 μr./h, the magnitude of the ionization measured in this mine was 0·05 μr./h, with approximately a 100 per cent error (Table 1). Even 0·1 μr./h is a remarkably low value for terrestrial ionization. In New York City, for example, the average background is at least two orders of magnitude larger. Oddly enough, radon was detected during these measurements, and there is the possibility that it originated outside the mine[8], in which case the ionization levels associated with this mine may be even lower.

We also conducted ionization measurements at the Pacific Asbestos open-strip mine at Copperopolis and obtained a value of 3·94 μr./h. This is the same (to within the ±0·09 μr./h error) as the value reported by Shamos and Liboff[7] for the cosmic ray ionization at this pressure (734·7 torr), again implying quite small terrestrial levels.

Table 1. SELECTED IONIZATION INTENSITIES OVER SOIL, NOT INCLUDING COSMIC RADIATION

Location	Exposure rate (μr./h)	Reference
Manitoba	≧1·4	1
Red Mountain mine, Santa Clara County, California	∼0·05	
Pacific Asbestos mine, Copperopolis, California	≲0·09	
Ruberoid mine, Eden Mills, Vermont	1·04 ± 0·21	
Jeffrey mine, Asbestos, Quebec	≲0·09	
Jeffrey mine (after heavy rain)	0·57 ± 0·14	
King Beaver mine, Thetford, Quebec	0·10 ± 0·17	
Salt mine, Houston	∼0·2	*
Denver, Colorado	12·4 ± 1·7	5
Pelham, New York	6·8 ± 0·4	6
Courtright Reservoir, California	20·7 ± 1·4	8
Guarapari, Brazil (monazite sand beaches)	≲2,000	9

All sites are above ground except for the Red Mountain mine, which is 400 ft. below the surface.

* Personal communication from W. Lowder, describing measurements made by H. Beck and W. J. Condon in 1965.

Three other asbestos strip mines have been studied, the Ruberoid mine in Hyde Park, Vermont, the Johns Manville (Jeffrey) mine in Asbestos, Quebec, and the (King Beaver) mine in Thetford, Quebec. These sites, too, exhibited far less than "normal" exposure rates. A summary of the findings is given in Table 1. The comparatively higher reading at the Ruberoid mine may reflect the fact that data were obtained for only 1 day at this location, and this in rainy conditions. Furthermore, the practice at this mine is to rebuild the excavated areas with processed rock and soil, with the inherent possibility of including foreign materials. Compare the two readings for the Jeffrey mine. The second set was taken after an extended rainy period.

As well as these sites, underground salt mines apparently also exhibit rather low levels of radiation. For example, in a mine near Houston, an approximate level of 0·2 μr./h has been determined. It is interesting that the contribution of potassium-40 was negligible, and that there was a substantial concentration of bismuth-214.

To scale the set of values in Table 1 properly, we have included some others. The first, an average of readings taken at an Atomic Energy Commission site in Pelham, New York, is a representative sample of the magnitude encountered over soil. The next value listed, on the other hand, is rather high. It was obtained over a granite outcrop at Courtright Reservoir in the Sierras. (This site serves as a standard for the University of California LRL Health Physics group.) In addition there are, of course, "hotter" regions, the best example of which is the monazite sands area on the Brazilian East Coast, where levels up to 2,000 μr./h are observed[9].

The ultra-low levels listed in Table 1 are almost certainly associated with ultramafic rocks. This is entirely reasonable considering that the concentrations of the principal radioactive contaminants, uranium, thorium and potassium, vary, in the words of Wollenberg and Smith[8], "with the acidity of igneous rocks, being practically nil in the ultramafic rocks". Davis and Hess[10] showed that as the mafic nature of igneous rocks varied between felsic and ultramafic, the concentration of uranium changed from $3·0 \times 10^{-6}$ to $0·03 \times 10^{-6}$ g/g of rock. Included in their samples, coincidently, were specimen rocks from Belvidere Mountain in Vermont, the site of the Ruberoid mine already mentioned. In addition to reporting that gravimetric determinations show "generally between 0·00 and 0·03% for the alkalis in ultramafic rocks", Hamilton and Mountjoy[11] also list the results of an extensive series of flame spectrophotometric analyses on alpine ultramafic rocks, in which the median concentration of potassium oxide was 0·003 per cent. Dunite and serpentine were said to be virtually free of potassium[12]. It is interesting that a constant ratio of potassium to uranium is said to occur[13] in ultramafic rock. In addition, the fact that relatively large regions, either on the Earth's surface or close to it, show ultra-low radioactivity may have significance in connexion with (*a*) the hypothesis[14,15] that the mantle immediately below the Mohorovičić discontinuity consists chiefly of ultra basic (peridotitic) rock, and (*b*) the fact that there are probably "quite severe restrictions . . . on the absolute concentrations of radioactive elements existing in the earth's upper mantle"[16].

Apart from geochemical considerations, perhaps the most interesting aspect is the possible exploitation of such areas for research which requires extremely low backgrounds. Consider, for example, what information can be derived from Fig. 1, which shows the ionization level in Red Mountain mine recorded for 10 min. Full scale is approximately equivalent to 4·0 μr./h. The electrometer zero is shown at the end of the record. Zero drift was measured during this period and found to be negligible.

The large spikes represent bursts of ionization from alpha particles emitted by contaminants in the chamber walls. Some are shown in greater detail in Fig. 2, in a portion of record taken at a higher speed. For those times long after an alpha emission and before another, the mean signal level approaches a net deflexion of about 3/4 of a small division. The cosmic ray level at the mine elevation (3,040 ft.) is 4·3 μr./h, but is greatly attenuated by the 400 ft. of vertical thickness of rock above the ionization chamber. Assuming a specific gravity of 2·5, this corresponds to a depth of $3·1 \times 10^{5}$ g/cm^{2}, enough to reduce the cosmic ray contribution to the order of 0·01 μr./h, and the net magnitude to the previously stated 0·05 μr./h.

By direct counting of pulses for 30 min it was established that there were 0·036 ± 0·003 alphas/sec. This, divided by the interior surface area of the chamber, is equivalent to $1·2 \times 10^{-5}$ alphas/cm^{2}/sec. Sharpe[17] has reported that aquadag coatings (which constituted the interior electrodes in our chamber) emit about $1·9 \times 10^{-5}$ alphas/cm^{2}/s. It is seen, therefore, that the residual contaminantion of the chamber itself is determined in a straightforward way. More elaborate schemes have been used in the past, notably the extrapolation techniques of Hess and Vancour[18].

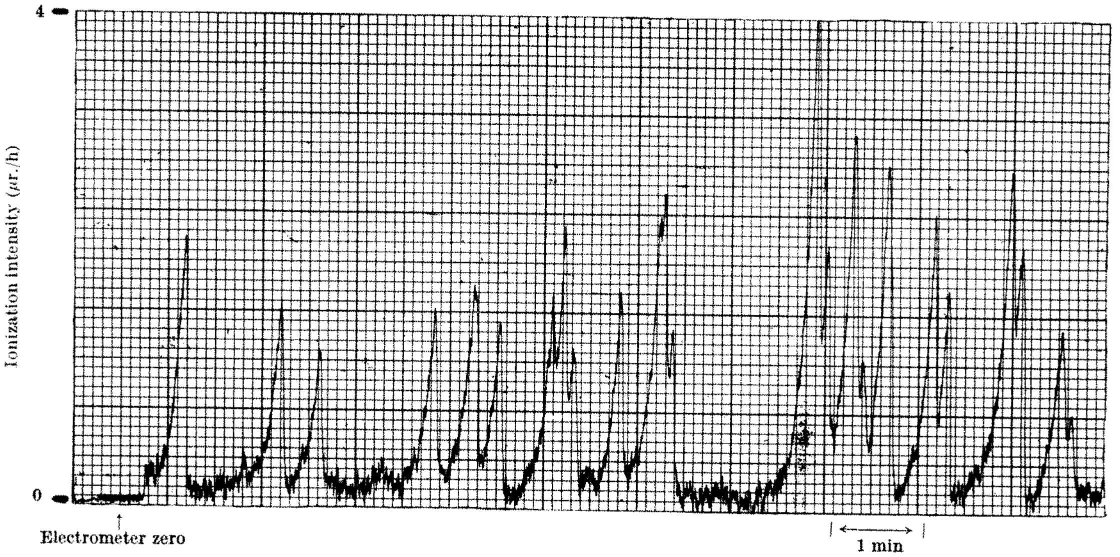

Fig. 1. Intensity of background ionization in the Red Mountain mine, showing the effect of wall alphas.

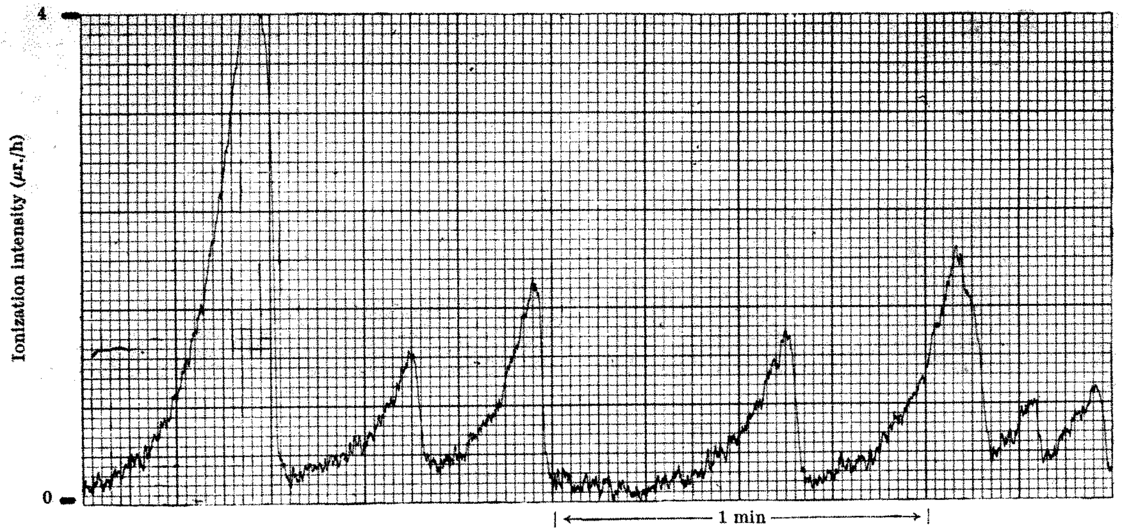

Fig. 2. The resolution of alpha activity in an ionization chamber.

As an example of the sort of experiment which could be carried out in the Red Mountain mine or, for that matter, in the mines in the Thetford region of Quebec, it has been shown[19] that certain anomalous results in the cosmic ray depth-intensity relation below ground[20] can be explained as resulting from multiple Geiger counter coincidences caused by local gamma radiation. A substantial decrease in local background would enable such work to be carried out with much less shielding, which, as an added benefit, would yield more data about the low energy end of the underground cosmic ray spectrum. Cosmic ray surface measurements could probably be performed over such regions with little or no shielding, and with perhaps little in the way of anticoincidence protection. Other possibilities include the use of such areas for research in those problems in genetics and archaeological dating where very low background levels are required.

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[1] Neher, H. V., *Science*, **125**, 1088 (1957).

[2] Solon, L. R., Lowder, W. M., Zila, A. V., Levine, H. D., Blatz, H., and Eisenbud, M., *Science*, **127**, 1183 (1958).

[3] Solon, L. R., Lowder, W. M., Shambon, A., and Blatz, H., *US Atomic Energy Comm. Health and Safety Lab. Report*, No. 73 (November 1959).

[4] Hultqvist, B., *Kingl. Svenska Vetenskapsakademiens Hadlingar*, **6**, No. 3 (1956).

[5] Beck, H. L., Condon, W. J., and Lowder, W. M., *US Atomic Energy Comm. Health and Safety Lab. Report*, No. 145 (April, 1964).

[6] Wollenberg, H. A., and Smith, A. R., *Earth Materials for Low Background Radiation Shielding* (Univ. Calif. Radiat. Lab., 9970, May 24, 1962).

[7] Shamos, M. H., and Liboff, A. R., *J. Geophys. Res.*, **7**, 19 (1966).

[8] Shamos, M. H., *Ann. Report, US Atomic Energy Comm. Div. Biol. and Med.*, 33 (1966).

[9] Roser, F. X., and Cullen, T. L., in *The Natural Radiation Environment* (edit. by Adams, J. A. S., and Lowder, W. M.), 825 (University of Chicago Press, 1964).

[10] Davis, G. L., and Hess, H. H., *Amer. J. Sci.*, **247**, 856 (1949).

[11] Hamilton, W., and Mountjoy, W., *Geochim. Cosmochim. Acta*, **29**, 661 (1965).

[12] Ahrens, L. H., in *Nuclear Geology* (edit. by Faul, H.), 131 (John Wiley, New York, 1954).

[13] Wasserburg, G. J., MacDonald, G. J. F., Hoyle, F., and Fowler, W. A., *Science*, **144**, 465 (1964).

[14] Ringwood, A. E., *J. Geophys. Res.*, **67**, 857 (1962).

[15] Ringwood, A. E., *J. Geophys. Res.*, **67**, 4473 (1962).

[16] Lovering, J. F., and Morgan, J. W., *Nature*, **197**, 138 (1963).

[17] Sharpe, J., *Nuclear Radiation Detectors*, 98 (Methuen, London, 1955).

[18] Hess, V. F., and Vancour, R. P., *Phys. Rev.*, **76**, 8, 1205 (1949).

[19] Barton, J. C., and Michaelis, E. G., *Proc. Phys. Soc.*, **77**, 377 (1960).

[20] Barnothy, J., and Forro, M., *Phys. Rev.*, **74**, 1300 (1948).

PHYSICS

Stimulated Emission and Laser Action from the Organic Ligand of Gadolinium Chelates

WE wish to report evidence for stimulated emission and laser characteristics from liquid solutions of gadolinium organic chelates at room temperature. To our knowledge this is the first time that electronic transitions in organic chromophores have performed laser action under direct flash in liquid solution. The stimulated emission is reproducibly obtained at room temperature in conditions in which no fluorescence or phosphorescence is usually detected. With identical pumping energy, the coherent emission from the organic ligand in the Gd chelate is comparable with that obtained from solutions of europium[1-3] and terbium[4] chelates in which the emitting species is the complexed lanthanide ion.

Three gadolinium chelates exhibited laser action at room temperature in solution: *tris*(theonyltrifluoroacetonate)-Gd III (GdT$_3$), α,α′-dipyridyl-*tris*(theonyltrifluoroacetonate)-Gd III (GdT$_3$Dipy), and 1,10-phenanthroline-*tris*(theonyltrifluoroacetonate)-Gd III (GdT$_3$Phen). The procedures for their preparation have been described previously[5-7]. Solutions of 1×10^{-3}–3×10^{-3} M in spectrograde acetonitrile (Columbia Organic Chemicals) were prepared and tested for laser action at room temperature. Removal of oxygen (deaeration) or prolonged storage did not significantly change the laser performance of the material.

The liquid laser cavities and the alignment techniques employed in this work have been described in detail before[4,8].

Fig. 1 shows the relaxation oscillations (spiking) observed above threshold from GdT$_3$Dipy in acetonitrile. The optimum concentration (lowest threshold) was found to be around $2{\cdot}5 \times 10^{-3}$ M for GdT$_3$Dipy, the most efficient of the three chelates reported here. The spectral distribution of the laser lines is displayed in Fig. 2. The broad band centred at 394 mμ is caused by the background light from the xenon flash lamp corresponding to a "window" in the filter system and is easily reproduced when the liquid cell is empty or filled with pure solvent. The very narrow laser lines are superimposed on the background emission. Beam collimation has also been observed above threshold on far-field images of the output obtained on Polaroid film. These results are reproducible.

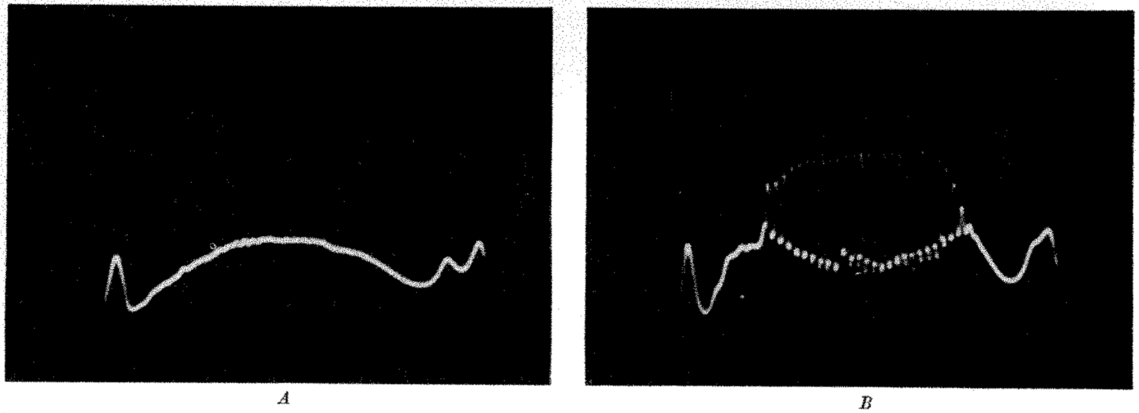
A *B*

Fig. 1. Relaxation oscillations in the output of the GdT_3Dipy laser, $2·5 \times 10^{-3}$ M, in acetonitrile at room temperature. *A*, Oscilloscope trace below threshold, 100 μs/cm sweep; *B*, same, above threshold.

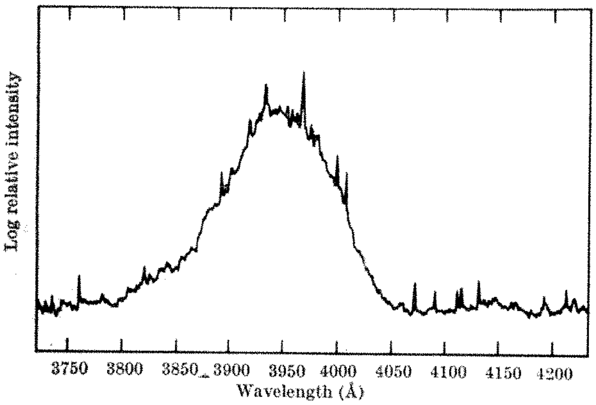

Fig. 2. Microdensitometer trace of the spectral output from the GdT_3Dipy laser. The broad band centred at 3940 Å is from the flash lamp; the narrow laser lines are superimposed on the background.

The same solution can be flashed repeatedly up to about twelve times before its laser activity diminishes. Between the flashes we allowed several minutes for cooling by equilibration with environmental temperature. The time elapse between flashes can be reduced using external cooling with cold nitrogen gas or recirculation[8].

There are at least fourteen clearly defined laser transitions detectable in the spectral range 3750–4200 Å. At present we are unable to make definite assignments of these transitions, but a brief discussion of the existing possibilities is warranted. The stimulated emission does not originate in the Gd^{3+} ion, which has the lowest resonance level at about 32,100 cm^{-1}. Emission spectra of the chelates investigated in this work have been reported recently[7]. The phosphorescence and phosphorescence excitation spectra illustrated in Fig. 3 were obtained in rigid matrix (EPA) at liquid nitrogen temperature. A detailed analysis of Fig. 3 can be found in ref. 7. It is obvious that the laser lines do not coincide with the phosphorescence of either T or Dipy chromophores (curves *A* and *C*, respectively). But the triplet–singlet emission in Fig. 3 was obtained in a different environment and at much lower temperature. It is possible that in acetonitrile the lowest triplet of the Dipy ligand is destabilized more than the ground singlet, in which case the laser lines would correspond to stimulated emission originating at the lowest triplet in the Dipy chromophore. The $n \rightarrow \pi^*$ transition in some compounds has been blue-shifted as much as 725 cm^{-1} in acetonitrile[9]. Nothing is known about the polarity or solvation properties of the excited states in the Gd chelate. But the possibility of a hypsochromic shift of the $0 \rightarrow 0$ band in the Dipy phosphorescence such as to overlap with the observed laser lines cannot be ruled out. Usually only very few compounds exhibit phosphorescence in liquid solution because of collisional deactivation (or chemical reaction) by solvent molecules or other cosolutes. This is a result of the relatively long lifetime of the lowest triplet. In a Fabry Perot resonator, however, the population inversion attained under a powerful flash may well lead to a stimulated radiative deactivation of the triplets.

The second alternative seems to be a stimulated emission from the lowest excited singlet in the T chromophore, for the lines in Fig. 2 could form a "mirror image" band with the excitation (absorption) curve *D* in Fig. 3. Nonetheless, we do not consider this alternative very probable because the rate of intersystem crossing in aromatic β-diketones is several orders of magnitude above the rate of emission from S_1, and in Gd chelates the crossover to the triplet should be even faster.

The third possibility is a photochemical process in which the intermediate or product is formed in an excited state which can be the origin of the lasing transitions. The decrease in lasing activity after repeated (about ten to twelve times) flash pumping tends to support this possibility. But the same effect may be the result of other photo-degradative processes.

The vibrational structure of the lines in Fig. 2 cannot be satisfactorily correlated with the absorption and emission spectra of the two organic ligands in the infrared (and Raman) and ultraviolet-visible. We are also considering the energy transfer between the two different

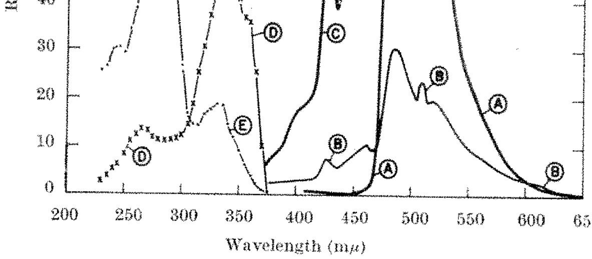

Fig. 3. Phosphorescence and excitation spectra of GdT_3Dipy 10^{-5} M in ethanol-2-methylpentane-ether (EPA) at 77° K: (*A*) phosphorescence of T excited at 345 mμ where only T absorbs; (*B*) excitation at 234 mμ in the Dipy absorption band, emission from both Dipy and T chromophores; (*C*) Dipy phosphorescence excited at 280 mμ; (*D*) excitation (corrected) of the T phosphorescence at 489 mμ; (*E*) excitation (corrected) of the Dipy phosphorescence at 426 mμ.

ligands[7] as well as other flash-induced alternatives in a detailed study to elucidate the origin of the lasing transitions.

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[1] Schimitschek, E. J., *Appl. Phys. Lett.*, **3**, 117 (1963).
[2] Lempicki, A., Samelson, H., and Brecher, C., *J. Chem. Phys.*, **41**, 1214 (1964).
[3] Bhaumik, M. L., Fletcher, P. C., Nugent, L. J., Lee, S. M., Higa, S., Telk, C. L., and Weinberg, M., *J. Phys. Chem.*, **68**, 1490 (1964).
[4] Bjorklund, S., Kellermeyer, G., Hurt, C. R., McAvoy, N., and Filipescu, N., *Appl. Phys. Lett.*, **10**, 160 (1967).
[5] Bauer, H., Blanc, J., and Ross, D. L., *J. Amer. Chem. Soc.*, **86**, 5125 (1964).
[6] Melby, L. R., Rose, N. J., Abramson, E., and Caris, J. C., *J. Amer. Chem. Soc.*, **86**, 5117 (1964).
[7] Filipescu, N., Bjorklund, S., Hurt, C. R., and McAvoy, N., *Canad. J. Chem.*, **45**, 1714 (1967).
[8] Bjorklund, S., Kellermeyer, G. L., McAvoy, N., and Filipescu, N., *J. Sci. Instrum.*, **44**, 947 (1967).
[9] Becker, R. S., *J. Mol. Spectros.*, **3**, 1 (1959).

Cavitation induced by Vorticity at a Smooth Flat Wall

An interesting example of cavitation, apparently induced by vorticity, has arisen at the plane side walls of a small cavitation tunnel during studies primarily concerned with the behaviour of large cavitating eddies.

A cylinder in a water tunnel has been made to generate, at high frequency and from alternate sides, a stream of cavitating eddies[1]. When viewed under stroboflashing light, cavitation bubbles are seen to stem from one or other side of the cylinder and in each case are carried by the stream into a rapidly growing but relatively short-lived eddy with a large amorphous central cavity. Each elongated, large-cavity eddy ultimately becomes detached from the cylinder; the amorphous core subsides quickly into two (or occasionally more) minor eddies of high angular momentum and each of these eddies briefly sustains a mist, or cloud, of minute cavities. Eventually each cloud collapses with extreme rapidity and may cause severe damage at adjacent boundaries[1].

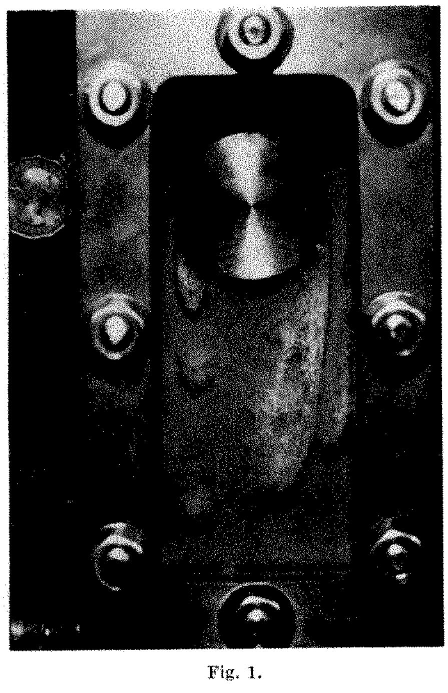

Fig. 1.

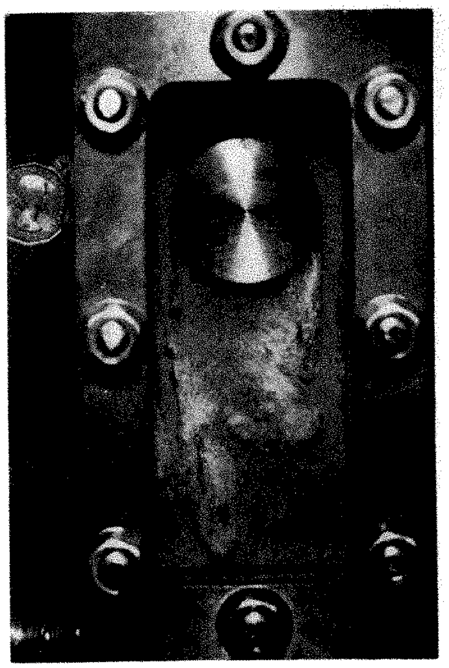

Fig. 2.

With the formation of each of the large cavity eddies, additional (and concurrent) cavitation has been observed, downstream of the cylinder, at the tunnel side wall adjacent to the large-cavity eddy. Flow is contained momentarily by the tunnel wall and by the free streamline of the large-cavity eddy. The free streamline, which is at cavitation pressure, curves away from the tunnel wall and the radial pressure gradients associated with the eddy might therefore be expected to keep the tunnel wall pressures above cavitation pressure. But substantial vorticity seems to be generated within the boundary layer at the tunnel wall and it seems that this vorticity is responsible for the low pressures actually occurring near the tunnel wall and observed by means of the induced tunnel-wall cavitation.

Figs. 1 and 2 show cavitation induced by vorticity near the tunnel walls downstream of the cylindrical obstruction. In this test, water at 66·5° Fahrenheit flowed past a 1·50 in. diameter cylinder in a tunnel which was 9 in. long and $2{\cdot}00 \times 0{\cdot}25$ in. in cross-section. The measured eddy-shedding cycle frequency was 6,600/min when the water approached the cylinder with a mean velocity of 17·2 ft./sec and a pressure of 74·7 ft. of water (absolute).

Tunnel-wall cavitation with associated cylinder-wake cavitation has also been observed (for example, Fig. 3) in the relatively steady flow situation obtained by closing one of the flow passages between the cylinder and the tunnel side walls. It thus seems that cavitation can be induced at a plane surface by the vorticity generated within the boundary layer and the vorticity may be detected and viewed, for example, under stroboflashing light, by its induced cavitation.

Cavitation inception at the centre of a boundary layer near a smooth wall has been reported by Daily and Johnson, who found that such cavitation always occurred when the wall pressure was greater than the water vapour pressure. This was attributed to pressure reductions in the boundary layer caused by vortical eddies and turbulence[2]. Arndt and Ippen have also observed cavitation inception in the centres of turbulent boundary layers at

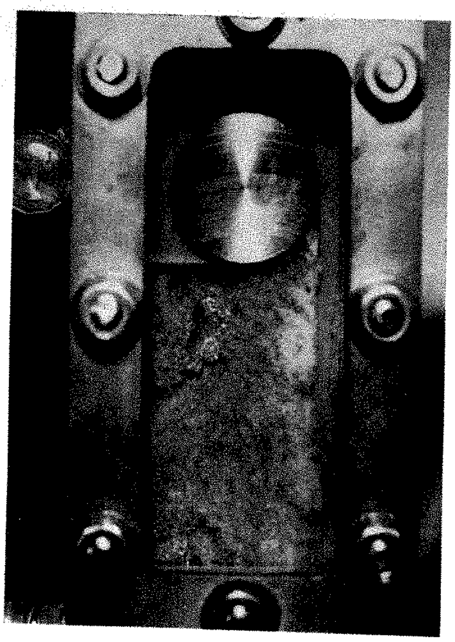

Fig. 3.

smooth and rough surfaces and have correlated the cavitation inception with the wall shear[3]. Cavitation has also been observed and studied at other regions of high shear, for example, by Rouse in the mixing zone of a submerged water jet[4]. Such regions of shear generate vorticity and may therefore be expected to produce local zones of low pressure and, in appropriate conditions, cavitation.

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[1] Kenn, M. J., *Civil Eng.*, **61**, 1404 (1966).

[2] Daily, J. W., and Johnson, V. E., *Trans. Amer. Soc. Mech. Eng.*, **78**, 1695 (1956).

[3] Arndt, R. E. A., and Ippen, A. T., *M.I.T. Hydrodynamics Laboratory Rep. No.* 104 (1967).

[4] Rouse, H., *La Houille Blanche* (January/February, 1953).

THE SOLID STATE

Mechanisms of Fatigue Damage in Face Centred Cubic Metals

The work of Wood *et al.*[1,2] has shown that fatigue damage occurs by different mechanisms in different regions of the *S/N* curve, these being designated as the *H* mechanism at high stress levels where fatigue life increases slowly with decreasing stress amplitude, and the *F* mechanism at lower stress levels where fatigue life increases rapidly with decreasing stress. There is also a pseudo-safe or *S* region. A number of workers[3-6], however, have suggested that there is a less clear-cut distinction between the mechanisms occurring at the different stress levels than the results of Wood *et al.* indicate, and we discuss here the role of material properties such as texture and ductility on the operative mechanisms of fatigue damage.

Fatigue tests have been carried out on three face centred cubic (FCC) materials, an aluminium–magnesium alloy (Alcan *CA*-57*S*), 99·99 per cent pure aluminium, and electrolytically pure tough-pitch copper, the plate being first cold-rolled by approximately 90 per cent to obtain sheets with a high degree of preferred orientation, the texture approximating to the $\{110\}<112>$ ideal orientation. Sheet with cube texture ($\{100\}<001>$) was produced by annealing the cold-rolled, high-purity aluminium and the copper, and material with a random texture by annealing the cold-rolled 57*S* alloy. Fatigue tests were made on all materials, the specimens first being carefully finished and checked to ensure that they were free from scratches. Stressing was by reversed bending and tests were made over a wide range of stress levels. After testing to failure, the specimens were examined under the optical microscope to determine the nature of the fatigue damage which had occurred.

The damage to the cold-rolled material tested at high and low stress amplitudes occurred by the formation of sub-boundaries and pores corresponding to Wood's *H* mechanism normally found at high stress amplitude, and Fig. 1 shows such damage on the surface of a sample of high-purity aluminium tested at a low stress amplitude, the photograph having been taken some distance from the main crack. In this sample the surface has been electro-polished and lightly etched to show the structure more clearly. It was noted that the fatigue cracks, which developed by the continued production and linking of the pores, propagated in a direction approximately normal to the specimen axis in all the cold-rolled samples. In the sheet metal specimens with cube texture, fatigue damage at all stress amplitudes occurred along slip bands, which broadened until cracks formed and propagated at approximately 45° to the specimen axis. This corresponds to Wood's *F* mechanism, and the surface of a specimen of annealed high-purity aluminium tested at a high stress amplitude and exhibiting damage along slip bands is shown in Fig. 2: again the specimen has been electro-polished and lightly etched and the region shown is some distance from the fracture. It is also of interest that the annealed material with random texture did not show a distinct difference between the mechanisms of fatigue at high and low stress levels, damage occurring predominantly by the *H* mechanism at high stress and by the *F* mechanism at low stress with a gradual transition at intermediate stress levels. The observations made from all the tests are summarized in Table 1, the high and low stress levels being defined in terms of the number of cycles to failure (*N*).

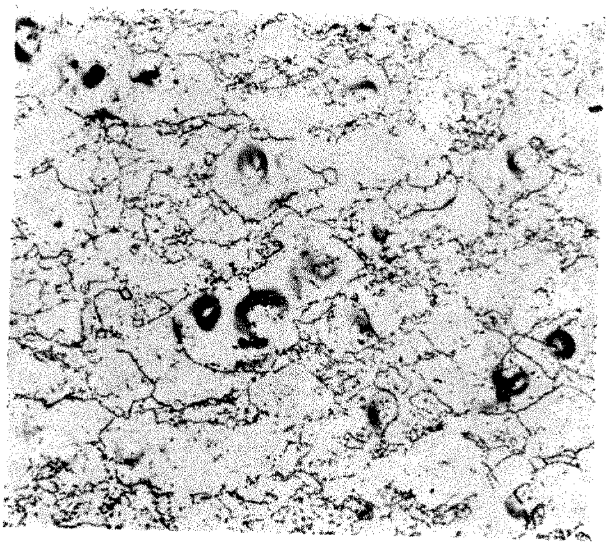

Fig. 1. Cold-rolled, high-purity aluminium fatigued at a low stress amplitude showing Wood's *H* mechanism of fatigue damage (× 240).

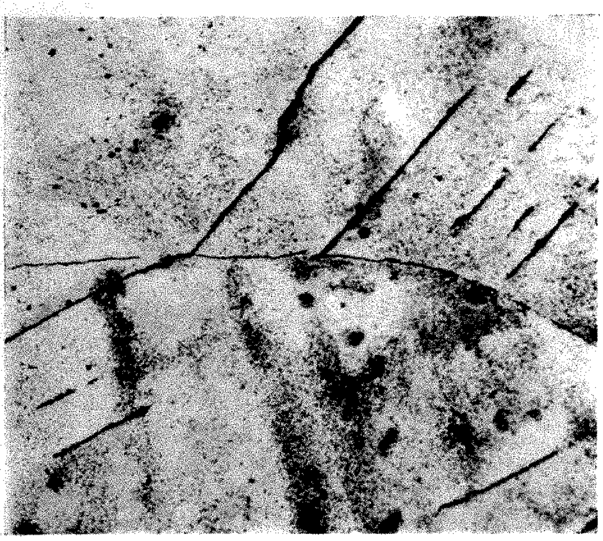

Fig. 2. Annealed, high-purity aluminium fatigued at a high stress amplitude showing Wood's *F* mechanism of fatigue damage (× 240).

The results indicate that there is not necessarily a correlation between high stress amplitude and *H* mechanism, and low stress amplitude and *F* mechanism, and that the operative mechanisms of fatigue damage may be influenced to a great extent by texture and ductility. Although it is difficult to assess the separate effects of these two factors, because the ductility depends on texture to a large extent, it may be seen from Table 1 that the annealed aluminium 57*S* with random texture and the annealed high-purity aluminium with cube texture have similar ductility, but the fatigue damage at high stress occurred predominantly by the *H* mechanism in the former and by the *F* mechanism in the latter. We are now making a more detailed correlation between fatigue mechanisms and texture.

Table 1

Material	Condition	Tensile ductility (% elongation at fracture)	Texture	Fatigue damage mechanism	
				High stress ($N = 10^4$–10^5 cycles)	Low stress ($N = 10^6$–10^7 cycles)
Al 57*S*	Cold-rolled 93%	6	{110} < 112 >	*H*	*H*
Al 57*S*	Annealed	28	Random	*H* > *F*	*F* > *H*
Al (99·99%)	Cold-rolled 90%	6·5	{110} < 112 >	*H*	*H*
Al (99·99%)	Annealed	30	{100} < 001 >	*F*	*F*
Cu (tough pitch)	Cold-rolled 90%	6	{110} < 112 >	*H*	*H*
Cu (tough pitch)	Annealed	49	{100} < 001 >	*F*	*F*

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[1] Wood, W. A., Cousland, S. McK., and Sargant, K. R., *Acta Metall.*, **11**, 643 (1963).

[2] Wood, W. A., *Tewksbury Symposium on Fracture*, 1963, 63 (Eng. Faculty, University of Melbourne, 1965).

[3] Laird, C., and Smith, G. C., *Phil. Mag.*, **7**, 847 (1962).

[4] Stubbington, C. A., and Forsyth, P. J. E., *J. Inst. Metals*, **90**, 347 (1961–62)

[5] Forsyth, P. J. E., *Acta Metall.*, **11**, 703 (1963).

[6] Kennedy, A. J., *Brit. J. Appl. Phys.*, **15**, 229 (1964).

MOLECULAR STRUCTURE

NMR Studies of Gramicidine *S* in Solution

GRAMICIDINE *S* is a cyclic polypeptide containing ten amino-acid residues arranged in two identical groups. This molecule has attracted considerable interest as a model related to the more complex globular proteins.

Recently attempts have been made[1,2] to derive a model of gramicidine *S* on the basis of energy considerations. The two models are schematically drawn in Fig. 1*a*, *b* and *c*. The two conformations are markedly different in spite of the similar overall shape and dimensions of the molecules. The difference probably arises because the energy criterion was applied in different ways. Sheraga *et al.* have tried to calculate the potential energy of the molecule as a function of all the angles of rotation about the non-conjugated bonds (including the side chains), but Liquori *et al.* used their own stereochemical code for the amino-

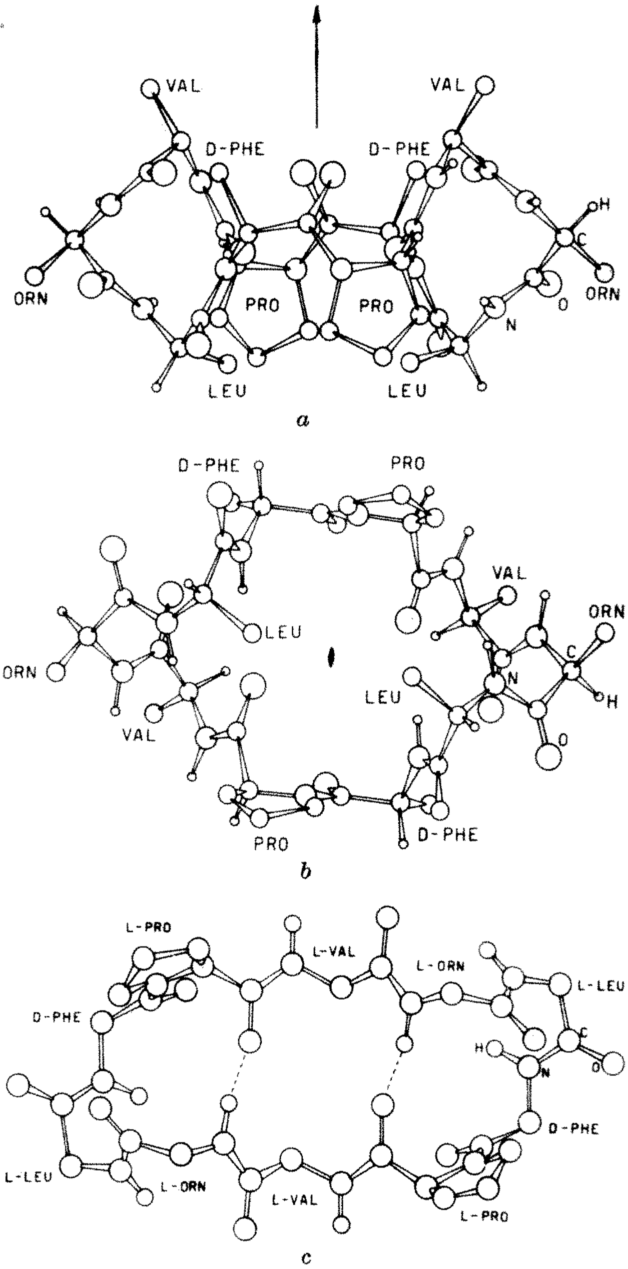

Fig. 1. Models of the molecule of gramicidine *S* due to Liquori *et al.*[1] (*a*) and (*b*) and Sheraga *et al.*[2] (*c*).

acid residue with standard bond lengths and bond angles* which can only have five conformations defined by the angles of rotation $\Psi_{C\text{-}C}$, $\Psi_{N\text{-}C}$, $\omega_{C\text{-}N} = 0$, about the skeleton bonds Cα–C, N–Cα, and C–N. These conformations are indicated by *Ra*, *b*, *c*, *d*, *La* for an L-residue (or Ra^+, b^+, c^+, d^+, La^+ for a D-residue). Identical repetitions of these five conformations generate the five helices *R*α, β, γ, δ, *L*α energetically allowed for a homopolypeptide chain.

The problem of deriving the conformation of gramicidine *S* was therefore treated as a "stereochemical translation" which involves assigning to each amino-acid residue a "stereochemical codon" with the restrictions imposed by the closure of the ring and by the condition of conformational equivalence between identical residues. As a result the skeleton of the molecule was "coded" as follows

$$\begin{array}{ccccccccc} d & & Ra & & Ra & & Ra & & c^+ \\ \text{Pro} & - & \text{Val} & - & \text{Orn} & - & \text{Leu} & - & \text{DPhe} \\ | & & & & & & & & | \\ \text{DPhe} & - & \text{Leu} & - & \text{Orn} & - & \text{Val} & - & \text{Pro} \\ c^+ & & Ra & & Ra & & Ra & & d \end{array}$$

The two segments Val–Orn–Leu can therefore be described as two anti-parallel right handed α-helical segments which are connected by two "non-helical" segments DPhe–Pro. Two hydrogen bonds between the C=O groups of two Val and the N–H groups of the two DPhe are present in this structure as in an α-helix. As well as testing this conformation by X-ray diffraction, we have carried out NMR studies in solution. One aim of this investigation was to estimate the relative ability of the peptide hydrogens present in the molecule to exchange for deuterium. Furthermore, we have tried to obtain NMR data about the correctness of our stereochemical codification of the skeleton of gramicidine *S*. This test is based on the very close similarity between the local conformation of an amino-acid residue of gramicidine *S* corresponding to a given stereochemical codon in the proposed model and that of the same amino-acid residue in the corresponding helix. It has been shown (unpublished work of Liquori, Conti and Ferretti) that the chemical shift of a CH proton in a homopolypeptide seems to be strongly dependent on the backbone conformation of the amino-acid residues, to a smaller extent on the nature and conformation of the side chains, and it is fairly insensitive to the solvent. Thus the chemical shift of the CHα of α-helical poly-L-leucine, α-helical poly-L-ornithine and δ-helical *trans* poly-L-proline (polyproline II) should not differ much from the chemical shifts of the CHα protons of leucine, ornithine and proline residues in gramicidine *S*, which, according to our model, should have local conformations *Ra*, *Ra* and *d* respectively.

The preliminary results obtained on a sample of gramicidine *S* (Mann Research Lab.) using an *HA* 100 Varian NMR spectrometer are summarized here.

(*a*) *Deuterium exchange experiments*. In Fig. 2 are shown the NMR spectra of gramicidine *S* recorded in pure dimethylsulphoxide (DMSO) and 10 min after addition of different amounts of D_2O and trifluoroacetic acid. This region contains peaks corresponding to phenyl and NH protons. The CH protons of the benzene rings of the two groups of DPhe (7·25 p.p.m.) are magnetically equivalent This effect implies that the two rings are probably free rotating in solution and is fully compatible with the presence of a dyad axis relating the two amino-acid residues. In pure DMSO, the NH protons give rise to three different signals respectively at 8·05, 8·30 and 8·60 p.p.m., which are called HN_I, NH_{II} and NH_{III}. It can be deduced from the peak areas that the ratio $NH_I : N_{II} : NH_{III}$ between these peaks is 4 : 2 : 2. The low peak at ~9·1 p.p.m. is probably caused by an impurity, as may be judged from the value of the peak area and from the unusual chemical shift. On progressive additions of D_2O, the peak NH_I tends to disappear, and in the presence of traces of trifluoroacetic acid the peak NH_{II} also disappears and NH_{III} is the only peak left. There are therefore two equivalent NH protons which exchange at a significantly slower rate than the others. Such a conclusion is fully consistent with our model of gramicidine *S* which contains two hydrogen bonded NH groups. It should, however, be noted that this does not rule out Sheraga's model, as can be seen in Fig. 1*c*. The NH_I peaks at higher field might be identified with the NH protons of two Leu and two Orn residues in Liquori's model which are less accessible and the NH_{II} peak might be identified with the NH protons of two Val residues which are more readily accessible and are magnetically equivalent.

(*b*) *Tentative assignments*. The 0–5 p.p.m. region of the NMR spectrum of gramicidine *S* in DMSO is shown in Fig. 3. The positions of the peaks corresponding to the protons in the NMR spectra of α-helical leucine and ornithine and of polyproline II (δ-helical) (unpublished results) are indicated schematically at the top of Fig. 3. It can be seen that the position of the CHα peak of polyproline II agrees very closely with the band at 4·80 p.p.m. in gramicidine *S* which according to the integral of the peak area contains two protons. The positions of the CHα peaks of α-helical poly-L-leucine and poly-L-ornithine fall within the band at 4·25 of the NMR spectrum of gramicidine *S*.

This peak corresponds to six protons and the shoulder, and so at a lower field (at 4·50) corresponds to two protons; the assignment of the two CHα protons of Phe and of the two CHα protons of Val presents some ambiguity.

It should be mentioned that the addition of $CDCl_3$ gives better resolution of the αCH regions in gramicidine *S* and that the shoulder at 4·50 becomes much more obvious. There is, however, a strong indication that an intermolecular association takes place and this is being studied.

This preliminary assignment therefore seems to be consistent with Liquori's model of gramicidine *S*, and we hope to reach more definite conclusions on the basis of NMR spectra at higher resolution.

Very little can be said about the consistency of Sheraga's model with the αCH proton region of the NMR spectra of gramicidine *S* because of the lack of data concerning suitable reference systems. The marked broadness of all CH protons, except the phenyl and methyl protons, which is commonly observed in the NMR spectra of α-helical homopolypeptides in solution, however, is in more general agreement with a compact conformation like the Liquori model than with an open structure like the Sheraga model.

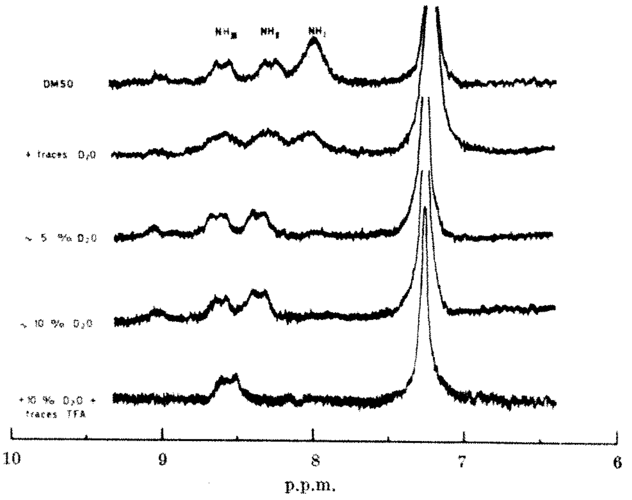

Fig. 2.

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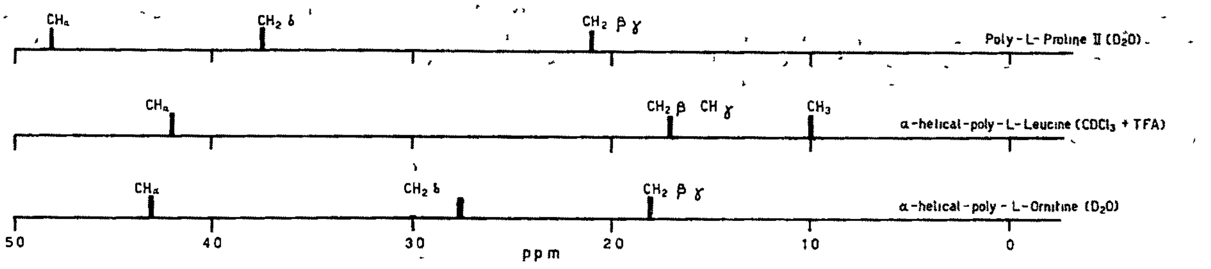
* Whereas previously[1] the best set of Corey and Pauling[3] for the bond angles and bond lengths could be adopted for all the amino-acid residues, the model has now been slightly refined by using bond angles found by Leang and Marsh[4] for the geometry of the L-proline residues (unpublished work of Liquori and Santis).



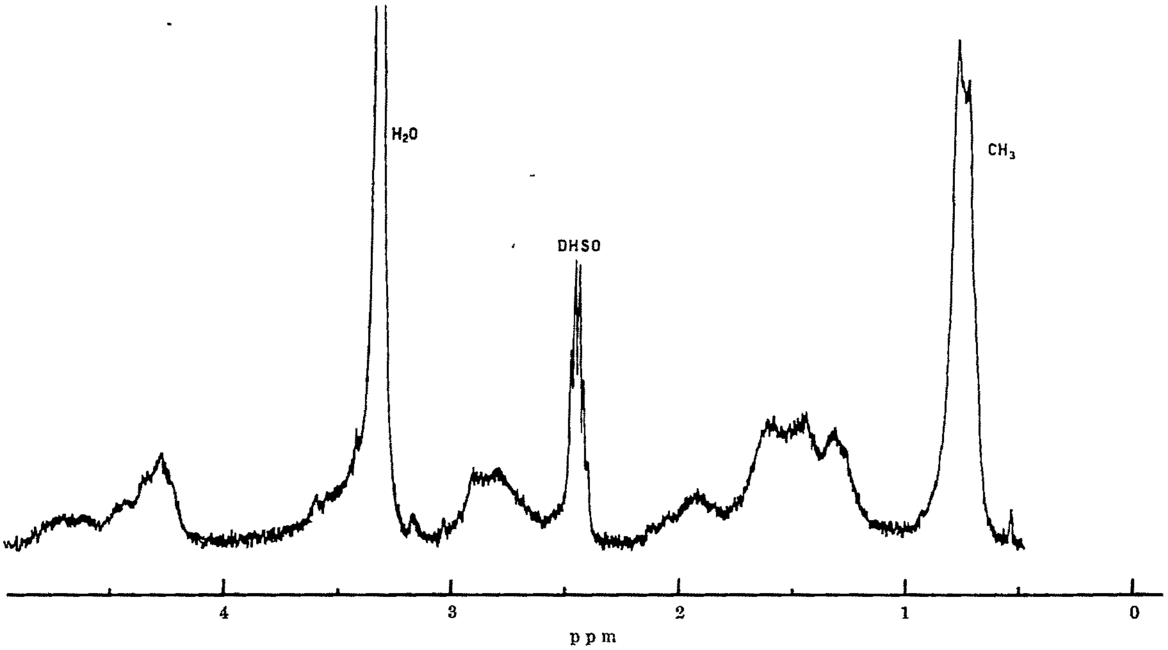


Fig 3

All our conclusions therefore contrast with those of Balasubramanian[5] and are in line with the results of optical rotation studies[6] It should, however, be mentioned that Balasubramanian's discussion of the experimental results reveals that he has not inspected the molecular models Typical in this connexion is the claim that Liquori's model is not compatible with the infrared data, because it contains peptide bonds in a *cis* conformation, which is not true[1]

The NMR experiments were carried out at the Istituto Superiore di Sanità We thank Dr G Conti for technical assistance

Note added in proof A recent calculation of the chemical shifts of the protons of gramicidine *S* carried out in our laboratory (to be published) gives results which confirm this tentative assignment of the NMR spectra according to Liquori's model and seem to be entirely inconsistent with Scheraga's model

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[1] Liquori, A M, De Santis, P, Kovacs, A L, and Mazzarella, L, *Nature*, 211, 1039 (1966)

[2] Vanderkoi, G, Leach, S J, Nemethy, G and Sheraga, H A, *Biochemistry*, 5, 2991 (1966)

[3] Corey, P B, and Pauling, L, *Proc Roy Soc*

[4] Leang, Y C, and Marsh, R E, *Acta Cryst*, 11, 17 (1958)

[5] Balasubramanian, D, *J Amer Chem Soc*, 89, 5445 (1967)

[6] Quadrifoglio, F, and Urry, P K, *Biochem Biophys Res Comm* (in the press)

CHEMISTRY

Laser Raman Spectra of Sorbed Species

WE have previously reported[1] that spectra of adsorbed species can easily be obtained with a laser excited Raman spectrometer We now wish to extend the field to the case in which there is the possibility of chemisorption

Young and Sheppard[2,3] have used infrared techniques to study a series of aliphatic aldehydes adsorbed onto silica gel and have concluded that at room temperature they are, at least initially, physically adsorbed Spectra obtained after desorption were not identical with those of the clean gel They showed neither aldehydic ν C—H nor carbonyl ν C=O bands and were considered to be caused by a new surface species It was thought that two distinct types of species might be formed, the first of these would have an alkoxide type structure (Fig 1) and the second would be either a linear or cyclic polymeric aldehyde, but Young and Sheppard were unable to discriminate between the various possibilities

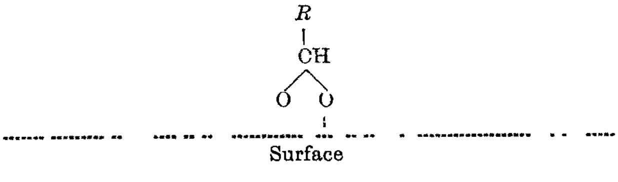

Fig 1

Using the sampling arrangements described earlier[1], we have investigated the Raman spectrum of acetaldehyde adsorbed onto silica gel The spectrum showed seven bands between $\Delta\nu = 80$ cm^{-1} and 2,000 cm^{-1}, none of which were found to coincide with the emissions caused by liquid acetaldehyde[4] and in particular the absence of the carbonyl ν C=O band at 1,720 cm^{-1} was most noticeable (Table 1)

Table 1

Adsorbate/ adsorbent	Paraldehyde (cm^{-1})	Acetaldehyde (cm^{-1})	Distillate (cm^{-1})
cm^{-1} *240 m			
	274 w		
	372 w		
477 s	474 s		474 s
		515 vs	515 s
527 vs	526 vs		528 vs
	575 w		
	755 w		
839 m	839 s		843 m
	857 w		
		887 s	892 m
		914 s	916 m
	945 w		
	1,101 w		
		1,119 s	1,117 m
1,174 m	1,169 w		
	1,192 m		1,184 m
	1,345 w		
		1,355 s	1,358 m
	1,372 w		
		1,392 s	1,398 s
		1,431 s	1,430 s
1,459 m	1,452 s		1,454 s
		1,720 vs	1,723 s

* Due to the instrument[5]

The spectrum was found to resemble that due to paraldehyde (Fig 2) because the stronger emissions of this cyclic molecule occur in the adsorbate–adsorbent system Subsequent desorption into a cold trap produced a liquid the spectrum of which is consistent with the circumstance of it being a mixture of acetaldehyde and paraldehyde

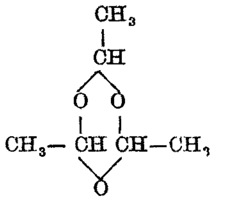

Fig 2.

Although overall similarities between the spectra of the structures shown in Figs 1 and 2 would be expected, the close resemblance between the spectra of paraldehyde and the adsorbate–adsorbent system strongly suggests that condensation of acetaldehyde has been catalysed at the surface, leaving a physically adsorbed cyclic product

It may be objected that the laser beam itself could raise the temperature of the sample locally and evaporate off the adsorbate and its reaction products This possibility has been discussed[1], but it is considered significant that the spectrum of the adsorbed layer is not identical with that of the distillate and bands characteristic of the volatile acetaldehyde are absent from it

It is evident that the use of Raman spectroscopy has considerable potential in the study of adsorbed species The small amount of interference from the substrate does not prevent the convenient recording of data over a wide and continuous frequency range ($\Delta\nu = 3,600$ cm^{-1} to 80 cm^{-1})

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[1] Hendra, P J , and Loader, E J , *Nature*, **216**, 789 (1967)
[2] Young, R P , and Sheppard, N , *J Catalysis*, **7**, 223 (1967)
[3] Young, R P , and Sheppard, N , *Trans Faraday Soc* , **63**, 2291 (1967)
[4] Wood, R W , *J Chem Phys* , **4**, 536 (1936)
[5] Beattie, I R , *Chemistry in Britain*, **3**, 347 (1967)

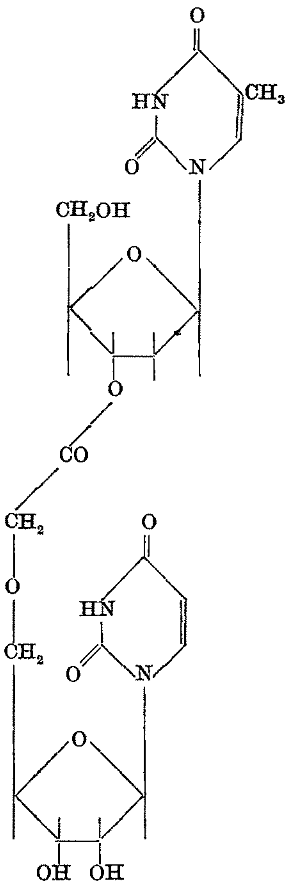
Synthetic Analogues of Polynucleotides

Analogues of purines and pyrimidines have been used as antimetabolites[1] Some of these have been converted into their nucleosides or nucleotides which also act as antimetabolites[2] Analogues of nucleosides in which D-ribose or 2-deoxy-D-ribose is replaced by another carbohydrate or carbohydrate analogue have also been extensively studied[2] Some of the nucleotide analogues have been converted into polynucleotide analogues by the use of suitable enzymes[3–6] and by taking advantage of the fact that certain micro-organisms will incorporate the nucleotide analogue into DNA or RNA (refs 7–13) The synthesis of polynucleotide analogues in which the purine or pyrimidine side chains are linked by a type of backbone different from the sugar phosphate backbone of the natural polynucleotides has been reported in previous work from this laboratory One type was obtained by copolymerizing 5′-*O*-acryloyluridine with acrylamide[14] and another by reacting adenosine dialdehyde with polyacrylic acid hydrazide[15] Both these polymers contained a fraction which hybridized with denatured DNA trapped in agar and with denatured DNA in solution

Another type of polynucleotide analogue is one in which the carbohydrate and purine and pyrimidine bases are the same as those of natural polynucleotides but in which the internucleoside linkage is not a phosphodiester linkage A dinucleoside phosphate analogue of this type has been synthesized in which the nucleoside units were linked by a phosphorothioate group[16] We were interested in synthesizing analogues in which the linkage between the nucleoside units was a carboxylic ester linkage This would give an uncharged molecule which might be expected to penetrate membranes of living cells more easily than a charged oligonucleotide From the chemical point of view, one of the easiest types of monomeric derivatives to prepare seemed to be the carboxymethyl derivatives of the nucleosides The linkage obtained by joining units of this type contains one more atom than does the phosphodiester linkage Examination of molecular models has shown, however, that the carboxymethyl ester linkage is fairly flexible so that the nucleoside units can become similarly spaced to those of the natural polynucleotides (7 1 Å) (ref 17)

As a first stage, an analogue of a dinucleoside phosphate was synthesized The carboxymethylnucleoside chosen was 5′-*O*-carboxymethyl-2′,3′-*O*-isopropylidineuridine (I) This was obtained as the sodium salt by treating the disodio-derivative of 2′,3′-*O*-isopropylidineuridine with sodium chloroacetate in dimethylsulphoxide In addition to the desired product, small amounts of 3-carboxymethyl-2′,3′-*O*-isopropylidineuridine (II) and 3-carboxymethyl-5′-*O*-carboxymethyl-2′,3′-*O*-isopropylidineuridine were formed If the monosodio-derivative of isopropylidineuridine was used the major product was II I, as the pyridinium salt, was condensed with 5′-*O*-tritylthymidine in the presence of dicyclohexylcarbodiimide and the trityl and isopropylidine protecting groups removed with 80 per cent acetic acid to give the dinucleoside phosphate analogue, 3'-*O*-(uridin-5'-yl-glycollyl)-thymidine (III).

III had λ_{max} 264 mμ in 0.01 N hydrochloric acid and an extinction coefficient of 18.3×10^3. The corresponding value for 5'-*O*-carboxymethyluridine (obtained from I by removal of the isopropylidene group with dilute acid) was 10.3×10^3, and those for uridine and thymidine were 10.1×10^3 and 9.65×10^3, respectively. Thus the extinction coefficient of III is 7–8 per cent lower than that calculated by summation of the corresponding values for the constituents of the molecule. This hypochromic effect is larger than that of about 3 per cent found for dithymidine phosphate in acid solution[18], and greater than the value of about 5 per cent found for polyuridylic acid[19]. This can be explained by the fact that the internucleoside linkage in III is more flexible than the phosphodiester linkage and therefore allows greater interaction between the bases than is the case with the oligonucleotides. It may be noted that the hypochromic effect for p^1,p^2-diadenosine-5'-pyrophosphate is considerably greater than that for diadenylic acid and that this has been attributed to the same effect[20].



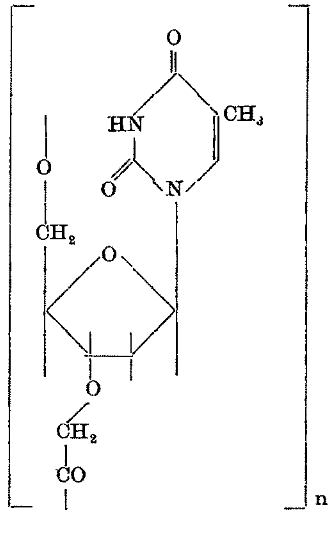
III

3'-*O*-(uridin-5'-yl-glycollyl)-thymidine

Solutions of III and polyadenylic acid in buffer, *p*H 6.3, of various ionic strengths showed no hypochromic effect when mixed. This result was not surprising because thymidine-containing oligonucleotides of chain length less than five showed no hypochromic effect with polyadenylic acid[21].

Because of these results we decided to synthesize a polymer of a carboxymethylnucleoside. We used 3'-*O*-carboxymethylthymidine, which was obtained as follows. The disodio-derivative of 5'-*O*-tritylthymidine was treated with sodium chloroacetate in dimethyl sulphoxide to give the sodium salt of 3'-*O*-carboxymethyl-5'-*O*-tritylthymidine (IV) as the major product. Some 3-carboxymethyl-5'-*O*-tritylthymidine was also produced.

The compound IV was obtained pure by fractional crystallization and the trityl group removed with dilute acetic acid to give 3'-*O*-carboxymethylthymidine. This was polymerized in the presence of a tenth molar proportion of IV (to act as a chain terminator) in concentrated solution in pyridine by the use of dicyclohexylcarbodiimide. The terminal trityl group was removed by treatment with dilute acetic acid, and the product dissolved in dimethylformamide, dialysed exhaustively against dimethylformamide and finally against water or salt solution to give a polymer of structure V. Although the solubility of the polymer in aqueous solution was low (about 4 μg/ml), this was sufficient for hybridization experiments with polynucleotides. The extent of hybridization was determined by measuring the hypochromic effect obtained on mixing solutions of the polymer with solutions of the polynucleotide. With polyadenylic acid a hypochromic effect of about 8 per cent was shown at room temperature in 1 M sodium chloride and in water, but there was no effect with polyuridylic acid or with denatured DNA.



V

A solution of the polymer in dimethylformamide was fractionated on 'Sephadex *LH*-20'. The results showed that part of the material was excluded and part just included in the gel. The material that was excluded therefore had a molecular weight greater than about 4,000. Both the fraction excluded from the 'Sephadex' and that included showed a hypochromic effect of 7–8 per cent with polyadenylic acid in water. A solution of the excluded fraction in water was allowed to stand at room temperature for two weeks. On refractionation in dimethylformamide solution on 'Sephadex *LH*-20', all the material was still excluded from the gel which showed that the polymer was stable in these conditions.

These results indicate that the polymeric thymine derivative was interacting with polyadenylic acid probably by means of specific hydrogen bonding as in the case of natural polynucleotides. An interesting feature was that this interaction occurred in water as well as in salt solution, in contrast with the natural polynucleotides which do not interact at low ionic strengths[19,22]. This suggests the possibility that polymers of this type might interfere with the function of messenger RNA in biological systems.

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[1] Bendich, A , *Nucleic Acids* (edit by Chargaff, E , and Davidson, J N), 1, 101 (Academic Press, 1955)
[2] Michelson, A M , *The Chemistry of Nucleosides and Nucleotides*, 54 (Academic Press, 1963)
[3] Shapiro, L , and August, J T , *J Mol Biol* , **14**, 214 (1965)
[4] Wahba, A J , Gardner, R S , Basilio, C , Miller, R S , Speyer, J F , and Lengyel, P , *Proc US Nat Acad Sci* , **49**, 116 (1963)
[5] Michelson, A M , and Pochon, F , *Biochim Biophys Acta*,**114**, 469 (1966)
[6] Pochon, F , and Michelseon, A M , *Biochim Biophys Acta*,**145**, 321 (1967)
[7] Dunn, D B , and Smith, J D , *Nature*, **174**, 305 (1954), *Biochem J* , **67**, 494 (1957)
[8] Prusoff, W H , *J Biol Chem* ,**226**, 901 (1957)
[9] Smith, J D , and Matthews, R E F , *Biochem J* , **66**, 323 (1957)
[10] Mandel, H G , Markham, R , and Matthews, R E F , *Biochim Biophys Acta*,**24** 205 (1957)
[11] Mandel, H. G , and Markham, R , *Biochem J* **69**, 297 (1958)
[12] Gordon, M P , and Staehelin, M , *J Amer Chem Soc* , **80**, 2340 (1958)
[13] Zamenhof, S , Rich, K , and Giovanni, R de, *J Biol Chem* , **234**, 2960 (1959)
[14] Cassidy, F , and Jones, A S , *Europ Polymer J* ,**2**, 319 (1966)
[15] Jones, A S , and Taylor, N , *Nature*,**215**, 505 (1967)
[16] Eckstein, F , *J Amer Chem Soc* , **88**, 4292 (1966), *Tetrahedron Lett* ,1157 (1957)
[17] Franklin, R F , and Gosling, R G , *Nature*, **171**, 740 (1953), Watson, J D , and Crick, F H C , ibid ,**171**, 964 (1953)
[18] Gilham, P T , and Khorana, H G , *J Amer Chem Soc* , **80**, 6212 (1958)
[19] Felsenfeld, G , and Rich, A , *Biochim Biophys Acta*, **26**, 457 (1957)
[20] Michelson, A M , *Biochim Biophys Acta*, **55**, 841 (1962)
[21] Naylor, R , and Gilham, P T , *Biochemistry*, **5**, 2722 (1966), Rich, A , and Tinoco, I , *J Amer Chem Soc* , **82**, 6409 (1960)
[22] Warner R C , *J Biol Chem* ,**229**, 711 (1957)

Laser Heating of Coal Particles in the Source of "Time-of-Flight" Mass Spectrometer

During general investigation of the processes that occur in the combustion of pulverized coal, experiments have been undertaken to develop methods for determining the nature of products formed by the rapid thermal decomposition of coal particles, especially those evolved in the very early stages of decomposition In previous work[1] the yields of the stable decomposition products evolved from small coal particles during the first millisecond of flash heating were determined by quenching the products in an inert atmosphere Using this method, however, it was not possible to determine whether free radical fragments constituted part of the primarily evolved species Recent work on the pyrolysis of lump coal using a laser beam has suggested that free radicals of molecular weight of about 100 are evolved[2], but in these experiments the products arose both from complete vaporization within the crater formed by the direct beam and from relatively slow low-temperature pyrolysis around this crater

In the experiments reported here, coal particles were heated in the source of a Bendix time-of-flight mass spectrometer and the spectra of the materials evolved have been recorded

A thin layer of particles of coal (Coal Rank Code No 902· C, 80 9, H, 4 8, O, 12 1 per cent dmmf basis), smaller than 10 μm in size, was placed on the upper polished end of a quartz rod that projected vertically through a vacuum seal in the base of the source-housing of the spectrometer, the upper end of the rod being located just below the ion grids of the source A ruby laser was mounted above the housing and the focused beam entered the source vertically through a quartz window and passed between the grids to strike the coal particles within a circular area of about 1 mm diameter The output from the laser was about 0 7 J which corresponded to a radiant flux falling on the surface of about 100 kW/cm² The weight of coal heated in this way was approximately 1 μg

The pyrolysis products from the coal travelled only about 1 cm in the high vacuum of the mass spectrometer before reaching the ionizing electron beam, thus minimizing the chance of secondary reactions occurring before analysis The mass spectra of the reaction products were shown on an oscilloscope and recorded using a high-speed drum camera, the interval between successive recorded spectra was 200 μs The first spectrum of the products of laser pyrolysis, recorded within 200 μs of the laser pulse, showed an unusual distribution of peaks that could not be satisfactorily explained by the cracking pattern of the low molecular weight decomposition products that are normally found in coal carbonization, and it was noted that the higher molecular weight materials normally found in coal tars were absent A typical example of such a spectrum, obtained at 70 eV, is shown in Fig 1*a* From this spectrum, and from others produced at lower electron energies, the following observations have been made about the nature of the initial products from the laser pyrolysis of the coal

(1) *Radicals and atoms* In the spectrum produced at an electron energy of 70 eV the peak at mass 15 (CH_3) was higher than that at mass 16 (CH_4); at the much lower electron energy of 13 eV (Fig 1*b*), at which the mass 16 has almost disappeared from the spectrum, the 15 peak persisted This suggests that the 15 peak is derived from methyl radicals and not from the cracking of CH_4 or methyl substituted hydrocarbons because at 13 eV the ionization voltage was too low to rupture the C—CH_3 or C—H bonds Similarly, the peaks at masses 1 and 2, which are caused by H and H_2, show a much higher mass 1/mass 2 ratio than is usual for molecular hydrogen The conclusion is that some of the mass 1 peak is caused by hydrogen atoms in the pyrolysis products The peak of mass 12 (carbon atoms) is too high to be accounted for by the cracking patterns of the hydrocarbons present and it is concluded therefore that carbon atoms generated during the pyrolysis also contribute to this peak

(2) *Polyacetylenes* The 26 peak, attributed to acetylene, is the principal hydrocarbon product and is accompanied by peaks at 50 (diacetylene) and two peaks

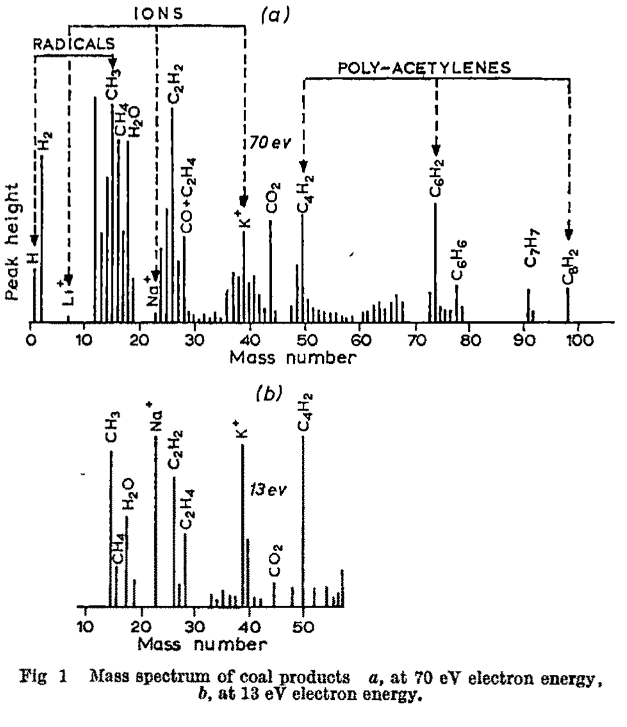

Fig 1 Mass spectrum of coal products *a*, at 70 eV electron energy, *b*, at 13 eV electron energy.

at 74 and 98 which are tentatively assigned to tri- and tetra-acetylene

(3) *Ions* The peaks at masses 7, 23 and 39 were detected even at zero electron energy and these are attributed to ions of the alkali metals, lithium, sodium and potassium Confirmatory experiments, in which carbon black and phenanthrene were heated with and without sodium and potassium salts, showed that these ions did not originate from the surface of the quartz rod

The results show that reactive species are evolved from coal on laser heating, but the temperature reached by the particles during the heat treatment is not known The height of the peak of mass 12 suggests the presence of a relatively high concentration of carbon atoms and indicates therefore that at some stage in the heating, temperatures of several thousands of degrees centigrade may have been reached The technique has, however, been shown to be suitable for detecting radicals and atoms, if present, and will therefore allow critical examination of spectra obtained by heating solids at a range of energies of laser radiation

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[1] Granger, A F , and Ladner, W R , *Combustion and Flame* (in the press)

[2] Vastola, F J , and Pirone, A J , *Amer Chem Soc , 151st Meeting Div of Fuel Chem , Symposium on Pyrolysis Reactions of Fossil Fuels*, Pittsburgh, **10**, No 2 (1966)

Preparation and Use of Porous Sheets with Enzyme Action

Two of the most important factors affecting the rate of catalysis by "insolubilized" enzymes are the rate of diffusion of substrate molecules from the bulk of the solution to the attached enzyme[1,2] and the interaction between the charged groups on the support material and the charged groups of the substrate molecules[3]

Many methods[4] are now available for coupling enzymes to insoluble supports, but they usually result in a support with a net negative charge Preparations of enzymes which attack neutral or negatively charged substrates such as ATP or NADH have activities which often seem to be low This is generally caused by electrostatic repulsion between the substrate and the support which results in the attached enzyme having a high apparent Michaelis constant compared with that of the free enzyme[1] It is therefore an advantage for such enzymes to be attached to supports with net positive charges In preparing these types of support, we have found that for attachment of enzymes to cellulose and cellulose derivatives it is an advantage if the net charge on the support is of opposite sign to that carried by the enzyme at the *p*H of attachment In most cases attachment is carried out in slightly alkaline medium (*p*H 7–9) Because the isoelectric points of many enzymes are less than *p*H 7, they are negatively charged above this *p*H and the support should therefore be positively charged

Kay and Crook[5] have attached chymotrypsin to mono and dichloro-*s*-triazinyl celluloses By modification of their technique it is possible to prepare support materials with net negative or positive charges and, if necessary, to alter the sign of the charge on the support after enzyme attachment, for example, by reacting the remaining chlorines of the support with a suitable amine Using the original method of Kay and Crook we have attached chymotrypsin to a dichloro-*s*-triazinyl cellulose paper (prepared from Whatman No 1 filter paper) and to a dichloro-*s*-triazinyl filter cloth To attach β-galactosidase and pyruvate kinase to these papers, however, it was first necessary to react the dichloro-*s*-triazinyl cellulose with a 0 2 N solution of N-(3-aminopropyl)-diethanolamine (Aldrich Chemical Co) at *p*H 7 0 for 7 5 min This results in a positively charged cellulose derivative It also has the effect of removing any free cyanuric chloride still remaining in the dichloro-*s*-triazinyl cellulose Very small amounts of cyanuric chloride can inactivate some enzymes presumably by reacting with certain groups (for example, sulphydryl) in their active sites After this pre-treatment, the paper was reacted with the enzyme for 10 min at *p*H 7 2 and 25° C

We have also attached β-galactosidase, creatine kinase and lactate dehydrogenase to chloro-amino-*s*-triazinyl derivatives of DEAE-cellulose paper (Whatman *DE* 81) Dichloro-carboxymethyl amino-*s*-triazine was reacted with *DE* 81 paper at *p*H 11 0 for 3 min and, after thorough washing at *p*H 2 0, the resulting product was reacted with β-galactosidase at *p*H 8 for 2 h at 25° C This technique was slightly modified for the attachment of creatine kinase Lactate dehydrogenase was attached to *DE* 81 paper by a similar technique (unpublished work) using dichloro-*s*-triazine 'Procion' dye 'Brilliant orange MGS' (Mayborn Products) The behaviour of these sheets has been investigated by passing substrates through them in various conditions Some examples are shown in Fig 1 All these preparations, with the possible exception of pyruvate kinase, were stable for at least several months

In most conditions, packed beds with enzyme action are theoretically more efficient than continuously fed stirred tanks for performing biochemical conversions[2] Porous sheets are a particular case of packed beds and have many operational advantages Reactors using several sheets stacked in series are easily set up and

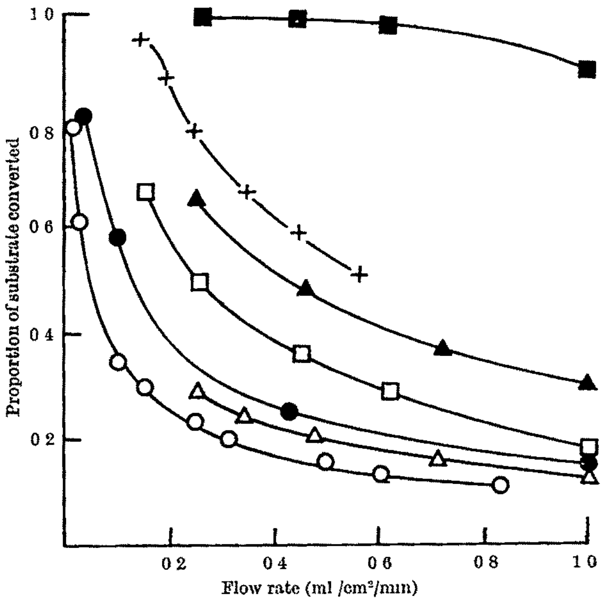

Fig 1 The effect of flow rate on the proportion of the rate-limiting substrate converted to product during passage through an enzymatically active paper sheet or filter cloth

Enzyme	Concentration of rate-limiting substrate	*p*H	Temperature (°C)
+ Lactate dehydrogenase	0 14 mM sodium pyruvate	7 4	25°
■ β-Galactosidase	2 5 mM *o*-nitrophenyl-galactoside	7 2	25°
□ β-Galactosidase	4 mM lactose	7 2	40°
▲ Pyruvate kinase*	0 26 mM ADP	7 4	25°
△ Creatine kinase	0 71 mM ATP	8 1	25°
● Chymotrypsin†	10 mM acetyltyrosine-ethylester	8 0	22°
○ Chymotrypsin	1 per cent (w/v) casein	8 5	22°

* Two sheets
† Attached to filter cloth

operated On an industrial scale, filter cloths with enzyme action can be mounted in a plate-and-frame filter press, in the laboratory scale, small pieces of active paper can be used by mounting them in a filter holder (Gelman Instrument Co , Michigan) In this way, good flow rates can be obtained without plugging or channelling, and the bed size can readily be altered by adding or removing sheets By using several sheets with different enzymes attached, it is possible to build a "multi-enzyme" reactor

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[1] Hornby, W E , Lilly, M D , and Crook, E M , *Biochem J* (in the press)
[2] Lilly, M D , and Sharp, A K , *The Chemical Engineer* (in the press)
[3] Goldstein, L , Levin, Y , and Katchalski, E , *Biochemistry*, 3, 1913 (1964)
[4] Silman, H I , and Katchalski, E , *Ann Rev Biochem* , 35, 873 (1966)
[5] Kay, G , and Crook, E M , *Nature*, 216, 514 (1967)

De-esterification using Ion Exchangers

Ion exchangers, as the name implies, are used principally for the removal of unwanted ions. but they have also been used for ionic catalysis. In particular, cation exchangers in the H^+ form have been employed to catalyse reactions requiring H^+ in which the product is required free from acid

The hydrolysis of esters can be catalysed by acids or bases, but the latter are more effective Esters of different types of alcohol (primary, secondary, hemiacetal) have different stability to base-catalysed hydrolysis This property was employed in the selective hydrolysis of peracetylated sugars with acetonic potassium hydroxide to give compounds which are esterified at the primary hydroxyl groups only[1-3] The sugar products of these reactions were accompanied by potassium acetate from which they had to be separated

This communication describes the use of anion exchange columns to accomplish two tasks first, to catalyse the hydrolysis of esters and, second, to absorb the acid so formed Peracetylated sugars and sugar alcohols were used as starting materials Preliminary experiments indicated that weak anion exchangers such as 'Dowex 3' or 'Duolite *A*6' were not effective and so 'Dowex 1' was used A column of this material effectively hydrolyses β-glucose pentacetate or β-galactose pentacetate to the free sugar, or sorbitol hexacetate to the alditol at room temperature If, however, the temperature is below 5° C, then the deacetylation is not completed Thus 'Dowex 1' × 1 50–100 mesh (400 g) was equilibrated with normal sodium hydroxide, washed three times with water and twice with actone water 1 1 and placed in a cooled Liebig condenser measuring 200 × 2 cm and washed with acetone–water until the *p*H of the eluant was below 8 Ten grams of β-glucose pentacetate or β-galactose pentacetate in 20 ml acetone was then applied to the column, which was maintained below 5° C The column was eluted with acetone–water The first 650 ml of eluate contained the products, which were a mixture of several acetates of the sugars, with 6-*O*-acetyl glucose or 6-*O*-acetyl galactose, respectively, predominating These were crystallized and identified When sorbitol hexacetate was applied, thin layer chromatography of the eluate (silica-gel *D*5 from Camag developed with chloroform methanol, 88 12, and made visible with iodine), showed the product to be a mixture of acetates Of these, the 1,6-di-*O*-acetyl sorbitol predominated Also present were monoacetyl sorbitol, esterified at a primary alcoholic group, and triacetyl sorbitol (esterified at C1, C6 and C2 or possibly C5) and some tetraacetyl sorbitol

At both room temperature and in the cold, the column absorbed the acetate that was formed Attempts to use the column for a third task, namely, that of separating the sugar–ester products from each other, were unsuccessful

Preliminary experiments indicate that the method of ion exchange de-esterification would be widely applied, not only for sugar esters but also for lipids Thus glyceryl tripropionate was hydrolysed to a mixture of glycerol and monoglycerate A paper chromatogram of the latter reacted slowly with alkaline permanganate–periodate spray[4] (for glycols), and weakly with the alternate periodate–*p*-anisidine spray[5] but strongly with alkaline hydroxylamine–acidic ferric chloride spray[6] (for esters) Quantitative periodate oxidation confirmed that it was a mixture of α- and β-monopropionin The mixture could have arisen as a result of acyl migration[7]

Polyacetylated oligosaccharides can also be deacetylated in this fashion, for the 'Dowex 1' column does not affect most glycosidic linkages Even the relatively labile β-fructofuranoside linkage is not hydrolysed, and, on deacetylation, sucrose octacetate gave sucrose admixed with sucrose monoacetate On the other hand, in suitable conditions, 'Dowex 50' and other cation exchangers hydrolysed glycoside bonds but not acetyl bonds

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[1] Frohwein, Y Z , and Leibowitz, J , *Nature*, 186, 153 (1960)
[2] Frohwein, Y Z , *Nature*, 196, 775 (1962)
[3] Frohwein, Y Z , and Leibowitz, J , *Bull Res Coun* , Israel, 11A, 330 (1963)
[4] Lemieux, R U , and Bauer, H F , *Anal Chem* , 26, 920 (1954)
[5] Bragg, P D , and Hough, L , *J Chem Soc* , 4050 (1958)
[6] Frohwein, Y Z , *Israel J Chem* , 2, 57 (1964)
[7] Borgstrøm, B , *Acta Physiol Scand* , 30, 231 (1954)

RADIOBIOLOGY

Attenuation in the Chest Wall of 20 keV X-rays from an Inhaled Radioactive Aerosol

External counting of the low intensity *L* uranium X-rays (energies 13 6, 16 9 and 20 2 keV) is a promising technique for the estimation of insoluble compounds of plutonium in the human lung These low energy radiations are, however, very easily absorbed in the tissues of the chest wall, the half-value thickness (HVT) is about 0 7 cm of soft tissue and only 0 03 cm of bone The effects of variations in body build are clearly important and difficult to assess, and calibration of the counting equipment is a severe problem One approach has been the use of a phantom[1] or cadaver[2] In neither of these cases is there any certainty that the radiation from plutonium inserted into the chest cavity will undergo the same attenuation by surrounding tissues as *in vivo*

There are now techniques for the preparation of monodisperse aerosols of polystyrene particles, labelled with radio-nuclides, and suitable for inhalation by volunteers[3]. This has made it possible to study the plutonium calibration problem *in vivo*, using radio-nuclides which decay by orbital electron capture, and so emit characteristic X-rays. As the first radio-nuclide for investigation we chose 17 day palladium-103, which emits the characteristic K X-rays of rhodium (K_α at 20·2 keV, K_β at 22·8 keV) and is thus very suitable as a component of "mock plutonium"[4].

Irradiated palladium was purified and converted into the bis-diethyldithiocarbamate, which was dissolved in xylene containing 0·2 per cent of polystyrene. Because the palladium-103 emits no significant gamma-radiation we used 27·9 day chromium-51 to provide a "label" (0·323 MeV gamma-rays). This was added to the polystyrene solution as the acetylacetonate, to give a mixture of approximately equal activities at the beginning of the experiments. A monodisperse aerosol of polystyrene particles 5 μm in diameter was generated by the spinning disk technique[3].

Seven healthy adult men volunteered to inhale the radioactive aerosol; the weights and heights of the subjects covered a wide range as shown in Table 1. The lung contents of chromium-51 were determined by gamma-ray spectrometry using two collimated crystals of NaI(Tl), 15 cm diameter by 8·9 cm thick, placed above and below the chest of the supine subject as described elsewhere[3]. From the estimated contents of chromium-51 (Table 1) the contents of palladium-103 were calculated from the known initial activity ratio and the half-lives.

The X-rays from the palladium-103 were measured inside a lead shield with a gas-flow (90 per cent Ar, 10 per cent CH_4) proportional counter, approximately 30 cm square, placed about 1 cm above the highest point of the chest of the supine subject. This counter, which will be described in detail elsewhere, uses the multi-wire method of internal anti-coincidence for background reduction[5]. The main counting volume of 9 l. is defined on four sides by 103 wires which also form the cathodes for thirty-nine anti-coincidence counters; these reduce the background response from about 725 c.p.m. to 13 c.p.m. above a threshold of 8 keV. A further reduction to 4 c.p.m. results from the use of pulse rise time discrimination[6], which also reduces the contribution at low energies from the chromium-51 gamma-rays, but without affecting significantly the response to 20 keV X-rays. For these experiments, an energy band of 14–29 keV was used and the background in this band was 2·3 c.p.m.

The counting rate per microcurie of palladium-103 was determined at various times after the inhalation, and in Fig. 1 the results are plotted as a function of the ratio of weight to height (a measure of the average cross-section area of the body) for the seven subjects. Two sets of results are shown—those obtained from the first measurement after inhalation and the average of those obtained after 48 h and up to 14 days, when the early phase of elimination from the lung was complete. At 48 h the average retention was 69 per cent (range 62–76 per cent), and in all subjects the retention was decreasing only slowly.

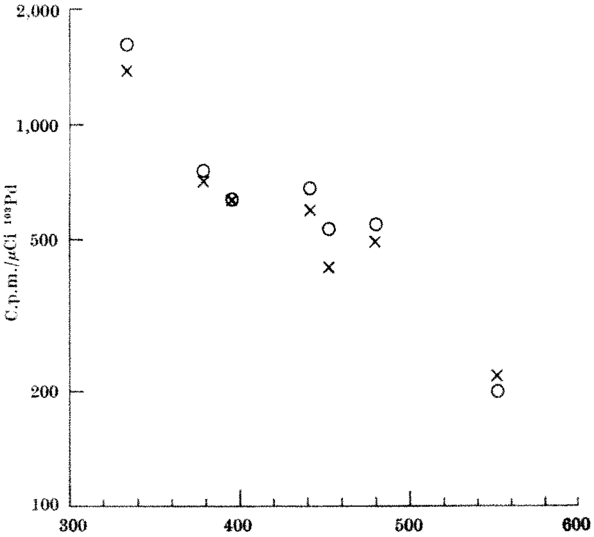

Fig. 1. Variation of counting efficiency with the ratio weight/height. ○, Measurements at about 30 min after inhalation; ×, average of measurements made between 2 days and 14 days after inhalation.

Table 1

Subject	Age	Weight (kg)	Height (cm)	Chest circumference (cm)	Radioactivity inhaled, μCi ^{51}Cr	Radioactivity inhaled, μCi ^{103}Pd
D.V.B.	46	61·5	184	88·6	0·304	0·226
D.N.	30	64·5	170	93·5	0·098	0·081
R.P.R.	40	70·5	178	95	0·278	0·172
J.E.M.J.	56	79·5	180	96·3	0·260	0·171
J.R.	42	80·6	178	99·7	0·367	0·273
N.G.S.	51	88·0	183	105	0·311	0·205
J.W.C.J.	47	107·0	194	114	0·315	0·195

There is a remarkable degree of correlation between the counting efficiency and the crude measure of body thickness represented by the ratio of weight to height. A very similar correlation was observed when the results were plotted against the chest circumference. There was only a small change in the counting efficiency as a result of the removal of some 30 per cent of the activity, presumably from the ciliated region of the lung. There was no significant systematic change after 2 days; when we ignore effects of statistical fluctuations, the range of all the values for any one subject corresponds to a change in average absorption of only 5 mm of soft tissue (about half one HVT at 20·2 keV).

The factor of eight between the efficiencies for the subjects at the extremes of build corresponds to a difference in absorption of about 3 cm of soft tissue, and this is in very good agreement with the results of investigations using ultrasonics for subjects of similar builds[7]. In that method, however, only the soft tissue thickness external to the rib cage was measured, whereas our measurements give results which include the effects of the ribs, and of differences in the size of the lungs which affect the geometrical efficiency.

Because the uranium L X-rays emitted by plutonium-239 have energies between 13·6 and 20·2 keV, the variations in absorption of that radiation among subjects will be even bigger than observed by us. It should also be pointed out that the fraction of counts attributable to K_β radiation (22·8 keV) from palladium-103 in the lung is higher than that from a bare source, so that this method probably under-estimates the effects of absorption at 20·2 keV. We propose to carry out further experiments using other radio-nuclides which emit X-rays of lower energies and with more efficient counters being developed commercially from our design.

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[1] Speight, R. G., Peabody, C. O., and Ramsden, D., in *Assessment of Radioactivity in Man*, **1**, 115 (Vienna, IAEA, 1964).

[2] Ehret, R., Kiefer, H., Maushart, R., and Möhrle, G., in *Assessment of Radioactivity in Man*, **1**, 141 (Vienna, IAEA, 1964).

[3] Booker, D. V., Chamberlain, A. C., Rundo, J., Muir, D. C. F., and Thomson, M. L., *Nature*, **215**, 30 (1967).

[4] Taylor, B. T., and Rundo, J., UKAEA Rep. *AERE-R* 4155 (1962). Also in *Radioactivity in Man* (Second Symposium), 52 (Thomas, Springfield, Illinois, 1965).

[5] Drever, R. W. P., Moljk, A., and Curran, S. C., *Proc. Roy. Soc.*, A, **239**, 433 (1957).

[6] Mathieson, E., and Sanford, P. W., *Proc. Intern. Symp. Nucl. Elect.*, 65 (Paris, ENEA, 1963).

[7] Ramsden, D., Peabody, C. O., and Speight, R. G., UKAEA Rep. *AEWE-R* 493 (1967).

Influence of Micro-organisms on the Distribution in Roots of Phosphate labelled with Phosphorus-32

MICRO-ORGANISMS have been shown to affect profoundly the uptake of phosphate by plant roots, especially from dilute solutions[1-6]. The way in which they influence the distribution of phosphate between different tissues of roots has now been examined in autoradiographs.

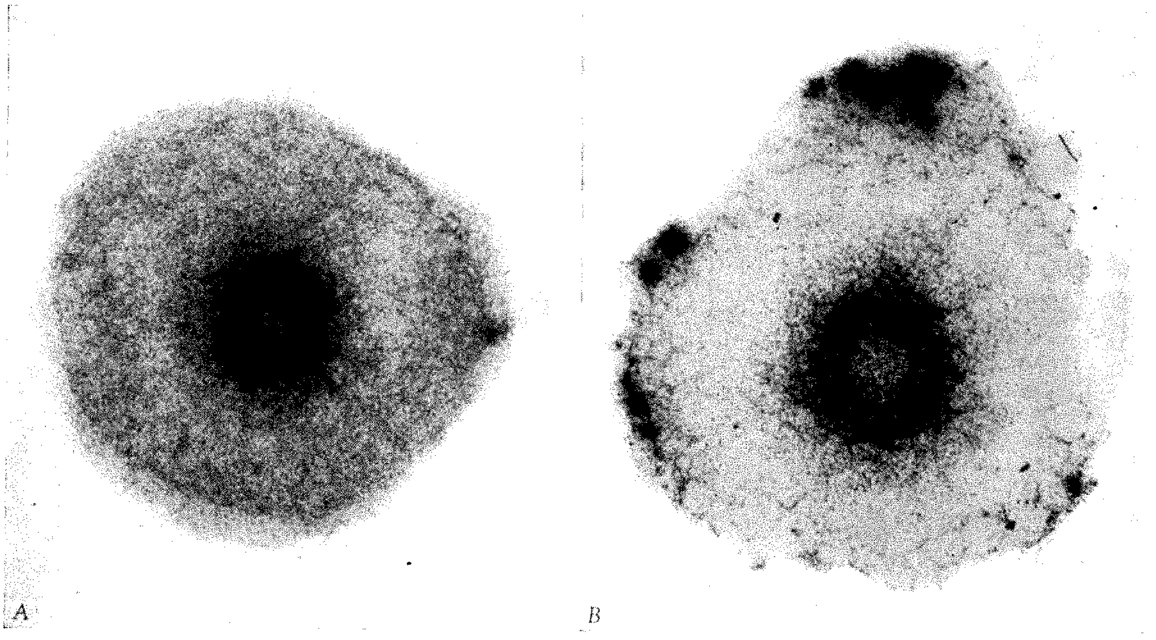

Fig. 1. Autoradiographs showing the distribution of phosphate in transverse sections of roots from barley plants which had been supplied for 6 h with 3×10^{-6} molar potassium phosphate containing 1 mc. of phosphorus-32/l. *A*, Plant grown in the absence of micro-organisms; *B*, plant infected at the ambient laboratory level.

Fig. 1 shows typical results for transverse sections of roots of 3 week old barley plants which were grown under sterile and non-sterile conditions and which were supplied for 6 h with 3×10^{-6} molar solutions of potassium phosphate containing 1 mc. of phosphorus-32. The procedures for plant culture have been described by Barber[4]; autoradiographs were prepared by the method of Crossett[7].

In roots which were grown in sterile conditions, phosphate was distributed relatively uniformly throughout the cortex and there was a marked accumulation in the stele. The presence of micro-organisms, however, led to considerable concentration of phosphate in irregular zones close to the root surface. These are attributed to the accumulation of the ion in colonies of bacteria, for microscopic examination showed that there was no fungal invasion of the cortex. This accumulation of phosphate in micro-organisms is readily compatible with the observation that nucleic acids account for a considerably larger fraction of the recently absorbed phosphate in infected than in uninfected roots[2,3,5].

The extent of microbial infection in the root (Fig. 1*B*) is likely to be less than the infection which frequently occurs in water culture because, in order to ensure comparability between sterile and non-sterile plants, all the seedlings were initially grown in sterile conditions, infection being brought about by aeration with unfiltered air. Autoradiographs in which the peripheral accumulation of phosphorus-32 was continuous have been prepared by Crossett[7] from the roots of maize plants which had been grown from germination without any attempt to exclude micro-organisms; he suggested that concentration of the ion in bacteria might be responsible.

It is thus evident that unless plants are grown under rigidly sterile conditions the observed distribution of phosphate between the root cortex and other tissues cannot be regarded as resulting solely from the metabolism of the plant. The extent to which the distribution of other ions is influenced by micro-organisms is not known, but a number of investigations have shown patterns of accumulation which are compatible with the view that microbial effects could be at least a contributory factor; for example, highly localized sites of accumulation of caesium in young barley roots[8] and the concentration of iron in the superficial tissues of pea roots[9].

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[1] Bowen, G. D., and Rovira, A. D., *Nature*, **211**, 665 (1966).
[2] Barber, D. A., *Nature*, **212**, 638 (1966).
[3] Rovira, A. D., and Bowen, G. D., *Austral. J. Biol. Sci.*, **19**, 1167 (1966).
[4] Barber, D. A., *J. Exp. Bot.*, **18**, 163 (1967).
[5] Barber, D. A., and Loughman, B. C., *J. Exp. Bot.*, **18**, 170 (1967).
[6] Barber, D. A., *Nature*, **215**, 779 (1967).
[7] Crossett, R. N., *Nature*, **213**, 312 (1967).
[8] Steward, F. C., *Symp. Soc. Exp. Biol.*, **8**, 393 (1954).
[9] Branton, D., and Jacobson, L., *Plant Physiol.*, **37**, 546 (1962).

MICROBIOLOGY

Stimulative Vaporization of Phenylmercuric Acetate by Mercury-resistant Bacteria

PHENYLMERCURIC acetate (PMA) has been widely used as a fungicide and as a disinfectant. It is well known that the antibacterial action of mercuric compounds results chiefly from their interference with essential

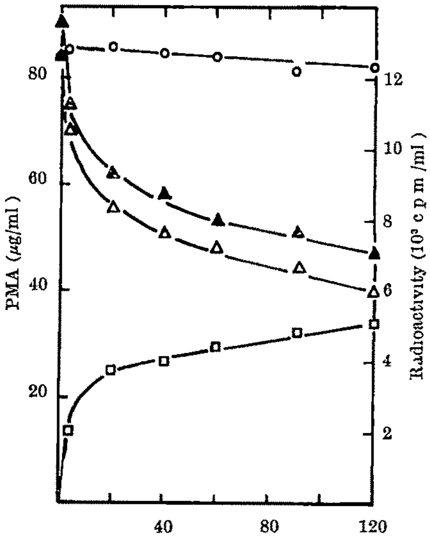

Fig 1 Uptake of ^{203}Hg-PMA by mercury-resistant bacteria The washed cells of the organism (8 8 × 10^8) were incubated in 1 ml cf 0 02 M phosphate buffer, *p*H 7 2, containing 90 p p m PMA Radioactivity in the whole medium (○), supernatant fluids (△), and cells (□) was assayed PMA in supernatant fluids (▲) was also assayed colorimetrically

sulphydryl groups of organisms There are, however, reports that some micro-organisms are resistant to mercuric compounds Russell[1] showed that a mercury-resistant strain of *Penicillium roqueforti* Thom was able to absorb a large amount of mercury from nutrient media containing PMA Stutzenberger and Bennett[2] have shown that *Staphylococcus aureus* is more resistant to mercuric compounds in the presence of *Escherichia coli* which produces extracellular glutathione and hydrogen sulphide This communication describes an experiment in which a large amount of PMA was absorbed by mercury-resistant bacteria The PMA was located chiefly on the bacterial surface and it was biologically stimulated to cause vaporization there

The mercury-resistant micro-organism was isolated from soil by enrichment culture containing glucose, mineral salts, vitamins and PMA The soil from which the micro-organism was isolated was heavily contaminated with PMA The micro-organism produced water-insoluble red pigment and possessed general characteristics of the genus *Pseudomonas* The growth of the organism was inhibited at concentrations of 450 p p m in mercuric chloride, 120 p p m in PMA and 20 p p m in ethylmercuric phosphate These concentrations were about a thousand times greater than those of other bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*

The organism was grown in medium containing glucose, mineral salts and vitamins on a shaker When the washed cells of the organism were incubated in 0 02 M phosphate buffer, *p*H 7 2, containing PMA labelled with mercury-203, PMA was taken up by the organism (Fig 1) PMA was assayed by the colour formed from reaction with dithizone in chloroform[3] Radioactivity in the cells, supernatant fluids and the whole medium was assayed with a scintillation counter The PMA-bound organism was disrupted by Braun's cell homogenizer with beads for 5 min so that the intracellular distribution could be studied, and then it was differentially fractionated by centrifugation Results obtained showed that 76 per cent of radioactivity was present in the pellet obtained after centrifuging at 2,000*g* for 15 min, although 12 per cent of protein was present in this fraction (Table 1) Bound PMA could be easily extracted with 0 1 M acetic acid A mercuric compound extracted from the cell was compared with standard PMA by thin-layer chromatography—no difference could be found between them

Table 1 DISTRIBUTION OF ^{203}Hg-PMA TAKEN UP BY MERCURY-RESISTANT BACTERIA*

Fraction	Radioactivity 10³ c p m	%	Protein mg	%
Homogenate	384	100	45 0	100
2,000*g*, 15 min, pellet 1	264	76	5 2	12
14,000*g*, 15 min, pellet 2	34	10	6 8	15
37,000*g*, 15 min, pellet 3	49	14	7 7	17
100,000*g*, 60 min, pellet 4	15	4	1 6	4
Supernatant 4	6	2	23 0	51

* The organism was disrupted after incubation for 2 h in the same conditions as in Fig 1

Table 2 ABSORPTION OF VAPORIZED RADIOACTIVE MERCURY BY ACTIVE CARBON

Organism	Added PMA		Radioactivity Medium		Carbon	
	c p m /ml	%	c p m /ml	%	c p m /ml	%
Not added	4,220	100	4,054	96	129	3
Added	4,220	100	1,527	36	2,800	67

To find the fate of cell-bound PMA, the PMA-bound organism was transferred to a fresh culture medium containing glucose, mineral salts and vitamins The composition of this medium was as follows 2 5 g of glucose, 0 2 g of KH_2PO_4, 1 6 g of K_2HPO_4, 1 g of $(NH_4)_2SO_4$, 0 2 g of $MgSO_4$ $7H_2O$, 0 02 g of $CaCl_2$ $2H_2O$, 0 1 g of NaCl, 0 01 g of $FeSO_4$ $7H_2O$, 0 5 mg of Na_2MoO_4, 0 5 mg of Na_2WO_4, 0 5 mg of $MnSO_4$, 400 μg of Ca-pantothenate, 200 μg of *p*-aminobenzoic acid, 400 μg of pyridoxine hydrochloride, 400 μg of thiamine hydrochloride, 400 μg of riboflavine, 2 μg of biotin, 0 5 μg of vitamin B_{12}, 1 l of distilled water, *p*H 7 2

It was found that cell-bound radioactivity disappeared rather rapidly from the culture before the beginning of growth (Fig 2) The *p*H of the culture medium changed only from 7 2 to 6 7 during this 53 h of incubation Free PMA, however, hardly disappeared from the solution in the same conditions When the organism was incubated directly with radioactive PMA in culture medium, the disappearance of radioactivity from the culture was also observed A modified type of Conway's microdiffusion apparatus[4] was used to trace this vaporized radioactive mercury It consisted of a glass container resembling a Petri dish (83 mm in inner diameter and 26 mm deep), within which was placed a smaller dish (50 mm in diameter and 13 mm deep) 1 g of active carbon was introduced into an inner dish In an outer dish which was tightly sealed, the organism was incubated in 10 ml of culture medium with 15 p p m PMA for 24 h at 25° C The result showed that the vaporized radioactivity was completely recovered by carbon (Table 2) It is obvious therefore that PMA was bound to the bacterial surface and then it was biologically stimulated to cause the vaporization of mercury The vaporization, however

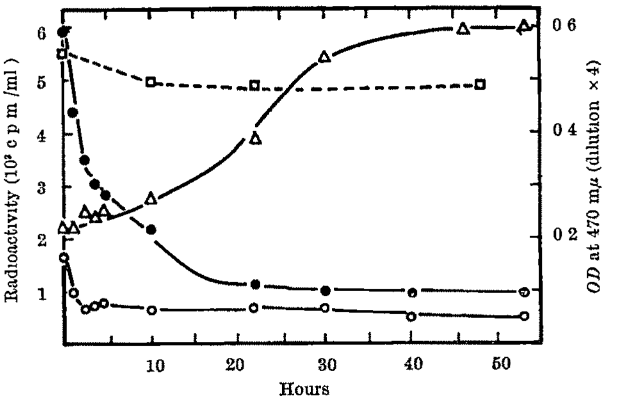

Fig 2 Vaporization of cell-bound ^{203}Hg-PMA PMA-bound cells were transferred to fresh culture medium and incubated Free PMA was also incubated in the same conditions as described here Radioactivity in the whole medium (●) and supernatant fluids (○) and in free PMA solution (□) was assayed The specific activity was 232 c p m per μg of PMA Growth (△) was measured by reading the optical density at 470 mμ

was not stimulated by heated cells, by cells treated with acetone or toluene, nor by disrupted cells The vaporization was inhibited at concentrations of PMA higher than 150 p p m and it was enhanced by cells pre-incubated with PMA The mechanism of the vaporization is not clear, but there are two possibilities The first is that the vaporization is stimulated by a gaseous substance evolved from the bacterial surface on which PMA is bound The second possibility is that there is a chemical conversion of PMA to a substance which is more volatile than PMA Evidence was obtained which suggested that the vaporized mercuric compound was different from PMA This idea was suggested when the vaporized compound absorbed to ferric hydroxide was examined by thin-layer chromatography after extraction with ethylacetate in the presence of hydrochloric acid This, however, does not preclude the first possibility It seems to be interesting that the environment contaminated with PMA is purified by the organism capable of removing PMA from its environment This then results in the proliferation of the organisms

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[1] Russell, P , *Nature*, **176**, 1123 (1955)
[2] Stutzenberger, F J , and Bennett, E O , *Appl Microbiol* , **13**, 570 (1965)
[3] Miller, V L , Polley, D , and Gould, C J , *Anal Chem* , **23**, 1286 (1951)
[4] Conway, E J , and Byrne, A , *Biochem J* , **27**, 419 (1933)

Action of Hydroxyurea on Multiplication of Influenza Virus in Mammalian Cells

HYDROXYUREA has been reported to be a specific inhibitor of DNA synthesis *in vitro* and *in vivo*[1,2] Inhibition results from interference with the reduction of ribonucleotides to deoxyribonucleotides[1,3,4] Hydroxyurea has also been shown to inhibit the multiplication of vaccinia virus[5] and T_4 bacteriophage[6] There has been some controversy concerning the part played by the host cell genome in the multiplication of the influenza virus[7,8] Hydroxyurea was used in this study to gain insight into this question

In 1961 Wong and Kilbourne[9] described a clone of a morphological variant of Chang's conjunctival cell which supported the complete growth of influenza virus These cells (clone 1-5c-4), grown in 60 × 15 mm Petri dishes[10], were used throughout our investigations Influenza *A/NWS* adapted to this cell line was provided by Dr E D Kilbourne The virus was propagated in embryonated eggs and stored at −70° C In all experiments, the input multiplicity was 8–10 PFU/cell and the monolayers were maintained under Eagle's medium containing two times vitamins, amino-acids, glutamine and 0 1 per cent bovine serum albumin fraction V After infection the cultures were incubated at 37° C in a humidified atmosphere containing 5 per cent carbon dioxide for 24 h The supernatant fluids were then removed, cleared by low speed centrifugation and titrated for haemagglutinating activity, using serial two-fold dilutions, and infectivity using half-log dilutions in embryonated eggs Hydroxyurea was found to inhibit the haemagglutinin production of virus infected cultures This effect was observed in cultures that were frozen and thawed (total virus) as well as those in which only the supernatant fractions (extra-cellular virus) were analysed

Table 1 EFFECT OF VARIOUS CONCENTRATIONS OF HYDROXYUREA ON THE HAEMAGGLUTINATION AND INFECTIVITY TITRES OF INFLUENZA *A/NWS* GROWN IN 1–5*c*–4 CELLS

Concentration of hydroxyurea (molar)	Haemagglutination* titre	Infectivity EID_{50}/ml †
10^{-2}	1 4	$10^{-7\ 13}$
10^{-3}	1 4	$10^{-7\ 13}$
10^{-4}	1 8	$10^{-7\ 25}$
10^{-5}	1 32	$10^{-7\ 25}$
10^{-6}	1 64	$10^{-7\ 38}$
Control	1 64	$10^{-7\ 25}$

* Two-fold dilutions using 0 5 per cent fowl red blood cells
† Negative logarithm to the base ten

The optimal concentrations of hydroxyurea producing this effect were 10^{-2} and 10^{-3} M as shown in Table 1 Both concentrations seemed to be equally effective in reducing the haemagglutinin yield, without an appreciable effect on the infectivity titre of the virus produced in this tissue culture system Higher concentrations of hydroxyurea were toxic to the cells during the experimental period, and lower concentrations did not produce an optimal effect When a sample of a standard preparation of virus was incubated with 10^{-2} M hydroxyurea for 1 h at 37° C and a similar sample without hydroxyurea was titrated, the haemagglutinating activity and infectivity titres were the same.

Fig 1 shows that the pretreatment of the cell culture for 1 h was essential for the inhibition of haemagglutinating activity, longer pretreatment periods did not alter the effect If hydroxyurea was added with the virus or any time after infection, a gradual decrease in the inhibition of haemagglutinating activity production was observed until 4 h post-infection when there was no inhibition of either the haemagglutinin or infectivity yields

The inhibitory action of hydroxyurea on DNA synthesis in this system was investigated by determining the rate of incorporation of ^{3}H-thymidine into acid insoluble material in treated and untreated cell cultures The reduction of ^{3}H-thymidine into hydroxyurea treated cultures was 85–95 per cent of the control cultures This inhibition indicates that DNA synthesis, as measured in this manner, was effectively inhibited by 10^{-2} and 10^{-3} molar hydroxyurea

The results already described and those reported for the action of cytosine arabinoside[11] suggest that DNA synthesis in the host cell is not a necessary condition for the replication of influenza virus Although studies with actinomycin *D* have shown that actinomycin *D* does interfere with influenza RNA synthesis[12], they cannot

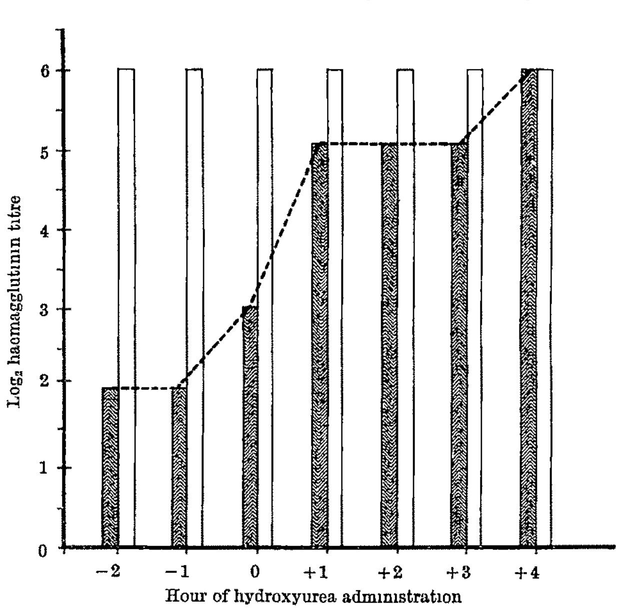

Fig 1 Temporal sequence of inhibition of influenza virus haemagglutinins by hydroxyurea White columns, control, shaded columns, treated

rule out the possibility that host cell genome function may be essential[7,13] This participation of the host cell in influenza replication may perhaps be reflected by the action of hydroxyurea on the production of functional viral haemagglutinin Further efforts at determining the effects of hydroxyurea on the multiplication of influenza virus in the conjunctival cell system are in progress

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Received December 11, 1967

[1] Young, C W, and Hodas, S, *Science*, **146**, 1172 (1964)
[2] Rosenkranz, H S, and Carr, H S, *J Bact*, **92**, 178 (1966)
[3] Adams, R L P, Abrams, R, and Lieberman, I, *J Biol Chem*, **241**, 903 (1966)
[4] Adams, R L P, Abrams, R, and Lieberman, I, *J Biol Chem*, **241**, 5777 (1966)
[5] Rosenkranz, H S, Rose, H M, Morgan, C, and Hsu, K C, *Virology*, **28**, 510 (1966)
[6] Margaretten, W, Morgan, C, Rosenkranz, H S, and Rose, H M, *J Bact*, **91**, 823 (1966)
[7] Barry, R D, *Ciba Foundation Symposium on Cellular Biology of Myxoviruses*, 51 (Little Brown, New York, 1964)
[8] Scholtissek, C, and Rott, R, *Z Naturforsch*, **16b**, 663 (1961)
[9] Wong, S C, and Kilbourne, E D, *J Exp Med*, **113**, 95 (1961)
[10] Sugiura, A, and Kilbourne, E D, *Virology*, **26**, 478 (1965)
[11] Nayak, D, and Rasmussen, A F, *Virology*, **30**, 673 (1966)
[12] Pons, M, *Virology*, **33**, 150 (1967)
[13] White, D O, and Cheyne, I M, *Virology*, **29**, 49 (1966)

Cercariae of *Schistosoma mansoni* and Plants: Attempt to penetrate *Phaseolus vulgaris* and *Hedychium coronarium* produces a Cercaricide

DURING the development of a method for evaluating the penetration of the cercariae of *Schistosoma mansoni*[1], a large percentage of the larvae were observed to penetrate the skin of dead as well as living mice. In addition, many cercariae attacked isolated mouse tissue, as had previously been reported[2] Furthermore, similar numbers of cercariae entered the skin of a wide variety of rodents regardless of whether or not the animals were hosts within which the larvae could complete their life cycle[3] It is well known that avian schistosomes penetrate mammalian skin where they die, in man this results in a severe dermatitis known as "swimmer's itch"[4] Thus these organisms seem to be bereft of any discriminatory mechanism in their attack on mammalian tissues In the plant kingdom, cercariae were observed to attempt to penetrate the integument of the green string bean, *Phaseolus vulgaris*, lose their tails in the process and thereby immobilize themselves Investigation of eighty-one aquatic plants failed to reveal a similar penetration phenomenon, but resulted in the discovery of a cercaricidal substance which exuded from the cut stem of the white butterfly lily, *Hedychium coronarium*

The method for evaluating the penetration of schistosome cercariae can be summarized as follows Using a bacteriologist's loop, forty cercariae were placed in non-wetting polystyrene containers After exposure of various materials to the cercariae, the contents of the container were poured into a Petri dish, and the intact cercariae, cercarial heads and tails were counted The number of intact cercariae and heads subtracted from the number of cercariae originally added provided the number of penetrants, the number of intact cercariae and tails provided a check on the cercariae placed in the containers In several hundred individual experiments the original count was verified in more than 90 per cent, and in the remaining 10 per cent it deviated by one or, rarely, two cercariae As well as using this technique, the behaviour of the larvae on contact with the various materials was observed under a dissecting microscope After exposure to large numbers of cercariae, samples of the plant material were preserved in buffered formalin for later sectioning and staining with haematoxylin and eosin

When the tails of anaesthetized living Swiss albino female mice were exposed to forty cercariae of a Puerto Rican strain of *S mansoni* in 5 ml of dechlorinated water for 30 min, penetration approached 90 per cent (Table 1), a high degree of penetration in similar conditions was also observed when the mice had been killed by cervical dislocation 3 days earlier, maintained at −30° C and warmed to room temperature before exposure (Table 1) In addition, the washed, isolated small intestine of freshly killed mice was also invaded by large numbers of cercariae (Table 1) A similar degree of penetration, as indicated by the disappearance of cercarial heads from solution, was observed when the green string bean, *Phaseolus vulgaris*, was exposed in the same conditions (Table 1)

Table 1 PENETRATION OF FORTY *S mansoni* CERCARIAE INTO LIVING AND DEAD ANIMAL AND VEGETABLE MATERIAL DURING A 30 MIN EXPOSURE IN 5 ML OF WATER

Material	No of specimens	Per cent penetration
Tails of living mice	40	87 5 ± 0 70
Tails of dead mice*	10	76 5 ± 2 18
Isolated mouse small intestine	10	61 3 ± 2 36
Green string bean, *Phaseolus vulgaris*	10	67 0 ± 2 32

* Dead 3 days (maintained in freezer), experiment performed at room temperature

Further studies, using the dissecting microscope, revealed that when the cercariae came into contact with the green string bean, they went through the series of penetrating motions described by Stirewalt and Hackey[2] and in the process they lost their tails Stained sections of the beans revealed cercariae in the process of penetration, but no cercariae were observed beyond the superficial outer layers of the vegetable (Fig 1)

In contrast, exposure of ten specimens each of two other vegetables, celery and scallions, and three aquatic plants, *Pomatogeton*, *Myriophyllum* and *Eichornia*, resulted in no penetration, nor did *S mansoni* cercariae attack specimens of seventy-eight other species of aquatic or semi-aquatic plants which were similarly tested *H coronarium*, however, had a profound effect on the cercariae When the stem of this plant was cut off just above the crown (the usual procedure for studying plants with root systems) and inserted into the plastic cup containing forty cercariae in 100 ml of water, the cercariae were attracted to the stem, the tails broke off, and it

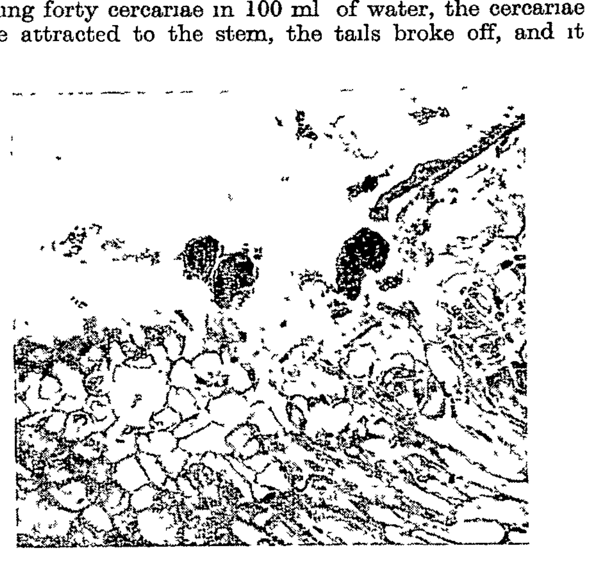

Fig 1 Cercariae of *S mansoni* attempting to penetrate the integument of the green string bean, *Phaseolus vulgaris* Stained with haematoxylin and eosin (×200)

seemed at first as though the heads were disappearing from the solution When a lily stem and the tail of a living anaesthetized mouse were placed at opposite ends of a container, the separated cercarial tails were divided equally between them Further study, however, revealed a situation which was completely different from that which had occurred with the string bean When the intact butterfly lily, complete with root system, was exposed to cercariae, the larvae did not attempt to penetrate the stem of the plant Within a few minutes, however, after cut pieces of stem were added to the water, the cercariae began to break apart, fell to the bottom of the cup and rapidly ceased all movement Even after the solid material had been removed by filtration, the solution remained toxic to the parasite larvae In contrast, when the cut ends of the stems were sealed with silicon stopcock grease, no cercaricidal effect was observed

The plant kingdom has been associated with schistosomiasis in several respects, for (*a*) plants are important in the ecology of snails, (*b*) the fruit of several plants are reported to show molluscicidal activity, and (*c*) various plants are widely used by practitioners of Chinese traditional medicine in the treatment of schistosomiasis and its complications[5] Our investigations reveal further associations between plants and schistosomes which have implications in relation to the distribution and control of the disease

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[1] Warren, K S , and Peters, P A , *Ann Trop Med Parasitol* (in the press)
[2] Stirewalt, M A , and Hackey, J R , *J Parasitol* , **42**, 565 (1956)
[3] Warren, K S , and Peters, P A , *Amer J Trop Med Hyg* ,**16**, 718 (1967)
[4] Cort W W , *Amer J Hyg* , **52**, 251 (1950)
[5] Warren, K S , and Newill, V A , *Schistosomiasis, A Bibliography of the World's Literature from 1852–1962*, **2** (Western Reserve University Press, Cleveland, Ohio 1967)

Filtration of Arboviruses through 'Millipore' Membranes

CURRENT attempts[1,2] to fit the arboviruses within a universal system of classification have revealed severe deficiencies in the knowledge of the basic physical structural and biochemical properties of these agents, on which proposed systems are chiefly based

Although virus size is not among the properties used for classification in one of the proposed schemes[3], it nevertheless seems logically connected with at least one element used in that scheme, for markedly dissimilar sizes within a collection of viruses may reflect differences in number of capsomeres as well as in the triangulation numbers of agents with cubic or icosahedral symmetry In addition, heterogeneity in size may correspond with heterogeneity in still other properties

The present observations regarding the behaviour of arboviruses on filtration through 'Millipore' membranes were made as part of a continuing investigation in this laboratory Properties other than size may affect the filterability of viruses through such membranes, and the commercially available membranes represent only a limited range of average pore diameter (APD), and so these observations are at best estimates of virus size within rather wide limits rather than accurate size determinations

The technique usually employed was similar in some respects to that used with simian viruses[4] Except for group *C* agents, each virus preparation was made up as a 10^{-2} suspension of infected newborn mouse brain tissue, using an electrical blender, with group *C* viruses (Nos 13, 14 and 15 in Table 1), a similar dilution of infected serum or whole blood was employed The diluent was 0 02 M phosphate buffer in physiological saline, *p*H 7 2, to which was added 0 75 per cent bovine plasma albumin (Armour fraction V) Between 50 and 60 ml of the freshly prepared virus suspension was spun in a Spinco preparatory centrifuge (Model *L*, Type 40 rotor) at 15,000 r p m for 30 min or, in the case of some of the larger viruses (Nyamanini, herpes, Newcastle disease), at 5,000 r p m for 30 min The supernatant fluid was then filtered by pressure (20 p s i) successively through filters of APD 450, 220, 100 and 50 mμ, the diameter of each filter was 47 mm and a microglass prefilter was used in all cases At each stage about 5 ml was reserved for infectivity titration The least volume emerging through the last filter was 10 to 15 ml The original crude suspension, the supernatant fluid and each successive filtrate were titrated by the intracerebral route of inoculation in 2–4 day old mice, eight mice being used for each dilution

Table 1 INFECTIVITY OF ARBOVIRUSES AFTER FILTRATION THROUGH 'MILLIPORE' MEMBRANES

Virus, strain	LD_{50} titre of filtrate (APD of membrane in mμ)				Estimated diameter of particle (EDP)*
	450	220	100	50	
(1) Dengue 2, *NGB*	4 8†	4 9	5 0	4 6	EDP < 50
(2) Ntaya, orig	5 3	5 3	5 5	5 3	EDP < 50
(3) *MVE*, French	7 0	7 0	7 2	6 4	EDP < 50
(4) *SLE*, Parton	7 0	6 9	7 0	6 6	EDP < 50
SLE, Parton	1 3	1 6	0 9	0 8	EDP < 50
(5) Chikungunya, Ross	5 6	5 5	5 6	5 2	EDP < 50
(6) Sindbis, *EgAr* 339	6 1	6 0	5 4	4 6	EDP ⩽ 50
(7) *EEE*, orig	7 4	7 4	6 7	5 9	EDP ⩽ 50
(8) *WEE* McMillan	7 0	7 5	6 6	4 6	50 < EDP < 100
WEE, McMillan	3 9	3 9	3 0	1 0	50 < EDP < 100
(9) Bunyamwera, orig	6 3	6 0	5 5	2 8	50 < EDP < 100
(10) Chenuda, *EgAr* 1152	5 0	4 5	3 5	0 6	50 < EDP ⩽ 100
(11) Kemerovo, *R*10	5 4	5 5	4 1	1 1	50 < EDP ⩽ 100
(12) Bwamba, *M*459	4 5	4 4	1 4	N‡	100 < EDP < 220
(13) Caraparu, *BeAn* 3994	3 1	1 9	N	N	100 < EDP < 220
(14) Murutucu, *BeAn* 974	4 7	4 1	1 5	0 8	100 < EDP < 220
(15) Oriboca, *BeAn* 17	2 5	2 0	N	N	100 < EDP < 220
(16) Bhanja, *IG* 690	4 2	3 1	1 2	1 1	100 < EDP < 220
(17) *CTF*, Condon	4 4	4 5	0 2	N	100 < EDP < 220
(18) Ganjam, *IG* 619	3 6	3 6	N	N	100 < EDP < 220
(19) Hughes, Dry Tortugas	3 4	2 4	0 4	N	100 < EDP < 220
(20) Kaisodi, *IG* 14132	3 8	3 6	1 7	1 2	100 < EDP < 220
(21) Quaranfil, *EgAr* 1095	2 8	2 1	0	N	100 < EDP < 220
(22) Silverwater, *M*3737	3 2	2 9	1 4	0 6	100 < EDP < 220
(23) Thogoto, 2*A*	3 1	2 6	0 6	N	100 < EDP < 220
(24) Tribec, orig	5 0	4 9	1 0	N	100 < EDP < 220
(25) Wad Medani, *EgAr* 492	5 0	4 5	2 6	N	100 < EDP < 220
(26) Nyamanini, *EgAr* 1304	4 4	2 7	N	N	220 < EDP < 450
Nyamanini, *EgAr* 1304	2 3	0 5	N	N	220 < EDP < 450
(27) Reovirus 3, *Tr* 23421	5 4	5 3	4 0	0 6	50 < EDP ⩽ 100
(28) Herpes, *HF*	2 7	0	N	N	220 < EDP < 450
(29) *NDV*, 98	1 9	N	N	N	220 < EDP < 450

MVE, Murray Valley encephalitis, *SLE*, St Louis encephalitis, *EEE*, *WEE*, eastern and western equine encephalitis, *CTF*, Colorado tick fever, *NDV*, Newcastle disease virus

* Based on the APD of the two consecutive filters between which dex 1 5 or more of virus was removed

† Expressed in dex/0 02 ml

‡ N indicates no virus recovered

With a few exceptions, the size of the viruses tested was such that they were neither sedimented by the centrifugation employed nor excluded by the filter of APD 450 mμ to a significant degree In nearly all instances, the infective titre of material passing the 450 APD membrane was not more than 0 7–1 0 dex[5] lower than the titre of the original suspension, and this loss was caused as much by centrifugation as by filtration For our purposes, therefore, the 450 APD filtrate has been adopted as the working suspension, and reduction of infective titres resulting from subsequent filtrations has been calculated with reference to it rather than to the crude original 10^{-2} brain suspension

Several viruses not arboviruses (Nos 27, 28 and 29 in Table 1) were included because their sizes have been reported[6–8], usually as determined by electron microscopy, similarly, for some of the arboviruses included there are reports of measurements, by the same and other techniques[9] It was presupposed that the observed degree of filterability of viruses of known size in the present system would permit certain inferences, by analogy, about the relative size of the other arboviruses

The results presented in Table 1 show, not unexpectedly, that the viruses tested differed considerably in degree of filterability, and presumably therefore in relative size, in our experimental conditions Considering a loss of 1 5 dex or more in infective titre as significant, viruses Nos 1–5, represented by group *B* agents and one from group *A* (chikungunya), were not affected by final filtration through the smallest APD used, 50 mμ Viruses Nos 6–9, however, suffered a loss of titre on filtration through the 50 mμ APD membrane, particularly pronounced in the case of *WEE* and Bunyamwera and less so for Sindbis and *EEE* Kemerovo virus (No 11) has been reported[10] to have a virion size, including its envelope, of between 60 and 100 mμ, and its behaviour on filtration corresponds well with this size The behaviour of reovirus type 3 (No. 27) on filtration similarly corresponded with its reported size of 70–77 mμ

The greatest number of viruses (Nos 12–25) experienced a significant loss of infectivity on filtration through the 100 mμ APD membrane but either no loss or only a moderate loss on filtration through a 220 mμ membrane These viruses comprised members of group *C* (Nos 13, 14 and 15), Bwamba (No 12) and tick-borne viruses not of group *B* (Nos 16–25)

Nyamanini virus (No 26) has repeatedly shown considerable loss of titre on filtration through a 220 mμ APD membrane, if particle aggregation can eventually be ruled out, this loss in titre may indicate an unusually large size of arbovirus

These observations seem to provide further evidence that the group of the animal viruses carried by arthropods is heterogeneous in physical properties, inclusion of the arboviruses in a universal scheme will have to acknowledge such heterogeneity

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[1] Casals, J , *Ninth Intern Cong Microbiol Moscow, Symposia*, 441 (1966)
[2] *WHO Scientific Group, Technical Report Series*, No 369 (1967)
[3] *Ann Inst Pasteur*, **109**, 625 (1965)
[4] Atoynatan, T , and Hsiung, G D , *Proc Soc Exp Biol and Med* , **116**, 852 (1964)
[5] Haldane, J B S , *Nature*, **187**, 879 (1960)
[6] Gomatos, P J , Tamm, I , Dales, S , and Franklin, R M , *Virology*, **17**, 441 (1962)
[7] Wildy, P , Russell, W C , and Horne, R W , *Virology*, **12**, 204 (1960)
[8] Waterson, A P , in *Newcastle Disease Virus* (edit by Hanson, R P), 119 (University of Wisconsin Press, 1964)
[9] Mussgay, M , *Prog Med Virol* , **6**, 193 (1964)
[10] Reingold, V N , Shestopalova, N M , Grachov, V P , Karpovich, L G , and Chumakov, M P , *Proc Third Europ Reg Conf Electron Microscopy*, Prague, 353 (1964)

CYTOLOGY

Gene Amplification in the Oocyte Nucleus of Mutant and Wild-type *Xenopus laevis*

During the development of the amphibian oocyte, the germinal vesicle becomes enlarged and can be seen to contain very many nucleoli[1,2] instead of the four which would be expected from the tetraploidy of the egg nucleus The RNA of these nucleoli is similar in base composition to ribosomal RNA[3] and each nucleolus contains a small amount of DNA[4–6] Amphibian oocyte nucleoli can be hypotonically disrupted to reveal DNP rings in the shape of beaded circles or necklaces[2,6] There is compelling evidence in amphibia[2,6,7] as well as in the insect *Tipula*[8] that this DNA is derived from the chromosomal nucleolar organizer region which contains the ribosomal cistrons[9,10] This conclusion is supported by the recent finding that in ovarian tissue of *Xenopus* there is a disproportionately greater synthesis of ribosomal cistrons[11] which leads eventually to a higher proportion of them than that found in DNA of somatic tissues[11,12] In *Bufo*, the nucleolar DNA migrates as granules from the chromosomal bouquet to the periphery of the nucleus where, at later stages, the granules give rise to nucleoli[5] A similar process occurs in *Xenopus* where Feulgen-positive material appears in the oocyte nucleus as a "nuclear cap" at leptotene[13] After zygotene this cap disperses and numerous nucleoli are formed which contain one or several DNA cores or rings[14]

Table 1 OCCURRENCE OF NUCLEOLI, NUCLEOLAR CORES AND NUCLEOLAR DNA IN THE OOCYTES OF MUTANT AND WILD-TYPE *Xenopus laevis*

Diameter of oocyte (mm)	No of oocytes examined Wild-type	Hetero-zygous	Average count Wild-type	Standard deviation Heterozygous
Nucleoli				
0 05–0 07	12	5	396 ± 45	435 ± 85
0 1 –0 3	12	15	1,206 ± 88	905 ± 71
0 4 –0 5	17	18	1,370 ± 38	1,450 ± 110
0 6 –0 8	8	12	1,420 ± 56	1,720 ± 190
0 9 –1 0	13	22	1,375 ± 58	1,640 ± 136
1 1 –1 4	18	10	1,470 ± 43	1,400 ± 134
1 5	6	7	920 ± 70	1,090 ± 100
Cores				
0 3 –1 4	10	36	5,265 ± 145	5,120 ± 125

Oocytes were removed from ovaries of seven wild-type and five heterozygous adult females in 0 1 M potassium chloride 0 1 M sodium chloride (5 1) The germinal vesicles were isolated manually, under a binocular microscope, washed several times in the same medium by repeated gentle syringing in a Pasteur pipette and, when free of all yolk, transferred into a well which contained 0 2 per cent $(NH_4)_2SO_4$ together with a trace of methylene blue The well consisted of a perforated microscope slide to which a coverslip had been fastened with paraffin–wax mixture The nuclear membrane was disrupted by means of two tungsten needles and the well covered with another coverslip to the exclusion of all air The slides were then kept for at least 24 h in the cold to allow settling out of the nucleoli The nucleoli were counted in a Wild inverted microscope fitted with a drawing tubus. In some experiments, in order to reveal the nucleolar cores, the isolated germinal vesicles were disaggregated in 0 02–0 05 per cent $(NH_4)_2SO_4$

We have counted nucleoli and nucleolar cores in *Xenopus laevis* and have found that the *Xenopus* oocyte nucleus contains some 1,400–1,600 nucleoli and some 5,200 nucleolar cores (Table 1) The nucleolar count remains constant for a range of oocyte diameters (0 4–1 0 mm) Contrary to our expectations, the number of nucleoli and the abundance of the nucleolar cores are unchanged in the mutant form of *Xenopus* in which one of the two chromosomal nucleolar organizers is deleted[15,16]

From the microspectrometric quantitation of the Feulgen stain in oocyte nuclei at post-zygotene—a time in development when all incorporation of ^{3}H-thymidine into the nucleus has ceased[13]—we deduce that mutant and wild-type nuclei both contain a similar amount of DNA (Table 2) This amount has been found to be about 42 pg for the wild-type oocyte[13] From this value we subtract 4C (12 pg (ref 9)) DNA in order to obtain a mass for the amplified DNA of 30 pg

Two molecular mechanisms have been suggested for the production of the extrachromosomal nucleolar DNA units[6] (*a*) the chromosomal organizer is the only template and (*b*) the product of endoduplication can serve as further template in this process The results with the mutant *Xenopus* do not exclude possibility (*a*) We now know, however, that in *Xenopus* the number of chromosomal organizers is in itself not a limiting factor in the production of these extra copies The controlling factors for amplification must obviously lie elsewhere The concept of a cascade effect in gene amplification (possibility (*b*)) is strongly favoured by the finding that the rate of incorporation of ^{3}H-thymidine is roughly proportional to the mass of the DNA already amplified[13] According to this scheme, the mutant nucleus could easily achieve the same degree of multiplicity by a single additional round of reduplication (twelve rounds rather than eleven)

In somatic tissues the ribosomal cistrons represent some 0 2 per cent of the genome, or some 0 1 per cent organ-

Table 2 FEULGEN DYE CONTENTS, IN ARBITRARY UNITS, OF POST-ZYGOTENE OOCYTE NUCLEI FROM WILD-TYPE *Xenopus* AND THEIR MUTANT SIBS

Wild-type (normal) *Xenopus*

Preparations	1	2	3	4	5
	120	146	135	153	151
	125	143	171	146	163
	173	132	131	149	129
	157	138	149	159	136
	147	140	152	166	156
	139			132	141
	157				150
	159				
	151				
	130				
	143				
Means and standard errors	145 ± 16	140 ± 5	147 ± 16	151 ± 12	147 ± 12

Mutant (mononucleolate) *Xenopus*

Preparations	1	2	3	4	5
	129	150	133	163	135
	164	145	161	140	150
	132	140	133	123	138
	158	153	136	129	136
	141	153	146	153	144
	143		152	154	136
	139		153	148	144
	156		141		157
	132				
	139				
	124				
	126				
Means and standard errors	140 ± 13	148 ± 7	144 ± 10	144 ± 14	143 ± 8

Measurements were obtained from five preparations Each preparation was made from a different pair of animals

Female *Xenopus* weighing between 0 4 and 1 g were used as a source of material for all photometric observations In each preparation an ovary from a wild-type animal was squashed, before hydrolysis and staining, on the same slide as an ovary from a mutant sib The two ovaries therefore underwent precisely the same hydrolysis, staining, washing, dehydration and mounting procedures Feulgen dye contents were measured in post-zygotene nuclei in which the cap of nucleolar DNA had more or less dispersed Details of the technique are described elsewhere[13]

izer[9,16] If gene amplification in the oocyte were concerned with the replication of the entire block of ribosomal cistrons, the 5,200 cores would be expected to represent 520 per cent of the diploid DNA mass The *Xenopus* nucleus contains some 6 pg of DNA[9], so 5,200 such DNA units would yield some 31 2 pg of DNA This is remarkably close to the amount determined in the spectrophotometric experiments We take this to mean that amplification in the oocyte is concerned with the entire cluster of ribosomal cistrons, and thus with the nucleolar organizer as a whole, resulting in an increase in the number of ribosomal cistrons per cell from 1,600 in somatic tissues to some 4×10^6 in the oocyte

It is easily demonstrated that an increase of this magnitude in the number of ribosomal cistrons is highly advantageous for the oocyte The mature *Xenopus* oocyte contains some $1\ 1 \times 10^{12}$ ribosomal 28*S* and 18*S* RNA molecules (4 µg (ref 17)), the somatic cell, on average, some $3\ 3 \times 10^6$ (12 pg (unpublished results of M L B)) In rapidly proliferating tissue with a generation time of 24 h, a complete set of ribosomes will be synthesized with every cell division, at a rate of the order of 1 4 ribosomes/cistron/min If ribosome synthesis occurred at this same rate in the oocyte, in the absence of gene amplification, some $1{\cdot}7 \times 10^5$ days would be necessary for the production of the oocyte ribosomal RNA Amplification of the genes by a factor of 2,600 would reduce the time interval to some 65 days, which is an interval commensurate with the time necessary for oocyte growth

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[1] Wilson, E B , *The Cell in Development and Heredity* (Macmillan, New York, 1925)

[2] Macgregor, H C , *Quart J Microsc Sci* , 106, 215 (1965).

[3] Edström, J E , and Gall, J G , *J Cell Biol* , 19, 279 (1963)

[4] Brachet, J , *Arch Biol* , 51, 151 (1940)

[5] Painter, T S , and Tayler, A N , *Proc US Nat Acad Sci* , 28, 311 (1942)

[6] Miller, O , *Nat Cancer Inst Monog* , 23, 53 (1966)

[7] Callan, H G , *J Cell Sci* , 1, 85 (1966)

[8] Lima-de-Faria, A , *Chromosoma*, 13, 47 (1962)

[9] Wallace, H , and Birnstiel, M L , *Biochim Biophys Acta*, 144, 296 (1966)

[10] Ritossa, F , and Spiegelman, S , *Proc US Nat Acad Sci* , 53, 737 (1965)

[11] Gall, J G , *Proc US Nat Acad Sci* (in the press)

[12] Brown, D D , Webster, C S , and Sinclair, J H , *Carnegie Inst Wash Yearbook* (1967)

[13] Macgregor, H C , *J Cell Sci* (in the press)

[14] Macgregor, H C , *J Cell Sci* , 2, 145 (1967)

[15] Kahn, J , *Quart J Microsc Sci* , 103, 407 (1962)

[16] Birnstiel, M L , Wallace, H , Sirlin, J L , and Fischberg, M , *Nat Cancer Inst Monog* , 23, 431 (1966)

[17] Brown, D D , *Nat Cancer Inst Monog* , 23, 297 (1966)

Epidermal Cell Population Kinetics in Albino Guinea-pig Skin

IN adult mammals the epidermal cell population is in a steady state—continuous cell loss by keratinization is balanced quantitatively by new cell production in the basal layer. We have found that the turnover time of albino guinea-pig epidermis (time required for the replacement of a number of cells equal to that present in the basal, spinous and granular layers) differed from that reported by Platt[1] and also from that found for other mammals[2–5]

To determine the turnover time of the epidermis by the method of Leblond *et al* [6], we needed to know the DNA synthesizing time, t_s Because t_s of many mammalian cells was reported to be 6–8 h, several workers have assumed t_s to be 7 h when calculating the turnover time for the human epidermis[1], rat oesophageal epithelium[5] and human sebaceous glands[7] It should be pointed out, however, that t_s for a number of mammalian cells is shorter or longer than 6–8 h, for example, t_s for mouse ear epidermal cells is 30 h (ref 5) We therefore determined the t_s of guinea-pig epidermal cells using double labelling with ^{3}H and ^{14}C-thymidine Hair was clipped from the flank of albino guinea-pigs when about 4–6 months old and weighing 500–800 g Three hours after intradermal injection of 2 5 µCi of ^{3}H-thymidine (5 0 Ci/mmole) in 0 1 ml of saline, 2 5 µCi of ^{14}C-thymidine (37 9 mCi/mmole) in 0 1 ml of saline was injected into the same sites, and the animals were killed 1 h later at 4 00 to 5 00 p m Labelled thymidine was assumed to remain available to cells synthesizing DNA for less than 1 h (ref 8) Pieces of skin, about 1 cm in diameter, were fixed in 10 per cent formalin, embedded in paraffin, and cut to 6µ thick The slides were dipped in NTB-3 liquid emulsion, dried in a horizontal position, developed with Kodak *D*-19 developer after exposure for 21 days, and stained with haematoxylin (Fig 1). Tritium gave silver grains in one focal plane immediately over the cell nucleus whereas carbon-14 produced a spray of tracks of silver grains in many focal planes above and around the cell nucleus, and so the cells labelled with tritium alone and the cells labelled with carbon-14 alone or with both isotopes were clearly distinguishable from each other The difference between labelling with carbon-14 and with tritium was much clearer under the microscope when there was a film of water between emulsion and cover glass. Two-emulsion autoradiography was also carried out according to the method described by Baserga[9], but we found this method no better than autoradiography with only one emulsion

The time of DNA synthesis, t_s, was calculated by the following formula[10]

$$t_s = \frac{N^{14}{}_{\mathrm{C}}}{N^{3}{}_{\mathrm{H}}} \times t_a$$

where $N^{3}{}_{\mathrm{H}}$ indicates the number of cells labelled with tritium alone, $N^{14}{}_{\mathrm{C}}$ the number of cells labelled with carbon-14 alone or with tritium, and t_a the time interval between labelling, which was 3 h in this study.

Table 1

Animal	Average No of ^{14}C-labelled cells in a section ($N^{14}{}_{c}$)	Average No of ^{3}H-labelled cells in a section ($N^{3}{}_{H}$)	$N^{14}{}_{c}/N^{3}{}_{H}$	DNA synthesizing time in h (t_s)
1	81	29	2 8	8 4
2	73	23	3 2	9 6
3	77	23	3 4	10 2
4	76	25	3 0	9 0
				Mean 9 3

One or two skin sites were used for each animal and at least eight sections were counted for each skin site to obtain the average number of labelled cells

Table 1 shows that the average t_s from four animals was 9 3 h. The 4 h interval between the injection of tritiated thymidine and the killing of animals in our determination of t_s could be too long, for some of the cells labelled with tritium could complete mitosis during this interval We found, however, only a few cells labelled with tritium which entered mitosis in the 4 h interval, and no labelled cells seemed to be the progeny of initially labelled cells This observation was similar to the findings with human and pig epidermis[2–4] in which paired labelled cells were first noted 12–24 h after injection of ^{3}H-thymidine Thus, it is very likely that the postsynthetic period (G_2) in epidermal cells is unusually long as compared with that of other cells[11], and all the cells labelled with tritium which we counted were likely to have been labelled originally

In the next series of experiments, animals received an intradermal injection of ^{3}H-thymidine in a dose of 5 μCi (5 Ci/mmole) in 0 1 ml of saline at various times between 1 h and 12 days before they were killed Autoradiographs of skin were made as already described

In the tissues obtained 1 h after injection of ^{3}H-thymidine, most labelled cells were found in the basal layer although there were a few in the transitional layer The total number of cells and the number of labelled cells in the interfollicular epidermis of these 1 h specimens were counted, and the values are shown in Table 2

The turnover time was calculated by the following formula[5]

$$T = \frac{N}{n_s} \times t_s$$

where T indicates the turnover time, N/n_s the ratio of the number of total cells to that of labelled cells in the slides obtained 1 h after injection of ^{3}H-thymidine, and t_s is the time of DNA synthesis

The average turnover time of guinea-pig epidermis obtained from six animals was 8 5 days (Table 2) Although this was very close to the turnover time for hairless mouse epidermis, 9–10 days (ref 12), it was shorter than that of human epidermis, 14–17 7 days (ref 2), or rat and mouse epidermis, 19–34 days (ref 8) The difference in epidermal turnover time between guinea-pig epidermis and other mammals was also obvious in the autoradiographs of skin specimens obtained at various time intervals after injection of ^{3}H-thymidine We found that by 6 days after injection of ^{3}H-thymidine some labelled cells had already appeared in the granular layer, and by 12 days most of the heavily labelled cells were in the granular layer (Fig 2), whereas labelled cells are reported to appear first after 10–14 days in the granular layer of human epidermis[1] and after 14 days in pig epidermis[3] Thus it seems that, contrary to previous conclusions of others[1,11], the turnover time of similar organs in different species is not always the same

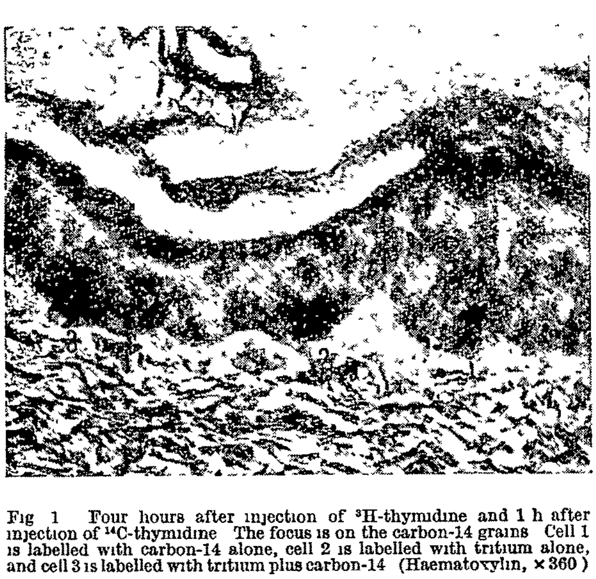

Fig 1 Four hours after injection of ^{3}H-thymidine and 1 h after injection of ^{14}C-thymidine The focus is on the carbon-14 grains Cell 1 is labelled with carbon-14 alone, cell 2 is labelled with tritium alone, and cell 3 is labelled with tritium plus carbon-14 (Haematoxylin, × 360)

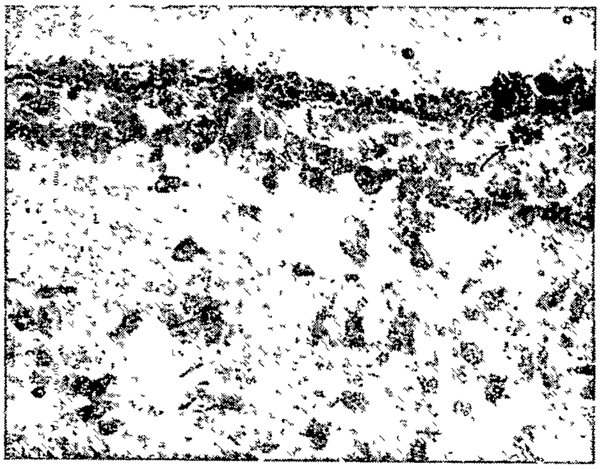

Fig 2 Twelve days after injection of ^{3}H-thymidine Heavily labelled cells are in the granular layer, and lightly labelled cells in the basal, spinous and granular layers are the progeny of the initially labelled cells (Haematoxylin, × 360)

Table 2

Animal	Average total No of cells in a section (N)	Average No of ^{3}H-labelled cells in a section (n_s)	N/n_s	Turnover time (h)	Turnover time (days)
1	1,228	49	25 1	233 4	9 7
2	1,712	77	22 2	206 5	8 6
3	1,047	50	20 9	194 4	8 1
4	1,110	59	18 8	174 8	7 3
5	1,059	52	20 4	189 7	7 9
6	1,231	51	24 1	224 1	9 3
					Mean 8 5 ± 0 4*

One or two skin sites were used for each animal and at least eight sections were counted for each skin site to obtain average N and n_s

* Mean ± standard error of the mean

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[1] Platt, H , *Nature*, **184**, 1654 (1959)

[2] Epstein, W L , and Maibach, H I , *Arch Dermatol* , **92**, 462 (1965)

[3] Weinstein, G D , and Van Scott, E J , *J Invest Dermatol* , **45**, 257 (1965)

[4] Weinstein, G D , *J Invest Dermatol* , **44**, 413 (1965)

[5] Sherman, F G , Quastler, H , and Wimber, D R , *Exp Cell Res* , **25**, 114 (1961)

[6] Leblond, C P , Greulich, R C , and Pereira, J P M , *Symp on Wound Healing* (edit by Montagna, W), 39 (Pergamon Press, New York, 1964)

[7] Epstein, jun , E H , and Epstein, W L , *J Invest Dermatol* , **46**, 453 (1966)

[8] Bertalanffy, F D , *Lab Invest* , **13**, 871 (1964)

[9] Baserga, R , and Nemeroff, K , *J Histochem Cytochem* , **10**, 628 (1962)

[10] Wimber, D E , *Symp on Cell Proliferation* (edit by Lamerton, L F , and Fry, R J M), 1 (F A Davis Co , Philadelphia, 1963)

[11] Pederson, T , and Gelfant, S , *Seventh Ann Meeting Amer Soc Cell Biol* , Denver, Colorado, Abstract No 209 (1967)

[12] Skjaeggestad, O , *Acta Pathol Microbiol Scand* , Suppl 169 (1964)

Lens Soluble Proteins: Correlation with the Cytological Differentiation in the Young Adult Organ of the Chick

THE lens of the eye is an outstanding example of cellular differentiation. At any moment the organ shows the complete sequence of its cellular ontogeny from epithelial cells to equatorial, cortical and nuclear fibre cells[1,2].

The protein components of the whole lens have been extensively analysed during pre-natal and post-natal development. This research has made available more knowledge of the molecular structure of this organ.

The lens therefore appears to be an ideal model for analysing the correlation between structural and biochemical differentiation. Some data on the correlation between developmental differentiation of the lens and its protein biosynthesis have been reported[3]. Information on the structural and cytological organization of the lens, synthesis of structural proteins, *m*RNA stability, oxidative metabolism and patterns of lactic dehydrogenase isozyme activity has also been collected[2,4–7].

Most immunological studies on chicken lenses have been on pre-natal development in order to establish the sequence of appearance of structural lens antigens[8–13]. In the adult chick the data on the correlation between cytological and macromolecular differentiation are very limited. This investigation was carried out to look for a correlation between the differentiating cells of the lens and their proteins and structural antigens. We attempted to interrelate the immunological results with data provided by other techniques of protein analysis.

Lenses from 6 week old White Leghorn chickens were separately dissected in the following way. A tungsten needle was used to make a circular cut along the equator of the capsule. The anterior portion of the capsule was pulled off together with the epithelial cells. The equatorial region, which histologically corresponds to the annular pad, was removed joined to the rest of the capsule. Superficial cortical and nuclear fibre cells were isolated and the intermediate cells were discarded. The material collected therefore corresponds to the cytological differentiation sequence: epithelial cells corresponding to the epithelium, elongating equatorial cells to the annular pad zone, superficial fibre cells to the cortex and the deeper fibre elements corresponding to the nucleus. Lens tissues from the different regions of the organ were identified by phase contrast analysis (Fig. 1).

The material was pooled, weighed and homogenized in 0·85 per cent sodium chloride solution at 4° C, and centrifuged at 17,000*g* for 15 min at the same temperature. The protein content of the supernatants was evaluated according to Lowry *et al.*[14]. Extracts gave a protein concentration of 32 mg/ml for the annular pad cells, 42 mg/ml for the cortical fibre cells, and 78 mg/ml for the nuclear fibre cells. These concentrations refer to a 25 per cent dilution of the tissue wet weight. Thus the normal differences in protein content of annular pad cells (129 mg/g), cortical fibre cells (168 mg/g) and nuclear fibre cells (312 mg/g) were maintained in all the subsequent electrophoretic procedures. Because of technical difficulties epithelial cells were directly diluted and the protein concentration was adjusted to 30 mg/ml. Antisera to the complete adult chick lens were produced by subcutaneous injections of a mixture of chick lens extract, containing 50 mg/ml., and Freund's adjuvant into rabbits. A sample (5 ml.) of this suspension was separately injected and this was repeated at weekly intervals for a month. The antisera were collected from the rabbits 5 days after the last injection. Three electrophoretic techniques were used.

Agar immunoelectrophoresis was carried out in barbital buffer (0·1 molar, *p*H 8·6) in a field strength of 6 V/cm and starch gel electrophoresis was carried out in a *tris*-discontinuous system for 16 h at 12 V/cm according to Poulik[15]. Lens extracts (50λ) were electrophoresed in cellulose acetate medium using barbital buffer (0·1 molar, *p*H 8·6), using a Beckman Microzone electrophoresis cell for 30 min. Strips were scanned on a model *RB* Beckman 'Analytrol'.

Protein nomenclature used follows the recommendations of Zwaan and Ikeda[16]. According to these authors, the chick lens contains three chief groups of soluble proteins, alpha, beta and delta crystallins. Delta crystallin, found only in the Sauropsida, has often been considered as beta crystallin[11] and it has also been called FISC (first important soluble crystallin)[12].

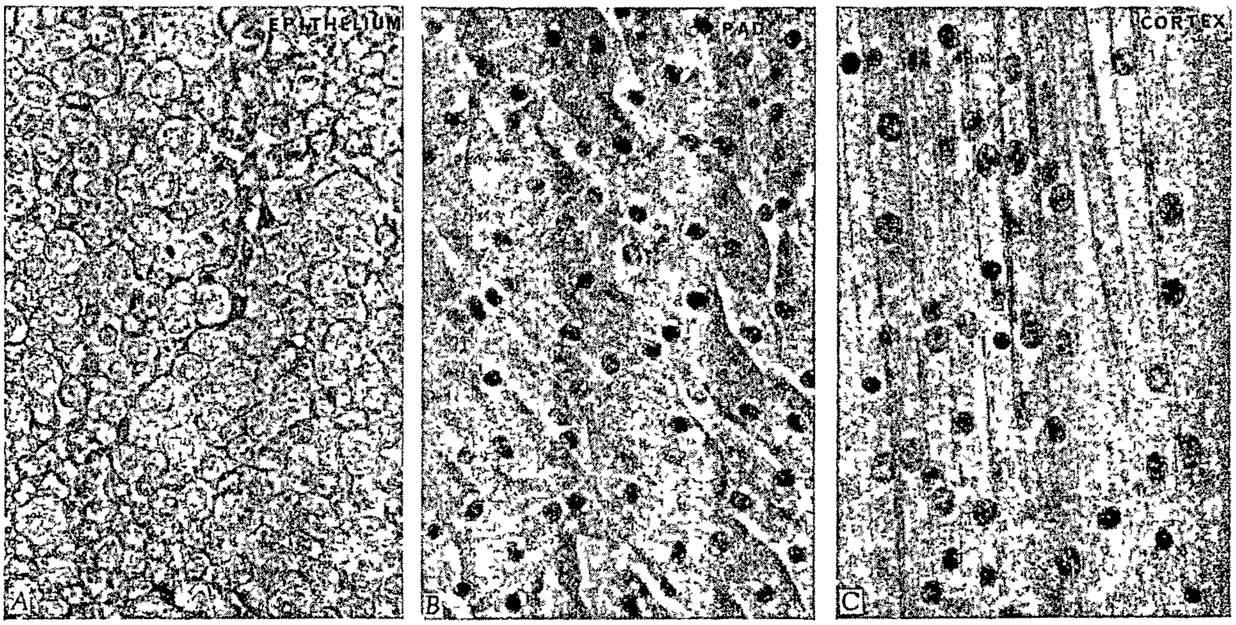

Fig. 1. Phase contrast analysis of lens tissues from different regions of lenses from 6 week old chicks (× 200). *A*, Epithelium; *B*, annular pad zone; *C*, cortex.

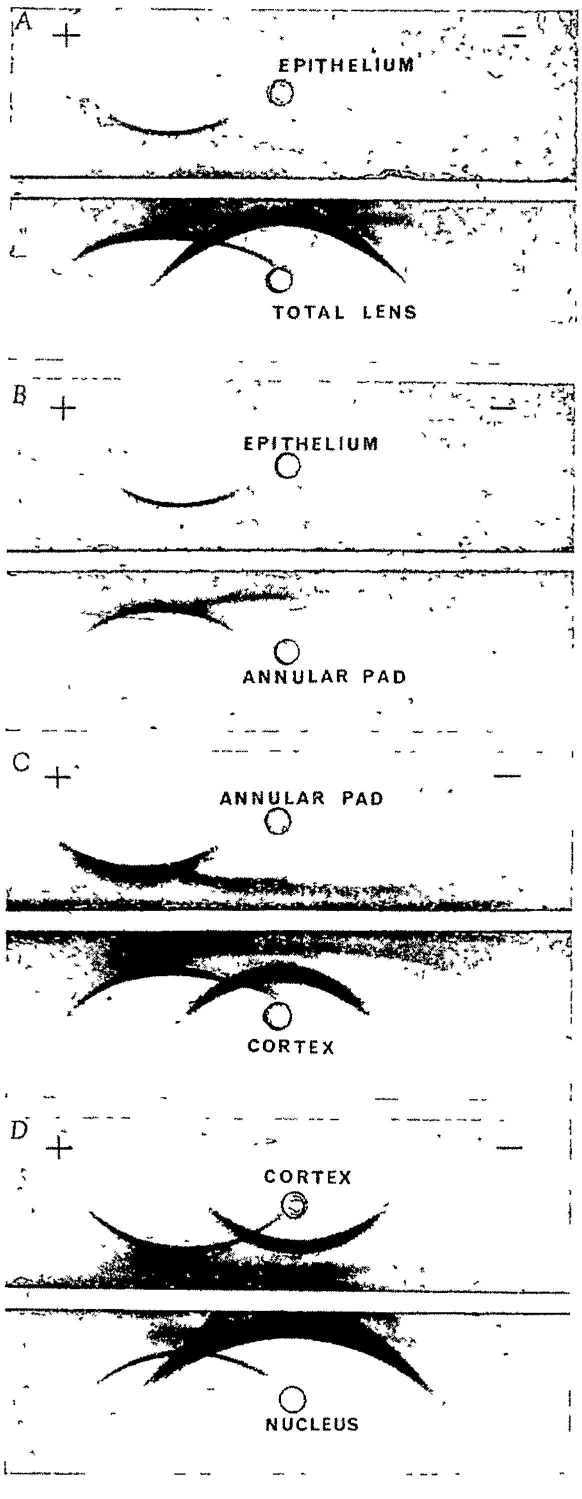

Fig 2 Immunoelectrophoretic patterns of extracts of total lens and differentiating lens parts *A*, Extracts of total lens and lens epithelium, *B* extracts of epithelial and equatorial cells, *C*, extracts of annular pad cells and cortex cells, *D*, extracts of lens cortex and nucleus

In this communication beta crystallin refers to all other protein families, irrespective of electrophoretic mobilities, with the exclusion of alpha and delta crystallin

The immunological pattern of extracts of total lens shows three principal precipitin reactions which correspond to the alpha, beta and delta groups of crystallins The precipitin arc for alpha shows the fastest mobility Beta antigenic determinants give rise to a long line which extends from the anode to the cathode Delta antigenic determinants delimit a sharply defined arc occupying an intermediate position A comparison between the patterns of total lens extracts and those detected in the differentiating lens parts clearly shows that antigenic determinants vary, both qualitatively and quantitatively, according to the cytological differentiation of the lens Thus the extracts of lens epithelium show two main precipitin areas with a mobility corresponding to the alpha and cathodal portion of the long line It also reveals that delta antigenic determinants are not present in the epithelial cells of the lens (Fig 2*A*)

The annular pad cells, which represent a more advanced degree of cytological differentiation and the beginning of cellular elongation, show two antigenic lines, which also correspond to alpha and beta crystallin determinants Differences between the precipitin patterns of the epithelial and equatorial cells are shown in Fig 2*B* The amount and mobility of alpha crystallin antigenic determinants are greater in the equatorial cells The intermediate portion of the long line is predominant in the annular pad cells Similar to the epithelial cells, the equatorial cells lack the reaction for delta crystallin, although a soft but faint antigenic arc with the mobility of delta crystallin was observed in some experiments

The simultaneous immunoelectrophoretic patterns of annular pad cells and superficial fibre cells show clearly that the elongated cells of the lens cortex have a marked reaction for delta crystallin The patterns also reveal a significant predominance of the anodic portion of the long line This is not present in either the epithelial or the equatorial cells The alpha antigenic determinants also have a higher mobility in the equatorial cells (Fig 2*C*) The innermost fibre cells of the lens nucleus show a pointed antigenic reaction for delta crystallin The antigenic determinants for alpha and beta crystallins are also present, but they are in low concentration (Fig 2*D*)

Similar results are obtained by subjecting the different lens extracts to starch gel electrophoresis (Fig 3) Thus band 3, which corresponds to alpha crystallin, is a predominant fraction at the epithelial, equatorial and cortical cells, although it can be detected in smaller quantities in the nuclear fibre cells There are differences in mobility between the alpha fractions of the pad and the other regions Band 4 was identified as delta crystallin This protein is significantly predominant in the nuclear fibre cells No traces of delta crystallin were found in the equatorial or in the epithelial cells Other bands which are present in the protein patterns of starch gel fractionation must be considered as beta crystallin (bands 1 2, 5, 6, 7) These protein fractions show different mobilities Thus bands 1 and 2 are in a cathodal position and predominant in epithelial and equatorial cells Band 5, with an intermediate mobility is mainly detected in

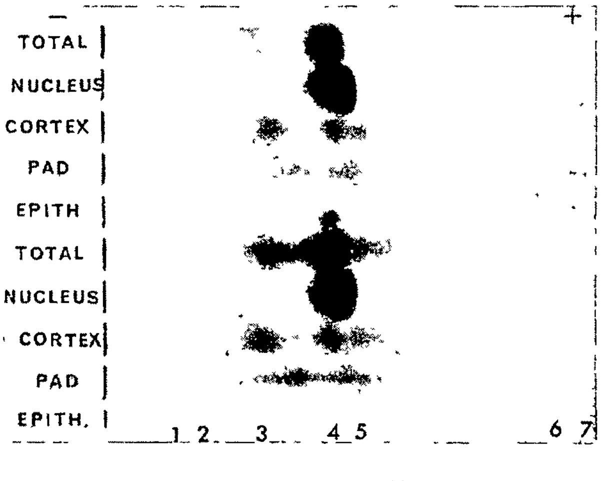

Fig 3 Starch gel electrophoresis of lens extracts

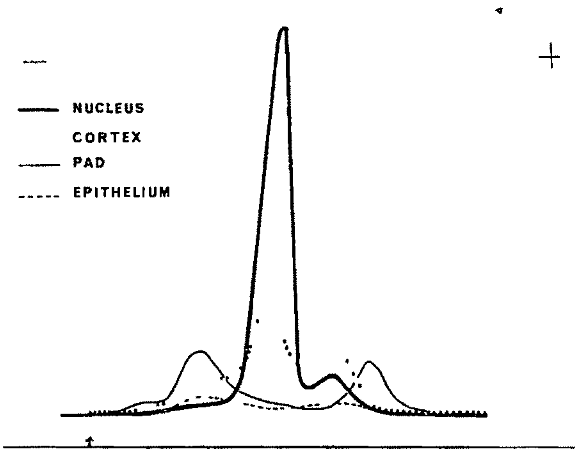

Fig 4 Cellulose acetate electrophoresis of lens extracts

the annular pad and cortical fibre cells Bands 6 and 7, with the fastest migration, can be registered in the cortical and nuclear fibres, but they were never detected in the eipthelial or equatorial cells

In cellulose acetate electrophoresis from the anode to the cathode, corresponding beta, delta and alpha crystallin were detected The nuclear fibre cells showed a marked concentration of delta crystallin (80 per cent) Alpha and beta crystallin were also detected but in low concentration (13 per cent and 7 per cent, respectively) Delta crystallin is still the predominant fraction (69 per cent) in the cortical fibre cells The concentration of alpha crystallin is clearly more than that of the beta fraction (47 per cent and 27 per cent, respectively) In the epithelial and annular pad cells two protein fractions were identified as alpha and beta crystallin, beta crystallin being in the higher concentration (66 3 per cent and 33 7 per cent) As it can clearly be seen, alpha crystallin molecules have a faster migration than alpha crystallin present in the nuclear or cortical fibre cells (Fig 4)

The analysis demonstrates that delta crystallin is mainly contained in the old central fibre cells and superficial cells of young animals, but it is no longer synthesized in the equatorial cells or in the epithelium Alpha crystallin seems to be produced in significant amounts in the epithelial, equatorial and cortical cells It is also present in low concentrations in the inner fibre cells Alpha crystallin is constantly more movable in the equatorial cells, suggesting physicochemical differences from the alpha crystallin of other differentiating lens cells Anodal beta crystallins are a typical characteristic of elongated lens fibres Medium migrating beta crystallins are a consistent feature of early cell differentiation at the equatorial region Cathodal beta crystallins seem to be synthesized in the young epithelial cells

The results, obtained by three different methods, are in general terms consistent They indicate clearly that antigenic determinants and protein patterns vary both qualitatively and quantitatively according to the sequence of differentiation and elongation of the lens cells

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[1] Ralb, C , *Z Wiss Zool* , **65**, 257 (1889)
[2] Papaconstantinou, J , *Science*, **156**, 338 (1967)
[3] Genis-Galvez, J M , and Maisel, H , *Life Sci* , **6** 197 (1967)
[4] François, J , Rabaey, M , Wieme, R J , and Kaminski, M , *Amer J Ophthalm* , **42**, 577 (1956)
[5] François, J , Rabaey, M , and Wieme, R J , *Arch Ophthalm Chicago*, **53**, 481 (1955)
[6] Maisel, H , and Goodman, M , *Anat Rec* , **151**, 209 (1965)
[7] Genis-Galvez, J M , and Maisel, H , *Nature*, **213**, 283 (1967)
[8] Ten Cate, G , *Natuurw Tijdschr* , **92** (1949)
[9] Ten Cate, G , and van Doorenmaalen, W J , *Proc Kon Ned Akad Wet* , **53**, 894 (1950)
[10] Clayton, R M , *Nature*, **174**, 1059 (1954)
[11] Maisel, H , and Langman, J , *J Embryol Exp Morph* , **9**, 191 (1961)
[12] Rabaey, M , *Exp Eye Res* , **1**, 310 (1962)
[13] Zwaan, J , thesis, Univ Amsterdam (1963)
[14] Lowry, O H , Rosebrough, N J , Farr, A L , and Randal, R J , *J Biol Chem* , **193**, 265 (1951)
[15] Poulik, M D , *Nature*, **180**, 1477 (1957)
[16] Zwaan, J , and Ikeda, A , in *The Structure of the Eye* (edit by Rohen, J W) (Schattauer–Verlag, 1965)

Plant Agglutinins and Mitosis

THE ability of saline extracts of certain plants to agglutinate human red cells was first noticed in 1888 (ref 1), and this property has since been found in many plants, particularly in the Leguminosae[2] Some plant agglutinins show blood group specificity[3–5]

Phytohaemagglutinin (PHA)—a plant agglutinin extracted from the red kidney bean, *Phaseolus vulgaris*—also agglutinates white cells[6,7] and stimulates lymphocytes to undergo mitosis when grown in tissue culture[8] Extracts of other leguminous plants[9] and of *Phytolacca americana*[10] also have mitogenic effects The nature of the relationship between the erythro-agglutinating, the leucoagglutinating and the mitogenic properties of plant agglutinins, however, is obscure

Attempts to fractionate the mitogenic and the agglutinating components of PHA have given variable results Punnett and Punnett[11], using fractional precipitation, were able to prepare a mitogenic fraction from PHA which was free from erythro-agglutinating activity, but, as they pointed out, it will not be certain that these two fractions are different components of PHA until a less drastic preparative procedure is developed Nordman *et al* [7], on the other hand, were unable to achieve separation of the erythro-agglutinating and leucocyte-mitogenic activities of PHA using ion exchange chromatography, gel filtration, starch gel and immunoelectrophoresis Similarly, Rigas and Johnson[12] prepared a purified protein fraction of PHA which had both agglutinating and mitogenic activities and which seemed to be homogenous after ultracentrifugation, a variety of electrophoretic and chromatographic techniques and N-terminal amino-acid analysis

Absorption experiments have also given differing results Elves and Israels[13] found that, when the erythro-agglutinating activity of PHA was reduced by absorption with red cells, the mitogenic effect was also diminished On the other hand, de la Chapelle *et al* [14] and Barkhan and Ballas[15] were able to remove most of the erythro-agglutinating activity from PHA by absorption with red cells and yet the mitogenic activity was unimpaired Nordman *et al.*[7], however, were unable to isolate erythro-agglutinins from PHA which lacked mitogenic activity, and they suggested that PHA contains a pure leuco-agglutinin and an erythro-agglutinin which also agglutinates leucocytes, and that both of these agglutinins are mitogenic

In an investigation of the properties of *Phytolacca americana*, Borjeson *et al* [16] were unable to separate chemically the agglutinating and mitogenic activities of the extract, although electrophoretic and chromatographic results indicated that the mitogen from *Phytolacca americana* has different mobility and charge properties from the mitogen of PHA In absorption studies of the extract of *Phytolacca* the haemagglutinin could be selectively removed with red cells or stroma without altering the

Table 1

Plant extract	Erythro-agglutination	Leuco-agglutination	Mitogenicity
Phaseolus vulgaris (seed)	+ + + +	+ + + +	+ + + +
Phytolacca americana (leaves)	+	+ +	–
(root)	+	+ +	+ + + +
Another species of *Phytolacca*			
(not classified) (root)	+	+ + + +	+ + + +
(berries)	+	+	+ + +
(stem)	+	+ +	+ + + +
Lentils (three varieties)	+ + + +	+ + + +	+ + + +
Ulex europaeus (seed)	+ + +	+ +	–
Dolichos biflorus (seed)	+ + + +	+ + +	–
(stem)	+ + + +	+ + +	–
(root)	+ + + +	+ + +	–
Potato	+ + +	+ + +	–
Pelargonium hortorum (root)	+ +	Not tested	–
Rose (garden variety) (petals)	+ +	+ + +	–
Rumex crispus (seed)	+ +	Not tested	–
(leaves)	+ + +	–	–
(stem)	+ + +	+ + +	–

mitogen, whereas absorption with leucocytes resulted in loss of both mitogenic and leuco-agglutinating activity

In the present study, the erythro-agglutinating, the leuco-agglutinating and the mitogenic activities of several plant agglutinins, including blood group specific ones, have been compared in an attempt to ascertain the relationship between these different properties

The extracts which were prepared are shown in Table 1. Approximately 400 mg of the plant material was crushed in 7 ml of 0 9 per cent saline, left overnight at 4° C, centrifuged and filtered through a sterile bacteriological filter (porosity 5) The extract of *Dolichos biflorus* was specific for A_1 cells, and that of *Ulex europaeus* was specific for *H* substance, but the other preparations were non-specific Serial saline dilutions were made up to 1 in 800, and were tested against cells from healthy donors Erythro-agglutination was examined by the standard tube method and leuco-agglutination by the method of Killman[17], positive and negative controls were included in each experiment In the agglutination tests four doubling dilutions of the samples were also examined to a titre of 1/16 The erythro-agglutination was assessed macroscopically and graded + to 4+ The leuco-agglutination was read microscopically and graded + to 4+ Most of the samples which gave a positive result did so to a titre of 1/16 The leucocyte cultures were prepared by the method of Hungerford, Donnelly, Nowell and Beck[18], except that PHA was not added in the isolation of the leucocytes A sample (0 1 ml) of the plant agglutinin extracts was added to 2 ml of the leucocyte suspension which contained about $1\ 5 \times 10^6$ leucocytes/ml , and had a serum concentration of 20 per cent A negative control and a positive control with PHA (Difco *M*) were included in each experiment The cells from each culture were treated with a hypotonic citrate solution, fixed with acetic alcohol (one part glacial acetic acid, three parts methanol), spread on a slide and stained In the control and negative cultures, less than three mitoses were seen on the whole slide and when 1,000 cells were counted the percentage of mitoses was less than 0 1 per cent In the positive cultures many mitoses were seen in percentages which varied from 1–14 per cent and were graded + to 4+ The lymphocytes of at least three donors were tested with each extract The extracts of *Ulex europaeus* and *Dolichos biflorus* were tested against the lymphocytes of three donors of group *O* and three donors of group A_1 The results of the agglutination and cell culture tests were all read blind by two observers and were closely reproducible

The results are shown in Table 1 With one exception, all the extracts agglutinated white cells as well as red cells The extract of *Dolichos biflorus* agglutinated group A_1 leucocytes but not those belonging to group *O*, and the extracts of *Ulex europaeus* agglutinated leucocytes from group *O* subjects more strongly than those from group A_1 individuals Similar specific leucocyte agglutination by plant extracts has been described by Hossaini[19] Most of the extracts, including the blood group specific ones, failed to induce mitosis of lymphocytes, however

The present study shows therefore that, although plant agglutinins will commonly agglutinate leucocytes as well as red cells, they seldom also induce mitosis of lymphocytes in tissue culture This suggests that the mitogenic property present in some plant agglutinins is independent both of the erythro-agglutinating and leuco-agglutinating activity

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[1] Stillmark, H , *Inaug Diss* , *Dorpat* (1888), quoted by Makela (1957)

[2] Makela, O , *Ann Med Exp Biol Fenn* , **35**, suppl 11 (1957)

[3] Renkonen, K O , *Ann Med Exp Biol Fenn* , **26**, 66 (1948)

[4] Boyd, W C , and Reguera, R M , *J Immunol* , **62**, 333 (1949)

[5] Bird, G W G , *Brit Med Bull* , **15**, 165 (1959)

[6] Hirschhorn, K , Kolodny, R L , Hashem, N , and Bach, F , *Lancet*, ii, 305 (1963)

[7] Nordman, C T , de la Chapelle, A , and Grasbeck, R , *Acta Med Scand* suppl , **412**, 49 (1964)

[8] Nowell, P C , *Cancer Res* , **20**, 462 (1960)

[9] Hashem, N , and Kabarity, L , *Lancet*, i, 1428 (1966)

[10] Farnes, P , Barker, B E , Brownhill, L E , and Fanger, H , *Lancet*, ii, 1100 (1964)

[11] Punnett, T , and Punnett, H H , *Nature*, **198**, 1173 (1963)

[12] Rigas, D A , and Johnson, E A , *Ann NY Acad Sci* , **113**, 800 (1964)

[13] Elves, M W , and Israels, M C G , *Brit J Haematol* , **9**, 406 (1963)

[14] de la Chapelle, A , Nordman, C T , and Grasbeck, R , *Proc Ninth Cong Europ Soc Haematol* , Lisbon (edit by Karger, S), 78 (Basel/New York, 1963)

[15] Barkhan, P , and Ballas, A , *Nature*, **200**, 141 (1963)

[16] Borjeson, J , Reisfeld, R , Chessin, L N , Welsh, P D , and Douglas, S D , *J Exp Med* , **124**, 859 (1966)

[17] Killman, S -Å , *Leukocyte Agglutinins* (Blackwell Scientific Publications, Oxford, 1960)

[18] Hungerford, D A , Donnelly, A J , Nowell, P C , and Beck, S , *Amer J Hum Genet* , **11**, 215 (1959)

[19] Hossaini, A A , *Amer J Clin Pathol* , **46**, 507 (1966)

Distribution of Some Dehydrogenase in the Cerebellar Cortex

Eccles and his colleagues have shown us that cerebellar cortical neurones are predominantly inhibitory elements, including those of Purkinje cells which inhibit the neurones of vestibular nuclei[1–4] The only excitatory elements known until now have been granular cells, and the individual structures and systems within the cerebellar cortex cannot be characterized according to their metabolic activity

We have tried to characterize histochemically the distribution of enzymes the functions of which are connected with glycolysis, the pentose shunt, the citric acid cycle, fatty acid oxidation and with a system which destroys gamma aminobutyric acid (GABA) Table 1 shows the methods which we used

Table 1

Enzymes detected	Incubation time (min)	Incubating medium, *p*H 7 2	Density of Purkinje cell/glomerulus / Geometrical thickness
Glucose-6-phosphate dehydrogenase	120	Substrate (0 1 M)	0 89
Isocitrate dehydrogenase	90	NADH/NADP 3 mM	0 95
Malate dehydrogenase	65	NBT/TNBT 0 025%	0 96
α-Glycerophosphate dehydrogenase	65	NaN_3 10 mM	0 65
β-Hydroxybutyrate dehydrogenase	65	$MgCl_2$ 7 mM	0 80
Lactate dehydrogenase	65	0 05 M *tris*-HCl buffer	1 11
Glutamate dehydrogenase	65	Cryostat sections	0 86
Succinate dehydrogenase	90	– 20° C	0 92
NADH-diaphorase	20	3 mM NADH	0 97
GABA-transaminase	90		1 03

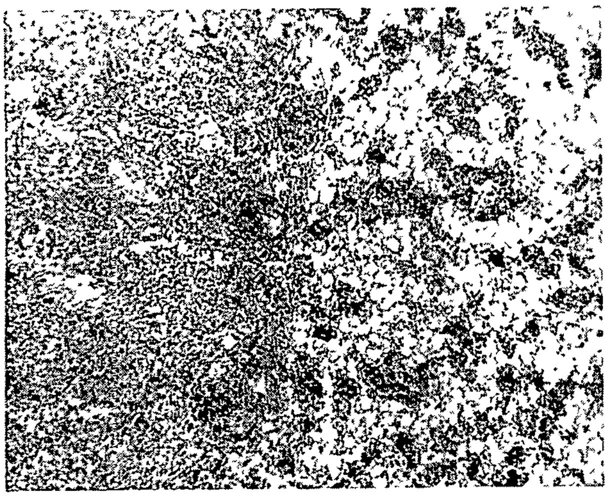

Fig. 1. Glucose 6-phosphate dehydrogenase (stained with TNBT, ×12.5).

Although the general patterns of distribution of the ten enzymes which we studied (in the rabbit cerebellar cortex) are all similar, there are some interesting differences. A high density of formazan deposits was found in the glomeruli of the granular layer as well as in Purkinje cells. The neuropil in the granular layer shows a diffused distribution of formazan deposits with a slightly lower density (Fig. 1). All enzyme reactions which we carried out were positive in Purkinje, stellate and basket cells and also in Golgi neurones. These results contradict the suggestion that succinate dehydrogenase is not active in small neurones of the molecular layer and in the Golgi cells[5]. Most important seems to be the question of whether there are any differences between the metabolic activity of excitatory and inhibitory neurones. While comparing slides which showed different histochemical reactions, we found that this question could be answered only qualitatively. All enzymes which we determined are present in the plasma of the granular cells. In cryostat sections, the cytoplasm of these cells fills a narrow space 0.3–0.5μ wide. The evidence of formazan deposits lies just on the resolution limits of the optical microscope and depends on results of the histochemical technique which is used. This is probably the reason why the ideas which have been published about enzyme activity in granular cells are so different[5–7]. In these cells, the degree of the enzyme activity which is detected histochemically probably depends on the dimensions of the cytoplasm and on its ultrastructural organization. We could find only six to ten mitochondria in the cytoplasm of an ultrathin section of a granular cell. Histochemical evidence methods indicate that there are no qualitative differences in dehydrogenase activity between excitatory and inhibitory neurones.

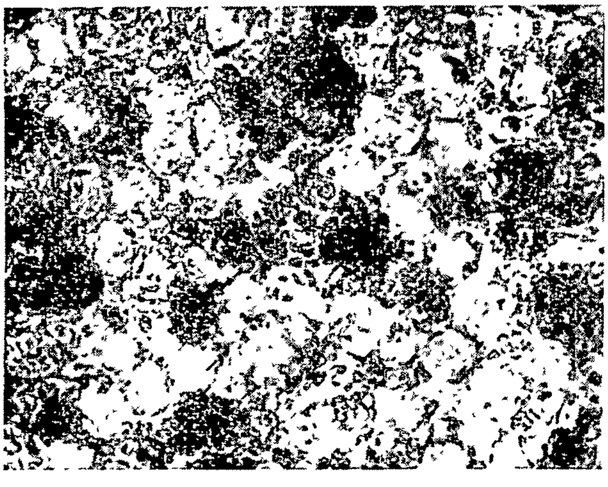

Fig. 2. GABA transaminase, according to Van Gelder (stained with TNBT, ×50). Formazan deposits seen in cytoplasm of granular cells.

The distribution of the enzyme which destroys GABA could be expected to be a reflexion of its function. The GABA transaminase succinic semi-aldehyde system has been assumed to exert its effect on inhibitory neurones postsynaptically, where GABA is destroyed in large quantities. This enzyme was also shown to be absent from granular cells[8–10]. We have used the same histochemical technique and been able to show evidence of significant activity of this enzyme in granular cells (Fig. 2). At least a part of this enzyme might be localized in the mitochondria. (The same picture was obtained with TNBT and NBT.) The enzyme shows remarkable activity in the glial cells of the white and grey matter (Fig. 3). This pattern of distribution of enzyme activity is very similar to that of other hydrogenases of the Krebs cycle.

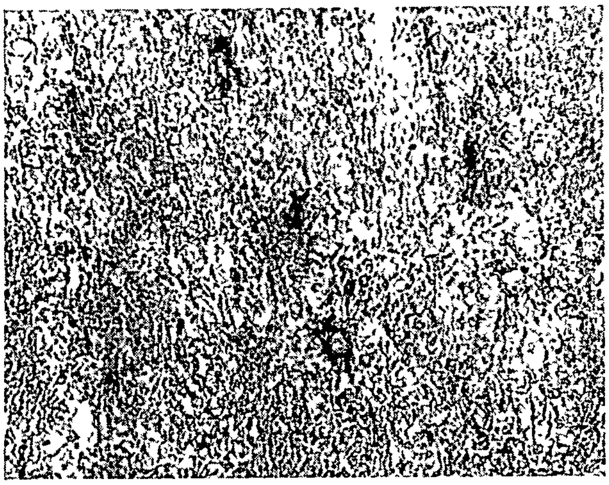

Fig. 3. Activity of GABA transaminase in glial cells of cerebellar white matter (×20).

Very high activity of dehydrogenases was observed in the spaces of the granular layer—the glomeruli cerebellosi. These glomeruli are structures where fibre terminals form a large presynaptic block which has contact with numerous granular cell dendrites and with dendrites of Golgi cells. Enzyme activity in these spaces seems to be an example of metabolic activity in a multisynaptic space. We tried to obtain more exact information about the activity of dehydrogenase in this space and in the cytoplasm of Purkinje cells, using a cytophotometer (wavelength 5600 Å) to compare the distribution of activity with the density of formazan deposits in these places. The extinction value divided by the measured geometrical thickness gives the degree of the density of formazan. Results are recorded in Table 1 and are expressed as the ratio of the density of Purkinje cells to that of glomeruli. The ratio of densities is very similar for the different dehydrogenases which are functionally connected with the citric acid cycle and NADH diaphorase. The activity of these enzymes is also very similar in Purkinje cells and glomeruli. (The ratio is near to 1.0.) Glutamate dehydrogenase, glucose-6-phosphate dehydrogenase and β-hydroxybutyrate dehydrogenase show higher activity in glomerular structure whereas GABA transaminase and chiefly lactate dehydrogenase show higher activity in the cytoplasm of Purkinje cells. The activity of α-glycerophosphate dehydrogenase in Purkinje cells is low, and this enzyme usually shows less activity in neurones and glial cells than in the neuropil

These results indicate that there are differences in the generally similar distribution pattern of different dehydrogenases Histochemical evidence is that glomerular structures show more activity of the dehydrogenases which we have investigated The relatively high activity of lactate dehydrogenase in the cytoplasm of neurones is interesting

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[1] Eccles, J C, Llinás, R, and Sasaki, K, *Nature*, **204**, 1265 (1964)
[2] Eccles, J C, Llinás, R, and Sasaki, K, *Exp Brain Res*, 1, 17 (1966)
[3] Eccles, J C, Llinás, R, and Sasaki, K, *Exp Brain Res*, **3**, 81 (1967)
[4] Ito, M, Joshida, M, and Obata, K, *Experientia*, **20**, 575 (1964)
[5] Tewari, H B, and Bourne, G H, *J Histochem Cytochem*, **10**, 619 (1962)
[6] Thomas, E, and Pearse, A G E, *Histochemie*, **2**, 266 (1961)
Friede, R L, *Topographic Brain Chemistry* (Academic Press, New York, 1966)
[8] Van Gelder, N M, *J Neurochem*, **12**, 231 (1965)
[9] Van Gelder, N M, *J Neurochem*, **12**, 279 (1965)
[10] Kuriyama, K, Haber, B, Sisken, B, and Roberts, E, *Proc US Nat Acad Sci*, **55**, 846 (1966)

IMMUNOLOGY

Passive Sensitization of *Salmonella adelaide* to the Bactericidal Action of Antibody and Complement

Red blood cells, onto which various unrelated antigens have been adsorbed or coupled chemically, may become sensitized to lysis by antibody against the adsorbed antigen in the presence of complement[1] In other words, lysis of red cells in this manner results from a heterologous antigen–antibody reaction with fixation of complement occurring on its surface The component requirements for cell damage by this system of antibody and complement seem to be identical irrespective of the nature of the cell involved, whether it is a red cell, a tissue cell or a Gram negative bacterium Passive sensitization to complement-induced lysis has been achieved using red cells coated with lipopolysaccharides or with bovine serum albumin (BSA)[2], with mast cells[3–5] and with lymphocytes[6,7] among others

We wished to know whether the similar behaviour of Gram negative bacteria as regards their killing and lysis by antibody and complement also extended to passive sensitization The plan was to couple various proteins to the surface of *S adelaide* through the agency of *bis*-diazotized benzidine in a manner analogous to that used by Rangel and Repka[2] with sheep red cells The sensitivity of the organisms to killing by the antibody–complement system was then determined by an assay similar to that described by Michael *et al*[8] Fresh guinea-pig serum was used as a source of complement and was absorbed for 60 min in an ice-water bath each day before use with approximately 10^9 living *S adelaide*/ml to remove any specific *S adelaide* antibodies After absorption the serum was sterilized by filtration through a 0 8 mμ 'Millipore' membrane Rabbit antisera against bovine serum albumin, mouse gamma globulin and aggregated human gamma globulin were all absorbed in this way to remove any naturally occurring anti-*S adelaide* antibodies The antibody contents of the various antisera were assayed by quantitative precipitation in the conventional manner[9]

Crystalline BSA was purchased from the Commonwealth Serum Laboratories, Australia, the mouse gamma globulin was prepared by fractionation on DEAE cellulose[10] of the serum globulins precipitated at 16 per cent sodium sulphate and the aggregated human gamma globulin by heating human gamma globulin (immune serum globulins from the Commonwealth Serum Laboratories) at 63° C for 20 min[11] Bacteria were coupled to proteins in the way described by Rangel and Repka[2] A volume (100 mg) of benzidine dihydrochloride was dissolved in 10 ml of 0 25 normal hydrochloric acid The solution was cooled to 0° C and 2 0 ml of precooled 0 5 normal sodium nitrite was slowly added with continuous stirring which continued while the solution was maintained at 5° C until no test for free nitrite was obtained with potassium iodide and starch This solution (*bis*-diazotized benzidine) was then diluted 1 30 with chilled 0 15 molar phosphate buffer, *p*H 7 4, and 5 ml was immediately added to 5 ml of a suspension in the same buffer of 10^8 *S adelaide*/ml which contained 10 mg of the protein to be coupled The suspension was stirred for 15 min at 4°–7° C, centrifuged once and the deposit diluted to a concentration of 2×10^4 bacteria/ml in 0 15 molar phosphate buffer, *p*H 7 4, containing 0 02 per cent magnesium chloride

After coupling in this manner, bacteria showed large and inexplicable variations in viability, in some instances only 1 per cent remained viable while in other experiments 90 per cent viability was obtained The only bactericidal assays considered to be satisfactory were those in which the original coupling reaction allowed more than 10 per cent of the organisms to survive

For the bactericidal assays, a suspension of 10^4 protein-coupled bacteria in 0 5 ml of a 1 10 dilution of absorbed guinea-pig serum was added to 0 5 ml dilutions of the appropriate antiserum at 4° C All dilutions were made in the 0 15 molar phosphate buffer, *p*H 7 4, containing 0 02 per cent magnesium chloride Plate counts of the numbers of bacteria/ml in each dilution were prepared at this time and again after 150 min at 37° C The number of colonies appearing after overnight incubation of the agar plates at 37° C was noted The magnitude of killing was assessed by comparison with dilutions of control coupled bacteria incubated for 150 min in the absence of antisera In order to establish that the killing of coupled bacteria in the presence of complement involved antibody directed specifically against the foreign protein, normal *S adelaide* was incubated as before with dilutions of the *S adelaide* absorbed rabbit anti-BSA

Fig 1 shows a typical relationship between the killing of both BSA-coupled and normal *S adelaide* as a function of the concentration of rabbit antiserum against BSA Although 50 per cent of the coupled bacteria were killed at a dilution of 1/5,000 the uncoupled bacteria required a dilution of 1/380 for the same degree of killing Coating the bacteria with BSA, however, did not impair the efficiency of killing by rabbit antiserum to *S adelaide* plus complement

The average results obtained from three separate experiments for each antiserum are shown in Table 1 Two findings emerged from these results first, that passive sensitization to complement-induced cytotoxicity occurs with Gram negative bacteria, and second, that the efficiency of killing by antibody plus complement seems to be related to the physical dimensions of the coupled foreign protein

The finding that bacteria, like other cells, can be killed by heterologous antigen–antibody reactions occurring on their surface in the presence of complement serves to emphasize the fundamental similarity by which these reactants act on all susceptible cells Moreover, it strongly

Table 1 BACTERICIDAL STUDIES WITH PROTEIN-COUPLED *S adelaide*

Foreign protein on *S adelaide* Nature	Molecular wt	Rabbit antibody used	Precipitable antibody protein (mg/ml)	Maximum dilution of antiserum producing 2 $\log_{10}$ kill	Conc of antibody (μg/ml at maximum dilution producing 2 $\log_{10}$ kill)
Uncoupled	—	Anti-*S adelaide*	0 26	1/10^7	2 6 × 10^{-5}
BSA	55,000	Anti-*S adelaide*	0 26	1/10^7	2 6 × 10^{-5}
		Anti-BSA	1 1	1/570	1 9
Mouse gamma globulin	160,000	Anti-mouse gamma globulin	4 8	1/270	18
Agg human gamma globulin	1–2 × 10^6	Anti-agg human gamma globulin	7 9	No kill	No kill

suggests that the appearance of holes in red cells or bacteria[12,13] after complement attack is not the result of a direct action on the antigen substrate but is a secondary effect consequent on the antigen–antibody fixation of complement as suggested by Muschel[15] The finding that the efficiency of killing seems to be related to the physical dimensions of the foreign protein is particularly apparent when one considers the concentration of specific antibody in μg/ml necessary to give a 2 $\log_{10}$ kill of the bacteria A 10^5-fold increase in micrograms of precipitable antibody/ml is necessary to kill *S adelaide* if the antibody is directed against the BSA coating rather than against the organism itself This is even more striking when the bacteria were coupled with mouse gamma globulin while coating with aggregated human gamma globulin failed to sensitize the organisms to killing by antibody plus complement

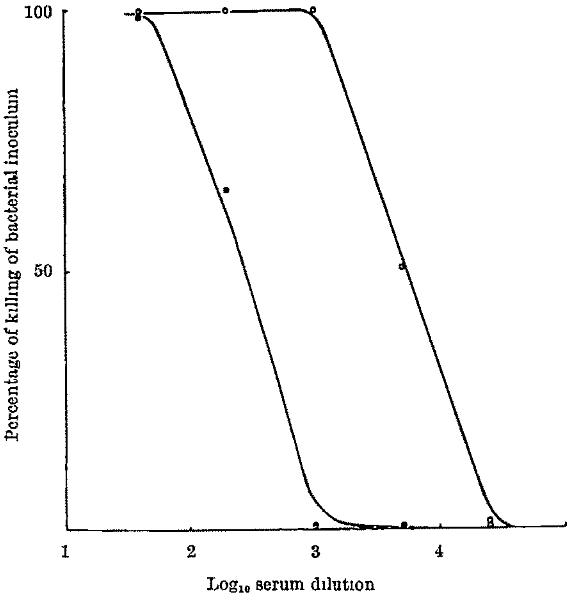

Fig 1 Bactericidal action of rabbit anti-bovine serum albumin serum (absorbed with *S adelaide*) against *S adelaide* (●) and *S adelaide* coupled to bovine serum albumin (○)

It seems unlikely that this great reduction in efficiency using antibody against coupled antigen could be explained by a paucity of antigenic sites In some of our experiments, BSA labelled with iodine-131 was used and this indicated that more than 10^6 molecules of BSA were coupled to each bacterial cell Moreover, with a strain of bacteria highly sensitive to antibody and complement it has been shown that the number of antibody molecules which need to react with the surface in order to kill the bacterial cell may be as low as thirty in the case of γM (ref 14)

These observations suggest that the efficiency of bactericidal effects which are mediated by complement is dependent on the effective distance at which complement is fixed from the bacterial surface Muschel[15] has offered a similar explanation for the fact that, while *P ballerup* is refractory to the bactericidal action of serum, spheroplasts of *P ballerup* are as sensitive to lysis, mediated by the antibody–complement system, as those of the moderately serum-sensitive *S typhosa* 0 901

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[1] Neter, E , *Bact Rev* ,**20**, 166 (1956)

[2] Rangel, H , and Repka, D , *Immunology*, **8**, 618 (1965)

[3] Archer, G T , *Austral J Exp Biol Med Sci* , **38** 147 (1960)

[4] Keller, R , *Pathol et Microbiol* ,**24**, 932 (1961)

[5] Keller, R , *Nature*, **193**, 282 (1962)

[6] Favour, C B , *Proc Soc Exp Biol and Med* , **65**, 269 (1947)

[7] Waksman, B H , *Amer Rev Tuberc* , **68**, 746 (1953)

[8] Michael, J G , Whitby, J L , and Landy, M , *J Exp Med* ,**115**, 131 (1962)

[9] Kabat E A , and Mayer, M M , *Experimental Immunochemistry* (C C Thomas, Springfield, Illinois, 1961)

[10] Turner, K J , Jenkin, C R , and Rowley, D , *Austral J Exp Biol Med Sci* , **42**, 229 (1964)

[11] Ishizaka, T , Ishizaka, K , and Borsos, T , *Immunology*, **87**, 433 (1961)

[12] Rosse, W F , Dourmashkin, R , and Humphrey, J L , *J Exp Med* ,**123** 969 (1966)

[13] Bladen, H A , Gewurz, H , and Mergenhagen, S E , *J Exp Med* ,**125**, 767 (1967)

[14] Sterzl, J , Kostka, J , and Lauc A , *Fol Microbiol* , **7**, 162 (1962)

[15] Muschel, L , *Ciba Foundation Symposium on Complement* (edit by Wolstenholme, G E W , and Knight J) (J and A Churchill, London, 1965)

In vitro Detection of Immunosuppressive Activity of Anti-lymphocyte Sera

THE problem of *in vitro* evaluation of anti-lymphocyte sera (ALS) has not yet been solved The tests used, such as leuco-agglutination, cytotoxicity and lymphocyte stimulation in culture, do not detect small amounts of anti-lymphocyte antibodies and, furthermore, the results of these tests are not regularly correlated with graft survival time[1,2] We propose a new *in vitro* test, based on inhibition of an active function of the lymphocytes

Immunologically active cells can be detected by an immunocyto-adherence technique—rosette formation[3,4] One rosette-forming cell (RFC) is found for every thousand lymphocytes in the spleen of Swiss and *C57Bl6* non-immunized mice incubated with sheep red blood cells (RBC) We have studied the *in vitro* action of ALS on this background of RFC

Ten heterologous anti-mouse ALS from rabbits (Table 1) were obtained as previously described[5] In four cases, immunization was achieved using rabbits which were previously tolerant to blood and kidney of mice[6] (ALS Nos 4, 7, 9, 10) These sera showed no activity or only weak activity against erythrocytes, serum and kidney of mice The technique of immunocyto-adherence was a modifica-

Table 1 INHIBITION TITRES OF TEN ALS

ALS	Antigen used for ALS production	Inhibition titre Without complement	With complement
1	Lymph node cells	1/600	1/10,000
2	,, ,, ,,	1/1,200	1/20,000
3	,, ,, ,,	1/1,200	1/20,000
4	,, ,, ,,	1/2,500	1/20,000
5	,, ,, ,,	1/600	1/1,200
6	,, ,, ,,	1/1,200	1/80,000
7	Homogenized lymph node cells	1/1,200	1/20,000
8	Insoluble fraction from homogenized lymph node cells	1/10,000	1/40,000
9	Thymic cells	1/2,500	1/20,000
10	Splenic cells	1/2,500	1/10,000

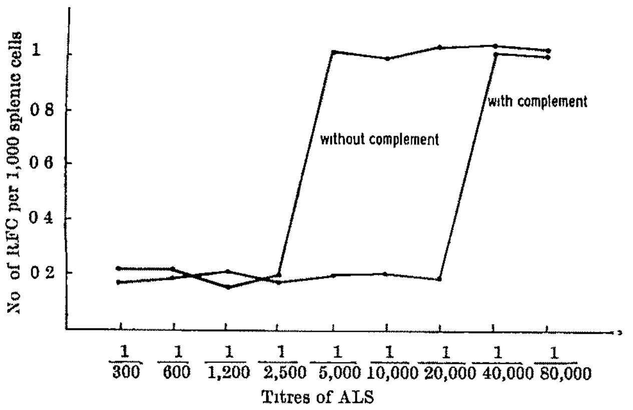

Fig 1 *In vitro* inhibition of rosette-forming cells with ALS ALS 4, leuco-agglutination titre, 1/16, cytotoxicity titre, 1/1,024, rosette inhibition titre without complement, 1/2,500, rosette inhibition titre with complement, 1/20,000

tion of Biozzi's method[7] 6 x 10[6] spleen cells were washed twice in Hanks BSS and were incubated for 90 min at 37° C, with two-fold dilutions of inactivated ALS After addition of 25 x 10[6] sheep RBC and centrifugation (200*g* for 5 min) the number of rosette-forming cells was counted in a haematocytometer

Results are shown in Fig 1 After incubation with ALS the number of RFC is 0 2/1,000 spleen cells as compared with the control value of 1 0/1,000 cells without ALS When ALS is diluted to 1/5,000, the number of RFC increases suddenly to 1/1,000 cells The test could not be performed with serum dilutions of under 1/300 because of leuco-agglutination Identical figures were obtained for all sera but one, the exception was serum 1 for which an intermediary plateau was observed over two dilutions From these experiments, the "inhibition titre" has been defined as the highest dilution for which the number of RFC is 0 3/1,000 cells or lower The inhibition titre was increased by complement (1/500 dilution of guinea-pig fresh serum) which was added before splenic cells were incubated with ALS For example, the inhibition titre of ALS 6 was 1/80,000 with complement as compared with 1/1,200 without complement Inactivated guinea-pig serum did not increase the inhibition titre

The mechanism of the inhibition of RFC by ALS is unknown The anti-sheep RBC antibodies contained in ALS are not concerned because the titre of rosette inhibition was not modified after absorption of these antibodies The anti-mouse immunoglobulin antibodies which are present in ALS do not seem responsible either, first, because anti-immunoglobulin sera inhibit the RFC[7] but not so strongly as ALS, second, because rosette formation is inhibited by ALS without any anti-immunoglobulin precipitating activity (ALS 2, 4, 6, 7, 9, 10), and third, because when complement is added the inhibition of RFC by anti-immunoglobulin sera is not increased Inhibition is not caused by cytotoxicity, as defined by the trypan blue exclusion test, because the titres for cytotoxicity are smaller than the inhibition titres

We are currently studying the possible correlation between this *in vitro* test and homograft survival Human application is feasible because inhibition of human lymphocytes with horse anti-human ALS has been found in preliminary experiments

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[1] Jeejeebhoy, M F, *Transplantation*, 5, 273 (1967)

[2] Antoine, B, Neveu, T, Bach, J F, and Dardenne, M, *First Inter Cong Transplan Soc*, Paris (1967) (in the press)

[3] Nota, N R, Liacopoulos Briot, M, Stiffel, C, and Biozzi, G, *CR Acad Sci*, Paris, 259, 1277 (1964)

[4] Zaalberg, O B, *Nature*, 202, 1231 (1964)

[5] Bach, J F, Neveu, T, Klein, M, Watchi, J M, Dardenne, M, and Antoine, B, *Cell Bound Immunity with Special Reference to Antilymphocyte Serum and Immunotherapy of Cancer* (Les Congrès et Colloques de l'Université de Liège pub, in the press)

[6] Antoine, B, and Neveu, T, *Ann Inst Pasteur*, 113, 509 (1967)

[7] Biozzi, G, Stiffel, C, and Mouton, D, *Immunity, Cancer and Chemother* (edit by Mihich, E) (Academic Press, New York, 1967)

BIOCHEMISTRY

Inhibition by Lasiocarpine of RNA Synthesis, RNA Polymerase and Induction of Tryptophan Pyrrolase Activity

Lasiocarpine a pyrrolizidine alkaloid derived from *Heliotropium lasiocarpium* or from *Heliotropium europaeum*, produces chronic liver damage and cancer in rats and other laboratory animals[1-3], and it induces nucleolar "capping"[4,5] (Fig 1) The functional significance of nucleolar "caps" is poorly understood They are probably related to alterations in DNA-directed RNA synthesis and RNA polymerase activity[6-8]

The studies of Christie[9] and Gallagher[10] deal with the effect of heliotrine and lasiocarpine on the activity *in vitro* of certain mitochondrial enzyme systems, but no information is available on the effect of pyrrolizidine alkaloids on RNA metabolism or on enzyme induction

During a comparative study of RNA synthesis after administration of selected hepatocarcinogens (unpublished work of J Reddy and D Svoboda), it was observed that lasiocarpine markedly inhibited the incorporation *in vivo* of ^{3}H-uridine into rat liver nuclear RNA and significantly lowered the RNA content of the nuclear and ribosomal fractions (Table 1) To elucidate further the mechanism of inhibition of ^{3}H-uridine incorporation by lasiocarpine and the significance of the concomitant morphological abnormalities in the nucleolus and cytoplasm, the effect of lasiocarpine or tryptophan pyrrolase induced by steroids and RNA polymerase activity was investigated

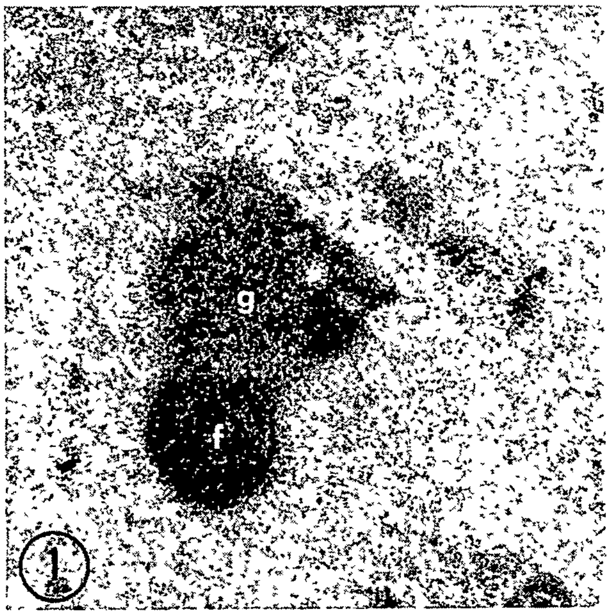

Fig 1 The nucleolus of a rat liver cell, 24 h after the injection of lasiocarpine, showing separation of the granular (*g*) and fibrillar (*f*) components to form two distinct zones (x 49,000)

With intraperitoneal injection (80 mg/kg body weight), lasiocarpine produced characteristic nuclear and cytoplasmic alterations in rat liver as early as 30 min after administration By 24 h, the nucleoli of almost all hepatic cells showed typical separation of the granular and fibrillar components (Fig. 1), which recovered by 72 h (ref 4)

For estimation of steroid-induced activity of tryptophan pyrrolase, adrenalectomized male *F*-344 rats weighing 200–300 g were injected with lasiocarpine (80 mg/kg body weight, intraperitoneally) and killed at 6, 24, 72 and 120 h Hydrocortisone acetate (2 5 mg/100 g body weight, intraperitoneal) was injected 4 h before death Tryptophan pyrrolase assays were performed according to the method of Knox and Mehler[11]

Table 1 EFFECT OF LASIOCARPINE ON LIVER NUCLEAR AND RIBOSOMAL RNA CONTENT, AND INCORPORATION *in vivo* OF ^{3}H-URIDINE

Group	RNA content*		^{3}H-uridine incorporation†	
	Nuclear RNA/DNA ratio (μg/μg)	Ribosomal RNA (mg/g liver)	Nuclear fraction (d p m / μg RNA)	Ribosomal fraction (d p m /100 μg RNA)
Control	0 196 ± 0 001 ‡	6 33 ± 0 06	2,166	1,230
Lasiocarpine 24 h (80 mg/kg body weight)	0 120 ± 0 006 (*P* > 0 001)	4 71 ± 0 08 (*P* > 0 001)	255	540

* Each group consists of five animals

† 24 h after the administration of lasiocarpine, two animals in each group were given 15 μCi of ^{3}H-uridine/g body weight intraperitoneally and killed after 30 min Results are expressed as average of two animals

‡ Mean ± standard error

The activity of RNA polymerase was determined on intact isolated nuclei[12] from livers of rats injected with a single dose of lasiocarpine at 2, 4, 6, 12, 24, 48 and 72 h before death Two rats were used for each interval Pure nuclear preparations were assayed in duplicate at each interval in the presence of 0·32 M ammonium sulphate, using the standard assay mixture for nuclear RNA polymerase based on the method of Gelboin *et al* [8] Nucleic acids were estimated by the method of Schneider[13] and the radioactivity was determined by using a Packard 'Tri-carb' liquid scintillation spectrometer

In the livers of adrenalectomized rats, lasiocarpine caused significant inhibition of steroid-induced tryptophan pyrrolase (Fig 2) at 6 h Even at 72 h after a single dose of lasiocarpine there was substantial reduction of tryptophan pyrrolase activity Recovery was apparent, however, at 5 days

The studies of *in vivo* incorporation of a 30 min ^{3}H-uridine pulse into rat liver nuclear RNA showed marked inhibition 24 h after lasiocarpine administration (Table 1)

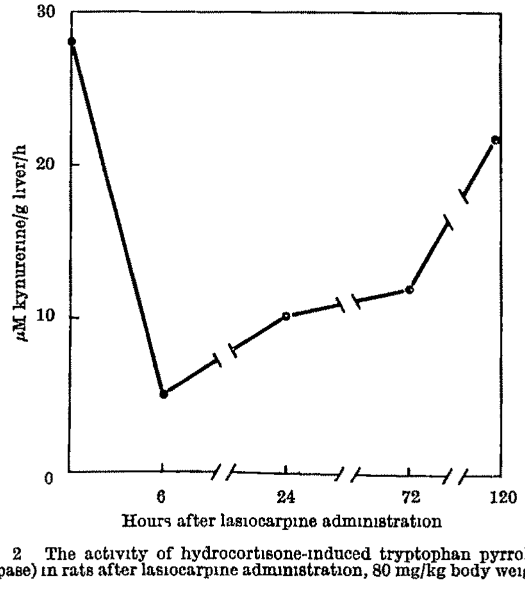

Fig 2 The activity of hydrocortisone-induced tryptophan pyrrolase (T-pase) in rats after lasiocarpine administration, 80 mg/kg body weight

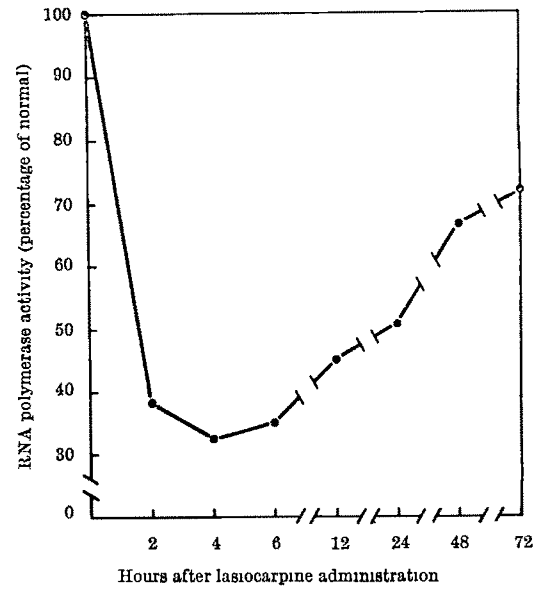

Fig 3 Rat liver RNA polymerase activity after lasiocarpine treatment The animals were injected with lasiocarpine 80 mg/kg body weight and killed at 2, 4, 6, 12, 24, 48 and 72 h

Likewise there was also a significant reduction in the labelled ribosomal RNA The depression of incorporation was accompanied by a 40 per cent reduction in the nuclear RNA/DNA ratio

RNA polymerase activity in the hepatic nuclei of rats treated with lasiocarpine for 2 and 4 h before they were killed was significantly decreased Fig 3 summarizes the data of incorporation of cytidine triphosphate into acid-insoluble material, in terms of percentage of normal Maximum inhibition (about 65 per cent) of RNA polymerase activity was still seen after 24 h, and nearly normal levels were noted by 72 h. Inhibition of RNA polymerase activity by lasiocarpine suggests that this agent, like aflatoxin B_1 (refs 8 and 14) and actinomycin *D* (ref 15), could interfere with transcription of the DNA molecule by inhibiting RNA polymerase activity. The analogy between lasiocarpine, aflatoxin B_1 and actinomycin is extended further by the similarity of the early morphological abnormalities in nucleoli caused by all three[4,16,17]

Because the nucleolus plays a significant part in the synthesis of ribosomal RNA, the derangements of RNA synthetic activity and the inhibition of tryptophan pyrrolase induction after lasiocarpine treatment may be related, in part, to the loss of structural integrity of the nucleolus The reversal to normal of the morphological changes 72 h after administration of lasiocarpine is accompanied by restoration of RNA polymerase activity Steroid induction of tryptophan pyrrolase has been shown to be dependent on intact systems for transcription and translation of protein synthesis[18,19], processes which are reflected in part by nucleolar and cytoplasmic morphology, respectively Significant restoration of tryptophan pyrrolase induction by hydrocortisone accompanies reversal to normal of the cytoplasmic changes 120 h after lasiocarpine This further supports the contention that aberrations in the nucleolar morphology reflect or occur concomitantly with biochemical lesions and decreased RNA polymerase activity related to transcription (DNA-dependent RNA synthesis)

Because the biochemical and ultrastructural effects of lasiocarpine on nuclei of rat liver cells are analogous to those of actinomycin *D* (refs 15, 16 and 19) and aflatoxin[8,14,17], it is suggested that inhibition of nuclear RNA synthesis, steroid induction of tryptophan pyrrolase and RNA polymerase activity are important biochemical defects in lasiocarpine injury and probably reflect the change termed nucleolar segregation or "capping"

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[1] Schoental, R., and Magee, P. N., *J. Pathol. Bact.*, **74**, 305 (1957).
[2] Bull, L., and Dick, A., *J. Pathol. Bact.*, **78**, 483 (1959).
[3] Schoental, R., and Magee, P. N., *J. Pathol. Bact.*, **78**, 471 (1959).
[4] Svoboda, D., and Soga, J., *Amer. J. Pathol.*, **48**, 347 (1966).
[5] Svoboda, D., Racela, A., and Higginson, J., *Biochem. Pharmacol.*, **16**, 651 (1967).
[6] Simard, R., and Bernhard, W., *Intern. J. Cancer*, **1**, 463 (1966).
[7] Friedman, M. A., and Wogan, G. N., *Fed. Proc.*, **25**, 662 (1966).
[8] Gelboin, H. V., Wortham, J. S., Wilson, R. G., Friedman, M., and Wogan, G. N., *Science*, **154**, 1205 (1966).
[9] Christie, G. S., *Austral. J. Exp. Biol. Med. Sci.*, **36**, 413 (1958).
[10] Gallagher, C. H., *Biochem. Pharmacol.*, **3**, 220 (1960).
[11] Knox, W. E., and Mehler, A. H., *J. Biol. Chem.*, **187**, 419 (1950).
[12] Widnell, C. C., and Tata, J. R., *Biochem. J.*, **92**, 313 (1964).
[13] Schneider, W. C., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **3**, 680 (Academic Press, New York, 1957).
[14] Clifford, J. I., and Rees, K. R., *Nature*, **209**, 312 (1966).
[15] Reich, E., and Goldberg, I. H., in *Progress in Nucleic Acid Research and Molecular Biology* (edit. by Davidson, J. N., and Cohn, W. E.), **3**, 183 (Academic Press, New York, 1964).
[16] Schoefl, G. I., *J. Ultrastruct. Res.*, **10**, 224 (1964).
[17] Svoboda, D., Grady, H., and Higginson, J., *Amer. J. Pathol.*, **49**, 1023 (1966).
[18] Greengard, O., Smith, M. A., and Acs, G., *J. Biol. Chem.*, **238**, 1548 (1963).
[19] Greengard, O., and Acs, G., *Biochim. Biophys. Acta*, **61**, 652 (1962).

Urinary Peptides derived from the Cross-linked Regions of Connective Tissue Proteins

We have used 'Sephadex *G*-15' to examine the molecular weight distribution of urinary peptides obtained from normal males, aged between 22 and 24 yr, by a method designed to protect labile groups within peptides. We obtained a peptide fraction, of approximate molecular weight 600, which gave several slowly developing yellow Ehrlich positive zones after electrophoresis in pyridine acetate buffer at *p*H 5·5. This staining property was lost after chromatography in systems containing acetic acid, presumably because of oxidative decomposition on the paper surface in acetic acid. High voltage electrophoresis of this peptide fraction in pyridine acetate buffer at *p*H 3·7 showed that at least fifteen peptides were present.

Total hydrolysis of the whole peptide fraction in sealed tubes containing 6 N hydrochloric acid under nitrogen at 100° C for 24 h yielded a complex mixture of amino-acids. These amino-acids were separated with the Technicon autoanalyser system under nitrogen using the sodium buffer elution technique[1]. The hydrolysate contained the following. (*a*) Desmosine, isodesmosine and lysino-norleucine which are thought to be unique to insoluble elastin where they are known to be the acid stable cross-linkages[2,3]. (*b*) Hydroxyproline and hydroxylysine, the latter of which is known to be involved in glycosidic linkage with galactose and glucose in collagen[4] and has only been isolated from connective tissue proteins. (*c*) Two unidentified amino-acids, described as V* and Hi*, which have recently been observed for the first time in a protein fraction of the human intervertebral disk (unpublished results of F. S. Steven, D. S. Jackson and K. Broady). This protein is very firmly bound to collagen in the disk. The quantity of V* in the urinary peptide fraction is equivalent to 0·3 mg/l. of urine. (*d*) Several unidentified ninhydrin positive peaks which seem from their positions on the chromatographic elution pattern to be derived from basic amino-acids.

Table 1. R_F VALUES OF COMPOUNDS GIVING A YELLOW EHRLICH REACTION

Solvent	Iso-propanol : water 160 : 40	*n*-Butanol : ethanol : water 120 : 30 : 50	Colour development
Urea	62	41	Fast
Homocitrulline	27	17	Fast
Unknown	39	31	Slow

Total hydrolysis of the disk protein fractions referred to in (*c*) also yielded a yellow Ehrlich positive spot after two dimensional chromatography in systems which did not contain acetic acid. This yellow spot again failed to appear in hydrolysates which had been subjected to chromatography or electrophoresis in systems containing acetic acid. Table 1 shows the R_F values of urea, homocitrulline and the unknown Ehrlich positive spot.

Hydroxyproline peptides are commonly found in urine, but hydroxylysine has only once been observed[5] in normal adult urine. Desmosine, isodesmosine, lysino-norleucine and the unidentified amino-acids have not previously been reported in urine. The modified amino-acids have probably escaped detection in previous studies of urine, because little work has been carried out on these low molecular weight peptide fractions.

The presence of this peptide fraction suggests that the catabolism of connective tissue proteins proceeds normally by proteolytic digestion until a cross-linked region is reached. At this stage a fragment is released which contains the cross-link plus perhaps two amino-acid residues adjacent to the cross-link on each peptide chain. This fragment might be an enzyme-resistant H-peptide. The urinary peptides containing desmosine, isodesmosine or lysino-norleucine as cross-linkages would be derived from catabolism of elastin. Those peptides containing hydroxylysine would probably be derived from collagen catabolism and those peptides containing V* and Hi* might be expected to be derived from the disk type protein complex with collagen.

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[1] Steven, F. S., and Jackson, D. S., *Biochem. J.*, **104**, 534 (1967).
[2] Thomas, J., Elsden, D. F., and Partridge, S. M., *Nature*, **200**, 651 (1963).
[3] Franzblau, C., Sinex, F. M., Faris, B., and Lampidis, R., *Biochem. Biophys. Res. Commun.*, **21**, 575 (1965).
[4] Butler, W. T., and Cunningham, L. W., *J. Biol. Chem.*, **241**, 3882 (1966).
[5] Cunningham, L. W., Ford, J. D., and Segrest, J. P., *J. Biol. Chem.*, **242**, 2570 (1967).

Sedimentation Behaviour of Rapidly Labelled RNA from *Escherichia coli*

Rapidity of labelling after exposure to radioactive precursor is one of the distinctive characteristics of bacterial messenger RNA (*m*RNA)[1]. In early studies, rapidly labelled RNA prepared from bacterial extracts obtained by alumina grinding was of small size and sedimented as a broad peak at about 8–10*S* (ref. 1). When steps were taken to reduce enzyme degradation, rapidly labelled RNA showed a very heterogeneous distribution with fractions sedimenting at 6–8*S*, 14–16*S* and heavy chains at 16–30*S* (refs. 2 and 3). The heavy chains of rapidly labelled RNA could be dissociated to 18*S* by centrifugation in gradients of sulpholane, a compound known to decrease interactions between ribosomal components[4]. There is also evidence that *m*RNA may become fragmented by sonication. Thus *m*RNA labelled with ^{3}H-uridine from *E. coli* which sedimented at 10–12*S* became fragmented by sonication to chains sedimenting at 4*S* (ref. 5).

Chromatography of RNA on columns of methylated albumin (MAK)[6] resolved rapidly labelled RNA from *E. coli* into several fractions which eluted around the

16*S* and 23*S* peaks of ribosomal RNA (*r*RNA) (refs. 2 and 7). It has since been shown, however, that the use of methylated albumin columns for the determination of the size of *m*RNA is unreliable. *m*RNA molecules have been found to complex with *r*RNA molecules at sodium chloride concentrations needed for elution of the latter[3].

We have found that the sedimentation behaviour ascribed to *m*RNA from *E. coli* is that of chains of rapidly labelled RNA fragmented by mechanical shear, and that sedimentation properties of non-fragmented rapidly labelled RNA chains are similar to those of ribosomal RNA.

We used *E. coli B/u* in growth conditions similar to those described before[8]. The pulse-labelled cells (see figure legends for details of labelling) were poured over frozen and crushed 10^{-2} M *tris* buffer, *p*H 7·75, containing $1·5 \times 10^{-2}$ M magnesium ions and collected by centrifugation in the cold. For the preparation of RNA we used spheroplasts and extracts obtained by alumina grinding or sonication.

The spheroplasts were prepared by a slight modification of the procedure of Kohn[9]. The cells with rapidly labelled RNA were washed by centrifugation in the cold in 10^{-2} M *tris* buffer, *p*H 7·75, containing $1·5 \times 10^{-2}$ M magnesium ions and 30 per cent sucrose, and resuspended in the same buffer (0·5 ml. of the buffer per 6×10^{10} cells). Lysozyme (1 mg/ml. of the cell suspension) was added and the cells were twice frozen in a solid carbon dioxide–acetone bath and thawed in an ice bath. After centrifugation in the cold the spheroplasts were gently resuspended using a wide bore pipette in 10^{-2} M *tris* buffer, *p*H 7·4, containing 10^{-2} M magnesium ions (*tris*-Mg buffer) and lysed by adding 0·1 of their volume of 15 per cent sodium dodecyl sulphate and incubating the mixture for 1 min at 0° C (3 ml. of the lysing solution per 6×10^{10} of the cells).

For sonication, spheroplasts or whole cells in *tris* Mg buffer or purified RNA in 5×10^{-3} M *tris* buffer, *p*H 7·4, containing 10^{-1} M NaCl and 10^{-3} M ethylenediamine tetraacetic acid (EDTA), were routinely disrupted for 1·5 min at 4° C in the 20 kHz MSE ultrasonic disintegrator with an output of 60 W using the 19 mm probe with a 9·5 mm tip. For alumina grinding, cells were ground with twice their weight of alumina in the cold and extracted as described previously[8].

RNA from the lysed spheroplasts and bacterial extracts obtained by alumina grinding or sonication was prepared as follows. To 3 ml. of the cell lysates or extracts, 3 ml. of freshly distilled 90 per cent (v/v) phenol was added. The phenol mixture in stoppered glass centrifuge tubes was gently agitated by turning the tubes upside down for 2–5 min in the cold and centrifuged at 10,000*g* for 10 min. The upper aqueous layer was carefully collected and freed from phenol by repeated ether extractions. The dissolved ether was removed by air blowing and the solution was incubated with 10 μg/ml. of RNase-free DNase for 20 min at 4° C. Thereafter 100 μg/ml. of pronase was added, the mixture was reincubated at 37° C for 20 min and the RNAs were precipitated with two volumes of ice-cold 95 per cent (v/v) ethanol. The product was stored at −20° C.

Sedimentation behaviour of RNA was studied by centrifugation in linear sucrose density gradients 5–20 per cent (w/v) in suitable buffer and ionic conditions (as listed in the figure legends). Gradients were run in the Spinco model *L* ultracentrifuge with the *SW* 39 rotor at 37,000 r.p.m. for 4 h at 4° C. From each gradient, between twenty-five and thirty fractions were collected. Optical density and trichloroacetic acid precipitable radioactivity of each fraction were determined[8].

We determined the recovery of radioactivity in the final RNA product, to make sure that our methods do not cause selective extraction of a special class of rapidly labelled RNA. If the total trichloroacetic acid-insoluble radioactivity in whole cells is taken as 100 per cent, the RNA isolated from lysed spheroplasts and from extracts obtained by alumina grinding and sonication contained 92 per cent, 84 per cent and 80 per cent of radioactivity, respectively.

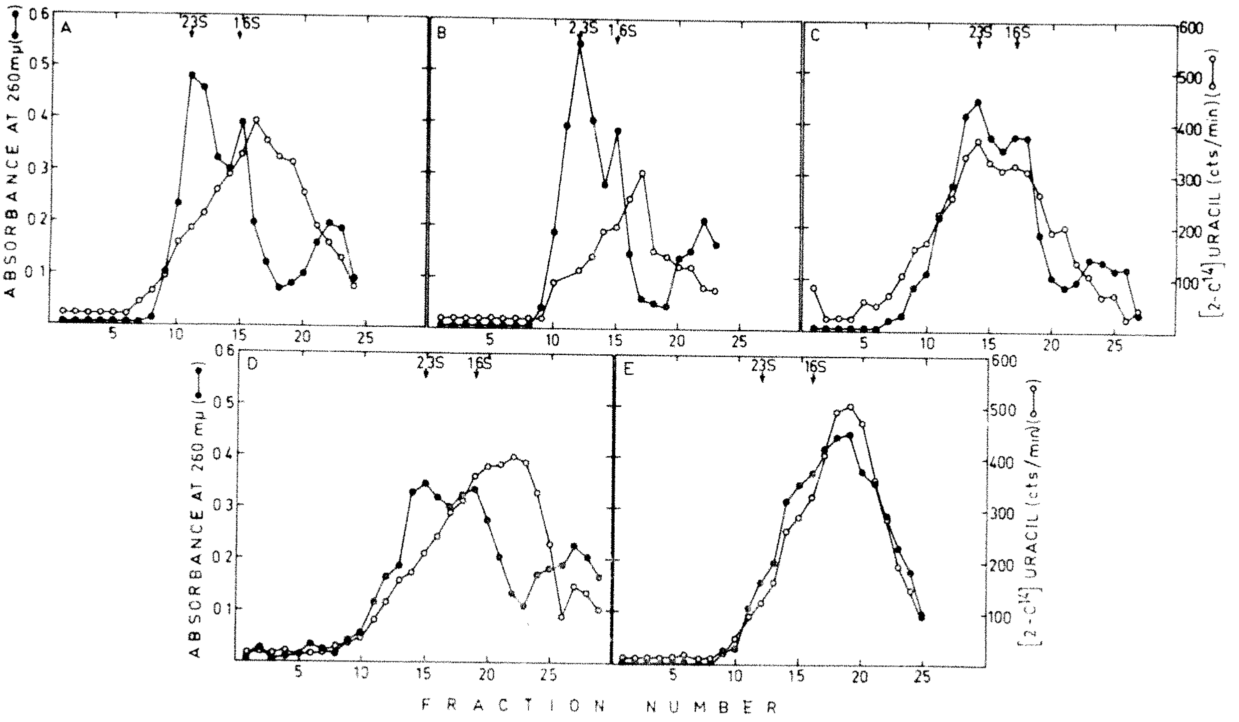

Fig. 1. Sedimentation behaviour of rapidly labelled and ribosomal RNA from *E. coli*. Influence of the method of RNA preparation. Cells were pulse-labelled with 0·1 μCi/ml. of 2-^{14}C-uracil for 1/140th of the generation time. The RNA samples were dissolved in 5×10^{-3} M *tris* buffer, *p*H 7·4, containing 0·1 M sodium chloride and 10^{-3} M EDTA. Sucrose solutions used to prepare the gradients were made in the same buffer. RNA obtained from: *A*, sonicated cells; *B*, cells ground with alumina; *C*, spheroplasts; *D*, sonicated spheroplasts; *E*, spheroplasts sonicated after phenol extraction and ethanol precipitation.

Figs. 1*A* and *B* show the sedimentation behaviour of ribosomal and rapidly labelled RNA prepared from bacterial extracts obtained by sonication and alumina grinding, respectively. The ultraviolet-absorbing material comprising the two *r*RNA components and transfer RNA is well resolved into three fractions sedimenting at 23*S*, 16*S* and 4*S*. The rapidly labelled RNA has a mean sedimentation coefficient in the range of 10–12*S* with a second rather ill-defined peak at about 8*S*.

Fig. 1*C* shows the sedimentation behaviour of a similar RNA preparation obtained from *E. coli* spheroplasts. Isotope distribution is now coincident with the distribution of material which absorbs at 260 mμ, and both sediment as two peaks with sedimentation coefficients of 23*S* and 16*S*. When the spheroplasts were subjected to sonication, rapidly labelled RNA fragmented (Fig. 1*D*).

Fig. 1*E* shows the sedimentation behaviour of RNA preparations obtained from *E. coli* spheroplasts and subsequently sonicated. It is evident that deproteinized ribosomal RNA is as susceptible to mechanical shear as is rapidly labelled RNA. It can be seen that purified ribosomal RNA was fragmented by sonication and sedimented as a broad peak at about 10–12*S*.

Although the sedimentation behaviour of ribosomal RNA is largely unaffected by the method of extraction, ionic strength and bivalent cation concentration of the medium and of sucrose gradients strongly influence the tertiary and/or secondary structure of ribosomal RNA components and their capacity to form complexes between themselves and with rapidly labelled RNA[10-12]. We have therefore compared the sedimentation behaviour of non-fragmented ribosomal RNA with that of non-fragmented and sheared rapidly labelled RNA chains in media which induce changes in the sedimentation behaviour of the former.

Fig. 2*A* shows that in media of moderate ionic strength, fragmented rapidly labelled RNA obtained from sonicated cells of *E. coli* sediments as a single peak at about 10–12*S*. In the presence of magnesium ions (Fig. 2*B*), complex formation between ribosomal RNA components and between ribosomal RNA and rapidly labelled RNA chains occurred. The rapidly labelled RNA gave a poorly resolved profile in which the radioactivity was spread along the entire sucrose gradient. The same conclusions based on similar experiments have recently been reported by Asano[3] and Hayes *et al.*[12].

Fig. 2*C* shows the sedimentation behaviour of a similar RNA preparation run in sucrose density gradient at low ionic strength in the presence of EDTA. Ribosomal RNA sediments as a single peak at about 16*S*. The behaviour of fragmented rapidly labelled RNA, however, remained unaltered and it sedimented as a broad peak at about 8–10*S*.

Figs. 2*D*, *E* and *F* show the sedimentation behaviour of non-fragmented rapidly labelled RNA in sucrose density gradients of moderate ionic strength, in the presence of magnesium ions and at low ionic strength in the presence of EDTA, respectively. In these conditions, the isotope distribution is coincident with the distribution of the material which absorbs at 260 mμ and both sediment as two peaks with sedimentation coefficients of 23*S* and 16*S* in gradients of moderate ionic strength, as 39*S*, 28*S* and 16*S* in the presence of 10^{-2} M magnesium ions and as a single peak at about 16*S* at low ionic strength in the presence of EDTA.

Figs. 1*C* and 2*D* show that in addition to the bulk of rapidly labelled RNA which sediments at 23*S* and 16*S* there is a fraction of radioactivity sedimenting as a broad zone at about 10–12*S*. This may indicate that in spite of strict precautions a small proportion of rapidly labelled RNA became fragmented. On the other hand, the material sedimenting behind the 16*S* peak may represent RNA chains in the process of being synthesized. To distinguish experimentally between the two possibilities, we have examined the sedimentation behaviour of non-fragmented RNA chains labelled during pulses of various length.

The results of a typical experiment are shown in Fig. 3. Most RNA chains labelled during 10 seconds (Fig. 3*A*) are already resolved into two fractions which sediment at 23*S* and 16*S*. A fraction of radioactivity (about 20 per cent

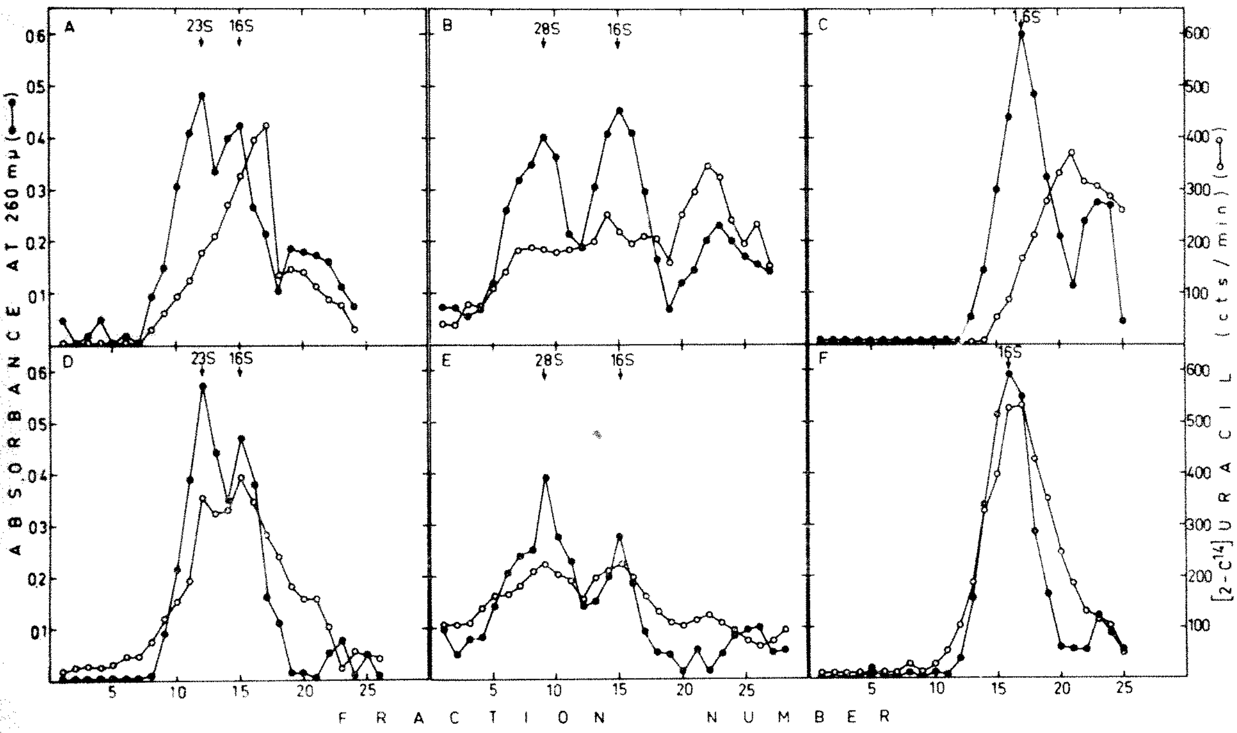

Fig. 2. Sedimentation behaviour of rapidly labelled and ribosomal RNA from *E. coli*. Influence of ionic strength and bivalent cation concentration. Cells were pulse-labelled with 2-^{14}C-uracil for 1/140th of the generation time. RNA was prepared from sonicated cells (*A*, *B* and *C*) and from spheroplasts (*D*, *E* and *F*). The RNA samples were dissolved and centrifuged in sucrose gradients containing: *A* and *D*, 5×10^{-3} M *tris* buffer, *p*H 7·4, 0·1 M NaCl and 10^{-3} M EDTA; *B* and *E*, 5×10^{-3} M *tris* buffer, *p*H 7·4, and 10^{-2} M magnesium ions; *C* and *F*, 5×10^{-3} M *tris* buffer, *p*H 7·4, and 10^{-3} M EDTA.

of total) sediments as a broad peak behind 16*S*. After longer periods of labelling the fraction of rapidly labelled RNA of low sedimentation coefficient decreases with a concomitant increase in the radioactivity sedimenting at 23*S* and 16*S*. These results suggest that the non-fragmented rapidly labelled RNA of low sedimentation coefficient represents incomplete RNA chains in the process of being synthesized.

The data presented in this work indicate that: (1) unlike *r*RNA, rapidly labelled RNA becomes fragmented by exposing cells to mechanical shear; (2) deproteinization makes ribosomal RNA susceptible to mechanical shear; and (3) the sedimentation behaviour of non-fragmented rapidly labelled RNA is similar to that of non-fragmented ribosomal RNA.

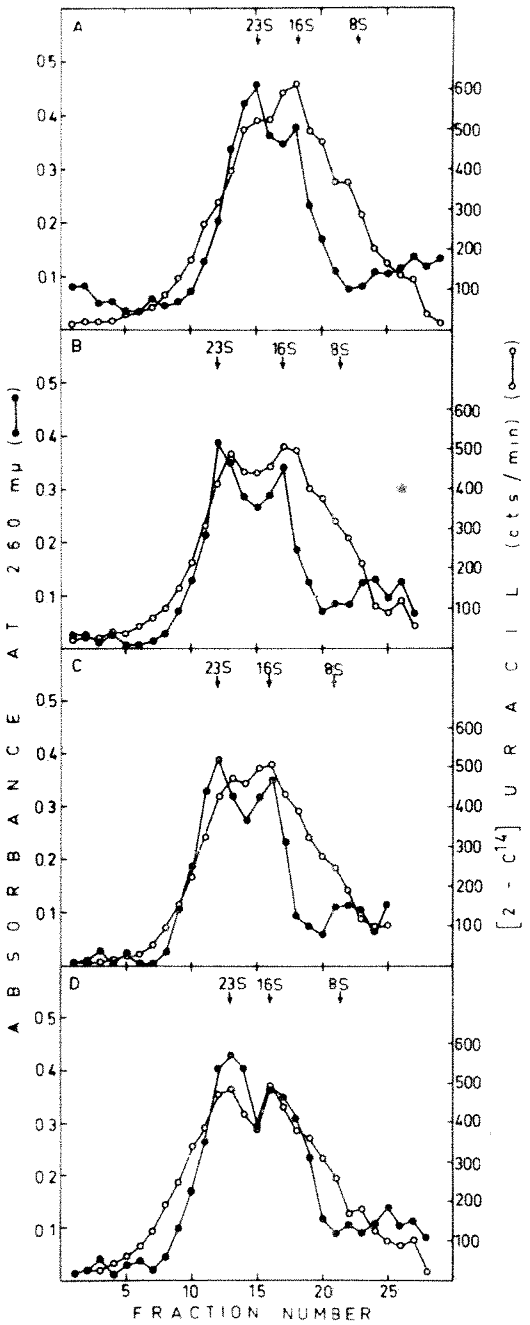

Fig. 3. Sucrose gradient profiles of RNA labelled for different periods of time. RNA was extracted from spheroplasts. Cells were labelled with 2-^{14}C-uracil: *A*, 0·3 μCi/ml. of cells for 10 s; *B*, 0·15 μCi/ml. of cells for 20 s; *C*, 0·1 μCi/ml. of cells for 30 s; *D*, 0·066 μCi/ml. of cells for 40 s. The RNA samples were dissolved in 5×10^{-3} M *tris* buffer, *p*H 7·4, containing 0·1 M sodium chloride and 10^{-3} M EDTA. Sucrose solutions used to prepare the gradients were made in the same buffer.

It has been shown that a large percentage of the rapidly labelled RNA moiety represents newly synthesized ribosomal RNA[13,14] associated with 30*S* and 50*S* ribosomal sub-units[15,16], the remaining apparently being *m*RNA. The data presented here indicate that non-fragmented rapidly labelled RNA cannot be resolved into *r*RNA and *m*RNA components by sedimentation analysis, because they both sediment at a similar rate in sucrose gradients of different ionic composition.

Ribosomal RNA in mature ribonucleoprotein remains intact in conditions when newly synthesized RNA is sheared and becomes susceptible to mechanical shear after deproteinization. Similar results on the resistance and susceptibility of these two classes of RNA towards nucleases were recently reported[8,17]. It should therefore be assumed that the interaction of protein and RNA in mature ribonucleoprotein gives protection to the RNA moiety against mechanical shear and the action of degradative enzymes, and that maturation of the ribosome involves qualitative and quantitative alterations or both in the protein–RNA interaction. These alterations lead to resistance of *r*RNA to agents which may endanger the integrity of the ribosome and impair its function.

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[1] Gros, F., Hiatt, H., Gilbert. W., Kurland, C. G., Risebrough, R. W., and Watson, J. D., *Nature*, **190**, 581 (1961).
[2] Monier, R., Naono, S., Hayes, D., Hayes, F., and Gros, F., *J. Mol. Biol.*, **5**, 311 (1962).
[3] Asano, K., *J. Mol. Biol.*, **14**, 71 (1965).
[4] Parish, J. H., and Kirby, K. S., *Biochim. Biophys. Acta*, **129**, 554 (1966).
[5] Das, H. K., Goldstein, A., and Lowney, L. I., *J. Mol. Biol.*, **24**, 231 (1967).
[6] Mandell, J. D., and Hershey, A. D., *Anal. Biochem.*, **1**, 66 (1960).
[7] Ishihama, A., Mizuno, N., Takai, M., Otaka, E., and Osawa, S., *J. Mol. Biol.*, **5**, 251 (1962).
[8] Artman, M., Silman, N., and Engelberg, H., *Biochem. J.*, **104**, 878 (1967).
[9] Kohn, A., *J. Bact.*, **79**, 697 (1960).
[10] Rodgers, A. R., *Biochem. J.*, **100**, 102 (1966).
[11] Marcot-Queiroz, J., and Monier, R., *J. Mol. Biol.*, **14**, 490 (1965).
[12] Hayes, D. H., Hayes, F., and Guerin, M. F., *J. Mol. Biol.*, **18**, 499 (1966).
[13] Bolton, E. T., and McCarthy, B. J., *Proc. US Nat. Acad. Sci.*, **48**, 1390 (1962).
[14] Midgley, J. E. M., *Biochim. Biophys. Acta*, **61**, 513 (1962).
[15] Artman, M., Silman, N., and Engelberg, H., *Nature*, **213**, 39 (1967).
[16] Nakada, D., and Kaji, A., *Proc. US Nat. Acad. Sci.*, **57**, 128 (1967).
[17] Artman, M., and Engelberg, H., *Biochim. Biophys. Acta*, **95**, 687 (1965).

Inhibition of Leucine and Lysine Aminopeptidase by Atopic Allergens

MANY atopic allergens—the biologically active substances involved in the syndrome of atopy in man (asthma, hay fever, atopic eczema)—have been shown to incorporate 1-deoxy-2-ketose side chains attached at the sugar carbon C1 to the ε-amino groups of lysine residues in the peptide chain of the protein or glycoprotein carrier molecules[1-3]. Recent syntheses have shown that the selective coupling of reducing sugar to the ε-amino functions of lysine side chains in the β-lactoglobulin molecule does in fact increase the specific activity of the protein during skin tests in milk-sensitive individuals[4]. The blockade of a few amine or amide groups by combination with monosaccharides would also provide a plausible explanation for the occurrence of, and for the physicochemical difference between, the "iso-allergens" in extracts of grass pollen[5].

The initial allergen–reagin interaction in hypersensitive individuals ultimately causes the local or systemic release of a number of pharmacologically active substances, among which are the peptides kallidin-10 and bradykinin. These kinins, which are vaso-active and which stimulate smooth muscle, are usually rapidly inactivated by specific enzymes, that is, by the converting enzyme aminopeptidase *B*, and by the kininase carboxypeptidase *N* which cleaves the C-terminal arginine of bradykinin[6]. The aminopeptidase is capable of releasing the N-terminal lysine of kallidin-10, converting it into bradykinin, but it has been reported to be unable to hydrolyse the arginine–prolyl bond at the N-terminal end of bradykinin[7]. At the site of inflammation, proteolytic enzymes (presumably activated by the antigen–antibody complex) are thought to act on an α_2-globulin substrate to release the active peptide kallidin-10, which must first be converted into bradykinin before the pharmacological activity can be completely abolished[8].

On the hypothesis that the active site of aminopeptidase *B* might be susceptible to inhibition by large molecules carrying sugar-substituted lysine residues, we have studied the influence of several atopic allergens on the reaction between the substrate L-lysyl-β-naphthylamide and commercial preparations of leucine aminopeptidase as a source of the enzyme. Although they are highly purified, these preparations (extracted bovine lens and extracted pig kidney, Seravac Laboratories) hydrolysed all three substrates which were tested—the β-naphthylamides of L-leucine, L-lysine and L-arginine—the turnover rates being approximately in the proportion 5 : 6 : 4, respectively.

The reaction system contained 0·1 mg of the lyophilized enzyme, 0·2 ml. of L-lysyl-β-naphthylamide carbonate (0·2 μmole) and quantities up to 1 mg of allergen in a final volume of 2·3 ml. of *tris*(hydroxymethyl)aminomethane–hydrochloric acid buffer (0·1 molar, *p*H 7·0). The tubes were shaken for 1 h at 37° C and the reaction was stopped by the addition of 1 ml. of 0·1 per cent fast garnet GBC salt in 1 molar sodium acetate buffer *p*H 6·5. Exactly 30 min was allowed at room temperature for full colour development, and the extinction of the samples was finally read at 525 nm. Sets included blanks for enzyme and reagent absorption, for allergen absorption at 525 nm and for spontaneous decomposition of the substrate. A series of standard solutions containing the substrate in different concentrations served for calculation. The degree of enzyme inhibition was plotted against allergen concentration and the point of 50 per cent inhibition was read from these curves by intrapolation.

All atopic allergens which were investigated were found to inhibit the hydrolysis of L-lysyl-β-naphthylamide by the enzyme samples, the degree of inhibition being less pronounced with the leucyl- and arginyl-β-naphthylamides as the substrates. The inhibiting capacity for a given amount of enzyme varied considerably, however, among different atopic allergens. A plot of the quantity of allergen causing 50 per cent inhibition against the ratio of the extinction coefficients in the ultraviolet *E*305/*E*280 (Fig. 1) shows at once that the inhibiting power is a function of the number of lysine–sugar determinants in the peptide moieties of the respective allergens; the extinction coefficient at 305 nm has been shown to be indicative of the 1·2-enol tautomer of the 1-deoxy-2-ketose blocking the lysine residues[1–3]. The most highly pigmented allergens—kapok, feathers and house dust—were among the most potent inhibitors. Assuming a molecular weight of 30,000 for the kapok allergen and of 300,000 for the leucine aminopeptidase, the molecular ratio of allergen : enzyme for 50 per cent inhibition was calculated to be approximately 2 : 1. Conversely, the inhibiting capacity decreases in a hyperbolic function with a diminishing number of blocked lysine ε-amino groups in the allergens and model compounds (Fig. 1). The observation that the synthetic model compound 1-(*N*)-deoxy-ribulose bovine serum albumin inhibited the enzyme, in contrast with the crystalline parent protein, indicates that the active site of the aminopeptidase is indeed sensitive to the lysine–sugar conjugate.

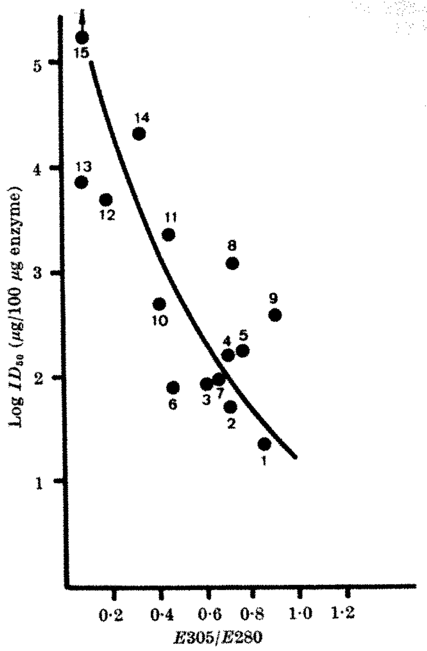

Fig. 1. Graph showing the relationship between inhibiting capacity (dose for 50 per cent inhibition in μg/100 μg of leucine aminopeptidase, log scale) and the approximate number of lysine-sugar residues of atopic allergens (see text). Key: (1) kapok KP-C; (2) feathers FE-B; (3, 4, 5) house dust (fractions E, B and D, respectively); (6, 7, 8) human dandruff (fractions E, D and B respectively); (9) pyrethrum (dialysed extract); (10) ipecac (fraction D); (11) timothy pollen (precipitate 0·3–0·8 saturated ammonium sulphate); (12) horse dandruff (dialysed extract); (13) ovomucoid (extracted hen's eggs); (14) deoxyribulose bovine serum albumin; (15) crystalline bovine serum albumin. For allergens and fractions see refs. 1–3.

Although identical results were obtained during studies of aminopeptidase inhibition in human sera, inactivation of the enzyme converting kallidin-10 into bradykinin can only be a side-effect of the biological action of allergens in man, if only because the most strongly pigmented allergens do not necessarily exhibit the highest reactivity in skin tests. Moreover, no significant differences were detected in the level of serum aminopeptidases between groups of normal and allergic human donors, nor in the behavioural pattern of these enzymes with regard to inhibition by allergens. Both normal and atopic individuals are, of course, exposed to environmental allergens to the same extent, and this would cause no difference in the degree of enzyme inhibition between the two groups of subjects. The interference of atopic allergens with the action of an enzyme which is essential for the rapid breakdown of active kallidin-10 might, however, conceivably intensify the traumatic response of allergic individuals to the triggering allergen–reagin interaction.

No results have yet been obtained with the kininase carboxypeptidase *N*, but experiments with crystalline pancreatic carboxypeptidase *B* hydrolysing hippuryl-L-lysine, a substrate of carboxypeptidase *N*, showed that the enzymatic activity was unimpaired in the presence of any of the atopic allergens which were investigated.

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[1] Berrens, L., and Bleumink, E., *Intern. Arch. Allergy*, **28**, 150 (1965).

[2] Berrens, L., *Immunochemistry*, **4**, 37 (1967).

[3] Berrens, L., *Immunochemistry*, **4**, 81 (1967).

[4] Bleumink, E., and Berrens, L., *Nature*, **212**, 541 (1966).

[5] Johnson, P., and Marsh, D. G., *Nature*, **206**, 935 (1966).

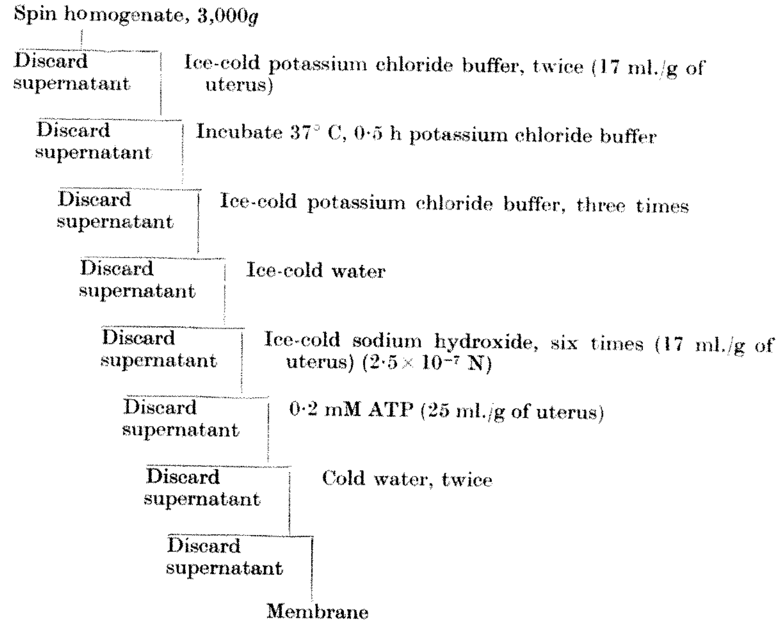
[6] Erdös, E. G., Yang, H. Y. T., Tague, L. L., and Manning, N., *Biochem. Pharmacol.*, **16**, 1287 (1967).

[7] Hopsu-Havu, V. K., Mäkinen, K. K., and Glenner, G. G., *Nature*, **212**, 1271 (1966).

[8] Webster, M. E., and Pierce, J. V., *Ann. NY Acad. Sci.*, **104**, 91 (1963).

Cell Membrane of Uterine Smooth Muscle

We wished to isolate the cell membranes of the smooth muscle of the uterus for investigation of the sialic acid components (see following communication) and have therefore applied the method of Rosenthal, Edelman and Schwartz[1], which is a modification of McCollester's method for emptying broken skeletal muscle cells[2]. The uterine muscle cells are small, tapering and enmeshed in collagen so that the tissue is too tough to be disintegrated in a blender. We therefore protected the uterine horns against the effects of freezing by impregnating with glycerol[3], and then froze them between slabs of solid carbon dioxide and scraped off the endometrium. Frozen muscle segments are made into a block with drops of 50 mM calcium chloride, and sectioned on a freezing microtome. The mush on the knife was periodically transferred to 50 mM calcium chloride at 0° C and this homogenate was freed of cell contents by modifying Rosenthal's method as in Fig. 1. The membranes sedimented readily during centrifugation because the collagen fibres were retained; if they did not settle after the ATP treatment, but remained as a fluffy dispersion, then phase-contrast microscopy showed that they were still surrounded by extruded protein containing nuclei and mitochondria, and a second ATP treatment was necessary.


Spin homogenate, 3,000g
Discard supernatant
Ice-cold potassium chloride buffer, twice (17 ml./g of uterus)
Discard supernatant
Incubate 37° C, 0·5 h potassium chloride buffer
Discard supernatant
Ice-cold potassium chloride buffer, three times
Discard supernatant
Ice-cold water
Discard supernatant
Ice-cold sodium hydroxide, six times (17 ml./g of uterus) ($2{\cdot}5 \times 10^{-7}$ N)
Discard supernatant
0·2 mM ATP (25 ml./g of uterus)
Discard supernatant
Cold water, twice
Discard supernatant
Membrane

Rats were treated with diethylstilboestrol 75 μg/day for 6 days before killing.

Fig. 1. Procedure for isolating myometrial cell membrane

Absence of mitochondria in the final membrane product was confirmed by electron microscopy[4]. By phase-contrast microscopy, the emptied tissue sections resembled a network of holes, as would be expected for rings of empty membrane held together by the surrounding collagen fibres, and did not resemble skeletal muscle sarcolemma tubules[1,2,5,6]. This is not surprising because of the method of comminution and the structural differences between large, easily separated skeletal muscle fibres and small tapering smooth muscle cells enmeshed in collagen. The ratio of cell surface area to cell volume is ten times greater for uterine muscle than for skeletal muscle[7] and so contamination of the membrane preparation is less of a problem.

Table 1. COMPOSITION OF MYOMETRIAL MEMBRANE

	% Dry weight of total membrane Average values
Nitrogen	13·4
Phosphorus	1·2
Collagen	58·1
Hydroxyproline	4·9
Lipid	13
Total sialic acid	0·52
Non-lipid sialic acid	0·43
Phospholipid phosphorus	0·27
Cholesterol	1·6
Proteolipid protein	0·5

Nitrogen, phosphorus, collagen[9] and hydroxyproline[10] were estimated in the dried membrane. The lipid was obtained by extracting dried membrane with chloroform–methanol 2 : 1 v/v (0·5 ml./mg) for 3 h at 24° C and, after removal of the solvent, the lipid was analysed for phospholipid phosphorus[11,12], cholesterol[13] and proteolipid protein[14]. Sialic acid was estimated by direct Ehrlich reaction[15].

Table 1 gives analyses of this membrane preparation. The chloroform–methanol soluble portion (13 per cent) was shown to be predominantly lipid, not proteolipid, by the occurrence of only 4 per cent protein in this fraction. Wallach has expressed serious doubt that the lipoprotein plasmalemma is obtained in McCollester's method, because of the use of low ionic strength solutions[8]. That this doubt is unfounded is shown by the analyses for cholesterol and phospholipid in the uterine smooth muscle membrane; the molar ratio of cholesterol to phospholipid averaged 0·47. Table 2 shows the relative proportions of the various phospholipids. The analyses for this membrane preparation are in reasonable accord with the results for various other plasma membranes.

The electrolyte composition of the membrane is interesting because of the high ratio of sodium to potassium, and this was independent of the use of the sodium salt of ATP in the last stage of the preparation (Table 3). Even if the membrane had bound every sodium ion available during the preparation with sodium hydroxide, it could have

Table 2. PHOSPHOLIPID COMPOSITION OF MYOMETRIAL MEMBRANES

Phospholipid	% Total recovered phosphorus
Solvent front (everything above P.E.)	2·3
Phosphatidyl ethanolamine (P.E.)	24·8
Phosphatidyl serine	6·4
Phosphatidyl choline	50·3
Sphingomyelin (S)	13·8
Everything below S	2·5

The phosphorus content of each spot on a silica gel thin layer chromatogram of the lipid extract was estimated by the molybdate method[11,13].

Table 3. ELECTROLYTE COMPOSITION OF MYOMETRIAL MEMBRANES

	Membrane preparation		
Electrolyte	ATP sodium salt	ATP *tris* salt	
Mg	3·0	3·6	3·3
Ca	4·4	4·1	3·9
K	2·1	1·5	1·0
Na	74·7	71·0	78·0

Expressed as mM/kg dry weight of membrane. Tissues were dried, digested with nitric acid and analysed by flame photometry.

obtained only approximately 1 mM/kg dry weight. The preceding stages involved potassium chloride buffer, and so we must conclude that the membrane strongly retained sodium ions despite the opportunity to exchange them for potassium ions.

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[1] Rosenthal, S. L., Edelman, P. M., and Schwartz, I. L., *Biochim. Biophys. Acta*, **109**, 512 (1965).

[2] McCollester, D. L., *Biochim. Biophys. Acta*, **57**, 427 (1962).

[3] Carroll, P. M., and Lin, J. H., *Cryobiology* (in the press).

[4] Sjöstrand, F. S., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **4**, 391 (Academic Press, New York, 1957).

[5] Kono, T., and Colowick, S. P., *Arch. Biochem. Biophys.*, **93**, 520 (1961).

[6] Westort, C., and Hultin, H. O., *Anal. Biochem.*, **16**, 314 (1966).

[7] Kao, C. Y., in *Cellular Biology of the Uterus* (edit. by Wynn, R. M.), 388 (Appleton Century Crofts, New York, 1967).

[8] Wallach, D. F. H., in *The Specificity of Cell Surfaces* (edit. by Davis, B. D., and Warren, L.), 136 (Prentice Hall, Englewood Cliffs, New Jersey, 1967).

[9] Robinson, D. S., *Biochem. J.*, **52**, 621 (1952).

[10] Miyada, D. S., and Tappel, A. L., *Anal. Chem.*, **28**, 909 (1956).

[11] Chen, P. S., Toribara, T. Y., and Warner, H., *Anal. Chem.*, **28**, 1756 (1956).

[12] Baginski, E. S., Foa, P. P., and Zak, B., *Clin. Chim. Acta*, **15**, 155 (1967).

[13] McIlwain, H., and Rodnight, R., *Practical Neurochemistry*, 101 (Churchill, London, 1962).

[14] Hess, H. H., and Lewin, E., *J. Neurochem.*, **12**, 205 (1965).

[15] Werner, I., and Odin, L., *Acta Soc. Med. Uppsala*, **57**, 230 (1952).

Sialic Acid of Uterine Muscle Membrane: Reappraisal of the Serotonin Receptor of Smooth Muscle

WOOLLEY suggested that the serotonin receptor of uterus and stomach is a ganglioside, because neuraminidase destroys the sensitivity of those tissues to serotonin, and the sensitivity can be partially restored by the addition of brain gangliosides[1]. This assumption that a material which restores activity is the naturally occurring receptor can be questioned, and we have therefore investigated the sialic acid of the uterine muscle membrane and the occurrence or otherwise of gangliosides.

The direct Ehrlich reaction[2] for estimating sialic acid lacks sensitivity but has a high degree of specificity[3]. Applying it to gelatine (as a membrane substitute) plus *N*-acetylneuraminic acid in the appropriate ratio, we found only slight interference (2 per cent) and therefore used this reaction to estimate the membrane sialic acid; (the thiobarbituric acid method applied to mucosubstances is unreliable[4–8]). The uterine muscle membrane was prepared and the lipid portion extracted (see preceding communication) from uteri which had been preincubated in physiological salt solution, either with or without neuraminidase.

The lipid-soluble portion of sialic acid was approximately one-seventh of the total membrane sialic acid and *Vibrio cholerae* neuraminidase acted only on the non-lipid portion of the membrane sialic acid, without detectable activity on the lipid-soluble sialic acid (Table 1). With *Clostridium perfringens* neuraminidase, a difference of 1 μg of sialic acid was not significant in relation to the total quantity of sialic acid and it is not correct to consider that one-third of the neuraminidase action was on the lipid-soluble sialic acid, for it may have removed no lipid sialic acid. Sigma no longer supplies this enzyme, so we cannot confirm that *Clostridium perfringens* neuraminidase does not act on the lipid-soluble sialic acid if used for a longer time.

Table 1. ACTION OF NEURAMINIDASE ON SIALIC ACID OF MYOMETRIAL MEMBRANES

	Control	Enzyme treated	Calculated removed by enzyme
		V. cholerae	
Complete membrane	54	44	10
Lipid-free membrane	46	36	10
Calculated in lipid	8	8	
		C. perfringens	
Complete membrane	55	52	3
Lipid-free membrane	47	45	2
Calculated in lipid	8	7	

Control and enzyme-treated groups incubated for 75 min in physiological salt solution containing 1·4 mM calcium chloride, *p*H 7·15. *Vibrio cholerae* (Calbiochem) and *Clostridium perfringens* (Sigma type V) neuraminidase were added with 1·5 mM EDTA. After incubation the membranes were isolated and results expressed as μg of *N*-acetylneuraminic acid in 10 mg of complete membrane.

The release of only 3 μg of sialic acid from a total of 55 μg by *Clostridium perfringens* might explain why Woolley found this particular neuraminidase essential for his method of estimating the serotonin receptor and why the conditions he used were so critical[9]. If only a very small proportion of the membrane sialic acid was removed, then only slight conformational changes might occur in the serotonin receptor area, and gangliosides might succeed in partially reversing these conformational changes. *Vibrio cholerae* neuraminidase was more active in our experiment, removing 10 μg of sialic acid from a total of 54 μg, and presumably greater conformational changes therefore occur with the use of this enzyme, although Wesemann and Zilliken[10] could completely restore the sensitivity to serotonin of tissue treated with this enzyme by the addition of *N*-acetyl, *N*-glycolyl or *N*-carbobenzoxyneuraminic acid. These facts argue against the importance of gangliosides, as such, in restoring the sensitivity to serotonin, and suggest that it is the acidic sialic acid group which is important.

Gangliosides had not been detected in rat uterus[11], and so, to obtain a greater quantity of tissue, we used bovine uteri. Bovine myometrium, dried with acetone, was extracted with chloroform–methanol, partitioned and dialysed by standard methods for preparing gangliosides. Analysis by a modification of Long's method[11] showed that the dried myometrium contained 0·017 per cent sialic acid attributable to gangliosides and thin-layer chromatography revealed spots, some corresponding with bovine brain gangliosides (Sigma type II). Thus bovine myometrium contains small quantities of gangliosides and human uterus also appears to contain them[12]; if sufficient rat uteri were used, one could probably also find gangliosides there.

Woolley's colleagues reported that the rat brain ganglioside most active in restoring the serotonin response of neuraminidase-treated smooth muscle contained a molecular ratio of galactose : glucose : stearic acid 1 : 2 : 1 (ref. 13). Gielen found only one ganglioside reversibly combined with serotonin; it had the molecular ratio 1 : 1 : 1 and bound an equimolar quantity of serotonin, but had a greater affinity for calcium[14]. Even if there should be a trace of this latter ganglioside in rat uterus and stomach, the fact that it complexes *in vitro* with serotonin does not imply that it is the natural receptor of uterine and stomach smooth muscle.

Consideration of the observations of Wesemann and Zilliken[10], and of our finding that the sialic acid of uterine membrane is chiefly in the form of glycoproteins, not lipid-soluble gangliosides, and that neuraminidase acts predominantly on the non-lipid sialic acid, leads us to believe that serious consideration should be given to the possibility that the smooth muscle receptors for serotonin are not gangliosides, but are glycoproteins containing sialic acid. Loss of sialic acid from the glycoproteins would probably result in conformational changes in the receptor areas, and it may well be that addition of sialic acids can reverse those conformational changes.

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[1] Woolley, D. W., and Gommi, B. W., *Nature*, **202**, 1074 (1964).
[2] Werner, I., and Odin, L., *Acta Soc. Med. Uppsala*, **57**, 230 (1952).
[3] Gottschalk, A., *The Chemistry and Biology of Sialic Acids*, 45 (Cambridge University Press, London, 1960).
[4] Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).
[5] Booth, D. A., *J. Neurochem.*, **9**, 265 (1962).
[6] Hess, H. H., and Rolde, E., *J. Biol. Chem.*, **239**, 3215 (1964).
[7] Brunngraber, E. G., and Brown, D. B., *Biochem. J.*, **103**, 65 (1967).
[8] Gibbons, R. A., *Biochem. J.*, **89**, 380 (1963).
[9] Woolley, D. W., and Gommi, B. W., *Arch. Intern. Pharmacodyn.*, **159**, 8 (1966).
[10] Wesemann, W., and Zilliken, F., *Liebigs Ann. Chem.*, **695**, 209 (1966).
[11] Long, C., and Staples, D. A., *Biochem. J.*, **73**, 385 (1959).
[12] Howse, D. C., and Wherrett, J. R., *Proc. Canad. Fed. Biol. Soc.*, **10**, 157 (1967).
[13] Wild, G., Woolley, D. W., and Gommi, B. W., *Biochemistry*, **6**, 1671 (1967).
[14] Gielen, W., *Z. Naturforsch.*, **21 b**, 1007 (1966).

Isolation of a New Toxin from Cultures of *Aspergillus flavus*

THE mould *Aspergillus flavus* produces a wide variety of toxic metabolites and, because of its role as a frequent contaminant of some foods and feeds, its metabolites have been investigated both chemically and toxicologically[1]. Recent research on the toxic metabolites of this mould has focused on the compounds known as aflatoxins[2].

Work in our laboratories directed toward the preparative separation of the aflatoxins has resulted in the isolation of an unreported metabolite of *A. flavus* to which we have tentatively assigned the name "aspertoxin". *A. flavus* was grown on shredded wheat and the metabolites were extracted with chloroform. Addition of hexane to the chloroform extract yielded a crude precipitate which contained the aflatoxins, aspertoxin, and several highly coloured compounds. Chromatography of the crude precipitate of *A. flavus* on an acid alumina column with benzene/chloroform (1/1, v/v) gave a colourless fraction containing aflatoxins B_1, G_1 and B_2. Aflatoxins G_2 and M_1 were removed with chloroform/acetone (9/1, v/v). When the eluate containing aflatoxins G_2 and M_1 was concentrated, aspertoxin rapidly crystallized as tiny, colourless needles.

Aspertoxin was originally thought to be aflatoxin M_1 because of an equivalence of R_F values ($R_F = 0{\cdot}32$) on silica gel thin-layer chromatography plates when chloroform/acetone (9/1, v/v) was used as the developing solvent. Development of the plates with chloroform/pyridine (9/1, v/v) or chloroform/acetic acid (9/1, v/v), however, provided criteria for distinguishing the two compounds. In each case, aspertoxin moved at an R_F of 0·55–0·60 whereas aflatoxin M_1 appeared at an R_F of 0·40.

The new isolate was quite pure without further crystallization; however, it could be recrystallized from hot dioxane–water. The compound decomposed at 240°–280° C without melting, and yielded a red compound(s); the resulting mixture melted at 297°–304° C. By high resolution mass spectrometry aspertoxin was shown to have the molecular formula $C_{19}H_{14}O_7$. The compound exhibited a molecular ion peak at $m/e = 354{\cdot}074200$; the mass calculated for $C_{19}H_{14}O_7$ is 354·073949.

The infrared spectrum of aspertoxin has bands at 3,354 cm^{-1} (hydrogen-bonded OH), 1,656 cm^{-1} (C=O), 1,630 cm^{-1} (hydrogen-bonded C=O), 1,587 cm^{-1} and 1,575 cm^{-1} (phenyl), 1,133 cm^{-1} (C—O—C) and other strong bands at 1,460 cm^{-1}, 1,269 cm^{-1} and 1,103 cm^{-1}.

The ultraviolet spectrum of aspertoxin has $\lambda_{\max}^{\mathrm{MeOH}}$ 310 mμ (ε 11,400) and 241 mμ (ε 34,000). The spectrum was not affected by the addition of acid or base.

A complete structure for aspertoxin cannot be assigned on the basis of the limited chemical evidence so far elaborated. The compound is not difficult to isolate and purify; however, typical crude precipitates of *A. flavus* extracts usually contain less than 0·5 per cent aspertoxin by weight, whereas aflatoxin B_1, the most abundant aflatoxin, usually accounts for about 30 per cent of the total weight of crude precipitate. The chemical data obtained thus far show that aspertoxin is not one of the previously known metabolites of *A. flavus*.

Aspertoxin was tested for biological activity and found to have pronounced toxic effects on the developing chicken embryo. In preliminary experiments, the injection of 2·0 μg/egg into either the yolk or air cell of fresh fertile eggs killed 100 per cent of the embryos; a concentration of 0·7 μg/egg killed 50 per cent of the embryos. Aflatoxin B_1 is an unusually potent toxin in the chicken embryo, and it produces comparable mortalities in lower concentrations (LD_{50}, air cell route, 0·025 μg/egg)[3]. Nevertheless, aspertoxin must also be considered highly toxic in this system. Macroscopic examination of non-viable embryos revealed beak malformations, generalized oedema, loss of muscle tone and haemorrhage from the umbilical vessels. Whereas lethal doses of aflatoxin B_1 are effective within 3 or 4 days after injection, lethal doses of aspertoxin produce no mortality until late in the incubation period. A more detailed investigation, including histopathology, of the toxic effects of this new metabolite in the chicken embryo is in progress.

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[1] Wilson, B. J., *Bact. Rev.*, **30**, 478 (1966).
[2] Wogan, G. N., *Bact. Rev.*, **30**, 460 (1966).
[3] Verrett, M. J., Marliac, J., and McLaughlin, J., *J. Assoc. Offic. Agr. Chemists*, **47**, 1003 (1964).

Catabolic Rate of Body Protein in Rat during the Earlier Stages of Protein Depletion

THERE have been many attempts to determine the magnitude of protein reserves or endogenous nitrogen excretion in protein-depleted or starved animals[1–5]. Previous results, however, have not clearly stated the fractional rate of catabolism of body protein itself, either for a whole body or for an individual organ or tissue. Borsook[6,7] presented the metabolic concept as a development of Martin and Robison's concept[2] that the cytoplasmic proteins constituting reserve proteins are broken down at a rate depending on their mass, or in a first-order reaction.

In this study, ten adult male Wistar rats, weighing about 450 g, were fed a protein-free diet after preliminary feeding on a 20 per cent casein diet. The percentage changes in body weight and body nitrogen during the first 16 days are shown in Fig. 1; the second set of values derived from the final body nitrogen and the nitrogen balance during experimental periods. As Fig. 1 shows, total body protein decreases exponentially with extremely little variance between individuals at any time, whereas the changes in body weight show a sigmoidal decrease with a greater variance. Such a constancy in the catabolic rate of body protein as a whole after protein deprivation could provide a useful basis for estimating protein requirements. In Fig. 2, the percentage changes in body protein, together with those in the total nitrogen content of liver and muscle, are plotted logarithmically against time for the first 40 days of the depletion. This figure seems to show that the labile body protein is composed of at least two components, both almost completely catabolized in a first-order reaction.

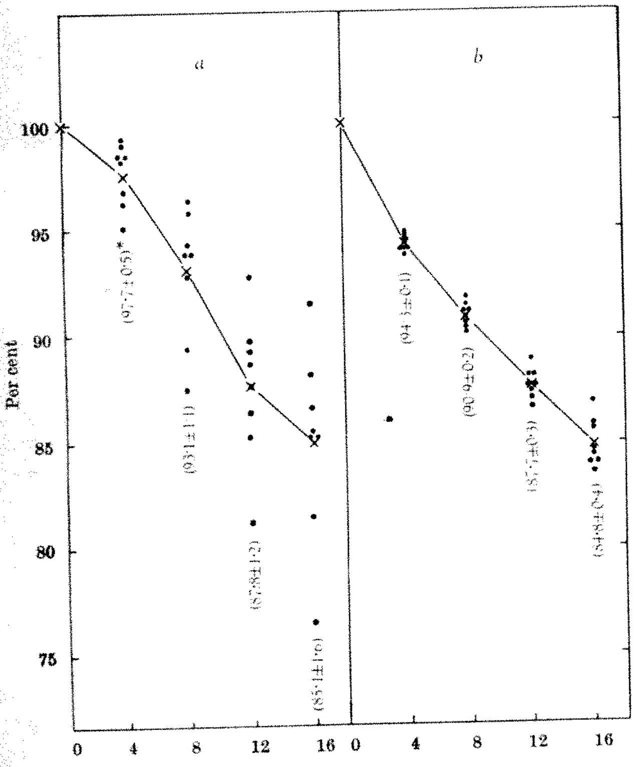

Fig. 1. Percentage changes in body weight (*a*) and body nitrogen (*b*) of adult rats during the initial stages of protein depletion. * Mean ± standard error.

By a general kinetic analysis[8], the catabolic rate per day and the content of the less labile component were first estimated to be 0·79 per cent and 97·2 per cent, respectively. Then those of the more labile component were estimated to be 83·3 per cent and 2·8 per cent, respectively. The content of the more labile component almost agrees with the so-called extra nitrogen of urine estimated by Campbell[3] and Lusk[9]. Its catabolic rate, equivalent to only 0·83 day (0·693/0·833) of half-value time, implies that this labile component should be represented by the transitive or mobile body nitrogen which could be much influenced by daily intake of protein and bear a close relation to the amount of reserve protein. The contents of the more labile component in liver and muscle were similarly estimated to be 30·6 per cent and 6·6 per cent, respectively.

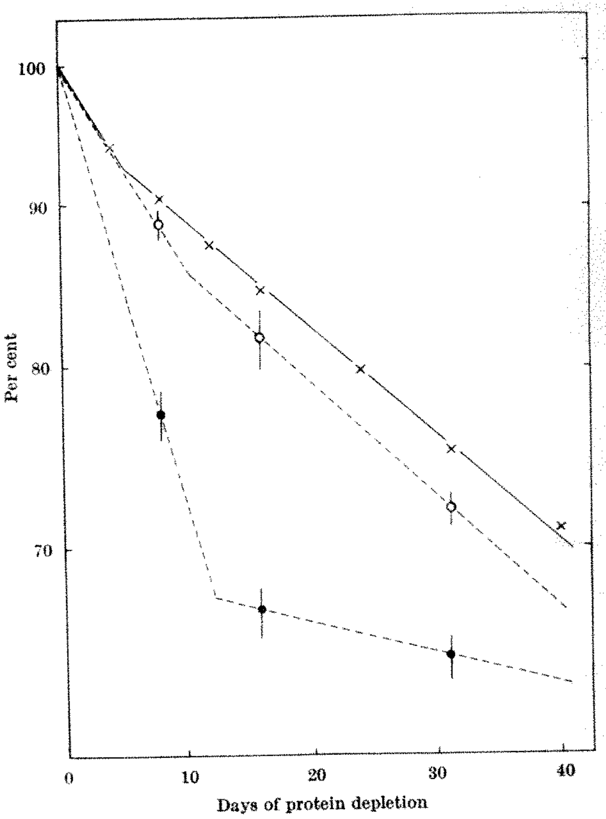

Fig. 2. Logarithm of total percentage changes in body, liver and muscle nitrogen in adult rats during the earlier stages of protein depletion. ×, Body nitrogen; ○, muscle nitrogen; ●, liver nitrogen. Vertical lines represent standard error.

We have tried to estimate roughly the protein requirements for maintaining such a normal level of body protein. Because the content of body nitrogen of rats was approximately 3·00 per cent of body weight, their protein content is 18·75 per cent. We therefore suggest that 437 mg (18·75 g × 0·028 × 0·833) of body protein is catabolized each day as a more labile component, and 144 mg (18·75 g × 0·972 × 0·0079) as a less labile component. A total of 581 mg of protein should therefore be supplied in a diet for each 100 g of body weight, corresponding to 830 mg if the value for net protein utilization[10] of the dietary protein is 70. In this experiment, the rats consumed about 25 g of diet daily, so that these protein requirements correspond to about 15 per cent of the diet (0·830 g × 4·5/25 g).

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[1] Voit, C., *Z. Biol.*, **2**, 307 (1866).
[2] Martin, C. J., and Robison, R., *Biochem. J.*, **16**, 407 (1922).
[3] Campbell, R. M., and Kosterlitz, H. W., *Biochem. J.*, **43**, 416 (1948).
[4] Holt, L. E., and Halac, E., *J. Amer. Med. Assoc.*, **181**, 699 (1962).
[5] Allison, J. B., and Wannemacher, R. W., *Amer. J. Clin. Nutrit.*, **16**, 445 (1965).
[6] Borsook, H., and Keighley, G. H., *Proc. Roy. Soc.*, B, **118**, 488 (1935).
[7] Borsook, H., and Dubnoff, J. W., *Ann. Rev. Biochem.*, **12**, 183 (1943).
[8] Comar, C. L., *Radioisotopes in Biology and Agriculture*, 18 (McGraw-Hill, New York, 1955).
[9] Lusk, G., *The Elements of the Science of Nutrition*, fourth ed., 358 (W. B. Saunders Co., Philadelphia and London, 1928).
[10] Bender, A. E., and Miller, D. S., *Biochem. J.*, **53**, vii (1953).

Changes in Collagen Cross-linking in Rats with Reduced Food Intake

THERE have been several reports that decreased food intake blocks collagen cross-linking in rats[1,2]. The resulting collagen network is described as younger tissue. Comparing this observation with the classical experiments of McCay[3], who increased the life span of experimental animals by reducing their food intake, it seems that a more detailed study of the cross-linking processes is desirable. As far as we know, no data are available which specify the cross-links that are blocked in animals whose food is restricted. Even the fact of whether this change in collagen structure involves only some of the cross-links between the three polypeptide chains of the tropocollagen molecule, or whether it results in a random decrease in cross-link formation, has not yet been investigated.

Two approaches were chosen to answer these questions: (*a*) chromatographic separation of the alpha collagen polymers; and (*b*) estimation of the free carbonyl compounds in the soluble and insoluble collagen. The second approach was partly influenced by the findings of Milch *et al.*[4] on the role of some products of intermediary metabolism (chiefly aldehydes) as potential cross-linking agents and partly by our own results[5,6] on the presence of keto-acids and an unknown aldehyde component in collagen. It was reported earlier that the molar ratio of these compounds is changed, as the soluble collagen is converted into the insoluble network[6].

The average food intake of male Wistar rats was estimated at monthly intervals in rats of different ages. An experimental group of animals was restricted to 60 per cent of the control intake of food, and the control group was given standard pelleted (Larsen) food freely. The animals were 60 days old at the beginning of the experiment and were killed when they were 210 days old.

To prepare skin collagen, the hair was shaved off, the skin was freed of subcutaneous tissue and acid soluble collagen was prepared according to the procedure of Drake *et al.*[7]. Acid soluble collagen was denatured by heating to 50° C just before analysis, and the denatured product was separated by column chromatography on carboxymethyl cellulose. The separation technique used was essentially the same as that described by Veis and Anesey[8].

In the estimation of carbonyl compounds, both acid soluble and insoluble collagen were used. Insoluble collagen had to be dissolved before analysis; this was done by pronase treatment according to the procedure of Drake *et al.*[7]. Carbonyl compounds in the dialysable fraction after treatment with pronase were converted into the corresponding 2,4-dinitrophenylhydrazones and were separated on silica gel chromatographic sheets (Eastman Kodak *K* 301 *M*) in the following solvent systems: (*a*) chloroform–benzyl alcohol–acetic acid (70 : 30 : 3); (*b*) toluene–pyridine–ethylene–chlorhydrine–0·8 molar ammonia (100 : 30 : 60 : 60, upper phase).

A typical carboxymethyl cellulose chromatogram of the acid soluble collagen preparation from food restricted animals and animals fed freely is shown in Fig. 1. If the individual peaks are compared it is evident that there is no qualitative difference between both preparations; there are, however, differences in the quantity of individual polymers. The amount of all polymers except beta-22 and gamma-222 is clearly much smaller in the collagen preparation from food restricted animals and the so-called delta fraction is also slightly increased. This indicates that the formation of all cross-links is decreased, with the exception of those between two alpha-2 fractions which are not affected. The role of the cross-link between alpha-2 fractions has already been shown in hypophysectomized rats, where this type of cross-link is the only one which persists[9]. If the results of food restriction are compared with those of hypophysectomy, it is evident that whereas hypophysectomy results in an almost complete block of cross-link formation, which is very specific in allowing only gamma-222 formation, the effect of food restriction seems to be more random. We tried to classify the nature of this cross-link. Table 1 shows that the total number of carbonyl compounds is decreased from seven to almost five per mole and that this decrease occurs because of the free keto-acids. The total number of aldehyde components is not changed. Our results show the following. (*a*) Food restriction results in a less polymerized acid soluble collagen; the polymerization of alpha-2 fractions is apparently not affected whereas the cross-link formation between alpha-1 and alpha-2 and two alpha-1 fractions is markedly decreased. (*b*) The formation of the alpha-2–alpha-2 cross-link is either independent of the carbonyl compounds present in the tropocollagen molecule, or the presence of the carbonyl compound involved in cross-linking cannot be influenced by restricted food intake. (*c*) On the contrary, the total number of carbonyl compounds with free carbonyl groups tends to decrease to five in the insoluble fraction which is the number found in acid soluble collagen. This means that

Table 1. DINITROPHENYLHYDRAZINE-REACTIVE SUBSTANCES PRESENT IN THE COLLAGEN PREPARATIONS FROM CONTROLS AND FOOD RESTRICTED ANIMALS (MOLE PER MOLE OF TROPOCOLLAGEN, MOLECULAR WEIGHT OF TROPOCOLLAGEN CONSIDERED 260,000)*

Sample	Dialysable fraction after pronase treatment					Non-dialysable residue† (aldehyde)	Total carbonyl compounds
	Aldehyde	α-Ketoglutaric acid	Pyruvic acid	α-Ketoisovaleric	Total keto-acids		
Controls	(1·15)‡	(2·30)	(0·80)	(2·37)		(1·40)	
	(0·75) 1	(2·15) 2	(0·65) 1	(2·28) 2	5	1 (1·00)	7
	(1·27)	(1·65)	(0·90)	(1·70)		(0·65)	
Food restricted	(0·75)	(1·15)	(0·75)	(1·15)		(1·35)	
	(0·65) 1	(0·80) 1	(0·75) 1	(1·35) 1	3	1 (1·20)	5
	(0·75)	(0·80)	(1·20)	(0·65)		(0·65)	

* Data presented refer to 3 collagen preparations done from 20 skins each.

† The amount of the aldehyde component present in the non-dialysable fraction was estimated by measuring the optical density at 360 nm assuming the constancy of the extinction coefficient.

‡ Numbers in parentheses are actual experimental data.

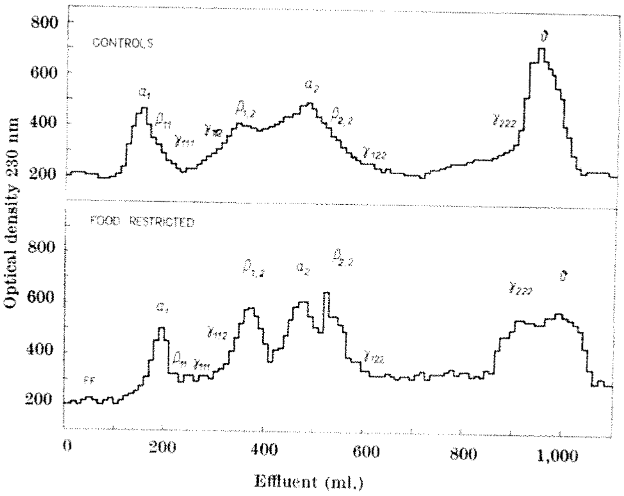

Fig. 1. Chromatographic pattern of the denaturation products of the acid soluble collagen from animals kept on a restricted diet compared with controls: starting buffer 0·04 M acetic acid and 0·06 M sodium acetate *p*H 4·8, limiting buffer prepared by adding 0·14 moles of sodium chloride to the starting buffer. After fraction No. 50 (500 ml. of effluent) the continuous gradient was suddenly changed with the starting buffer containing 0·44 M of sodium chloride. Another sudden change was introduced after fraction No. 68 when a 6 M urea starting buffer containing 1 M sodium chloride was used thereafter.

the amount of carbonyl compounds (keto-acids) in the collagen structure can be influenced by the food intake.

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[1] Chvapil, M., and Hruza, Z., *Gerontologia*, **3**, 241 (1959).
[2] Giles, J. S., and Everitt, A. V., *Gerontologia*, **13**, 65 (1967).
[3] McCay, C. M., Maynard, L. A., Sperling, G., and Barnes, L. L., *J. Nutrit.*, **18**, 1 (1939).
[4] Milch, R. A., *J. Surg. Res.*, **3**, 254 (1963).
[5] Rosmus, J., Trnavsky, K., and Deyl, Z., *Biochem. Pharmacol.*, **15**, 1405 (1966).
[6] Rosmus, J., and Deyl, Z., *Experientia*, **23**, 610 (1967).
[7] Drake, M. P., Davison, P. F., Bump, S., and Schmitt, F. O., *Biochemistry*, **5**, 301 (1966).
[8] Veis, A., and Anesey, J., *Biol. Chem.*, **240**, 3899 (1966).
[9] Deyl, Z., Everitt, A., and Rosmus, J., *Exp. Gerontol.* (in the press).

Anti-metabolic Extract from the Brain of *Protopterus aethiopicus*

It has been suggested that the brain of the aestivating lung-fish might contain an anti-metabolic agent[1], perhaps not unlike the mono-amines secreted in the pons and medulla of cats as active agents in the control of sleep[2].

Large lung-fish (2–6 kg) from Uganda were flown by jet to Denver, then placed in aquaria at Fort Collins, Colorado, in water 4–16 in. deep over specially collected mud. The constant temperature room was maintained at 24°–25° C. When the fish were accustomed to their new environment and were eating well, they were forced to encyst in the underlying mud by simply withdrawing a portion of the water from the aquarium each day until it was quite dry and the mud was caked hard. The fish dug their chambers at the bottom of tunnels, coiled up and entered a state of profound torpor, in every way similar to their behaviour in their normal environment in Africa during the dry season.

After the fish had been immobile for a month to 6 weeks, the mud was chipped away and an attempt was made to kill the fish abruptly without arousal. It is presumed that all fish had reduced metabolism. The oxygen consumption of one was successfully measured. In the resting–active state before encystment, the oxygen consumption was 30·7 ml./h/kg, whereas after 30 days in the mud, the consumption was 4·6 ml./h/kg, a drop of 85 per cent.

The head was severed from the body with a meat cleaver, and the brain immediately dissected from the skull and placed in acetone in a bottle packed in chipped ice. The entire brain was extracted on the same day using the methods described by Guillermin *et al.*[3]. The extract emerged as a buffered solution (pH 7·0) of 2 ml. volume.

The extract was injected intravenously into fasting white Wistar rats, contained in a 2 l. dark glass bottle with constant forced ventilation at 200 ml./min. The state of consciousness, rectal temperature, oxygen consumption and carbon dioxide production were monitored. Heat production was calculated from the amounts of oxygen consumed per unit time and the RQ according to the formula: HP (kcals/mol O_2) = 87 + 27 RQ. Readings were continued hourly or at irregular intervals for as long as was deemed desirable.

The following experiments were performed. (*a*) For the controls 2 ml. was injected of: (1) the extracting fluid; (2) and (3) extracts from the brains of two active fish (No. 1 and No. 2); (4) and (5) extracts from the brains of two torpid fish which were aroused and struggled for several minutes before being subdued and killed (No. 3 and No. 4). (*b*) For the protocols 2 ml. was injected of: (6) extract of brain of torpid fish No. 5; (7) combined extracts of brains of fish No. 6 and No. 7. The metabolic data are presented as a family of curves in Fig. 1. Each curve represents observations of 1 h plotted against time for each test animal, numbered as listed here. The "injection period" was variable, but represents the period of time the rat was out of the bottle for the injection procedure.

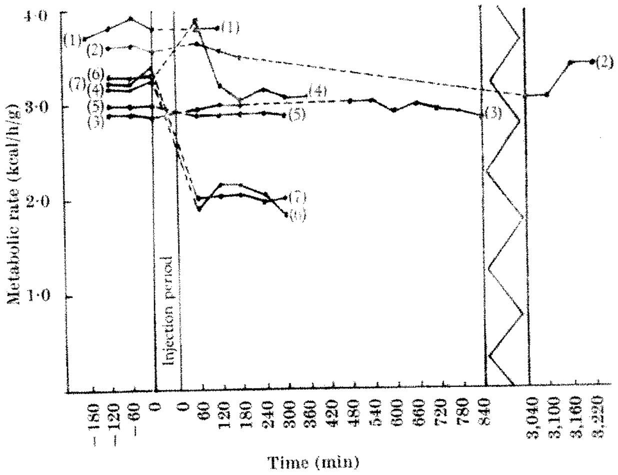

Fig. 1. Curves representing the data obtained from injection of rats.

None of the control extracts affected the injected rat in any observable or measured way. The response to extracts from the brains of fish which had been removed before the animal was aroused from deep torpor was profound and dramatic. The rats became lethargic and did not respond normally to irritating stimuli. They refused food, the body temperature fell 5° C and the measured metabolic rate fell to 65 per cent of the pre-injection fasting level. The respiration quotient did not change significantly (0·80). The effect lasted several hours.

Although the experiments are few in number, all those in which the protocol was successfully completed induced an unequivocal response: lethargy, a 35 per cent drop in metabolic rate and a fall of 5° C in body temperature.

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[1] Swan, H., *Amer. Med. Assoc. Arch. Surg.*, **87**, 715 (1963).
[2] Kleitman, N., *Sleep and Wakefulness* (Univ. of Chicago Press, 1963).
[3] Guillermin, R., Schally, A. V., Lipscomb, H. S., Andersen, R. N., and Long, J. M., *Endocrinology*, **70**, 471 (1962).

Maximum-intensity Fluorescence Microscopy

DESPITE the high intrinsic brilliance of some modern light sources, there are many occasions on which the intensity of an image viewed through a fluorescence microscope is inadequate, leading to inability to appreciate details or to inconveniently long exposures in photomicrography. This situation is likely to become increasingly important with the further development of more specific immunofluorescence methods and of fluorescence histochemistry. The system described here approaches the maximum theoretical efficiency and the image intensity is limited only by the intrinsic brilliance of the source and by the transmission or reflectivity of the optical components. At the same time this system opens up a number of new possibilities for viewing two different components simultaneously.

In recent years several vertical-incidence illuminators have been developed or adapted for fluorescence microscopy. One such system is shown in Fig. 1. The light source (LS) (usually a high pressure mercury lamp such as the Osram *HBO* 200) is focused by means of the collector lens (L_1) onto the aperture stop of the objective O. F_1 is a suitable exciter filter (usually passing long wave ultraviolet or blue light) and M_4 is a beam-splitting mirror. For maximum efficiency, M_4 should be a dichroic (spectrum-splitting) interference filter which reflects ultraviolet and blue and transmits visible light. In this case the specimen S will be illuminated with ultraviolet or blue light and the emitted fluorescence (usually of longer wavelength) will be transmitted through M_4 to the eyepiece E. A suitable barrier filter (B) is interposed to absorb any exciting light and provide a black background. This system has the advantage that most of the unabsorbed exciting light passes through the specimen and the little that is reflected by it or by the coverglass will be reflected away by M_4. Only a relatively light barrier filter is therefore required at B. The chief disadvantage is that the objective acts as its own condenser, so that the illuminating numerical aperture varies with the objective, whereas with a conventional transmitted light system the specimen can be illuminated by means of a single condenser of high numerical aperture. Maximum illumination in the vertical incidence system usually occurs with high power objectives, for which it is most needed. It is evident that vertical incidence fluorescence illumination can be combined with transmitted light illumination through a sub-stage condenser, using a second light source. This was carried out in preliminary experiments using a second *HBO* 200 lamp, collector lens and exciter filter. Measurements carried out with a photomultiplier photometer showed that the specimen illuminations obtained were additive. A simpler and economically more attractive method using a single source is illustrated. A second collector lens L_2 is used to image the back of the mercury lamp onto the condenser aperture stop. M_1, M_2 and M_3 are fully reflecting front surface aluminized mirrors, F_2 an exciter filter. M_4 now acts as a barrier filter and reflects away any exciting light transmitted by the specimen. The Zeiss large fluorescence microscope with vertical incidence illuminator proved particularly suitable for this modification; the beam-splitter M_4 in this instrument is of the dichroic type. The rear end of the lamp housing was removed and replaced by a collector lens. Only the mirrors M_1 and M_2 had to be added. When both objective and condenser are high-aperture immersion types, the specimen is illuminated by a solid cone approaching the theoretical maximum. With smaller apertures, some increase might be obtained by using auxiliary oblique or annular illumination, but it is doubtful whether the gain would be worth while.

This system can, of course, be used for combining fluorescence with other methods of illumination, such as phase contrast. Suitably placed polarizing components make it possible to pick out birefringent elements in a fluorescent specimen. A less obvious application, not hitherto described, is to use different exciter filters F_1 and F_2 so that, for example, substances excited by ultraviolet light (such as many carcinogenic hydrocarbons) can be viewed against fluorochromed tissues excited by blue light and one or other component can be rapidly extinguished. It should also be possible to view simultaneously two different antigens stained by different immunofluorescent techniques and excited by light of different wavelengths.

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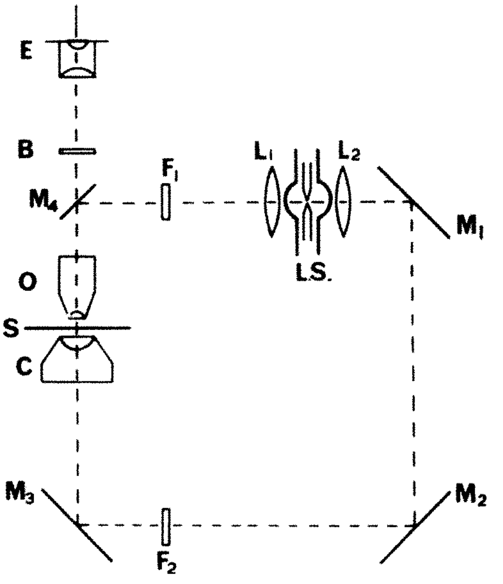

Fig. 1. Simultaneous vertical and transmitted light illumination using single source L.S. M_1, M_2, M_3 are fully reflecting mirrors. M_4 is a dichroic beam-splitter, F_1, F_2 exciter filters. B barrier filter, C condenser, O objective, S specimen, E eyepiece.

Photochemical Production of Quinoid Structures in Wood

DEGRADATION of wood by a number of agencies (for example, white rot fungi[1], hot alkaline sulphite[2] or light[3]) results in a decrease in the methoxyl content of the lignin. Thus after irradiation for 1,000 h using an enclosed 1 kW carbon arc, glass-covered newspaper manufactured predominantly from *Pinus radiata* seemed to have lost about one-third of its methoxyl groups. Such a high loss suggests that, despite the very complex and variable type of structure proposed for lignin[4], a fairly simple molecular unit, which can be formed from at least 30 per cent of the lignin building stones, is involved. Evidence is presented here which indicates that this intermediate is of quinoid structure; either an *o*-quinone, a *p*-quinone, or a *p*-quinone methide.

Of these three quinoid structures, a *p*-quinone could be eliminated if the degradation did not involve atmospheric oxygen, as I have suggested[3], but more recent experiments show that, if precautions are taken to exclude oxygen, wood is not yellowed by light and retains its methoxyl content. This means that the yellowing of wood by light is a photo-oxidative process and so *p*-benzoquinones could be formed. Indeed, 2,6-dimethoxybenzoquinone shows a significant (30 per cent) decrease in methoxyl content when it is irradiated for 1,500 h in a solid film of cellulose acetate. It is not yet known whether analogous *p*-quinone methides also show a decrease in methoxyl content on irradiation, and both they and *o*-quinones, from which the methoxyl group has already been lost, could also be formed by photo-oxidation of guaiacyl–propane systems.

The spectral region[3] (3550–4000 Å) which causes yellowing of wood confirms that quinones, which are able to absorb this light, could be responsible for the degradation. The most significant evidence for the photochemical production of quinoid structures in wood, however, comes from experiments in which the free phenolic hydroxy groups in wood lignin have been protected with acetyl or methyl groups. Wood or newspaper which has been acetylated by successive immersion in aqueous sodium hydroxide (1 N) and acetic anhydride yellows slowly (Fig. 1) and does not initially (100 h) show a loss of methoxyl groups when irradiated. Similarly, wood which has been acetylated by immersion in acetic anhydride containing a trace of perchloric acid, although initially coloured by the reaction, undergoes bleaching rather than yellowing when irradiated (Fig. 1). Methylation of the wood by soaking first in aqueous sodium hydroxide (1 N) and then in warm dimethyl sulphate also markedly slows the yellowing process, particularly in the initial stages (Fig. 2). These results indicate that lignin only readily undergoes photochemical demethoxylation and yellowing when it contains free phenolic hydroxy groups. The same condition governs the formation of quinones and quinone methides and it thus seems very likely that these are formed at some stage when wood yellows. Being highly photo-reactive themselves, it is probable that they will be further degraded and are not themselves responsible for the final yellow colour of the wood. This is in agreement with attempts to remove the yellow colour of irradiated wood by reduction. While dilute aqueous sodium borohydride removes all the yellow colour, hot stannous chloride has no effect on it, and hot sodium dithionite is only partially effective.

Neither the mechanism of methoxyl loss nor the structure of the quinoid precursors in wood is known. Reflectance measurements on panels of *Pinus radiata* in this laboratory indicate that some of the suggested quinoid structures may already be present in wood, because irradiation for a few hours under glass causes an increase in the reflectance around 3700 Å. Most of the quinoid structures, however, must be generated during the process of yellowing. Because reduction of benzoyl groups in the lignin by soaking the wood in aqueous sodium borohydride does not hinder the yellowing process (Fig. 2), the suggested quinoid structures may be quinone methides formed from benzyl alcohol groups. Certainly, irradiation under glass of 4-hydroxy-diphenylmethanes in cellulose acetate films produces yellow colours attributable to stable quinone methides. On the other hand, production of *o*-quinones would neatly combine the demethoxylation and the formation of quinones in one step.

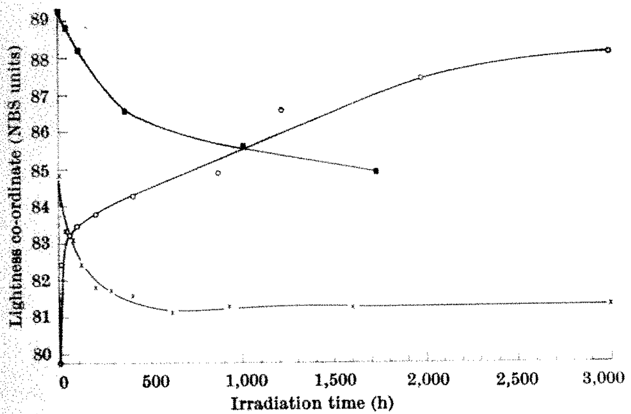

Fig. 1. Rates of yellowing of newspaper (1 kW carbon arc). ×, Untreated newspaper; ■, acetylated (Ac$_2$O/OH$^-$) newspaper; ○, acetylated (Ac$_2$O/H$^+$) newspaper. Lightness co-ordinates calculated according to Glasser and Troy[5].

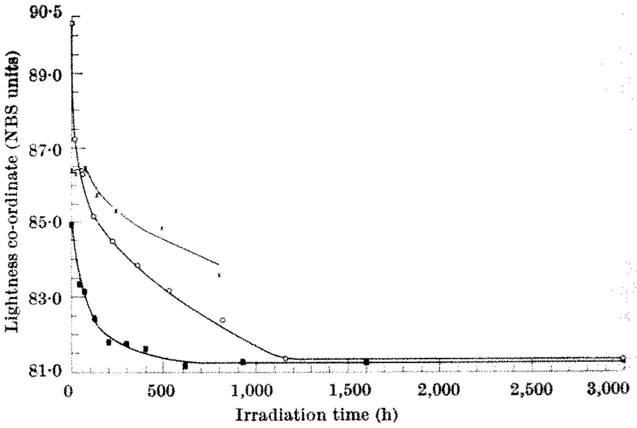

Fig. 2. Rates of yellowing of newspaper (1 kW carbon arc). ■, Untreated newspaper; ×, methylated newspaper; ○, reduced (NaBH$_4$) newspaper. Lightness co-ordinates calculated according to Glasser and Troy[5].

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[1] Nord, F. F., *Bull. Inst. Politeh. Iasi NS*, **11** (15), Spec. No. 35–46 (September 1965).

[2] Croon, I., and Swan, B., *Svensk Papperstidning*, **67**, 177 (1964).

[3] Leary, G. J., *Tappi*, **50**, 17 (1967).

[4] Brauns, F. E., and Brauns, D. A., *The Chemistry of Lignin*, Suppl. vol., 626 (Academic Press, New York, 1960).

[5] Glasser, L. G., and Troy, D. J., *J. Op. Soc. Amer.*, **42**, 652 (1952).

PHYSIOLOGY

Action of Procaine on Calcium Efflux from Frog Nerve and Muscle

A RECENT hypothesis about the mechanism of action of procaine and structurally similar depressants of excitation emphasizes their interaction with membrane calcium[1]. Procaine, it is suggested, replaces calcium at membrane anionic sites and this inhibits the influence of excitable stimuli on processes leading to sodium influx. The hypothesis is based, in part, on the assumed role of calcium in excitation, for the release of this cation is widely viewed as the first important step in membrane depolarization[2]. Furthermore, high concentrations of external calcium oppose the action of procaine on frog motoneurones[3] and lobster giant axons[1]. Evidence that procaine or other local anaesthetics actually increase the mobility of membrane calcium has not yet been reported. I have investigated the interaction between procaine and calcium in nerve and muscle and the question of whether procaine causes the calcium to be released from these tissues.

Sciatic nerves isolated from frogs (*Rana pipiens*) were desheathed and placed in a buffered Ringer solution (*p*H 7·2) of a composition already described[4]. Frog sartorius muscles were isolated and immersed in the same type of Ringer solution. After equilibration in normal Ringer solution at 22°–24° C, tissues were immersed for 4–8 h in Ringer solution (5° C) labelled with 10 μc./ml. of calcium-45. The tissues were then rinsed with 20 ml. of non-radioactive Ringer solution, and a single nerve or

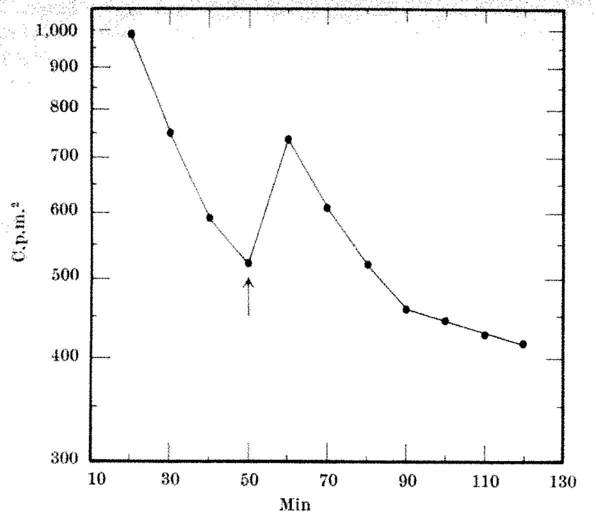

Fig. 1. Effect of 20 mM procaine on the time course of the rate of efflux of calcium-45 from isolated desheathed nerve. Procaine added at arrow. Each point is average of six nerves. Calcium-free Ringer solution used throughout.

muscle was placed in a chamber containing 2 ml. of calcium-free Ringer solution. After 10 min, the 2 ml. of collection fluid was placed in a scintillation vial and replaced by fresh calcium-free solution; this procedure was repeated every 10 min for the duration of the wash-out period, which was carried out at 22°–24° C. At the end of the wash-out, tissues were ashed and dissolved in acid. To each 2 ml. of collection fluid or 2 ml. of acid-dissolved ash was added 20 ml. of scintillation fluor. The radioactivity of each sample was measured in a liquid scintillation spectrometer, the efficiency for calcium-45 being 80 per cent. Two types of curves were plotted from the data: (*a*) a rate curve which describes the time course of the rate of calcium-45 efflux; and (*b*) a rate coefficient curve which is the percentage of the average tissue radioactivity during the time of collection that has emerged each minute during the collection interval.

The calcium-45 wash-out curve from nerve or muscle consists of an initial fast and a later slow component, and treatment with the local anaesthetic began during the slow phase. The slowly emerging calcium-45 probably derives from axons or muscle fibres but the specific locus of the cellular compartment remains uncertain. On the basis of results discussed below, we can at least conclude that the compartment(s) is readily accessible to the extracellular environment and is readily exchangeable with extracellular calcium, suggesting a membrane locus.

About 7 to 10 mM procaine is required to produce a measurable and consistent release of calcium-45 from the desheathed nerve, and 20 mM gives the maximum effect (Fig. 1). The maximum release occurs within the first 10 min of collection after procaine has been added to the nerve bath; this is followed by a rapid return of efflux to a rate measured in the absence of procaine. At 7 mmolar, procaine causes complete depression of the α-A fibre spike of desheathed nerve within 5 min (in normal or calcium free Ringer solution). Thus the calcium-releasing action requires relatively high anaesthetic concentrations. This may indicate that the calcium-releasing effect is not causally related to the anaesthetic effect of procaine or, as is more likely, that the amount of calcium-45 released is a very small fraction of the total emerging from the tissue.

If non-radioactive calcium (calcium-40) is added to the calcium-free nerve bath during the slow phase of calcium-45 efflux, a rapid and transient release of calcium-45 is produced. It is known that procaine depresses this so-called self-exchange reaction in frog skeletal muscle[5].

In frog desheathed nerve, 20 mM procaine depresses the influence of 5 mM calcium-40 on calcium-45 efflux. But this depression can be quantitatively accounted for by the calcium-releasing action of procaine (Fig. 2). This suggests that the calcium released by procaine and by calcium itself comes from the same cellular site, probably the membrane. Of even greater interest is the effect of 5 mM procaine on the calcium-releasing action of calcium-40. As Fig. 2 shows, this concentration of procaine has no effect on the rate of calcium-45 efflux, but it does enormously potentiate the exchange between calcium-45 and calcium-40. Rather than competing with extracellular calcium-40 for tissue calcium-45, procaine apparently weakens the association between calcium-45 and tissue binding sites.

In skeletal muscle, about 50 mM procaine is required to produce a consistent increase in the rate of calcium-45 efflux (Fig. 3), a concentration which causes complete loss of excitability within 3 min. The lower sensitivity of muscle to the calcium-releasing action of procaine may be related to the lower sensitivity of muscle (compared with nerve) to the anaesthetizing action of procaine. Of particular interest in Fig. 3 is the comparison between the action of procaine and the more potent local anaesthetic, tetracaine. At one-tenth of the concentration of procaine, tetracaine produces almost twice the increase in the rate of calcium efflux, and the effect is more enduring.

These results support the hypothesis that the action of local anaesthetics of the procaine type on excitable membranes is causally related to displacement of membrane calcium. We have recently tested the effect of 10^{-5} M tetrodotoxin on the desheathed nerve and found no influence of this maximally depressant concentration on the rate of calcium-45 efflux. This suggests some degree of specificity of the procaine type of local anaesthetic for the calcium-releasing mechanism. On the other hand, it seems paradoxical that procaine has an effect on calcium efflux qualitatively similar to that of other agents, such as calcium chelators[6], which lower excitability thresholds

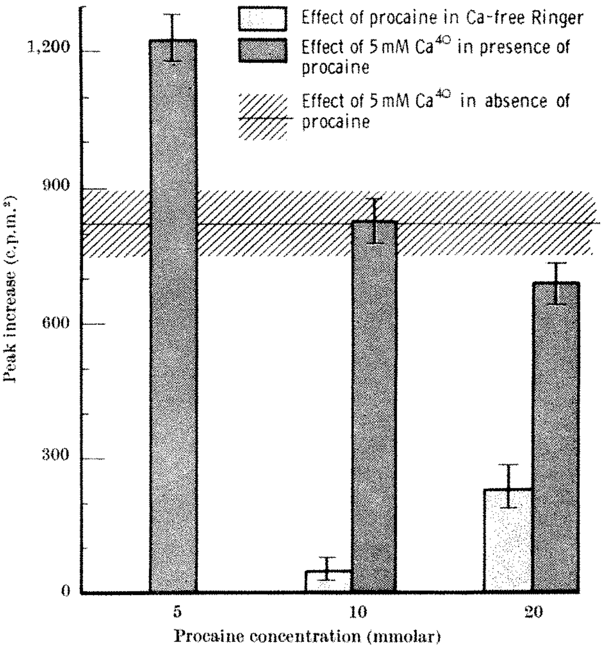

Fig. 2. Effect of various concentrations of procaine on release of calcium-45 from isolated desheathed nerve and on the exchange between calcium-45 and calcium-40. Each vertical bar represents an average of 6 nerves ± *S.D.* Peak increase is calculated as the difference between the maximum amount of calcium-45 released by procaine or calcium-40 and the amount of calcium-45 measured just before treatment. Procaine added at 50 min and calcium-40 added at 90 min from start of washout. Horizontal bar represents average peak amount (six nerves) of calcium-45 released by 5 mM calcium-40 in the absence of procaine. Note marked potentiation of effect of calcium-40 by 5 mM procaine.

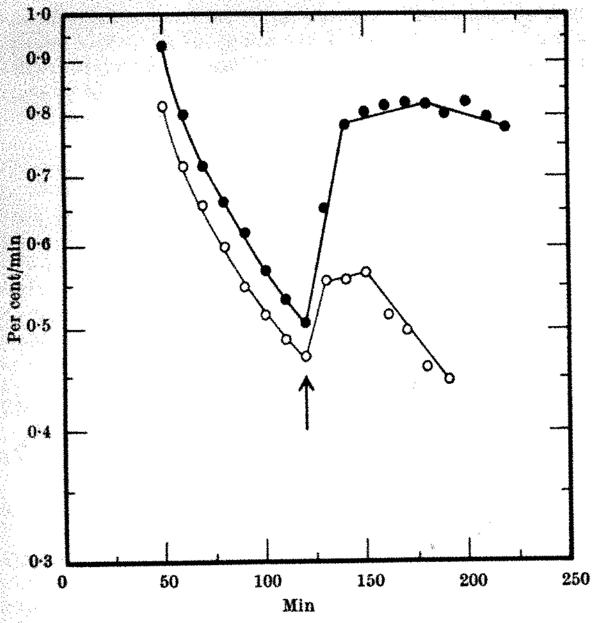

Fig. 3. Effect of procaine and tetracaine on the rate coefficient of calcium-45 release from isolated sartorius muscle. Each point is average of 6 muscles. Calcium-free Ringer solution used throughout. ●, 5 mmolar tetracaine; ○, 50 mmolar procaine.

and produce spontaneous impulses. Furthermore, procaine antagonizes the effects of these threshold-lowering agents at concentrations much less than those needed to release calcium[7]. Clearly there is much to learn about the roles of calcium in excitation and about the cellular localization of the different calcium-binding sites involved in excitation. Perhaps further investigation of the interaction between procaine and calcium will contribute to the solution of these problems.

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[1] Blaustein, M. P., and Goldman, D. E., *J. Gen. Physiol.*, **49**, 1043 (1966).
[2] Tobias, J. M., *Nature*, **203**, 13 (1964).
[3] Aceves, J., and Machne, X., *J. Pharmacol. Exp. Therap.*, **140**, 138 (1963).
[4] Kuperman, A. S., Okamoto, M., Beyer, A. M., and Volpert, W. A., *Science* **144**, 1222 (1964).
[5] Feinstein, M. B., *J. Gen. Physiol.*, **47**, 151 (1963).
[6] Koketsu, K., and Miyamoto, S., *Nature*, **189**, 402 (1961).
[7] Okamoto, M., Askari, A., and Kuperman, A. S., *J. Pharmacol. Exp. Therap.*, **144**, 229 (1964).

Photoinhibitory Function of the Dorsal Ocelli in the Phototactic Reaction of the Migratory Locust *Locusta migratoria* L.

AT least two physiological functions have been ascribed to the dorsal ocelli of locusts and insects in general. First, in response to light intensity they regulate the speed of movement (photokinesis)[1] and the threshold for the initiation of certain activities[2]; and second, coupled with the complex eyes, they are in some unknown way involved in angular orientation, both in the phototactic[1,3,4] and in the dorsal light reaction[5,6].

The following facts have led to a new hypothesis of co-operation between the three dorsal ocelli and the complex eyes in the control of the phototaxis of locusts. Locusts and grasshoppers which react phototactically can respond to changing light intensity in two ways: thus the strength of the phototactic turning tendency can either increase or decrease with increasing luminous intensity (that is, direct and inversely proportional reaction types). The discovery of the inversely proportional reaction, in which the strength of the response wanes with increasing intensity of stimulus, suggested an inhibitory mechanism which may be switched on and off[7]. In view of the electrophysiological evidence for the photoinhibition of the spike frequency of the ocellar nerve[8] and the observation that the menotactic angles of locusts can increase as a result of the occlusion of the ocelli[1], it seemed that the dorsal ocelli supply the input to this inhibitory mechanism[9]. To test this hypothesis we have investigated the relationship, in laboratory reared migratory locusts, between the occlusion of the ocelli and the relative strength of the phototactic turning tendency which is dependent on light intensity.

Two different methods of quantifying the strength of the phototactic turning tendency were employed—the geo-phototactic compromise experiment and the menotactic angle experiment. The equipment for the compromise experiment consisted of an inclined plane 1 m square with a slope of 30° ; the plane was illuminated from the side with a horizontal beam of light (Fig. 1). The light source, a Prado slide projector, 380 cm from the centre of the platform was visible as a disk of light with a luminous intensity of 200 stilb (full intensity) and subtended an angle of approximately 1°.

Locusts showing negative geotaxis and positive phototaxis which were placed head upwards in the centre of this plane assumed a compromise position between negative geotaxis and positive phototaxis. The stronger the turning tendency of the locust toward the light source, the greater the angle of compromise (α) will be. Locusts which show negative phototaxis behave similarly but turn away from the light source.

In testing the relative strength of the phototactic turning tendency, each animal was placed on the inclined plane and was allowed to orientate itself. The angle of the direction of the path of movement was then measured. Each individual tested was exposed five times in alternating sequence to each of the four following conditions: (1) ocelli intact and the light source at full intensity; (2) same as in (1) but with the light source reduced to 1/200 full intensity with a neutral grey filter; (3) all three ocelli occluded with opaque black shellac and the light source at full intensity; (4) same as in (3) but with the light source reduced to 1/200 full intensity.

Before the experiments were begun the reaction types of the test animals were determined and fourteen individuals of each type were selected for the experiments. The reaction was again checked at the conclusion of the

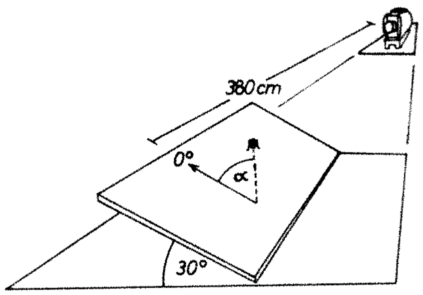

Fig. 1. Apparatus for geo-phototactic compromise orientation. The angle α represents the measurement of the relative strength of the phototactic turning tendency.

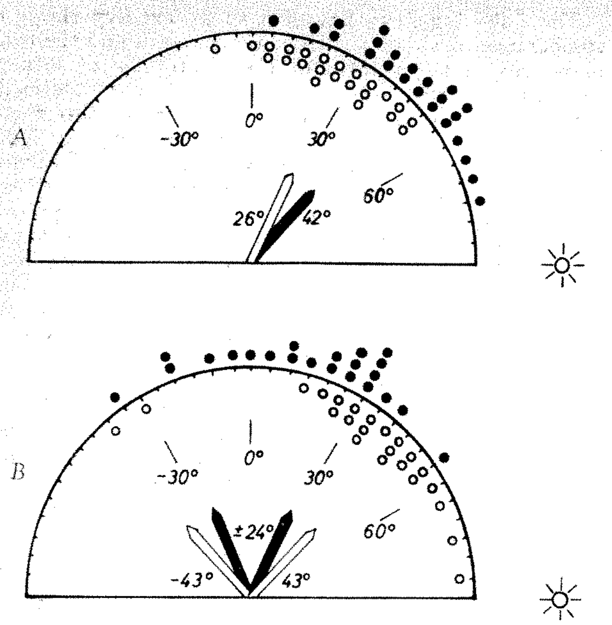

Fig. 2. Effects of changing light intensity on the relative strength of the phototactic turning tendency of inversely proportional reacting *Locusta migratoria* during the geo-phototactic compromise orientation. Each point represents three trials of a total of seventy runs executed by fourteen individuals. ●, Intensity of 200 stilb; ○, intensity of 1 stilb. The arrows indicate the mean angular deviation from 0° for both intensities; in (*B*) this was calculated irrespective of sign. (*A*) represents the results of tests in which the ocelli were intact, (*B*) represents tests in which the ocelli were occluded.

experiments to determine whether there had been a change from one to the other reaction type during the experiments.

The results show that the directly proportional reacting locusts maintained their reaction type after occlusion of the dorsal ocelli. The inversely proportional reacting individuals, however, reacted in a directly proportional manner when the ocelli were eliminated (Fig. 2). Thus the original photoinhibition of the phototactic turning tendency (Fig. 2*A*) was no longer evident (Fig. 2*B*). Both the photoinhibitory effect (inversely proportional reaction) before, and the photoexcitational effect (directly proportional reaction) after, occlusion were found to be statistically significant. (Fig. 2*A*, t test for mean angles, $P < 1$ per cent. Fig. 2*B*, χ^2 test, H_o: number of scores above and below the common mean angle (32·62°) are the same for both intensities, $P < 1$ per cent.) In control experiments in which animals were tested with clear shellac on the ocelli, the angular orientation of the inversely proportional reacting individuals was not affected.

The theory of the menotaxis of Jander[10] offered a second means of testing the relative strength of the phototactic turning tendency. In contrast to the first method in which the orientation arises as a balance between the opposing geotactic and phototactic turning tendencies, this method involves only the phototactic response. According to the menotactic theory the angular deviations of orientation pathways from the phototaxis (menotactic angles) are dependent on the strength of the phototactic turning tendency and must become smaller as this increases.

In this experiment, the same fourteen individuals from the previous test which reacted inversely proportionally were placed on a level platform to one side of which was a horizontal light source; the individuals were allowed to move towards the light. The light source was the same as that used in the first experiments. The parameter which was measured was the angular deviation of the direction of the positive phototactic orientation from the route which was aimed directly at the light source. Most individuals were tested five times in alternating sequence under the two light intensities with the ocelli either open or covered with black shellac as in the first experiment. During this experiment, several individuals with occluded ocelli walked in circles instead of executing directional orientations, indicating a breakdown in the orientation mechanism.

On decreasing the intensity of the light source, the variance of the menotactic angle decreased in locusts with intact ocelli (Fig. 3*A*); thus the strength of their phototactic turning tendency increased. The opposite results were, however, observed in the same experiments in which the ocelli were occluded; the strength of the phototactic turning tendency decreased when the intensity was lowered (Fig. 3*B*). The variances in the angular distribution in both experiments were significantly different under the two light intensities (F test, $P < 1$ per cent). Because essentially the same effect was observed using both this method (Fig. 3) and the first method (Fig. 2), we concluded that the ocellar nerve exerts a photoinhibitory (skotoexcitory) effect on the strength of the phototactic turning tendency of inversely proportional reacting migratory locusts.

Thus, in this behaviour, the intensity-dependent control of the phototactic turning tendency exerted by the ocelli is complementary to the corresponding control exerted by the complex eyes: photoinhibition through the ocelli, photoexcitation through the complex eyes. The results of this co-operation can be described as a form of central light intensity adaptation.

A possible biological meaning for this interaction is suggested by observations which were made during

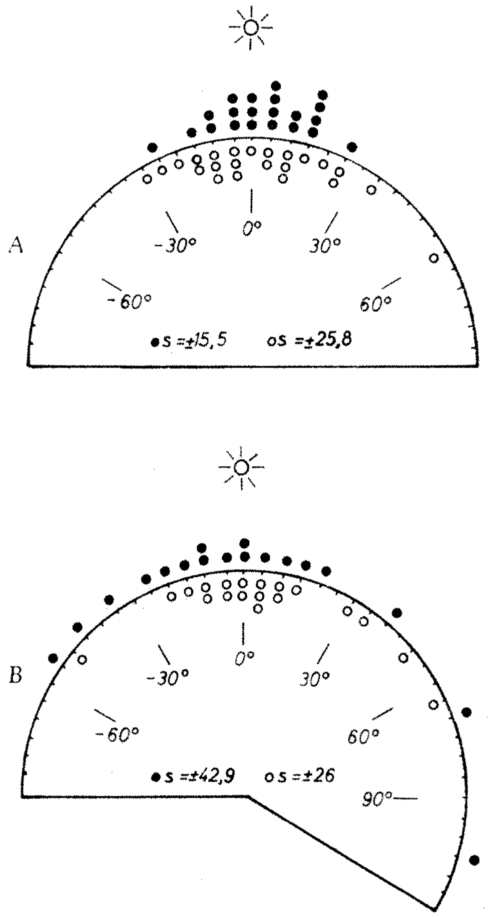

Fig. 3. Effects of changing light intensity on the variance of the menotactic angle of inversely proportional reacting *Locusta migratoria* during phototactic orientation. Each point represents three runs from a total of seventy runs in (*A*) and 67 and 59 in (*B*) carried out by fourteen animals. ○, Intensity of 200 stilb; ●, intensity of 1 stilb. In (*A*) ocelli were intact. in (*B*) they were occluded.

the second experiment. In conditions of weak intensity the ability of several individuals to orientate themselves with occluded ocelli broke down and they began to circle instead of progressing towards the light source. This was probably because the phototactic turning tendency without the darkness stimulation of the ocelli had become too weak. This effect was never observed in locusts with intact ocelli or in the control animals.

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[1] Cassier, P., *Ann. Sci. Nat. Zool.*, Paris, **12e** Serie, 7, 213 (1965).
[2] Schricker, B., *Z. vergl. Physiol.*, **49**, 420 (1965).
[3] Müller, E., *Z. vergl. Physiol.*, **14**, 348 (1931).
[4] Cornwell, P. B., *J. Exp. Biol.*, **32**, 217 (1955).
[5] Mittelstaedt, H., *Z. vergl. Physiol.*, **32** (1950).
[6] Goodman, L. J., *J. Exp. Biol.*, **42**, 385 (1965).
[7] Jander, R., *Z. vergl. Physiol.*, **47**, 381 (1963).
[8] Autrum, H. J., and Metschel, N., *Z. Naturforsch.*, **16**b, 384 (1961).
[9] Jander, R., *Verh. Dtsch. Zool. Ges.*, Jena, **266**, 1965 (1965).
[10] Jander, R., *Z. vergl. Physiol.*, **40**, 162 (1957).

Electroencephalogram Potentials evoked by Accelerated Visual Motion

CHANGES in the brightness and the spatial or temporal pattern of visual stimuli evoke small correlated changes in the electroencephalogram (EEG) potentials recorded from the occipital scalp and can be revealed by averaging the responses to repeated stimuli[1-5]. We have investigated the effects of changes in the velocity of patterned stimuli and have found what seem to be analogous evoked responses to acceleration of the retinal image.

It is well known that the perceived velocity of a moving figure is increased in the vicinity of a stationary reference point. Our first experiment was made to discover (*a*) whether any evoked potentials could be detected when a stationary visual stimulus was set in motion; (*b*) whether the proximity of a reference line affected the magnitude or form of any response detected.

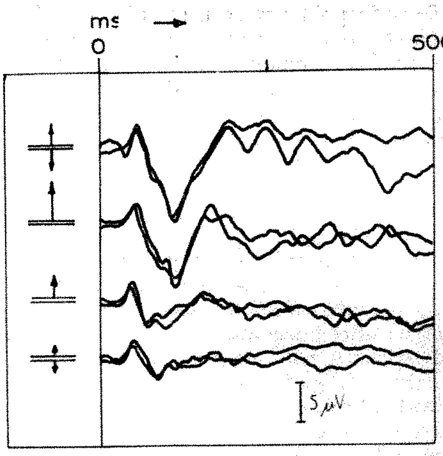

Fig. 2. Comparison of EPs for symmetrical and asymmetrical displacements giving relative velocities of 4 cm/s (upper) and 2 cm/s (lower).

The stimulus first used was a single horizontal line of light 7 cm long generated by an oscilloscope with a 100 kHz timebase and a normal green phosphor. The line was displaced vertically by a "ramp" function (Fig. 1*A*) so that it returned to start and repeated its course every 2 s. A second beam could be used to provide an adjustable stationary reference line on the same screen, parallel to the first. Alternatively, a front-silvered mirror parallel to the line direction and normal to the screen could enable an image of the stimulus line, moving in the opposite direction, to be added to the field of view at a varying initial distance from the first line. The viewing distance was 50 cm, and a faintly lit fixation point was optically superimposed.

The electrode placements used are shown in Fig. 1, which also shows some typical results, each obtained by averaging 100 responses on a Mnemotron *CAT* 400 computer. Fig. 1*B* shows that significant responses with a negative-peak latency of about 60 ms were obtained when an isolated stimulus line moved from rest with a velocity of 2 cm/s, and that the presence of a reference line enhanced the response, by an amount inversely related to the initial separation of the two lines (Fig. 1*C* and *D*). As a check of fixation, eye movements were monitored by electrical recording of trans-orbital potentials, and it was verified that any transient movements occurring were too small to contribute significantly to the average evoked potential.

As a further check, a symmetrical display (two lines diverging) was provided with the help of the mirror, so that fixation was more easily maintained. The results, shown in Fig. 2, seem to discount the likelihood that eye movements were responsible for the EPs. There is a clear similarity in magnitude and general form between the main components of the response to a line moving with a given velocity relative to a fixed line, and the response to two lines moving with equal and opposite velocities of half that amount.

It seemed possible that part of the evoked response might be an off effect caused by the removal of light from the foveal area when the line was displaced. Accordingly,

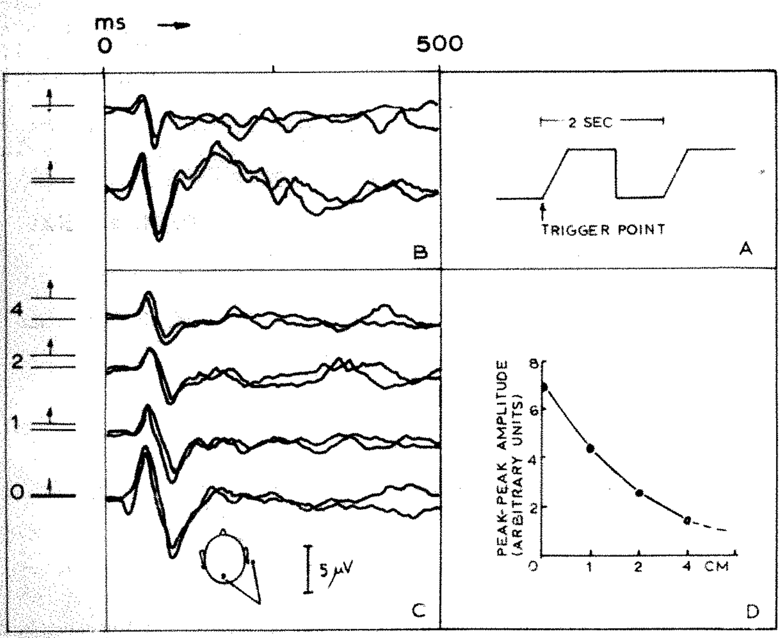

Fig. 1. *A*, Vertical deflecting waveform. *B*, Comparison of EPs to velocity step of 2 cm/s without reference line (upper) and with reference line (lower). *C* and *D*, Effects of distance of reference line (measured in cm) from starting point of moving line.

different fixation points were used, above and below the initial position of the accelerated line, so that motion of the line would in one case increase the total light on the fovea, and in the other case decrease it.

Although the EP drops off rapidly with eccentricity of fixation, it can be seen from Fig. 3 that there is no consistent asymmetry between the EPs with fixation above and below the rest position, such as would be expected if the change in total foveal illumination were responsible for the EP.

Partly in order to reduce any possible on or off effects as the moving line left the fovea, the spatial extent of the moving stimulus was increased by converting it into a raster of parallel horizontal lines, which was deflected up and down as a whole by the slow ramp waveform. The main effect (Fig. 4*A*) was a considerable increase in the amplitude of response. In order to minimize possible on or off responses as the individual raster lines appeared or disappeared at the edge of the field, the CRT face was masked by a sheet of photographic film having a circular aperture 5 cm in diameter with a blurred edge, but no significant change was produced by this.

The strong effects of reducing the spacing—and thus increasing the number—of the moving lines can be seen from Fig. 4*A*. Perhaps most striking is the finding (Fig. 4*B*) that with a many-line raster the introduction (by use of the mirror) of a second raster moving in the opposite direction now brings about an increase of only 30 per cent in EP.

With the help of a Dove prism, the effective orientation of the display (now viewed monocularly) was changed so that motion occurred in one of eight different directions. The ramp waveform was replaced by a sawtooth waveform with the gradients adjusted to be equal in both directions, so that the velocity of stimulus motion was simply reversed every 2 s. Electronic switching was used to gate the responses to alternate reversals into different channels of the *CAT* computer. The results showed no significant dependence of the evoked potential on the direction of stimulus motion.

The EPs recorded in these experiments seem clearly related to acceleration of a central portion of the retinal image. Their magnitude reflects both the change in velocity and the number, but not the direction, of accelerated contours.

They might therefore be parsimoniously interpreted as non-vectorial signs of the disturbance of the retinal population by the displacement of contours from stimulated to unstimulated areas; but it also appears from Figs. 1 and 2 that this disturbance is greatly increased (at least with small numbers of moving lines) by the proximity of other lines to serve as landmarks. The nature of the technique prevented the recording of signals indicative of steady velocity, so it remains doubtful whether these EPs reflect the activity of velocity-sensitive units in the visual cortex. It seems likely, however, that they may have a similar origin to the EPs recorded by Gaarder *et al.*[6] following saccadic eye movement, and to the EPs for pattern reversal recently described[7,8] which can be regarded as a limiting case for infinite image velocity.

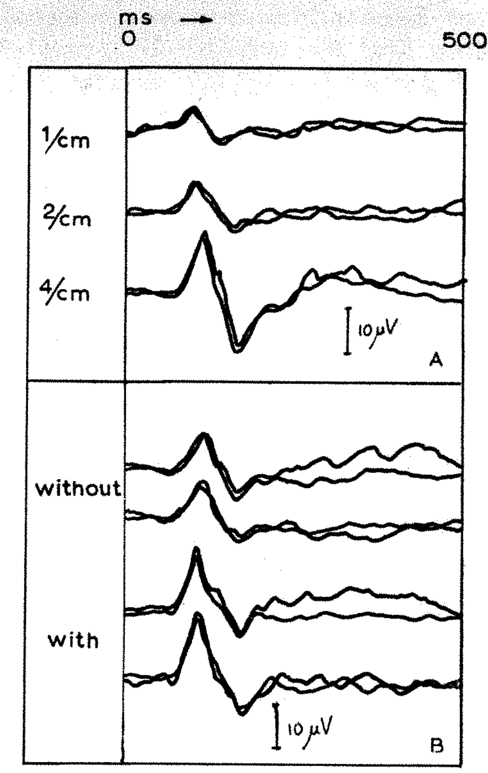

Fig. 4. *A*, Effects of increasing number of moving lines per cm. *B*, Effects of adding mirror image of moving raster.

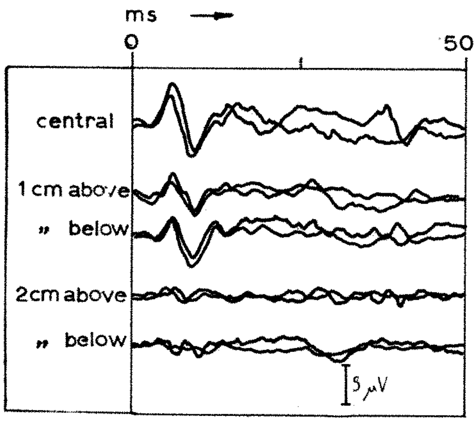

Fig. 3. Effects of off-centre fixation.

This preliminary study obviously raises a number of questions, which require a more flexible method of stimulation for their investigation. Some of these are suggested by the similarity between the present EPs and those evoked by stationary patterns[2-5]. An improved stimulator using continuously movable "visual noise" patterns has been constructed, and results obtained with it will be described elsewhere.

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[1] Rietveld, W. J., *Acta Physiol. Pharmacol. Néerl.*, **12**, 373 (1963).
[2] Spehlmann, R., *Electroenceph. Clin. Neurophysiol.*, **19**, 560 (1965).
[3] MacKay, D. M., Jeffreys, D. A., and Glover, R. R., *Final Scientific Report*, USAF Grant No. EOAR 65, June (1966).
[4] John, E. R., Herrington, R. N., and Sutton, S., *Science*, **155**, 1439 (1967).
[5] Rietveld, W. J., Tordoir, W. E. M., Hagenouw, J. R. B., and Spoor, T. A. C., *Acta Physiol. Pharmacol. Néerl.*, **14**, 259 (1967).
[6] Gaarder, K., Krauskopf, J., Graf, V., Kropfl, W., and Armington, J. C., *Science*, **146**, 1481 (1964).
[7] Gross, E. G., Vaughan, H. G., and Valenstein, E., *Electroenceph. Clin. Neurophysiol.*, **22**, 204 (1967).
[8] Cobb, W. A., Morton, H. B., and Ettlinger, G., *Nature*, **216**, 1123 (1967).

Effect of Excess Magnesium on Vagal Inhibition and Acetylcholine Sensitivity of the Mammalian Heart *in situ* and *in vitro*

It is known that an excess of magnesium ions blocks synaptic transmission through the ganglia of the sympathetic nervous system of mammals[1,2]. It is also accepted that peripheral parasympathetic pathways contain relay synapses at or near the target organ. The pharmacological properties of the junction between pre and post-ganglionic neurones in the parasympathetic system are believed to be similar to those in the ganglia of the orthosympathetic system. Stanbury[2], however, failed to observe blockade of the inhibitory effect of the vagus in cats treated with sufficient magnesium to block transmission in orthosympathetic ganglia.

We became interested in this problem during two experiments on the effect of intravenous administration of magnesium sulphate to human subjects[3]. In these experiments, sufficient magnesium was administered to cause complete paralysis of most skeletal muscles, but the two subjects remained conscious, and their EEG was unaffected. The only sign that could perhaps indicate that the central nervous system was depressed by the magnesium was the disappearance of (respiratory) sinus arrhythmia, which was noticed in both subjects. If the inhibitory effect of the vagus was transmitted normally in these subjects, the reflex modulation of the heart beat must have been blocked at a site "upstream" of the vagus, presumably in the central nervous system.

Since our report[3] has appeared, Toda and West[4] have shown that magnesium diminishes the negative chronotropic effect of vagal stimulation in isolated atria of rabbits. They used a higher concentration of magnesium on the isolated tissue than that which was effective in our human subjects. To determine whether the same dose of magnesium that paralyses skeletal muscle will also block vagal inhibition of the heart *in situ*, we carried out experiments with twelve unanaesthetized high spinal cats. One or both vagi were exposed in the neck and equipped with paired wire electrodes. Ten second trains of 0·5 ms shocks were delivered to the vagus at 1 min intervals. The intensity of the stimuli was kept above the maximum, but various frequencies were tried in most experiments. The arterial blood pressure and a lead II electrocardiogram (ECG) were recorded. In some experiments the isometric contractile force of the anterior tibial muscle, stimulated through its motor nerve, was also measured. The concentration of magnesium and calcium in plasma was measured in arterial blood samples as described earlier[5].

It is clear from Fig. 1 that during the infusion of magnesium chloride the effectiveness of vagal inhibition diminished. Partial relief was achieved by the injection of a small amount of calcium chloride. As with skeletal muscle, transmission of stimuli of low frequency was depressed earlier, and recovered later, than stimulation at a high frequency.

With the plasma magnesium elevated, high frequency stimulation of the vagus caused a partial block in atrio-ventricular conduction. Because of this, the ventricular beat was suppressed and the drop of blood pressure was almost as severe as in the control period, although the atrial frequency was much less affected by the vagus during infusion of magnesium than in the control state.

Similar results were obtained in every experiment. The effectiveness of vagal inhibition varies from cat to cat. On the whole, the magnesium block of vagal inhibition was more severe in animals where the vagal effect was weak.

Block of vagal inhibition could be a result of diminished acetylcholine output from presynaptic terminals, or of a decreased effect of the acetylcholine itself. A distinction between these two alternatives was possible by experiments on isolated perfused hearts. The heart of an anaesthetized rabbit was excised with the vagus nerve attached, and the coronary bed perfused with a constant-flow pump through the aorta. The Krebs–Ringer solution contained: Na^+ : 145 mequiv./l.; K^+ : 5·9 mequiv./l.; Ca^{++} : 5·0 mequiv./l.; Mg^{++} : 2·3 mequiv./l. ; Cl^- : 131 mequiv./l.; HCO_3^-: 24·0 mequiv./l.; SO_4^{--}: 2·3 mequiv./l.; $H_2PO_4^-$: 1·2 mequiv./l. Perfusion fluid with a high concentration of magnesium was prepared by adding isotonic (0·2 equiv./l.) magnesium chloride to the Krebs solution to bring the total magnesium concentration to 17 mequiv./l. Perfusion fluid with a high concentration of calcium contained 10 mequiv./l. calcium. The ECG was recorded differentially between right atrium and right ventricle using fine stainless steel hooks attached to the heart wall. The heart was immersed in Krebs solution; the temperature was kept at 37° C. Acetylcholine chloride was injected through a three-way tap close to the heart in volumes of 0·05 or 0·1 ml.

As shown on Fig. 2, the basal heart rate was slow during perfusion with Krebs solution containing an excess of magnesium but, relative to the basal rate, the effectiveness of vagal inhibition decreased. In contrast, injected acetylcholine caused a more pronounced inhibition of the heart perfused with Krebs solution containing a high concentration of magnesium than in the control state. Calcium, in the concentration used, enhanced the effect of vagal stimulation without much change in the effectiveness of injected acetylcholine.

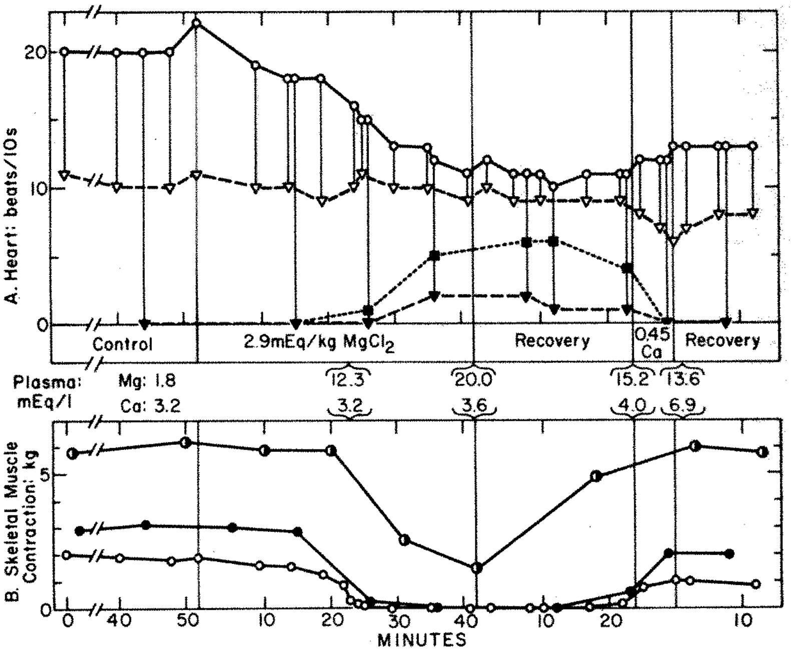

Fig. 1. Effect of intravenous infusion of 2·9 mequiv./kg body weight of magnesium chloride and 0·45 mequiv./kg of calcium chloride on heart rate and vagal inhibition of the heart (*A*); concentration of magnesium and calcium in plasma; and contractile force of anterior tibial muscle, stimulated via its motor nerve (*B*). *A*, Basal ventricular rate, counted in 10 s immediately before stimulation of vagus (○—○); ventricular rate during 10 s stimulation of vagus at 2/s (▽—▽); and at 20/s (▼—▼), atrial rate, when A-V conduction block occurred (■ . . . ■). *B*, Maximal force of contraction of muscle stimulated at 2/s (○—○); 20/s (●—●); and 200/s (◑—◑). During and after the infusion of magnesium tetanic tension was not well maintained, but only peak tension is shown on the chart.

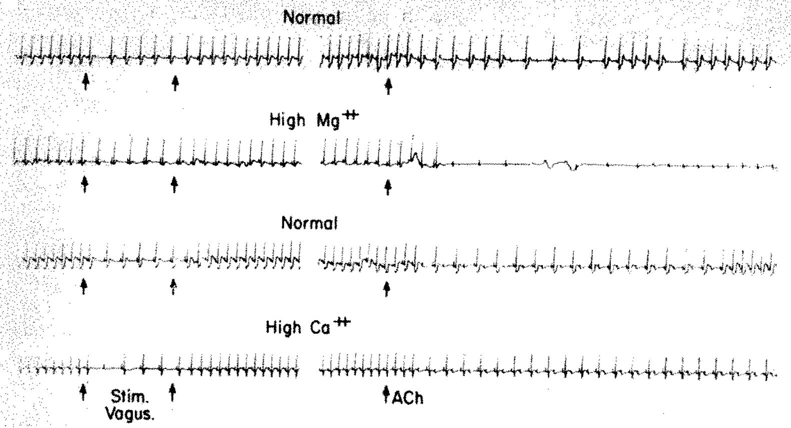

Fig. 2. The effect of stimulating the vagus at 25/s for 5 s, and of the injection of 2·5 μg of acetylcholine chloride, on an isolated rabbit heart perfused with Krebs solution; with solution containing 17 mequiv./l. of magnesium; and with solution containing 10 mequiv./l. of calcium.

It is clear that the partial atrio-ventricular conduction block, seen with vagal stimulation of a high frequency in the intact animal, is caused by the increased sensitivity of the conducting tissue of the heart to acetylcholine in the presence of excess magnesium. Infusion of calcium always caused an increase of the basal rhythm of the heart *in situ* (see Fig. 1*A*). The reason is not clear, but it could be caused by the release of adrenaline or noradrenaline. On pacemaker tissue which has been isolated, calcium has a negative chronotropic effect[4].

In conclusion, a rise in the magnesium content of plasma or perfusion fluid circulating in the coronary bed causes a decreased effectiveness of vagal inhibition. This is probably caused by a decrease in the release of acetylcholine. At the same time the effect of excess magnesium results in the increased sensitivity of the cardiac pacemaker and the atrio-ventricular conductile apparatus to the inhibitory effect of injected acetylcholine.

It is not clear from our observations whether the site of block is at the preganglionic, or the postganglionic terminals, or at both. Toda and West[4], who have studied isolated atria, concluded that magnesium had a blocking effect at both sites.

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Received December 1, 1967.

[1] Hutter, C. F., and Kostial, K., *J. Physiol.*, **124**, 234 (1954).
[2] Stanbury, J. B., *J. Pharmacol.*, **93**, 52 (1948).
[3] Somjen, G., Hilmy, M., and Stephen, C. R., *J. Pharmacol.*, **154**, 652 (1966).
[4] Toda, N., and West, T. C., *Amer. J. Physiol.*, **212**, 424 (1967).
[5] Hilmy, M. I., and Somjen, G. G., *Amer. J. Physiol.* (in the press).

Effect of Methionine Sulphoximine on Memory Transfer

Methionine sulphoximine influences the metabolism in the central nervous system in a number of different ways[1]. It affects the glutamine formation in the brain[2], decreases the incorporation of phosphorus-32 in cytoplasmic ribonucleic acid[3] and decreases the incorporation of methionine labelled with sulphur-35 into the brain proteins[4]. Methionine sulphoximine is an epileptogenic agent[5] and in subconvulsive doses it blocks alimentary conditioned reflexes[6]. Injection of this compound causes pathological changes in the synapses where considerable quantities of the drug are found[7].

In our experiments, methionine sulphoximine was used to study the mechanism of "transfer of memory trace" from one animal to another by means of brain extracts.

White mice were trained to open a transparent door in front of some food pellets, which were identical with those used in normal feeding. A light above the door acted as a conditioning stimulus accompanying the food. The mice were trained three times a day, each session consisting of ten trials with an interval between each trial of 80 s. The conditioning stimulus lasted 10 s and the opening of the door was recorded electrically. Mice were considered to be trained when they gave ten positive responses in a session, that is, they opened the door within the time of the conditioning stimulus and did not open the door more than ten times between trials.

D,L-Methionine sulphoximine, in a dose of 150 mg/kg of body weight, was injected intraperitoneally 20–24 h before the last training session. This dose does not cause convulsions but blocks the conditioned responses in trained donors. The injected trained donors were then tested in the experimental cage. The mice were killed within 1 h of testing and the brains were removed and homogenized in 2 ml. of distilled water. The homogenate was shaken for 4 h, left in the refrigerator at 1°–2° C overnight and centrifuged at 10,000 r.p.m. for 4 h. The supernatant was injected into the peritoneal cavities of the acceptor mice, which were tested in the experimental cage 24 h after the injection. The animals were starved between injection and testing. The number of conditioned reactions in the first testing session was calculated and the results were evaluated by means of rank correlation analysis for dichotomic ranks[8].

Experiments were performed with forty-seven acceptor and forty-seven donor mice, in six groups as shown in Table 1. Injections of extracts from an untrained brain into untrained animals did not improve the animals' response in the testing session, as compared with that of untreated animals ($S=50$, P not significant). Injections of extracts from trained brains increased the number of positive reactions in the first session when this group was compared both with controls without injection ($S=122$, $P<0{\cdot}001$) and with the results from acceptors injected with extracts from untrained brains ($S=73$, $P<0{\cdot}001$).

Injection of the subconvulsive dose of methionine sulphoximine blocks almost completely the alimentary conditioned reflex. The number of conditioned responses decreased from ten positive responses to none. Only for one animal were two conditioned responses recorded. This block could not have been caused by a decrease of appetite, because the animals ate normally at the end of the testing session.

When extracts from brains intoxicated by methionine sulphoximine were injected into the untrained acceptors, it caused a significant increase of positive conditioned responses in the first testing session of ten trials. The rank analysis showed $S=127$ ($P<0{\cdot}001$) when compared with the values from control uninjected animals, and $S=78$ ($P<0{\cdot}001$) when compared with the animals injected with extracts from untrained brains. No difference was noted between the performance of mice injected

Table 1. RESPONSE OF ACCEPTOR MICE TO TESTS IN EXPERIMENTAL CAGE

Donors	Injection of donors	Acceptors	No. of mice	Average No. of positive responses
Trained	—	Untrained	8	6·25
Trained	MSI	Untrained	8	6·38
Untrained	—	Untrained	10	2·70
Untrained	MSI	Untrained	15	0·07
Untrained	MSI	Trained	6	10·00*
Untrained	—	Untrained	16	1·88

MSI, Methionine sulphoximine.

* First session after the injection.

with extracts from brains from trained animals and mice injected with extracts from brains from trained animals treated with methionine sulphoximine ($S=0$, P not significant).

Injection of methionine sulphoximine into untrained animals caused no apparent changes of behaviour in normal animal house conditions. Motility was unchanged, there were no convulsions and no change of appetite was noticed. When extracts of the brains of these animals were injected into the untrained mice, three of the fifteen animals died; of the twelve remaining, only one was able to find the food in the first experimental session. The others did not find their way to the food and so the elaboration of the conditioned reflex could not be started. These mice were retested at weekly intervals. Four weeks after the injection, nine of the twelve mice showed response to training. These mice were then trained for positive conditioned responses. The other three mice did not respond during this period.

The six control mice were trained and then injected with extracts from the brains of untrained mice treated with methionine sulphoximine. Injection of this extract had no effect on the formed conditioned reflex. The performance of these six mice was not changed.

Methionine sulphoximine in subconvulsive doses blocks the alimentary conditioned reflex, but it does not disturb the "inter-animal memory transfer" mechanism. The substance responsible for the influencing of the higher nervous activity of acceptor mice is therefore not damaged by the methionine sulphoximine.

RNA (ref. 9) or small proteins[10] are thought to be responsible for memory transfer, but these experiments throw no light on the chemical nature of the phenomenon because of the complex effects of methionine sulphoximine on metabolism.

Injection of extracts from brains of untrained donors treated with methionine sulphoximine has a detrimental effect on the higher nervous activity of the acceptor animals. If this agent, responsible for blocking the ability to be trained, is injected into the trained animal, it has no effect. It is not formed if the donor mouse is trained first. This implies that this substance enters the chain of events leading to the formation of normal memory trace and is able to block it. This can only occur in the situation where the memory trace is not already formed. There is therefore some kind of competition between the formation of the normal memory trace and activity of the agent formed in the untrained brain under the influence of methionine sulphoximine.

The factor causing the deterioration of the higher nervous activity of the acceptor mice is probably not methionine sulphoximine itself, because this factor is, in contrast to the compound, ineffective when the acceptor is pretrained before the injection.

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Received December 12, 1967.

[1] Koloušek, J., *First Intern. Meeting Intern. Soc. Neurochem.*, Strasbourg, Abstracts, 120 (1967).

[2] Koloušek, J., and Jiráček, V., *J. Neurochem.*, **4**, 178 (1959).

[3] Žák, R., Klicpera, M., and Koloušek, J., in *Proc. Conference on Use of Radioactive Isotopes in Biology and Medicine*, Prague (1958).

[4] Koloušek, J., and Lodin, Z., *Physiologia Bohemoslovenica*, **8**, 129 (1959).

[5] Misani, F., and Reiner, L., *Arch. Biochem.*, **27**, 234 (1950).

[6] Lodin, Z., and Koloušek, J., *Physiologia Bohemoslovenica*, Suppl., **5**, 43 (1956).

[7] Robertis, E., De Sellinger, O. Z., De Lores, R., Arnaiz, G., Alberici, M., and Zieher, L. M., *J. Neurochem.*, **14**, 81 (1967).

[8] Kendall, M. G., *Rank Correlation Methods* (Griffin and Co., London, 1955).

[9] Hydén, H., and Egyhari, E., *Proc. US Nat. Acad. Sci.*, **52**, 1030 (1964).

[10] Rosenblatt, F., Farrow, J. T., and Herblin, W. F., *Nature*, **209**, 46 (1966).

BIOLOGY

Pre-gravid Phase of Ovarian Development in *Aedes detritus* Haliday

IN *Aedes detritus* (Diptera, Culicidae) Haliday, complete ovarian development to the mature egg state does not always occur after blood feeding. A second blood meal, however, was found to result in complete oogenesis. The occasional need for two blood meals for egg production has been noted in a few tropical mosquitoes[1-5], but has not been reported in any Palaearctic species.

Ae. detritus overwinters in its immature stages. In the Poole area of Dorset, where the investigations reported here were undertaken, the adult biting season was from May to November. A proportion of hungry females which was caught each month in human bait catches was allowed to engorge on the author in the laboratory. Batches of five to ten of these individuals fed on blood were held in paper lined plastic tubes and supplied daily with a wad of cotton wool soaked in water. They were kept outside the laboratory in normal climatic conditions, except that they were protected from direct sunlight and rain. They were examined daily, and 3 days after they appeared to have digested all their blood meal they were dissected and the ovaries classified according to their degree of ovarian development[6]. Any mosquitoes which died within this period were dissected, and if all blood had been digested they were included in the results.

Results in Table 1 show that, whereas all 350 adults fed and dissected from July to September developed fully mature ovaries after one blood meal, a proportion of those caught and fed at the beginning and end of the adult biting season failed to undergo complete ovarian development. These adults are referred to as pre-gravids. The degree of ovarian development after a blood meal is different from that observed in *Anopheles gambiae* Giles and *A. funestus* Giles. In these species, because the ovaries only proceed to stage II, all pre-gravids can be reliably separated from gravid adults without dissection[1,2]. In *Ae. detritus* there was no clear distinction in the external appearance of the two groups, except in those in which ovarian development had not gone further than stage III*b*. Pre-gravids, however, could, with the exception of the few with the ovaries in stage II*b*, be distinguished from unfed adults. It is apparent that at certain times of the year, some of the biting population require two blood meals for completion of the first gonotrophic cycle. It follows that adults at bait will, at least at certain times of the year, form three distinct age groups: (*a*) nulliparous adults with undeveloped ovaries feeding for the first time; (*b*) nulliparous adults with partially developed ovaries taking a second blood meal; and (*c*) parous adults with undeveloped ovaries returning for further feeds. It should be possible to distinguish most of those in the second group from the others, and thirty-seven such pre-gravids were recognized in bait catches. When these were given a blood meal, ovaries developed completely. A mean fertilization rate of 94·7 per cent was recorded in those caught at bait (during months in which pre-gravids were in evidence) which underwent complete ovarian development after one blood meal. In pre-gravids the fertilization rate was 84·8 per cent. The slightly but significantly ($P<0{\cdot}01$) higher fertilization

Table 1. OVARIAN DEVELOPMENT IN *Aedes detritus* AFTER A BLOOD MEAL

Month	Ovarian stages II*b*	III*a*-III*b*	IV*a*	V	Percentage pre-gravids (II–IV)	Percentage fertilization in pre-gravids	Percentage fertilization in older females	Mean monthly temperature ° C
May	0	8	13	58	26·6	85·7	95·5	12·9
June	0	2	2	64	5·9	100	100	14·4
July	0	0	0	98	0	—	95·1	17·1
August	0	0	0	153	0	—	71·1	16·1
September	0	0	0	129	0	—	100	14·9
October	5	12	11	98	22·2	89·3	100	9·5
November	3	25	31	109	35·1	81·4	87·3	8·4

rate in older females indicates that some mating of pre-gravids occurs. In the absence of pre-gravids, the fertilization rate in August was only 71·1 per cent, indicating that mating may occur in gravid individuals.

There is no indication of the proportion of *Ae. detritus* that in certain months require two blood meals for completion of the first gonotrophic cycle. Some adults may by-pass the pre-gravid stage. It was impossible to resolve this in the laboratory because none of the fifty and sixty-nine unmated newly emerged adults maintained, respectively, on tap water and 10 per cent cane sugar solution would take a blood meal. In the first group the ovaries remained in stages I*a*–I*b*, while 92·7 and 7·3 per cent of those fed on sugar solutions developed their ovaries to stages II*b* and III*a*, respectively. There was therefore no evidence of autogeny in the population, as was reported from North Africa[7].

It is not fully understood why pre-gravids seem to occur in the population only at certain times of the year, but similar seasonal occurrences have been reported in *A. annularis* Wulp and *A. culicifacies* Giles in India[6]. The proportion of pre-gravids is greater in cooler months so temperature may be involved. Temperature may affect the quantity of food available for the larval population, and poor larval nutrition has been suggested as a reason for the need for two blood meals during the first gonotrophic cycle in certain species[3,8].

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[1] Gillies, M. T., *Ann. Trop. Med. Parasitol.*, **48**, 58 (1954).
[2] Gillies, M. T., *Ann. Trop. Med. Parasitol.*, **49**, 320 (1954).
[3] Macdonald, W. W., *Ann. Trop. Med. Parasitol.*, **50**, 399 (1956).
[4] Roa, V. V., *Indian J. Malariol.*, **1**, 43 (1947).
[5] Singh, J., and Mohan, B. N., *Indian J. Malariol.*, **5**, 513 (1951).
[6] Macan, T. T., *Mem. Lond. Sch. Hyg. Trop. Med.*, **7**, 109 (1950).
[7] Vermeil, C., *Bull. Soc. Pathol. Exot.*, **46**, 971 (1953).
[8] Clements, A. N., *The Physiology of Mosquitoes* (Pergamon Press, London, 1963).

Ultrasound in Rodents

During a comparative survey among small mammals, I found that ultrasounds were produced by the young of all species of myomorph rodents studied. Ultrasounds were also associated with aggressive and submissive behaviour in adult laboratory rats and with aggressive behaviour in the adults of several other species[1]. Observations have now shown that ultrasonic pulses are produced by more species and in more situations than were previously mentioned, and these are reported here.

Ultrasounds have now been detected from the young of *Microtus agrestis* (L.) and *Mastomys natalensis* (A. Smith), both myomorph rodents. Measurements of the intensity of pulses as well as high speed tape recordings for later frequency analysis have now been made for the young of eight species of myomorph rodents. The effect has also been observed in five other species.

Two functions for these sounds produced by baby rodents have been suggested by other authors who reported that they were produced in conditions of stress. They suggested that ultrasounds both aid the retrieval of young to the nest by the parent animal[2,3] and inhibit the aggression of the parent animal to what may seem a strange animal outside the nest. The results of the present intensity measurements may be important in this respect.

For most of the species studied, the intensity of the ultrasonic pulses produced when the animals were left alone under the microphone was lower and decreased at an earlier age than that of the pulses produced when the animals were handled. The intensity of pulses produced by mice of the C_3H strain which were left alone decreased from about the fifth to seventh day until the twelfth to fifteenth day, after which no ultrasound was detected in this condition. This decrease in intensity coincides with the period of development of homoiothermy in mice[4]. For a strain of white mouse, E. Noirot and J. D. Pye (personal communication) have found that the intensity of ultrasonic calls decreases sharply on the seventh day, which is about the day that the development of homoiothermy begins. The termination of calling of *Peromyscus*, elicited by cold stress, apparently also coincides with the onset of thermoregulation[5].

The intensity of ultrasonic pulses produced when young C_3H mice were handled decreased sharply from the eleventh day until the thirteenth to eighteenth day, after which it remained at a low level until observations ceased between days 21 and 37. The pulses became more difficult to elicit as the young reached the age when they leave the family group. Noirot[6] has shown that there is a difference in the rate of calling between young mice left alone in the cage and those being retrieved. From the present study it seems as though there are also differences in the frequency patterns of pulses produced in these two conditions.

Meriones shawi females make occasional attacks on their young as they become old enough to fend for themselves, and Rowell[7] has reported similar attacks by hamsters.

The differing curves for the change of intensity in conditions of handling and non-handling may support the previous suggestion that the ultrasound produced by baby rodents has two functions[2,3]. Those pulses produced when the animals are not handled may be elicited by conditions of stress, cold and hunger, and ultimately result in retrieval and therefore restoration of warmth and food. Their intensity decreases with the decreasing dependence of the young on the mother. Those pulses produced when the young are handled may tend to inhibit the aggression of the retrieving adult. These persist at a higher intensity, but eventually decrease as the young become old enough to protect themselves.

It was reported previously that ultrasounds were associated with aggressive and submissive behaviour in laboratory rats, but in mice no ultrasound has been detected at any time during aggressive behaviour although twelve fights between male laboratory mice have been studied and tape recordings have been made of three of these. This may be significant because in mice no clearly defined submissive posture has been observed which compares with that which often ends bouts of fighting in rats. In *Acomys caharinus* (Desmarest) no ultrasound has been detected during aggressive behaviour, but in *Clethrionomys glareolus* Schr. a few low intensity pulses were recorded during fighting. Ultrasonic pulses at about 70 kHz were detected from *Apodemus sylvaticus* (L.) when one animal of a group faced an intruder, and a few pulses were produced during fighting.

In my earlier work[1], ultrasounds were detected from laboratory and wild rats (*Rattus norvegicus*) when a male mounted or attempted to mount a laboratory female rat or another male, and were detected from mice during mounting and sniffing of either sex by males. Observations of mating in laboratory mice, both C_3H and Noirot's impure strain, have shown that pulses at about 70 kHz were always produced during mounting and seemed to be correlated with the pelvic thrusts of the male. Audible sounds, clearly coming from the females, were often heard at the same time, but were not synchronous with the ultrasound. Ultrasound has also been detected during mating in *Clethrionomys glareolus* and *Acomys caharinus*, and in both it seems to be correlated with the pelvic thrusts of the male. The production of ultrasound during mating is thus common to four species of rodents which seem to differ in the production of ultrasound in other situations.

Purely ultrasonic pulses were detected from adult golden hamsters, *Mesocricetus auratus* (Waterhouse), when another hamster, either male or female, was introduced into the cage of a female on heat. Pulses of 22–30 kHz were heard when the resident female nosed the stranger. This occurred both immediately on introduction and also later during the observation if the female on heat had maintained the rigid mating posture for several minutes without being mounted. Female hamsters are usually very aggressive towards intruders, so the ultrasonic signals may indicate willingness to mate.

In *Acomys* ultrasound at about 50 kHz was detected from a lactating female after her litter had been removed from the nest box.

Another situation in which ultrasound is produced has been found in *Apodemus sylvaticus*. Ultrasonic pulses at 70 kHz have been detected as animals emerged from the nest after the cage had been disturbed. When two pairs of animals were separately put into new cages, no ultrasound was detected immediately in either case, but after 3–5 min pulses were heard as the animals moved around their cages and stood upright on their hind legs. They were often detected while juveniles, 2–3 weeks old, were seen out of the nest and running round the cage in which they were born. It seems possible that in this species ultrasound has an exploratory or territorial function.

These observations in addition to those previously reported[1] show that, among myomorph rodents at least, ultrasound probably plays an important part in several different aspects of social behaviour.

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[1] Sewell, G. D., *Nature*, **215**, 512 (1967).
[2] Zippelius, E., and Schleidt, W. M., *Naturwissenschaften*, **43**, 502 (1956).
[3] Noirot, E., *Ann. Soc. r. Zool. Belg.*, **95**, 47 (1965).
[4] Lagerspetz, K., *Experientia*, **18**, 282 (1962).
[5] Hart, F. M., and King, J. A., *J. Mammal.*, **47**, 287 (1966).
[6] Noirot, E., *Anim. Behav.*, **14**, 459 (1966).
[7] Rowell, T. E., *Behaviour*, **17**, 1 (1961).

Growth Lines in the Larvae and Adults of Bivalve Molluscs

THE existence of growth lines on the shells of adult bivalve molluscs is well known, but little attention has been given to the significance of similar features in the larvae, although they appear in illustrations, such as those of Ranson[1].

In adults, growth lines are the outer edges of growth layers within the shell. The cyclic nature of these layers has been studied by Barker[2], who concluded that the five orders of layers represented growth cycles with periods of approximately 6 h, 24 h, 15 days, 6 months and 1 yr. He suggested that the layers result from periodical changes in temperature, salinity, tidal level and other environmental factors.

Larvae of the European oyster *Ostrea edulis*, when grown in the laboratory and when taken from the plankton, show a series of concentric rings on the shell. The rings are of two orders: major rings which seem to correspond in number to the number of days since the larva was released from the parent, and several minor rings between adjacent major rings. This arrangement is like that described by Barker for adult shells, with the larval major and minor rings corresponding, respectively, to the fourth and fifth order rings of the adult. Both the fourth order adult ring and the major larval ring seem therefore to represent a 24 h rhythm. But in the larva, at least, the occurrence of rings does not seem to result from environmental changes. Typical rings were formed in larvae cultured at constant temperature and illumination, without mechanical disturbance. Furthermore, larvae taken from incubating females of the New Zealand species *Ostrea lutaria* Hutton and the Chilean species *O. chilensis* Philippi have growth rings which number twenty or more. It is significant that in both species the period of incubation is much longer than in *O. edulis*, and extends to about 20 days in *O. lataria* according to Hollis[3] and to 5–6 weeks in *O. chilensis* according to Walne[4]. The number of rings in these species also increases with the number of days of larval life, although the larva during incubation is possibly less subject to daily environmental changes than are the pelagic larvae of *O. edulis*. The larval lines may therefore result from an endogenous rhythm rather than an environmental one. If this is so in the larva, it may also be true of at least the daily (fourth order) lines of adult bivalves. Environmental factors may, of course, modify the expression of an endogenous rhythm.

Barker suggested "laboratory growth experiments and ecological investigations in order to test the hypothesis that growth layering reflects environmental periodicities", and I would add that the part played by endogenous rhythms should also be investigated.

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[1] Ranson, G., *Rev. Trav. Inst. Pêches Marit.*, **31**, 205 (1967).
[2] Barker, R. M., *Malacologia*, **2**, 69 (1964).
[3] Hollis, P. J., *Zool. Publ. Victoria Univ. Wellington*, **31**, 1 (1963).
[4] Walne, P. R., *Nature*, **197**, 676 (1963).

Female Copulatory Behaviour in Male and Androgenized Female Rats after Oestrogen/Amine Depletor Treatment

FEMALE sexual behaviour patterns are infrequent in adult castrated male rats unless very large doses of ovarian hormones are given[1,2]. The age at which the rats are castrated is important[3-5]. Male rats castrated later than 10 days of age and treated as adults with oestrogen in combination with progesterone displayed little feminine behaviour, but when castrated 1–5 days after birth the same hormone treatment caused more distinct female mating behaviour. Absence of androgen a short time after birth leads to behavioural feminization of the male rat.

Testosterone propionate administered neonatally to female rats abolished later feminine mating behaviour in the intact female, and oestrogen and progesterone activated sexual receptivity in ovariectomized rats[6-7].

Recent investigations suggest that in the female rat there are monoaminergic pathways which mediate inhibition of female mating behaviour. The oestrous behaviour activated by oestrogen and progesterone in spayed rats was inhibited by increased central nervous concentrations of monoamine[8-10], but amine depletors such as reserpine and tetrabenazine induced oestrous behaviour in rats treated with oestrogen even when there was no progesterone treatment, which is otherwise necessary for a response[11].

This work describes the extent to which female mating behaviour can be activated with oestrogen in combination with amine depletors in male rats castrated as adults, and in female rats treated neonatally with testosterone and spayed as adults.

Sprague–Dawley female and male rats were raised in a controlled temperature (21°–23° C) under reversed day–night illumination (light on 9 p.m.–9 a.m.). This regime was maintained throughout the experiment. The size of each litter was reduced to eight animals on the third or fourth day after delivery. Half the females in each litter were injected when 4 or 5 days old with 1 mg/0·025 ml. of testosterone propionate in olive oil per rat. The remaining females were injected with olive oil only. Castration and ovariectomy were performed at 90–100 days of age and the animals were used in the experiment 3–4 weeks later. The female or male was brought to a selected vigorous intact male (Wistar strain[8]) and tested for female mating behaviour on three or four occasions between 10 a.m. and 6 p.m. Tests for mating behaviour (lordosis reflex) were conducted as described earlier[8,9]. Each animal was offered at least five mounts with a maximum of eight mounts per test. A positive mating response was recorded when a clear cut lordosis reflex was observed on at least two occasions or on the last test. The hormones were dissolved in olive oil. Reserpine ('Serpasil') and tetrabenazine hydrochloric acid ('Nitoman') were diluted with or dissolved in distilled water.

The female mating pattern was not seen in the castrated males after oestradiol benzoate alone although large doses (50 or 100 μg/kg) were given (Table 1). The combination of oestradiol benzoate (25 μg/kg) and progesterone (0·4 mg/animal) elicited a weak response: only two out of twenty animals displayed the lordosis response when mounted by another male. Increasing the doses of oestrogen and progesterone to the maximal dose in the female ovariectomized rat did not lead to any further response in the castrated males. Oestradiol benzoate (25 μg/kg) followed 48 h later by reserpine (1 mg/kg) or tetrabenazine (40 mg/kg) activated an explicit female mating response in 40–50 per cent. The treated males accepted mounting by a normal male and displayed an evident lordosis reflex. No darting activity or ear movements typical of the female in heat were seen.

The gross time relationship between the injection of oestrogen and the ability of the males to respond is shown in Table 2. Reserpine was given at the same time as the oestrogen treatment. Most males which responded did so in tests performed 52–58 h after the hormone/amine depletor treatment and continued to respond 24 h later. The sedative effect of treatment with reserpine was very slight in the 52–58 h and 76–82 h tests. The males moved spontaneously with only a slight decrease in locomotor activity and exploring behaviour. They still had some ptosis.

Females treated with testosterone propionate 4 or 5 days after birth had a decreased ability to respond to oestrogen/progesterone treatment (Table 3, *A* and *B*). The number of females treated with androgen which came into heat after treatment with oestradiol benzoate (10 μg/kg) and progesterone (0·4 mg/animal) was about 50 per cent of that of the controls. This relationship persisted even when a larger dose of the hormones was used (Table 3, *B*) or when progesterone was replaced by one of the amine depletors, reserpine or tetrabenazine (Table 3, *C* and *D*). No qualitative differences were seen between the lordosis response displayed by the controls and the animals treated with androgen.

Table 1. LORDOSIS RESPONSE IN MALE RATS CASTRATED AS ADULTS

Treatment At 0 h	At 48 h	Lordosis response %	No.
OEB 50 μg/kg	Oil only	0	30
OEB 100 μg/kg	Oil only	0	10
OEB 25 μg/kg	Prg (0·4 mg/animal)	10	20
OEB 50 μg/kg	Prg (1·0 mg/animal)	10	21
OEB 25 μg/kg	Reserpine (1·0 mg/kg)	50	24
OEB 25 μg/kg	Tetrabenazine (40·0 mg/kg)	40	10
None	Prg (1·0 mg/animal) and reserpine (1·0 mg/kg)	0	20

OEB, Oestradiol benzoate; Prg, progesterone.

Table 2. LORDOSIS RESPONSE IN MALE RATS CASTRATED AS ADULTS

Treatment	Hours after oestrogen injection	Lordosis response %
OEB (50 μg/kg) and reserpine (3 mg/kg)	28–34	9
	52–58	57
	76–82	48
	Cumulated:	61 ($N=23$)

OEB, Oestradiol benzoate.

Table 3. LORDOSIS RESPONSE IN ANDROGENIZED, OVARIECTOMIZED FEMALES

Treatment		Lordosis response		
At 0 h	At 48 h	Controls %	Treated rats %	Treated as percentage of controls
A, OEB (10 μg/kg)	Prg (0·4 mg/animal)	61 (23)	28 (21)	46
B, OEB (25 μg/kg)	Prg (2·0 mg/animal)	80 (18)	40 (15)	50
C, OEB (10 μg/kg)	Tetrabenazine (40 mg/kg)	72 (18)	36 (14)	50
D, OEB (10 μg/kg)	Reserpine (1 mg/kg)	77 (13)	33 (15)	43
E, OEB (10 μg/kg)	Reserpine (1 mg/kg) and Prg (0·4 mg/animal)	69 (16)	42 (12)	61

OEB, Oestradiol benzoate; Prg, progesterone.

Numbers in parentheses are the numbers of animals tested. Treated rats each received 1·0 mg of testosterone propionate in olive oil at 5 days of age. Controls had the same volume of oil (0·025 ml.).

Data from previous experiments with female rats suggest the existence of central nervous monoaminergic pathways which mediate inhibition of mating behaviour. These observations show that it is possible to induce female copulatory pattern in adult castrated male rats with oestradiol benzoate in combination with the amine-depletors reserpine or tetrabenazine. Oestradiol benzoate alone or followed by treatment with progesterone was less effective. These results indicate that the relationship between activation or components of the female copulatory behaviour and central nervous monoamines exists in the male as well.

In the androgenized female it is possible to substitute the amine depletors reserpine and tetrabenazine for progesterone to activate sexual receptivity. The difference in ability to respond between androgenized animals and the control group was not overcome by treatment with the amine depletors. It seems that reserpine or tetrabenazine treatment is more effective than progesterone in inducing the lordosis response in the oestrogen-primed castrate male. In the oestrogen-primed androgenized female, progesterone and amine depletors gave similar results.

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[1] Ball, J., *J. Comp. Psychol.*, **28**, 273 (1939).
[2] Beach, F. A., *Endocrinology*, **29**, 409 (1941).
[3] Grady, K. L., and Phoenix, C. H., *Amer. Zool.*, **3**, 482 (1963).
[4] Young, W. C., Goy, R. W., and Phoenix, C. H., *Science*, **143**, 212 (1964).
[5] Feder, H. H., and Whalen, R. E., *Science*, **147**, 306 (1965).
[6] Harris, G. W., and Levine, S., *J. Physiol.*, **163**, 42P (1962).
[7] Barraclough, C. A., and Gorski, R. A., *J. Endocrinol.*, **25**, 175 (1962).
[8] Meyerson, B. J., *Arch. Int. Pharmacodyn.*, **150**, 4 (1964).
[9] Meyerson, B. J., *Acta Physiol. Scand.*, **63**, suppl. 248 (1964).
[10] Meyerson, B. J., *Acta Physiol. Scand.*, **67**, 411 (1966).
[11] Meyerson, B. J., *Psychopharmacologia*, **6**, 210 (1964).

Stomatal Movements: a Yearly Rhythm

WHEN the effects of air pollutants on plants were investigated, the sensitivity of the plants to ozone or irradiated automobile exhaust was found to decrease during the winter months regardless of whether the plants were grown in the greenhouse or in controlled environmental conditions. Because the degree of stomatal opening may influence the degree of injury caused by air pollutants, a year's study of daytime stomatal movements was begun. Light, temperature, atmospheric pressure, relative humidity, time of day and month of year were the parameters studied.

Little has been published about endogenous rhythms with other than 24 h cycles. Bünning found an endogenous annual rhythm in germination of seed of various plant species[1]. Further experiments of his with seed of *Senecio vulgaris* and *Fragaria vesca* have indicated that the embryo assumes the yearly rhythm of the mother plant and that the time of maximum germination capability is independent of the time of seed maturation[2,3]. Bünning and Müssle[4] have since shown a yearly cycle in seed germination which is not affected by storage temperature and cold treatment. Transfer of an endogenous cycle from seed to plant has not been shown.

Certified seeds of *Phaseolus vulgaris* L., variety 'Pinto' (Haney Seed Co., Idaho), from stock stored at room temperature were planted in a standard peat–perlite mix and grown in the greenhouse and in the growth chamber. Two plants were grown in each 10·2 cm plastic pot. The pots were placed in a pan of Hoagland solution. Plants in five pots were started in both the greenhouse and the growth chamber each day.

In the greenhouse, atmospheric pressure, light intensity, temperature and relative humidity varied from hour to hour during the day and from month to month during the year. In the growth chamber, light intensity, temperature and photoperiod were held constant. Air entering the growth chamber and greenhouse was filtered through activated charcoal. Carbon dioxide levels at all seasons were always considerably below the 1,000–1,500 p.p.m. necessary to induce stomatal closure[5]. If there are yearly rhythms in stomatal opening as a result of photoperiod, temperature or changes in light intensity only, no rhythms or a reduced rhythm in the growth chamber would be expected.

The plants in the growth chamber were kept on a 16 h photoperiod under 1,900–2,200 ft.-candles using cool white fluorescent lamps with supplementary incandescent lighting. The temperature was held constant at 27° C during the day and at 21° C during the night. The greenhouse plants were grown under greenhouse temperature and light conditions, which varied throughout the year. The greenhouse experiment lasted from June 1965 until May 1966, and the growth chamber experiment lasted from September 1965 until August 1966.

Stomatal opening was measured with a modified Alvim porometer[6] on the eleventh day after planting. The time required for the manometer to drop from 40 to 30 mm of mercury was taken as the rate of airflow through the leaf and as a measure of the degree of stomatal opening. The readings were taken on the lobe of each primary leaf. Two readings, one on each of the two plants in a pot, were made at 2 h intervals during the day. The first reading was at 8 a.m., and the last reading was at 4 p.m. A new pot was used for each set of two-hourly readings in the greenhouse and in the growth chamber.

Fig. 1 shows that the stomatal openings are the smallest in December on plants grown in both the greenhouse and in the growth chamber. For plants grown in the greenhouse, the small stomatal openings might be attributed to the short photoperiod, the low temperature, or the low light intensity in December. For plants grown in the greenhouse the points on the curve are the monthly average values adjusted for changes in light, temperature and relative humidity. For the plants grown in the growth chamber the values of these factors are constant. The points on the curve are monthly average values of individual measurements. There was no statistically measurable effect of atmospheric pressure on stomatal opening in the greenhouse or the growth chamber.

There is a definite yearly rhythm in stomatal movement in 'Pinto' bean plants grown in the greenhouse and in the growth chamber. In both the greenhouse and the growth chamber the yearly rhythm was statistically significant at the 95 per cent level at least (Fig. 1). All months were significantly different from December, which was taken as the base month. As Fig. 1 shows, stomata of plants grown from November to February do not open as wide as those of plants grown from March to October. The yearly patterns for plants grown in the greenhouse and in the growth chamber are similar. The scale in seconds has been inverted so that the graph can give a clearer picture of the rhythm in stomatal opening. The smaller the stomatal opening, the longer the time required for the manometer reading to drop from 40 to 30 mm of mercury.

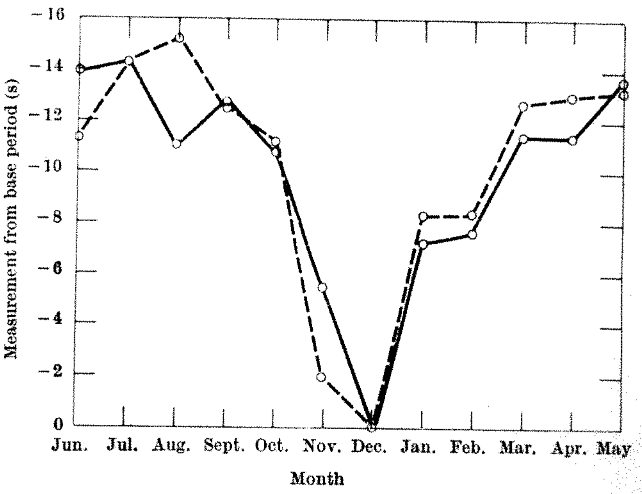

Fig. 1. Seasonal pattern in stomatal opening; —, growth chamber; - - -, greenhouse.

The question arises as to how the plants "know" when the seasons change and adjust their stomatal rhythm. Bünning's findings concerning endogenous rhythms passed on in seeds from the mother plant suggest that a "clock" set within the embryo at the time of seed formation continues to "keep time" as long as the seed remains viable and in this way the rhythm is passed on to the growing plant.

Our data suggest that the clock controlling the yearly rhythm of the stomata was set within the embryo at the time of seed formation. The results suggest also that this clock continues in the young 'Pinto' bean plant at least as long as the primary leaves remain attached.

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[1] Bünning, E., *Planta*, **35**, 352 (1947).
[2] Bünning, E., *Naturwissenschaften*, **35**, 221 (1948).
[3] Bünning, E., *Z. Naturforschung*, **4b**, 167 (1949).
[4] Bünning, E., and Müssle, L., *Z. Naturforschung*, **6b**, 108 (1951).
[5] Mansfield, T. A., *Physiol. Plantarum*, **18**, 79 (1965).
[6] Alvim, P. De T., *Physiol. Plantarum*, **19**, 308 (1966).

Red Pine Needle Structure

READERS of the recent article by Kozlowski and Clausen[1] on the anatomical responses of the needles of red pine (*Pinus resinosa* Ait.) to herbicides may have been confused by the extreme variations in needle structure which these authors described and illustrated. Kozlowski and Clausen attributed many of the anatomical differences between "needles" shown in their Fig. 1 to the effects of various herbicides applied to the soil in which the seedlings were grown. From what is known of the leaf structure of pine seedlings[2], however, we concluded that they had illustrated and described three different kinds of foliar appendages of red pine. Their Fig. 1*A*, a transection of a "needle" from a non-treated seedling, seems to be a primary leaf, as is Fig. 1*D* from a treated plant. Their Figs. 1*B*, *C* and *E* are cotyledons, and *F* resembles a shrivelled secondary leaf.

To verify our interpretation of their results, we have re-investigated the anatomy of red pine cotyledons and leaves. We tried to duplicate the experimental conditions of Kozlowski and Clausen, except that no herbicides were used. Red pine seedlings from two sources in Wisconsin were grown in soil in the greenhouse. The first seedlings appeared 3 weeks after planting. The first primary leaves developed 9 weeks after germination. They were followed about 5 weeks later by the first secondary leaves, which are borne in pairs on axillary short shoots. At 13 and 15 weeks after germination, cotyledons and primary leaves were collected from ten representative seedlings. Secondary leaves were collected from nine seedlings at 15 and 26 weeks. All samples were fixed in formalin–acetic acid–alcohol, embedded in tissuemat, sectioned at 12μ, mounted and stained with safranin and fast green.

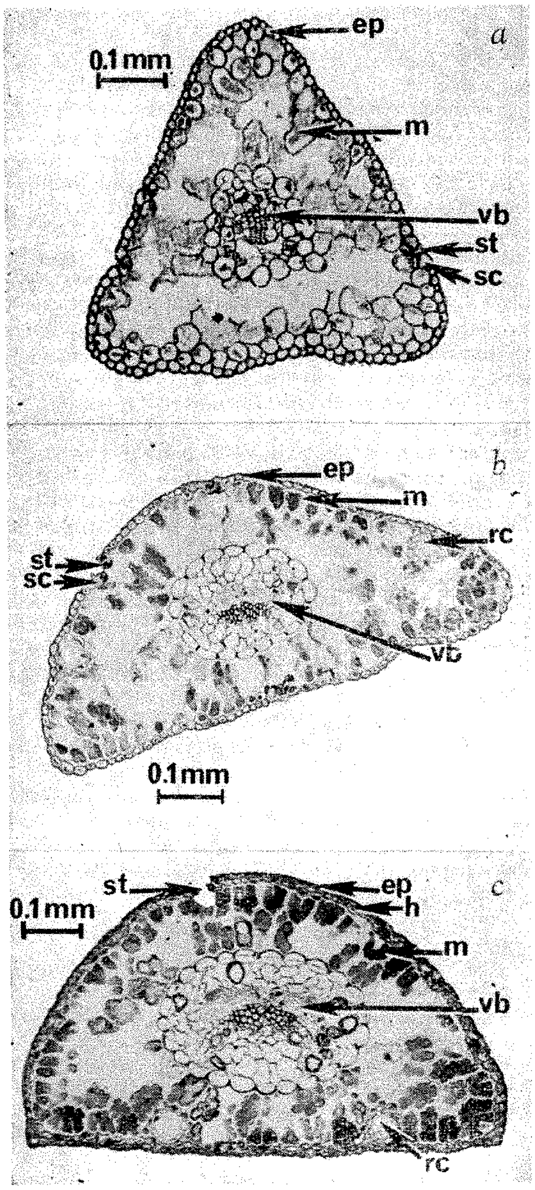

Fig. 1. Leaves of *Pinus resinosa* seedlings in transection: *a*, cotyledon of a 13 week old seedling; *b*, primary leaf of a 15 week old seedling; *c*, secondary leaf of a 26 week old seedling; *ep*, epidermis; *h*, hypodermis; *m*, mesophyll; *rc*, resin canal; *sc*, subsidiary cell; *st*, stoma; *vb*, vascular bundle.

The cotyledons of red pine are readily distinguished from all later-formed leaves by the absence of resin canals and by their triangular shape in transection (Fig. 1*a*). They also differ in having mesophyll cells which are usually rounded or undulate in transection, with most walls lacking internal ridges. The cotyledons have more extensive intercellular spaces than later-formed leaves, and the subsidiary cells of the stomata usually lack a flange. In common with primary leaves, the cotyledons have a single vascular bundle and thin-walled epidermal cells, and they lack a hypodermis.

The primary leaves differ from the secondary leaves of red pine in shape and in position of the resin canals, in addition to the features enumerated earlier: the single vascular bundle; thin-walled epidermis; and no hypodermis (Fig. 1*b*). The shape of the primary leaves in transection is variable, but the adaxial surface tends to be flattened and the abaxial surface rounded. The primary leaves lack the geometrically regular semicircular shape of the secondary leaves, however. The two external resin canals of the primary leaves are adjacent to the abaxial epidermis, in contrast to the adaxial position of the two principal resin canals in the secondary leaves.

Even the first-formed secondary leaves of seedlings have most of the features of the red pine needle illustrated, for example, by Esau[3]: semicircular shape; thick-walled epidermis; well-defined hypodermis; two principal resin canals in contact with the adaxial dermal layers; strongly ridged mesophyll-cell walls; and flanged stomatal subsidiary cells (Fig. 1*c*). Most of the secondary leaves of the seedlings have two vascular bundles—an invariable feature of the secondary leaves of older red pines.

It is clear from earlier work and from our observations that some of the anatomical effects which Kozlowski and Clausen attributed to herbicides are normal differences in structure between different kinds of foliar appendages. They considered these features to be the effects of herbicide treatments: the triangular shape of the treated leaves; the rounded shape of the mesophyll cells; the infrequency of internal ridges on the mesophyll-cell walls; and the extensive air spaces in the mesophyll. But these are all characteristics in which red pine cotyledons (see their Fig. 1*B*, *C* and *E*) usually differ from primary leaves which Kozlowski and Clausen used as a control (see their Fig. 1*A*).

Other effects that Kozlowski and Clausen reported may not have been a result of comparisons of different kinds of foliar appendages; some of these effects resemble herbicide-induced changes in the structure of secondary leavet of red pine reported by others[4]. Their conclusions make is evident, however, that future herbicide investigations which utilize an organism as complex as a pine seedling should be based on an understanding of the variety of foliar appendages that these plants can produce during their first season of growth.

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[1] Kozlowski, T. T., and Clausen, J. J., *Nature*, **209**, 486 (1966).

[2] de Ferré, Y., *Trav. Lab. Forest. Toulouse*, Tome II, Sect. I, **3**, Art. I (1952).

[3] Esau, K., *Plant Anatomy*, second ed.: Plates 78, 79 (1965).

[4] Shiue, C., and Hansen, H. L., *Hormolog*, **2**, 9 (1958).

BOOK REVIEWS

WEATHER LONG AGO

Histoire du Climat depuis l'An Mil

(Nouvelle Bibliothèque Scientifique.) By Emmanuel Le Roy Ladurie. Pp. 376+31 plates. (Paris: Flammarion, 1967.) n.p.

IT is now about a hundred and fifty years since the occurrence of ice ages at some time in the more or less distant past was first deduced from the identification of old moraines far down in the valleys of Switzerland and Scandinavia. The discovery was the product of an age that was anxious about current advances of the glaciers. The first arrangements by governments to organize and maintain daily weather observations, in France in 1775 and in Prussia in 1817, were inspired by anxiety over the behaviour of the seasons. There was also at that time an awareness in England and various other parts of Europe that the climate in the early middle ages (and to some extent in the first halves of the sixteenth and eighteenth centuries) had been more genial—enough to affect horticulture and the limits of the vine. It so happened that in the last quarter of the nineteenth century, when the first 100 year-long series of weather observations from a number of places became available for study, the prevailing temperatures after an interval of greater warmth between 1850 and 1875 had reverted to about the same values as when the series began. It was all too quickly concluded by the leading climatologists of the day that the climate was stable and that significant variability belonged only to the geological past. This view found its way into the textbooks; and it was not until about 1950 that the remarkable warming in the present century, which has produced historic retreats of the glaciers in most parts of the world and affected the prospects of agriculture and fisheries almost as widely and had a great impact on human habits, really brought the diagnosis into question. Since then the climatic trend seems to have reversed once more. So there is an urgent need for fuller knowledge and a better understanding of the phenomena of climatic change, to meet the demands of modern long-term economic planning.

Research has been proceeding on a small scale—a surprisingly small scale in relation to the economic stakes involved in forward planning of industrial and agricultural development—here and there wherever there happened to be interested individuals who could make an opportunity and had the necessary skills to pursue it. Institutions with departments fostering research in this field are rare: possibly the only ones deserving mention are the Center for Climatic Research established by Bryson in the University of Wisconsin Department of Meteorology and, for ancient climates, the Quaternary Research Sub-Department set up by Godwin in the Botany School at Cambridge—though other, smaller groups of interested palaeobotanists, meteorologists, oceanographers, geologists, glaciologists, radio-isotope physicists and others, in Scandinavia, central Europe, Russia and the United States have made important contributions to knowledge. The literature of the subject is correspondingly scattered. The quest for facts of past climates, delineation of the wind and ocean circulation patterns which controlled them, and physical interpretation of the development of climatic changes, require an exceptionally broadly based interdisciplinary enterprise which would properly demand the full resources of an interdisciplinary institute. This book is a remarkable testimony to what one brave man, possessed of furious energy and enthusiasm, can do; but it also reveals the breadth of the subject and the need for a co-ordinated attack on it.

Diverse researches on the evidence of the past have already uncovered the outline of a climatic sequence over the 10,000 years since the last ice age, which shows endless fluctuations of differing amplitude and on many time-scales, including a variation within the past thousand years such that the declining warmth and the precipitation changes between about AD 1300 and 1700 must have produced heavy strains on the primitive human economies of the time. Indeed, many of its impacts can be readily demonstrated, though historians have hitherto tended to attribute all such dislocations to the consequences of the Black Death and subsequent recurrences of the dreaded plague.

Historians have sometimes recently come under fire for working without reference to climatic stress except as an enemy the occasional intrusions of which on human affairs have been rare and entirely haphazard. Only in the history of Iceland and Scandinavia have the shifting climatic limits not been ignored—because they could not be. Professor Le Roy Ladurie, of the École Pratique des Hautes Études at Paris, the author of this book on climatic history from the year AD 1000, first found himself driven to the subject in his research on the agricultural history of the Languedoc, in the south of France. Now, after twelve years of wide study and reading, he has had the courage to produce not just a history of Europe which takes account of climatic events but a history of the climate itself.

The effort must have been enormous. The author has acquainted himself with most relevant matters from dendrochronology to radiocarbon and sunspot variation and has attained, on the whole, remarkably balanced judgments. The representative bibliography, like the meticulous documentation of his own historical researches, is a feature of the book. It is scarcely surprising that there are a few faults. One of the most glaring (on page 100), probably an accidental misquote or misunderstanding, concerns my own derivation of estimated average temperatures for the warmest period of the early middle ages. Quoting a summer temperature departure of 2° C above modern values for two centuries at that time, as in the much longer post-glacial warm epoch, Ladurie describes this as exaggerated: the actual published figure[1] derived by my analysis was an anomaly of +0·8° C, which I have mostly described as "about +1° C". Ladurie performs a valuable service to students in stressing the need for critical consideration of ancient texts before using them; but it is wrong to suggest that those meteorologists who have analysed the changing frequencies of various reported phenomena have omitted precautions about this. It is also untrue to say (page 22) that the wide agreement on the climatic sequence derived from these studies—for example, generally falling temperatures from the thirteenth to the seventeenth centuries and with sharper stages around 1315, 1430 and 1560–1600 and some let-ups in between—depends on the use and re-use of the same sources by successive investigators. It has received verification in recent years by the publication of previously unused source material—for example, two items in Ladurie's own bibliography: Titow's study of the weather recorded in the medieval rolls of the bishopric of Winchester[2,3] and Buchinsky's collection of reports contained in the chronicles of the medieval monasteries on the Russian plain[4]. In any case, Ladurie's own picture of the climatic sequence differs only marginally in the magnitude and duration of the intermission which culminated with the warm years of the 1520s and 1530s: on this last detail he produces perhaps the most useful analysis so far (page 236), including a demonstration of the biennial oscillation which is a subject of current interest in general circulation studies.

One regrets a certain undervaluing of the contributions of Flohn and Kraus (in these cases perhaps it is lack of thorough acquaintance), also of earlier pioneers like Huntington, Kinzl and Utterström; though C. E. P. Brooks comes in for legitimate criticism (page 213) for "romancing" about the so-called "little ice age" of recent centuries and putting an "interglacial" into it between 1680 and 1740 on little evidence—actually the 1690s are now known to have been about the coldest decade in Iceland, Norway and Britain, though not in the Alps. Any unfairness has clearly resulted only from the difficulty of attaining, or expressing, a balanced judgment quickly. There is some polemic, probably mainly intended to goad his fellow historians into tackling the subject and producing further critically examined data for scientific analysis. This style of presentation does mean, however, that one has to read a long way before discovering that the author's conclusion (for example, page 31) that the climate has been stable during the past two thousand years or more admits of oscillations great enough to allow several hundred years of disastrous advance of the glaciers (Brooks's "little ice age"—a convenient enough term) and of the polar sea ice.

Ladurie arrives at sensible estimates for the amplitudes and different period-lengths of the various fluctuations. The great glacier advances referred to were produced by a variation of average annual mean temperature amounting to only about 1° C. This should be sufficient warning of the precariousness of practical decisions based on an assumed constancy of climate.

The book makes no attempt at meteorological interpretation and contains not a single weather map. The author seems to have much less appreciation of shifts in world precipitation patterns than he has of temperature, though a selection of Fritts[5] maps of average tree-growth in different decades since 1576 in the western United States are reproduced. Chapters of the book are devoted to: (1) aims of his quest for facts of past climates, (2) the evidence of changes in the forests and vintages, (3) the warming of the climate from 1850 to 1950, (4) the problems and evidence of the "little ice age" which preceded the recent warming, (5) "working hypotheses" (in reality an assessment of the magnitude and course of the temperature changes which underlay the glacier changes of recent centuries), and (6) the turf moor at Fernau in the Austrian Alps. The last-named brings in a useful survey of the longer span of post-glacial climatic history and much little known detail on the sequence of glacier advances and retreats since 1500 BC in that locality; though it is an extreme course to generalize the history of Europe's glaciers from the record of a single one as registered in the peat bog near its snout.

I share the author's earnest hope that this book will serve to awaken interest in the climatic record and encourage many more historians to take it up. In the long run, the chief value of the book will, however, be its remarkable documentation, with the quoting or reproduction of many ancient texts and dated maps and pictures, of the glacier advance and retreat in Europe since AD 1200 as well as the tabulated data year by year since AD 1349 of the French vintages and its analysis of how far the vintage variations were controlled by weather.

It makes a fascinating book and one which will be bought for the charm of its remarkable sequence of pictures of the Rhône glacier and the Chamonix glaciers as well as for reference to the critically examined data it contains.

H. H. LAMB

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[1] Lamb, H. H., *Palaeogeogr., Palaeoclim., Palaeoecol.*, **1**, 13 (Elsevier, Amsterdam, 1965).

[2] Titow, J., *Econ. Hist. Rev.* (1960).

[3] *Proc. Conf. on the Climate of the Eleventh and Sixteenth Centuries—Aspen, Colorado, June 1962*, 38 (NCAR, Tech. Note 63–1, Boulder, 1963).

[4] Buchinsky, I. E., *O klimate proslovo Russkoi ravniny* (The past climate of the Russian plain), Leningrad (*Gidrometeoizdat*, 1957).

[5] Fritts, H. C., *Monthly Weather Rev.*, **93**. 421 (Tree-Ring Evidence for Climatic Changes in Western North America) (U.S. Dept. of Commerce Weather Bureau, Washington).

LABORATORY PHARMACOLOGY

Pharmacological Experiments on Isolated Preparations

By the staff of the Department of Pharmacology, University of Edinburgh. Pp. 131. (Edinburgh and London: E. and S. Livingstone, Ltd., 1968.) 21*s*.

THE publication of this new practical book from Edinburgh will be welcomed by most pharmacologists. Experimental procedures for setting up isolated tissue preparations are described in sufficient detail for a novice to attempt these with every expectation of success. The illustrations are simple and helpful.

There is a wealth of information of particular value to those concerned in the preparation of practical classes, notably about concentrations and doses of drugs, most of which are, quite rightly, expressed on a molar basis. The references have been selected for their relevance to practical details. One appendix tabulates molecular weights and solubilities of drugs. Others deal with the estimation of fiducial limits and the equations of drug-receptor interaction. These are all useful data. An appendix listing the basic apparatus required for the experiments might perhaps be included in a subsequent edition.

The use of capital letters to distinguish pharmacopoeial or approved names from "chemical names when these are simple" is quite unjustified. This convention, not always consistent, coupled with the use of italics for trade names (and for other drugs when these are part of a heading) will confuse rather than help the student.

The description of each experiment begins on a new page; the descriptions of the biological preparations themselves, some of which apply to more than one experiment, are less easily located—a bolder type rather than italics might be helpful. The table of contents is clearly laid out and obviates the necessity for an index.

All the experiments described are exclusively on isolated tissues and so can be performed by students without a Home Office licence. It is surprising how much pharmacology can be taught on tissues *in vitro*. Nevertheless, it is essential for pharmacologists to remember that the experiments in this book should form only a part of any practical course in pharmacology. They must be supplemented by demonstrations on anaesthetized animals and if possible by the students experimenting on decerebrate, spinal or pithed preparations and on themselves. Perhaps the authors could be persuaded to write a further volume dealing with these aspects of practical pharmacology. It would be as well received as I am confident this one will be.

E. W. HORTON

CANCER IN CANADA

Canadian Cancer Conference

Vol. 7: Proceedings of the Seventh Canadian Cancer Research Conference, 1966. J. F. Morgan *et al.* (editors). Pp. 432. (Pergamon Press, 1967.) $18; £6.

THESE volumes serve to bring together the many aspects of cancer research. This latest volume contains twenty-two papers and covers five major areas in the study of cancer. Discussion of cell division and proliferation and of metabolic regulatory systems account for more than half of the contributions. The properties of cell membranes, the effects of radiation on cells, carcinogenesis and cancer epidemiology take up the remainder of the volume. The papers are all of a review nature, but this has not restrained the authors from presenting their personal views and speculations. Each of the papers is so wide-ranging that it is a pity there is no index to help the reader find particular details quickly. The editors are to be congratulated for achieving a uniformly high standard in the presentation and content of the papers in this useful volume.

P. W. BARLOW

ALL WILD LIFE IS HERE

The Penguin Dictionary of British Natural History

By Richard Fitter. Assisted by Maisie Fitter. Pp. 348. (Harmondsworth, Middx.: Penguin Books, Ltd., 1967.) 8*s*. 6*d*.

THE naturalist in the field is the latest person to benefit from the valuable series of Penguin dictionaries.

In this case, as befits a dictionary of natural history, the greatest number of entries are names of animals and plants. There are descriptions of birds, butterflies, fishes, insects, reptiles, flowers, fungi and trees, entered under their common names. The binomial names of each species, and their vernacular equivalents, are given in an index of species at the end of the book. All phyla and classes are also entered and each animal or plant can be fully classified through two names which are given after each species entry. The student and amateur naturalist now have a readily available way of linking common names to scientific names, which they must find useful. The important task of identifying animals and plants is not, of course, aided at all, and the identification manuals are certainly not made redundant.

Although this is not a dictionary which is specifically concerned with biology, terms which are in use with the field naturalist are included. For example, mimicry, polymorphism, gene, polyploid, hetero- and homozygote are explained for the amateur—a mutation is described as "A change in one of the genes of a chromosome". Mitosis, meiosis and other technicalities are not here. Various other ecological and ethological terms are included when they are relevant to natural history. There are also entries dealing with physical, meteorological, geographical and geological phenomena—there is a geological time scale which should be very useful to all those who can never remember the order and dates of the various periods.

A paperback probably will not stand up well to life in the field, and so this dictionary has to be recommended as a useful addition to the naturalist's bookshelf.

MARY LINDLEY

MODERN ALGEBRA

Lectures on Modern Algebra

By P. Dubreil and M. L. Dubreil-Jacotin. Translated by A. Geddes. (University Mathematical Monographs.) Pp. xii + 364. (Edinburgh and London: Oliver and Boyd, Ltd., 1967.) 105*s*. net.

THERE are many textbooks on modern algebra. Most introduce the student to the usual structures, including groups, rings, fields, vector spaces and perhaps lattices. This book does this too; but there is a difference, in that there is a strong motivation coming from a hidden source. What is this source? It is the body of algebraic knowledge now referred to as commutative algebra. Even this subject is of recent origin, arising, as it does, from algebraic deficiencies of a fundamental character thrown up by a study of algebraic geometry. It is rather surprising then that nowhere in the book is there any mention of algebraic geometry or of commutative algebra. The nearest the author comes to this is on page 142 where, in an example, it is remarked that the set of polynomials (of a polynomial ring) "which vanish at a given point, or at k given points, or on a given set of curves and points, is an ideal". It is a pity that the contact with algebraic geometry is so marginal.

In spite of what has been said about motivation there is no doubt that the book is an excellent text for the student of modern algebra. It carefully develops the theory from the most elementary concepts, and, while it contains no exercises, there is a good sprinkling of illustrative examples. A particularly pleasing feature is the inclusion of a chapter on lattice theory (largely in order to discuss the lattice of ideals of a commutative ring) and a chapter on noetherian rings. The last chapter, essentially on field extensions, contains sections on integral dependence and on the famous *Nullstellensatz* of Hilbert. These remarks on content serve to indicate how closely the material in the book is oriented towards commutative algebra. The book is a translation of the second edition of *Leçons d'Algèbre Moderne*, and it is number six in the series of "University Mathematical Monographs". The usual high standard of printing by Oliver and Boyd is everywhere evident.

L. S. GODDARD

BOOK OF SPECTRA

Atlas of Electron Spin Resonance Spectra

By B. J. H. Bielski and J. M. Gebicki. Pp. xiii + 665. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 220*s*.

THE word "Atlas" is a bit misleading. This mammoth book, which must have taken a great deal of time and effort to compile, is more like a rambler's guide book—one of those interesting and often delightful works that take the reader down many quaint by-ways, thereby avoiding terrain that has become commonplace.

This book will appeal to the expert, both as a source of information and as something to browse in. Basically, it consists of well reproduced esr spectra together with the appropriate references, the source of the paramagnetic material, and its nature. The field of organic radicals in fluid solution is quite well covered, as is that of radicals in systems of biological interest. The field of organic or inorganic radicals in solids is very sparse and the huge fields of colour centres and transition metal complexes are almost entirely omitted. For example, there is just one lonely reference to F-centres, and I cannot help feeling that this should have been replaced by a clear statement that F-centres were not covered, together with a list of references to appropriate reviews on the subject.

More serious omissions arise in another way. In recent years many authors to save space, particularly in cases where good spectra have been obtained the interpretation of which is clear, have published the derived parameters rather than the actual spectrum. No mention of these studies is made and hence this atlas cannot in any sense be taken as being comprehensive even in the limited areas covered.

The work is completely uncritical and therefore often very misleading, for many incorrect or improbable "identifications" are quoted. Some of these relate to work of at least ten years ago, the authors of which would surely no longer hold to their original assignments.

A remarkable number of spectra are listed as "unpublished results", and in some cases one realizes why. However, because often no interpretation is then offered, the reader can amuse himself by testing his own interpretative skill.

The order of presentation is often most puzzling, and anyone who decides to read consecutively will be astonished at its vagaries. More serious, however, are the many errors.

The symbol a_{H} is a popular one for these authors and is applied in many cases to describe the hyperfine parameters for a large number of nuclei, not just hydrogen. Certain peroxy-radicals formed in the reaction of hydrogen peroxide with titanous salts are incorrectly described as the hydroxyl radical. The *N*-methyl pyridinium ion is said to be the source of an interesting esr spectrum and is described as the methylviologen cation radical.

The reader may be taken aback by the large number of spectra assigned to nitrogen dioxide. In fact, only two of these are anything to do with NO_2, the others being, in my opinion, various radicals formed by reaction between NO_2 and either the solvent or impurities contained therein.

Radicals described as NH_3^-, $C_2H_2^+$ and allyl alcohol diradicals are without doubt other species than these, and the presence of a hyperfine splitting from ^{129}Xe in irradiated CF_4 is certainly surprising !

It would scarcely seem necessary to illustrate spectra for polycrystalline diphenylpicrylhydrazyl, or indeed for aqueous Mn^{2+}, but to find both these spectra referenced as "unpublished results" is quite astonishing.

M. C. R. SYMONS

SENSE OF PERSPECTIVE

The Graphic Work of M. C. Escher

Pp. 24+69 plates. (London: Oldbourne Book Co., Ltd., 1967.) 42*s*. net.

"ALTHOUGH I am absolutely innocent of training or knowledge in the exact sciences," Escher writes in the introduction to this book, "I often seem to have more in common with mathematicians than with my fellow artists." In the principles of symmetry, inversion and topology that so dominate his work, Escher shares a common interest, superficially at least, with chemists and crystallographers as well as with mathematicians. Yet scientists no less than others will wonder whether the intriguing visual paradoxes that Escher conjures forth from the formal qualities of line and plane in fact transcend their own virtuosity.

Escher's impossible buildings, achieved by subtle perversions of the rules of perspective, are among the best known of his works. A genre that he has made his own is the exact filling of a space with two or more sets of discrete objects which interlock perfectly with each other. Another common theme is the blending into one composition of two mutually incompatible situations. "Day and Night", for example, shows on the left hand black birds flying over a daytime landscape and on the right hand white birds flying over the mirror-image landscape under cover of darkness; the centre of the picture, with remarkable dexterity, merges the two flocks of birds into each other and into the contraposed landscapes.

But after the tricks and the *trompe-l'oeil*, what remains ? Is there a serious substance in these drawings, or are they to be dismissed as amusing exercises in technique, no more significant than the visual illusions beloved of psychologists ? Escher himself points out that two of his motifs were taken from an article in the *British Journal of Psychology*, and the bizarre naivety of the grinning fishes and dwarflike men that populate many of the drawings cannot but confirm the impression of frivolity.

Beneath the pyrotechnics there is, perhaps, a profounder purpose. Lévi-Strauss, possibly with tongue untypically in cheek, has remarked of abstract art that it is concerned with style, not content, and that an abstract work shows the manner in which the artist would execute his pictures if he were by chance to paint any. In a similar way it seems that Escher is preoccupied with the conventions of representing three dimensions in two, and that his pictures are inquiries into the logic of perspective and the assumptions made both by the hand of the artist and the eye of the beholder. Escher says in his notes on the pictures, "The two dimensional is every bit as fictitious as the four dimensional, for nothing is flat". His work is an exploration of the absurdities that spring from this fiction and, it may seem, is a statement of the problems to be overcome before he could himself portray the world as he sees it. The plates grouped under the heading "Conflict between the Flat and the Spatial" are a remarkable and not merely entertaining exposition of these absurdities.

At two guineas this book is moderately priced, and to those who decline to accept it as art, its illusions should give a good run for their money. N. M. L. WADE

OBITUARIES

Professor J. L. B. Smith

PROFESSOR J. L. B. SMITH, the South African ichthyologist, died at his home in Grahamstown on January 7, 1968. At the time of his death he held the research professorship of ichthyology at Rhodes University.

Professor Smith's name will always be associated with the discovery and description of the extant coelacanth crossopterygian, *Latimeria chalumnae*, captured in December 1938 near East London. The circumstances surrounding this event, and the subsequent fourteen-year-long campaign to obtain a second specimen, were vividly described by Smith in his book *Old Fourlegs* (1956). This account revealed (as its author realized) a great deal of the man, and rarely can have been equalled as a frank character-study of a determined and dedicated man.

Born at Graaff Reinet in 1897, Professor Smith was educated at a number of schools including "Bishops" College, and the Victoria College, Stellenbosch. It was at Stellenbosch that his interest in chemistry was first aroused, and it was as a chemist that he joined Rhodes University College, Grahamstown, as a lecturer in 1923, after obtaining a Ph.D at Cambridge. During the First World War, Smith served with the South African Forces in East Africa.

From 1923 until 1947 Smith lectured in chemistry at Rhodes, producing several papers and three textbooks on chemical subjects. His spare time, however, was largely devoted to studying fishes. It was during this period that, in addition to his work on the anatomy of *Latimeria*, he laid the foundations for his most widely known work, *The Sea Fishes of Southern Africa*. In 1947, the South African Council for Scientific and Industrial Research awarded Smith a research fellowship which enabled him to resign his post in the chemistry department and thereby devote his full energies to ichthyology.

Smith's interest in fishes stemmed from an early contact with them as a sea-angler. This introduction left its mark on all his subsequent work which, as far as possible, he based on personal acquaintance with the fishes in the field. His love and enthusiasm for angling, and his appreciation of the angler's problems, led Smith into wide and profitable contact with the public. There is probably no other ichthyologist who can claim to have had queues forming outside bookshops on the day his regional monograph was published. Yet this was the reception that Smith's great work *The Sea Fishes of Southern Africa* received in 1949.

Essentially synoptic in its treatment, *Sea Fishes* was followed by a series of family and generic revisions of species from South African waters and the western Indian Ocean, as well as numerous shorter papers dealing with new taxa. In 1963, Smith and his wife published another important regional synopsis, *Fishes of the Seychelles*, covering an area the marine fauna of which had received scant attention since the mid-nineteenth century. In all, Smith produced more than two hundred papers and articles on ichthyological subjects.

Although there is a long history of ichthyological research in South Africa, Smith's investigations have undoubtedly added more to our knowledge of the fishes from this vast marine area than have those of any of his predecessors. Smith's work was essentially pioneering, and his attitude that of a field-worker faced with the task of mapping a complex and rich fauna still imperfectly known. At times this brought him into conflict with European and American workers, particularly on questions of nomenclature. The momentary heat-haze generated by these differences cannot, however, obscure the fact that J. L. B. Smith's drive and dedication resulted in a very significant contribution to ichthyology.

Professor Smith is survived by his wife, Margaret M. Smith, who was his close collaborator in the field and laboratory, and who was responsible for most of the illustrations in his many papers. P. H. Greenwood

Professor George Macdonald

Professor George Macdonald, one of the most dominant figures in the world of tropical medicine for twenty years, died on December 10, 1967, in University College Hospital.

The son of John Smyth Macdonald, FRS, Professor of Physiology at Sheffield and later at Liverpool, George Macdonald graduated M.B., Ch.B. at Liverpool University in 1924 at the age of 21 and took his D.T.M. in the same year. In 1925 he went as a research assistant to the Sir Alfred Jones Laboratory at Freetown, Sierra Leone, and in 1926 he published the first of his many papers on malaria. After graduating M.D. Liverpool and taking the London D.P.H. in 1932, he spent seven years in India and Ceylon and returned to England as assistant director of the Ross Institute in 1939. He joined the Royal Army Medical Corps and after a short period as D.A.D.H. Salisbury Plain Area he was appointed to form and command the first Malaria Field Laboratory. This unit had an adventurous career in Greece and Crete.

In 1942 he was appointed consultant malariologist to the Middle East Forces, and his advice saved the Eighth Army from burdening itself with hundreds of tons of antimalarial equipment during its advance to Tripoli. In 1947 he became the first professor of tropical hygiene at the London School of Hygiene and Tropical Medicine, and director of the Ross Institute.

He was a pioneer throughout his life. In Ceylon he devised an automatically flushing siphon for antimalarial drainage, the appearance of which was followed by a spate of "new and improved models". In London, when he turned his mind to the quantitative epidemiology of malaria, the answers to entomological questions he posed were not available; his reaction was to set up the Ross Institute's own entomological unit, now an established leader in research on anophelines. In 1957 he published his collected malariological work in *The Epidemiology and Control of Malaria* (O.U.P.), a book which mathematicians and others could read with equal pleasure and profit. He was a member of the World Health Organization's Expert Committee on Malaria from its inception, and (theoretical though his work seemed to some people) on several occasions he reasoned out epidemiological methods with strictly practical field application.

The epidemiology of malaria, an asexual infection in man, involved relatively simple mathematical calculations which were soluble with hand calculators—and with help from professional statisticians which he freely acknowledged. When he turned his mind to helminthic and therefore sexual infections the aid of a computer was needed. Characteristically, he learned to produce his own programmes and in due course published a mathematical model for the epidemiology of schistosomiasis. This too has thoroughly practical implications, not least in initiating healthy controversy in a new subject.

As a teacher Macdonald tended to lecture above cloud level. But his formidable brainpower and concentration were freely available to anyone who went to his office, and he solved the problems of hundreds of callers, correspondents and committees. He met the knowledge of impending death with great mental and physical courage, carrying on creative work and inspiring others to the last possible moment.

He was made C.M.G. in 1953, and received the Darling Foundation Award in 1954 and the Bernhard Nocht Medal in 1963. He was president of the Royal Society of Tropical Medicine and Hygiene in 1965–67.

B. B. Waddy

Professor Frank Raw

Professor Frank Raw died suddenly, in his forty-seventh year, on October 1, 1967, in Brisbane. He had been professor of entomology in the University of Queensland, Australia, for six months.

Graduating from Queen Mary College, University of London, in 1942, Raw began research in Cambridge under Salt and Hollick with the war-time team studying wireworms in agriculture, work which included pioneer studies of the general arthropod fauna of pasture and arable soils—a subject that remained Raw's chief interest. After two years he became an advisory entomologist at the Research Station, Long Ashton, later transferring to the National Agricultural Advisory Service, where he continued his work on insects of pasture soils with a broad ecological study of the garden chafer beetle.

In 1948 Raw joined the staff of Rothamsted Experimental Station, and remained there until 1967, specializing in the agricultural aspects of soil zoology. During this period, however, he spent two years at the West African Cocoa Research Station, in what was then the Gold Coast, studying the control of cacao capsids with chlorinated hydrocarbon insecticides; this early work has since led to much improvement in cacao growing.

Raw's work very successfully combined fundamental and practical approaches, and his zoological studies were always related to the effects of soil animals on the yield of crops, an aspect of research in agricultural entomology still much neglected. He continued to study the biology of wireworms at Rothamsted and he made experiments in collaboration with the insecticides department on the chemical control of these pests especially with chlorinated hydrocarbon seed-dressings; this notably contributed to current methods of control. Nevertheless, he also studied side-effects both of chlorinated hydrocarbon and of organophosphorus insecticides on the invertebrate animals in the soil. By improving methods of extracting these animals he revealed the unexpected abundance of Protura, a group that had before been considered as being sparse.

Raw's work on earthworms is well known and his technique of using formalin for sampling populations of worms is now a standard method. He showed, especially in apple orchards, how weather and chemical sprays affected the abundance and activity of worms, how these changes affected the burial of fallen leaves and the incorporation of leaves into the soil as nutrients for the trees; this work also raised problems of the removal from orchards of the spores of the fungus causing apple scab when worms buried dead leaves.

In his studies of the wheat-bulb fly, cut short at a most promising stage, he related the population dynamics of the insect to damage done to wheat crops. He studied the effects of fallow, both locally and nationally, of seed-rates, tilth, fertilizer treatment and different varieties of wheat on the response of the crop to attack by this pest. Raw was more aware than most workers that the abundance of a pest is only one of the many factors to be considered when studying loss of yield on crops caused by insect attack and that various ecological interactions between crop, pests and associated organisms are important in agricultural entomology. He had a special ability, not common among research entomologists, to see pests in a broad agricultural context. This wide approach is evident in Raw's publications and especially in his joint editing of the recent treatise, *Soil Biology*.

Raw was, for long, an active officer of the Association of Applied Biologists and for the past seven years its esteemed treasurer.

It was expected that he would influence the subject of agricultural entomology greatly in his new appointment, for his clarity of thought and of exposition was exceptional. C. G. Johnson

University News:

Glasgow

DR JOHN H. SUBAK-SHARPE, at present a member of the staff of the MRC Experimental Virus Research Unit in the Institute of Virology at Glasgow, has been appointed to the chair of virology in succession to Professor M. G. P. Stoker, who has been appointed to the directorship of the Imperial Cancer Research Fund Laboratories in London. Dr Subak-Sharpe will also succeed Professor Stoker as honorary director of the Experimental Virus Research Unit.

London

MR R. G. BURWELL, senior registrar at the Robert Jones and Agnes Hunt Orthopaedic Hospital, has been appointed to the chair of orthopaedics tenable at the Institute of Orthopaedics.

Newcastle upon Tyne

DR D. H. WHIFFEN, at present superintendent of the Division of Molecular Science in the National Physical Laboratory, has been appointed to the chair of physical chemistry.

Appointments

THE Royal Society has made its ninth appointment to a Royal Society research professorship and the holder of this appointment is Professor H. C. Longuet-Higgins, honorary professor in the Department of Machine Intelligence and Perception, University of Edinburgh, and until 1967 John Humphrey Plummer professor of theoretical chemistry in the University of Cambridge. Professor Longuet-Higgins will take up his appointment on April 1 and will work at the University of Edinburgh.

Announcements

THE Scientific Medal of the Zoological Society of London has been awarded to the following in recognition of their distinguished work in zoology: Dr J. B. Gurdon, Department of Zoology, University Museum, Oxford, for his work on nuclear transplantation; Dr J. E. Treherne, Department of Zoology, University of Cambridge, for his work on insect physiology, particularly the absorption of nutrients; Professor L. Wolpert, Middlesex Hospital Medical School, London, for his work on cell biology and embryology.

THE Institution of Mechanical Engineers has awarded the James Clayton Prize jointly to Professor F. T. Barwell of the Mechanical Engineering Department, University College, Swansea, and Mr Frank Nixon, chief consultant at Rolls-Royce Ltd., Derby.

THE following addendum has been sent by Dr Kenneth S. Warren and refers to the article "Cercariae of *Schistosoma mansoni* and Plants: attempt to penetrate *Phaseolus vulgaris* and *Hedychium coronarium* produces a Cercaricide", which appears on page 647 of this issue: Recently it was noted that cercariae are attracted to only certain varieties of green string beans. Although there were no obvious differences among the beans tested, this phenomenon is under investigation.

ERRATUM. In the communication "Solar Oblateness and the Perihelion Advances of Planets" by J. J. Gilvarry and P. A. Sturrock (*Nature*, **216**, 1283; 1967) the third paragraph on page 1284 should begin: "Because the accuracies in the determinations of the perihelion advances of Venus and the Earth are inadequate for the purpose, the only natural planet that could be conjoined with Mercury for a test equation (1) is the asteroid (1566) Icarus[9]. For this minor planet, the advance of the perihelion predicted by general relativity is 10·1 sec/century, exceeding that of any planet except Mercury[9], and the advance arising from solar oblateness would be 15·7 sec/century . . .". The sixth line of the second paragraph on page 1285 should read "with no observational disagreement", and not "with an observational disagreement". In the sixteenth line of the fourth paragraph on page 1285 reference 11, not reference 8, should be mentioned, and reference 16 should be inserted at the end of the following paragraph. This article was received on September 12, 1967, and not revised as indicated. The following note was added in proof: It has been pointed out recently by J. Ashbrook (*Sky and Telescope*, **34**, 229; 1967) that the results of Dicke and Goldenberg differ sharply with the careful observations of W. Schur and L. Ambronn by means of a heliometer over the period 1891–1902 (essentially a sunspot cycle) implying only an insignificant solar oblateness. Their values are less than one-hundredth, at most, of that reported by Dicke and Goldenberg. In reply, the latter value is defended by Dicke (*Sky and Telescope*, **34**, 371; 1967) as based on a differential measurement between the limbs, rather than on a visual measurement from limb to limb.

ERRATUM. In the communication "Imidazole and Sequestration of Calcium Ions by Sarcoplasmic Reticulum" by B. P. Yu, E. J. Masoro and F. D. DeMartinis (*Nature*, **216**, 822; 1967) the figures were wrongly numbered. The legend of Fig. 4 should accompany the graph of Fig. 5, the legend of Fig. 3 should accompany the graph of Fig. 4, and the legend of Fig. 5 should accompany the graph of Fig. 3. (A previous erratum to this communication should be ignored.)

ERRATUM. In the communication "Transducing Phage for *Pseudomonas putida*" by B. W. Holloway and P. van de Putte (*Nature*, **217**, 459; 1968) the phage *PMBL-4B*1 was incorrectly described as *PMBL-4Bl*.

CORRIGENDUM. In the communication "Mechanism of Olfactory Transduction" by Barnett Rosenberg, T. N. Misra and Robert Switzer (*Nature*, **217**, 423; 1968) the sentence beginning on line 23 of the right hand column of page 426 should read: "In the dark, the conductivity of crystalline chlorophylls was again higher, and the adsorbed gas could be easily desorbed. This process of irradiation and desorption could be repeated a few times, with diminishing effects, until it almost disappeared".

CORRIGENDUM. In the communication "Instability of Amitriptyline Base" by C. R. Henwood (*Nature*, **216**, 1039; 1967) Fig. 1*b* was incorrectly drawn. The carbonyl group should be attached to the ortho positions of the benzene rings as in Fig. 1*a*.

CORRESPONDENCE

Mink in the Wild

SIR,—In the issue of *Nature* of January 6 (**217**, 9; 1968), you published an interesting note on "Mink in the Wild".

Apart from a disagreement with the Ministry of Agriculture about the significance of the damage which "escaped" mink do, I want to point out a misunderstanding concerning the reproduction. On p. 10 it is mentioned that "The young are born in March . . .". This is completely wrong. The mink has its breeding season in March. The length of gestation period is on average 49 days, that is the young are born at the beginning of May, ranging from late April to middle of May.

Yours sincerely,

O. VENGE
prof. dr. agro.

The Royal Veterinary and
Agricultural College,
Department of Animal Genetics
and Animal Breeding,
Copenhagen V, Denmark.

FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, February 19—Wednesday, February 21

Materials Science Club (at the City University, St. John Street, London, EC1)—Informal Meeting on "Materials for Fuel Cells and High Energy Density Rechargeable Batteries".

Tuesday, February 20

Society of Chemical Industry, Agriculture Group (at 14 Belgrave Square, London, SW1), at 10.30 a.m.—Meeting on "Practical Problems in the Analytical Control of the Animal Food Trade".

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I.Mech.E., The Iron and Steel Institute, and the Institute of Measurement and Control, at Savoy Place, London, WC2), at 11 a.m.—Colloquium on "The Design of Mechanical and Electronic Equipment for Automatic Control in the Steel and other Heavy Industries".

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5.30 p.m.—Mr P. G. McLaren: "Static Sampling Distance Relays".

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr Naomi Datta: "The Vectors of Antibiotic Resistance in Bacteria". (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Electrical Auxiliary Systems for Nuclear Power Stations".

Society for Analytical Chemistry, Microchemical Methods Group (at "The Feathers", Tudor Street, London, EC4), at 6.30 p.m.—Discussion Meeting on "Automatic C.H.N. Analyzers".

Society for Analytical Chemistry, Special Techniques Group (in Lecture Theatre "C", Chemistry Department, Imperial College, London, SW7), at 6.30 p.m.—Professor W. Klyne: "Optical Rotatory Dispersion and Circular Dichroism; Applications in Structural and Stereochemical Studies"; Dr R. D. Gillard: "Circular Dichroism and its Analytical Potential".

Wednesday, February 21

Royal Institution, History of Science Discussion Group (at 21 Albemarle Street, London, W1), at 1 p.m.—Professor D. M. Newitt: "Origins and Growth of Chemical Engineering".*

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 2.30 p.m.—Dr H. A. Cole: "The Scientific Cultivation of Sea Fish and Shell Fish".

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5.30 p.m.—Mr H. G. Riddlestone: "Standardization and Approval of Electrical Equipment for Use in Hazardous Atmospheres".

Mental Health Research Fund (in the Edward Lewis Theatre, Middlesex Hospital Medical School, Cleveland Street, London, W1), at 5.30 p.m.—Dr Lyman C. Wynne (National Institute of Mental Health, Bethesda): "Schizophrenics and Their Families—Recent Research Findings and Implications" (Sixth Mental Health Research Fund Lecture).

Society of Instrument Technology (at the A.E.I. Cinema, 33 Grosvenor Place, London, SW1), at 5.30 p.m.—Mr B. W. Balls: "The Place of Computers in Process Control".

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Professor A. P. Waterson: "Viruses and Virus Diseases". (Second of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)*

Institution of Electronic and Radio Engineers, Aerospace, Maritime and Military Systems Group (at 9 Bedford Square, London, WC1), at 6 p.m.—Symposium on "Integrated Harbour Surveillance Systems".

University of London (at the Institute of Archaeology, 31–34 Gordon Square, London, WC1), at 6 p.m.—Mr G. P. B. Naish: "Sources of Maritime Archaeology—Early Ships in Britain".*

Wednesday, February 21—Friday, February 23

Institution of Mechanical Engineers (jointly with the National Council for Quality and Reliability, Ministry of Defence, at 1 Birdcage Walk, Westminster, London, SW1)—Conference on "The Reliability of Service Equipment".

Thursday, February 22

University of London (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 1.20 p.m.—Mr N. J. B. Plomley: "The Tasmanians—The Extinction of a Race".*

Society of Chemical Industry, Microbiology Group (joint meeting with the Food and Nutrition Subjects Group of the Royal Society of Health, at the Royal Society of Medicine, 1 Wimpole Street, London, W1), at 2.15 p.m.—Meeting on "Water Pollution and Food Production".

Royal Society (at 6 Carlton House Terrace, London, SW1), at 4.30 p.m.—Professor A. Neuberger, CBE, FRS, Mr A. Gorchein and Mr G. H. Tait: "The Formation of Chromatophores in *Rhodopseudomonas spheroides*; an Example of Cellular Differentiation".

University of London (in the Physiology Theatre, University College London, Gower Street, London, WC1), at 5 p.m.—Professor J. Baddiley: "Bacterial Cell Walls" (further lecture on February 29).*

Institute of Petroleum, Exploration and Production Group (at 61 New Cavendish Street, London, W1), at 5.30 p.m.—Mr W. H. Berry: "Construction of the Shell–Esso 30″/4″ Twin Submarine Pipeline from North Sea Block 49/26 to the Norfolk Coast".

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5.30 p.m.—Dr R. C. Valentine: "Virus Structure". (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)*

University of London (at the School of Pharmacy, Brunswick Square, London, WC1), at 5.30 p.m.—Dr G. J. Popjak: "Biosynthesis of Sterols and Polyisoprenoids. I. Biochemical Aspects" (further lecture on February 23).*

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (Fluid Machinery Section) (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p.m.—Discussion Meeting on "Hydraulic Stability of Pump Operations".

Society of Chemical Industry, Plastics and Polymer Group, Carbon and Graphite Group, and the London Section (joint meeting with the London Section of the Plastics Institute, at the Institution of Electrical Engineers, Savoy Place, London, WC2), at 6 p.m.—Dr L. N. Phillips: "Carbon Fibre Reinforced Plastics".

Friday, February 23

Royal Institution, Photochemistry Discussion Group (joint meeting with the University of Sussex, at the Royal Institution, 21 Albemarle Street, London, W1), at 10.30 a.m.—Meeting on "Spectroscopy and Photochemistry".

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5.30 p.m.—Discussion Meeting on "Operational and Economic Implications of Generating Plant Mix on Future Power Stations".

Royal Institution (at 21 Albemarle Street, London, W1), at 9 p.m.—Professor J. W. Boag: "Pulse Radiolysis".

Saturday, February 24

Inner London Education Authority (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3.30 p.m.—Mr William C. Sturtevant (Smithsonian Institution, Washington): "Indians of Eastern North America—Their History and Present State".*

Monday, February 26

University of London (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 5.30 p.m.—Professor A. W. Merrison: "The Science and Engineering of Elementary Particle Physics" (further lectures on March 4 and 11).*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Junior Research Associate or Research Associate (honours graduate in physics and/or geology) in the School of Physics for geophysical studies of the East African Rift System under the direction of Dr R. W. Girdler—The Registrar, The University, Newcastle upon Tyne 2 (February 26).

Lecturer and a Demonstrator (preferably applicants working in the fields of cellular and chemical biology, embryology and animal behaviour) in the Department of Zoology—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh, Scotland (February 28).

Lecturer (physicist or electrical engineer with a Ph.D. degree or similar research experience) in the School of Electrical Engineering, University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, February 29).

Senior Lecturer/Lecturer (with a good honours degree in psychology and research interests in the areas of social psychology and/or language development) in Education—The Secretary, The University, Stirling, Scotland (February 29).

Head (with high academic qualifications, several years' research experience, and preferably some knowledge of computing and programming languages) of the Statistics Section—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (March 2).

Lecturer (with previous research experience in aeronomy, preferably ionospheric physics) in Upper Atmospheric Physics in the Department of Environmental Sciences—The Secretary, University of Lancaster, University House, Bailrigg, Lancaster, quoting Ref. L 283/A (March 2).

Assistant Lecturer or Lecturer in Statistics or Operational Research in the Department of Theoretical Mechanics—The Registrar, The University, Nottingham (March 4).

Fellow in South-East Asian Studies, with specific reference to the regional geography of South-East Asia, in the Department of Geography—The Registrar, The University, Hull (March 4).

Lecturers (2) in Pathology at the Royal Infirmary, Glasgow—The Secretary of the University Court, The University, Glasgow (March 8).

Assistant Lecturer in Sociological Studies—The Registrar, The University, Sheffield (March 9).

Assistant Lecturer (with special qualifications in cellular, neuro-muscular or general physiology) in the Department of Zoology—The Registrar, University College, Cathays Park, Cardiff (March 9).

Senior Lecturer in the Department of Probability and Statistics—The Registrar, The University, Sheffield (March 11).

Chair of Morbid Anatomy at King's College Hospital Medical School—The Academic Registrar (A), University of London, Senate House, London, WC1 (March 15).

Chair of Numerical Analysis at Chelsea College of Science and Technology—The Academic Registrar (A), University of London, Senate House, London, WC1 (March 18).

Biochemist to assist with investigations in children in a busy routine laboratory; and a Chemist (with experience in gas chromatography) to join a team working on metabolic disorders in children—The Chemical Pathologist, Hospital for Sick Children, Great Ormond Street, London, WC1.

Lecturer (preferably with experimental interests) in Aeronautical Structures to assist in undergraduate and postgraduate teaching and research—The Professor of Aeronautical Structures, Department of Aeronautics, Imperial College of Science and Technology, London, SW7.

Research Demonstrator (with a good degree in botany or horticulture) in the Department of Horticulture, to assist with laboratory classes and carry out research in plant physiology—Professor O. V. S. Heath, FRS, University of Reading Horticultural Research Laboratory, Shinfield Grange, Reading, Berkshire.

Sedimentary Petrologist, an Exploration Geochemist, and an Exploration Geophysicist (all appointments involve instruction at the graduate and undergraduate level, and participation in research programmes)—The Chairman, Department of Geology, The University of New Brunswick, Fredericton, NB, Canada.

Solid State Physicists (Experimental and Theoretical) for teaching and research—Dr A. H. Morrish, Head, Department of Physics, University of Manitoba, Winnipeg 19, Manitoba, Canada.

Temporary Lecturer (with a good honours degree in psychology, some experience in teaching and research, and capable of taking charge of introductory courses in experimental psychology) in **Psychology**—The Registrar, University of Strathclyde, George Street, Glasgow, quoting Ref. 1/68.

Temporary Lecturers (2) (pure or applied mathematicians) in the **Department of Mathematics**—The Secretary, University College London, Gower Street, London, WC1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Potato Marketing Board, Sutton Bridge Experimental Station. Report No. 1: Investigations on Different Methods of Storing Potatoes. Pp. ii + 12. Report No. 2: Investigations on the Effects of Varying Degrees of Environmental Control on the Storage of Potatoes—Interim Report. Pp. ii + 18. Report No. 3: Studies on the Relative Efficiency of Different Systems of Harvesting and Handling Potatoes into Store—1965/66 and 1966/67. Pp. ii + 6. (London: Potato Marketing Board. 1967.) *Gratis.* [612

Ministry of Transport; Road Research Laboratory. RRL Report 95: The Glasgow Experiment—Schemes and Equipment. By J. A. Hillier. Pp. 15 + 3 plates. (Crowthorne, Berkshire: Road Research Laboratory, 1967.) *Gratis.* [612

Natural Environment Research Council. Report of the Council for the year 1 April 1966—31 March 1967. Pp. vii + 99 + 8 plates. (London: H.M. Stationery Office, 1967.) 11*s.* net. [712

The Commonwealth Foundation. First Progress Report. Pp. 24. (London: The Commonwealth Foundation, 1967.) [712

Building Research Station. Digest 88 (Second Series): Electrical Supplies for Construction Sites—2. Pp. 8. (London: H.M. Stationery Office, 1967.) 4*d.* [812

The BBC's Foreign Relations. By Donald Stephenson. (BBC Lunch-time Lectures Sixth Series—2.) Pp. 15. (London: British Broadcasting Corporation, 1967.) [1112

Royal Observatory Bulletins. No. 130: Orbital Elements of Nine Spectroscopic Binaries. By Edwin S. Barker, David S. Evans and J. D. Laing. Pp. E355–E367. (London: H.M. Stationery Office, 1967.) 2*s.* 6*d.* net. [1112

The Schools Council. Humanities for the Young School Leaver: An Approach Through Classics. Pp. ix + 30. (London: H.M. Stationery Office, 1967.) 4*s.* net. [1212

National Foundation for Educational Research in England and Wales. Twenty-first Annual Report, 1966–1967. Pp. 66. (Slough, Bucks: National Foundation for Educational Research in England and Wales, 1967.) [1212

Natural Environment Research Council. Annual Report of the Institute of Geological Sciences for 1966. Pp. viii + 197 + 8 plates. (London: H.M. Stationery Office, 1967.) 20*s.* [1312

Sulphur: The Essential Plant Food Element. Pp. 29. (London: The Sulphur Institute, 1967.) [1512

Ministry of Agriculture, Fisheries and Food, Fisheries Radiobiological Laboratory. Technical Report, FRL 1: Radioactivity in Surface and Coastal Waters of the British Isles. Pp. 45. (Lowestoft: Fisheries Radiobiological Laboratory, 1967.) [1512

Other Countries

Institut Royal Météorologique de Belgique. Bulletin Mensuel. Observations Ionosphériques, Septembre 1967. Pp. 26. (Ucce-Bruxelles: Institut Royal Météorologique de Belgique, 1967.) [412

United States Department of the Interior: Geological Survey. Bulletin 1242-E: Coal Resources of the Cape Lisburne–Colville River Region, Alaska. By Farrell F. Barnes. Pp. iii + 37 + plate 1. (Washington, D.C.: Government Printing Office, 1967.) [512

United States National Museum Bulletin 260: Bathyl and Abyssal Gammaridean Amphipoda of Cedros Trench, Baja California. By J. Laurens Barnard. Pp. vi + 205. (Washington, D.C.: Government Printing Office, 1967.) $1. [512

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Physics 1966–67. Pp. 39. (Sydney: Commonwealth Scientific and Industrial Research Organization, 1967.) [512

Directory of Physics and Astronomy Faculties 1967–1968–United States/Canada/Mexico. Pp. 201. (New York: American Institute of Physics, 1967.) $3. [512

Commonwealth of Australia: Department of National Development. Bureau of Mineral Resources, Geology and Geophysics. Bulletin No. 58: Upper Cretaceous Ammonites from the Carnarvon Basin of Western Australia. 1: The Heteromorph Lytoceratina. By R. O. Brunnschweiler. Pp. 58 + 8 plates. Bulletin 75: Mesozoic Mollusca from Australia and New Guinea. By S. K. Skwarko. Pp. 100 + 12 plates. Bulletin 83: Morphology and Sediments of the Timor Sea. By Tjeerd H. van Andel and J. J. Veevers. Pp. x + 173 + 5 plates. Bullletin No. 93: Geology of Bougainville and Buka Islands, New Guinea. By D. H. Blake and Y. Miezitis. Pp. vii + 56 + 15 plates. (Parkes, A.C.T.: Bureau of Mineral Resources, Geology and Geophysics, 1966 and 1967.) [612

International Atomic Energy Agency. Safety Series No. 6: Regulations for the Safe Transport of Radioactive Materials—1967 Edition. Pp. 117. 65 schillings; 17*s.* 8*d.*; $2.50. Safety Series No. 22: Respirators and Protective Clothing. Pp. 82. 52 schillings; 14*s.* 2*d.*; $2. Research Applications of Nuclear Pulsed Systems. (Proceedings of a Panel on Research Applications of Repetitively Pulsed Reactors and Boosters, held in Dubna, USSR, 18–22 July 1966. Panel Proceedings Series.) Pp. 234. 130 schillings; 35*s.* 4*d.*; $5. Recovery of Fission Products. (Bibliographical Series, No. 25.) Pp. 240. 130 schillings; 35*s.* 4*d.*; $5. (Vienna: International Atomic Energy Agency; London: H.M. Stationery Office, 1967.) [612

Proceedings of the Sixth Annual Tall Timbers Fire Ecology Conference, March 6–7, 1967, Tallahassee, Florida. Pp. v + 212. (Tallahassee, Florida: Tall Timbers Research Station, 1967.) [612

Geological Survey of Western Australia. Bulletin No. 118: Devonian Reef Complexes of the Canning Basin, Western Australia. By Dr Phillip E. Playford and David C. Lowry. Pp. 150 + 7 plates. (Perth: Geological Survey of Western Australia, 1966.) [612

Theoretical Foundations of Chemical Engineering, Vol. 1, No. 1 (January–February, 1967). Translated from the Russian. Pp. 1–104. Subscription (6 issues) $100. Single issue $30. (New York: Consultants Bureau, 1967.) [612

High Energy Chemistry, Vol. 1, No. 1 (January–February, 1967). Translated from the Russian. Pp. 1–75. Subscription (6 issues) $100. Single issue $30. (New York: Consultants Bureau, 1967.) [612

Yearbook of the Association of Pacific Coast Geographers, Vol. 28, 1966. Pp. 192. (Corvallis, Oregon: Oregon State University Press, 1967.) [712

U.S. Department of Agriculture. Agriculture Information Bulletin No. 237: Controlling Insects on Flowers. By Floyd F. Smith. Pp. ii + 81. (Washington, D.C.: Government Printing Office, 1967.) $0.45. [712

Wattle Research Institute (South African Wattle Growers' Union—University of Natal—Government Department of Forestry)—Report for 1966–1967 (Twentieth Year). Pp. 55. (Pietermaritzburg: Wattle Research Institute, University of Natal, 1967.) [712

Science Policy Bulletin, No. 1 (October 1967). Pp. i + 32. (Columbus, Ohio: Battelle Memorial Institute, Columbus Laboratories, 1967.) [812

Serial Publications, Monographs, and Pamphlets issued by CSIRO. Pp. 63. (East Melbourne: Commonwealth Scientific and Industrial Research Organization, 1967.) [812

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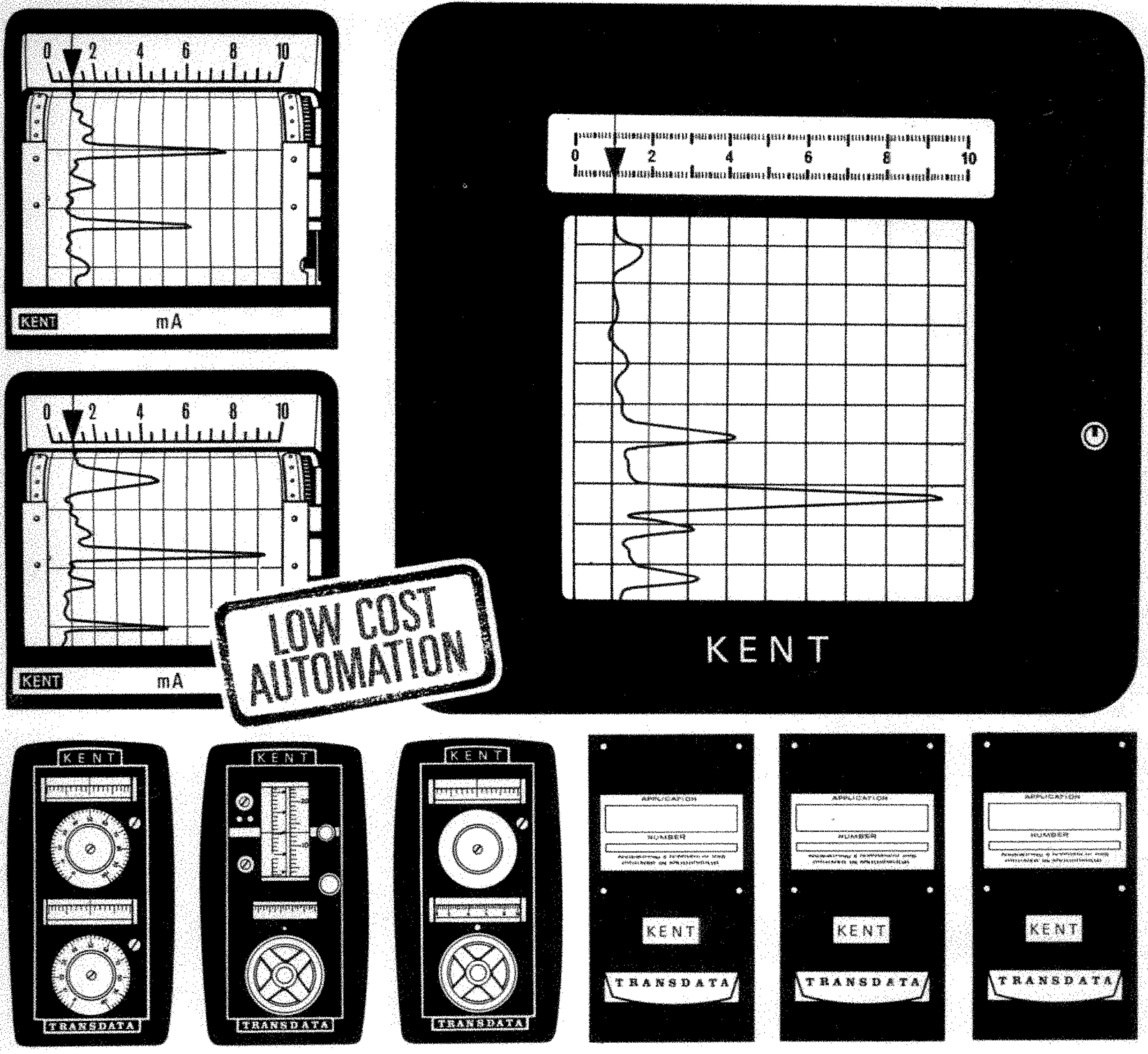

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NATURE Volume 217 FEBRUARY 24, 1968

What Price Roads?

ROMANTIC attitudes towards highways must now be almost extinguished in industrial societies, what with traffic accidents, air pollution and the like. Any doubts that may remain will, however, be dispelled by the appearance in the past week of a series of economic calculations by the Ministry of Transport which is intended as a basis for future attempts to apportion the costs of operating the highways system between the different classes of road users (*Road-Track Costs*, HMSO, 11*s*.). In practice, the study is an attempt to apply in British conditions some of the methods which have been developed elsewhere in the past decade or so, particularly in the United States and Canada. The British document is more than a mere academic study, however, for it is plainly intended as a theoretical justification of the decision by the Ministry of Transport to levy higher charges on heavy goods vehicles using British roads—one of the several controversial aspects of the traffic legislation with which the British Parliament is at present exercised. Indeed, there is some internal evidence that the economic argument, which is intricate enough to satisfy any taste, would have been more valuable if its authors had had more time to think about it.

The essence of the difficulty is that a road system is an integrated whole which is used as a whole by all kinds of users. The authors even use, as support for their argument, the old textbook example of how a sheep farmer cannot choose to produce wool alone, or mutton alone, but only some combination of these, but there are some readers who will ask whether there may nevertheless be a case for a less cursory examination of the economic advantages of traffic segregation. Most of the argument of the report is, however, taken up with a consideration of the various systems upon which the costs of operating roadway systems should be apportioned, and there is an air of didactic certainty about the way in which the report comes round, after much judicious consideration of other possibilities, to the view of what is called "long-run marginal cost pricing"—the impeccable principle that road users of different kinds should be saddled with that proportion of the total cost of building and maintaining the road system which is attributable to the use they make of it.

The trouble, as the report repeatedly acknowledges, is that there is an enormous gap between principle and practice. Thus in estimating the proportion of the capital expenditure on roads to be attributed to different kinds of vehicles, the ministry's argument has to rely on the rule of thumb that a heavy goods vehicle should bear twice as high a cost as a private car. Readers will be tempted to ask why this number is a better guide to good practice than the number three which the Ministry of Transport used in its evidence to the Geddes committee some years ago. Owners of heavy goods vehicles will more pertinently want to know how a calculation which involves such a large uncertainty can be used to justify an increase of about 5 per cent (£30 million a year) of the taxes levied on road users both directly and by means of fuel, especially when this increase falls exclusively on heavy goods vehicles. In the same way, there is more than a trace of arbitrariness in the way in which the ministry argues that if heavier vehicles tend to travel greater distances, there is a case for increasing the annual tax which they must pay. Is there not at least an equal case for putting up the fuel tax ?

These, however, are comparatively minor reasons for believing that the ministry has set out to design a basis for costing which is much more refined than circumstances will at present allow. The practical conclusion which is likely to be made much use of in the House of Commons in the weeks ahead is that the goods vehicles are responsible for 28·9 per cent of the cost of operating the British road system, but that they contribute only 24·8 per cent of the revenue raised from all road users. Its particular weakness is that the uncertainties of the calculation may be much greater than the difference between the two percentages. A more serious difficulty, however, is that the amount of revenue raised by taxation is roughly twice the cost of operating the road system in Britain. In circumstances like these, there is, of course, no point in seeking delicately to balance costs and receipts—and there is no serious hope of attempting to explain away the difference by vague talk about the "community cost" of air pollution and the like. The real truth is that British governments have decided to tax road transport of all kinds more heavily than costs alone would justify, and within this framework it is—and should be—a political matter to decide which kinds of road users should be let off most lightly. This, for example, is the spirit in which public service vehicles are undertaxed—and will continue to be. At a time at which the health of British industry is in everybody's mind, it would have been understandable if the Ministry of Transport had sought to reduce the costs of industrial transport. But it is, of course, well known that the ministry would like to divert some heavy road traffic to the railways (which at present cost a subsidy of nearly half the cost of running the British roads) and that is a legitimate intention if the object is really to make better use of under-used resources. And that, as it happens, would be a calculation of the kind to which the ministry's marginal costings could be much more accurately applied. In the homely language of the economists, this would not be a matter of asking sheep farmers to produce mutton and no wool, but of asking them whether they would like to produce cattle instead.

Making ESRO Ship-shape

THE European Space Research Organization is putting its house in order in preparation for the Third European Space Conference, which will take place in Bonn in June. The European Space Conference deals with the political aspects of a co-ordinated European space policy, and meets at least once a year at ministerial level. The previous meeting, at which Mr John Stonehouse, Minister of State for Technology, was Britain's principal representative, was held in Rome in July last year.

One of the outcomes of the Rome conference was that no new projects could be initiated by ESRO before the Bonn meeting, a decision expressing some dissatisfaction with the administrative and economic structure of ESRO. The new director-general of ESRO, Professor Hermann Bondi, who succeeded Professor Pierre Auger in November last year, sees as his main task the problem of getting ESRO into trim ready for the Bonn meeting. This involves the implementation of the findings of a report on the organization's structure, internal procedures and methods of work, prepared by a group of experts under the chairmanship of Mr. J. H. Bannier, a former chairman of the Council of CERN, the European nuclear research organization. The conclusions of the Bannier report included some reorganization of the managerial structure of ESRO, and the recommendation that the Council of ESRO should devote a greater proportion of its time to defining the policy and objectives of the organization. Professor Bondi is hoping that the reorganization at present under way will persuade the Bonn conference to lift the ban imposed last year.

Projects already in progress are not affected by the standstill. Three ESRO satellites are expected to be launched during 1968, and there is an extensive programme of small rocket launchings planned. A second attempt to launch the satellite ESRO-2 will be made in April—the first attempt failed because the third and fourth stages of the Scout launching rocket, fired from Vandenburg range in California, did not function. Although ESRO-2 was not placed in orbit, it transmitted signals up to eight minutes after the launch, an encouraging sign that the satellite itself was working correctly.

ESRO-1 will also be launched by a Scout rocket, probably in early October, followed in November by the launching of HEOS-A by a Delta rocket. HEOS-A will be ESRO's first satellite to be placed in a highly eccentric orbit, its purpose being the investigation of atomic particles and magnetic fields outside the magnetosphere.

Sounding rocket firings in 1968 will be based on ESRO's range at Kiruna, in northern Sweden. Most of the launchings will be from Kiruna, except four payloads which for scientific reasons cannot be launched at high latitudes. These experiments involve astronomical investigations which may be affected by auroras and studies of geophysical phenomena only observed at middle latitudes, and will be flown from a range in Sardinia.

The diverse experiments planned for the Kiruna flights include studies of auroral disturbances in the ionosphere, investigations of micrometeorites, and measurements of galactic X-rays. Perhaps the most interesting launching from the range in Sardinia will be an experiment to study the X-ray spectrum of the Sun, using a Skylark rocket fitted with a device that keeps the rocket pointing at the Sun.

Although the Rome conference recognized that a merger of the three European space organizations—ESRO, ELDO and CETS (the European Conference for Telecommunications by Satellites)—was not yet possible, a unified European space organization is likely to emerge in the future. This problem will also be receiving much of Professor Bondi's attention in 1968.

Threat to French High Energy Physics

from our Paris Correspondent

THERE are unaccustomed signs of discontent at present among the 600 French physicists who are specialists in high energy physics and who are preoccupied by the uncertainty hanging over the future of their subject. There are petitions circulating and collecting signatures, animated meetings in laboratories and a good deal of lobbying along the corridors of power. The publication next month of the report of the Consultative Committee for Research can doubtless only aggravate this state of affairs.

The twelve "wise men" (two doctors, two economists, a mathematician, a chemist, three physicists, a scientific agriculturist, a business man and a sociologist) who make up this committee responsible for advising the government departments on scientific policy were asked a year ago to furnish recommendations on the future of high energy physics. One of the important issues in this study was obviously the decision relating to the plan for a 45 GeV proton accelerator. The committee has just presented to the government a document of some 30 pages summarizing the results of its work. This report analyses the present situation and is most complimentary about the excellence of the French teams, which have, in the committee's view, assured for themselves an enviable position, particularly in CERN.

This expression of satisfaction for the men themselves is accompanied by rather more stringent comments on material resources. "French physicists, who for some time have been at an advantage by comparison with their European colleagues, now find themselves making use of more old-fashioned and generally less powerful machines." After such a preamble, the report's conclusions are extremely surprising. The committee judges, in effect, that high energy physics has for a long time benefited from a priority which, if continued, would compromise the progress of other subjects, and therefore urges that the increases of expenditure and in the number of research workers between now and 1980 should be curbed. The spending on fundamental research should be generally cut from 17·3 per cent to 15 per cent—indeed, even to 13 per cent of the research and development budget during this period—and the ceiling for expenditure on particle physics should be pegged at 5,400 million francs instead of the 6,645 million francs which was thought to be the necessary minimum sum by the working party on large accelerators. The rate of growth in the number of physicists would be limited to 3 per cent (those most concerned with the matter were asking for 5 per cent).

On the question of the 45 GeV machine, the wise men did not make any categorical statements: they are in favour of building it if it does not imperil the budgetary restrictions outlined here, which makes it something of a problem. On the other hand, the committee does emphasize the urgent need for drastic reorganization, bringing together again all the activity at present dispersed and, above all, recovering the work now under the wing of the Atomic Energy Commission.

The physicists are not unanimous in their reaction to these points, which cannot in any case be examined seriously from the point of view of France alone. The uncertainty hanging over the 300 GeV of CERN, the ambiguous attitude of Germany which had envisaged a Franco-German 45 GeV and which, tired of Paris beating about the bush, is now talking of building such an accelerator itself, has decidedly complicated the issue. However, whatever the position of the French particle physicists on the opportunity for building the 45 GeV machine and on the choice of technical solutions, they would, at all events, like the government to make a quick and clear decision. They are also surprised, and apparently justifiably so, that the question of priority from which their subject has benefited is being called into question at this point, particularly so because the committee of wise men states that the effort has been a "paying one". A change in policy will surely compromise the results so far achieved—these have already for some time been a bit precarious because of the antiquated equipment —without necessarily making certain of marked progress in other fields of research. However, "a fine sprinkling of funds" has so often been a characteristic of French research and development programmes.

Machine Minding

Next month the British Computer Society will have to decide whether to go ahead with the plan to turn itself into a professional body. The plan, put forward in June 1967, proposes that the society should become the learned institution for computer scientists, establishing four grades of membership—student, associate, member and fellow. Ultimately, admission to the society will be controlled by examination, and the society aims to do for the computer scientists what the engineering institutions have done for the engineers.

Not all the present members of the society are enthusiastic about the plan. Some say that computer science is still too young and diffuse a discipline to be easily examinable, others that experience will always be worth much more than paper qualifications. Many of the existing members of the society are computer users rather than scientists, and one member of the society compared the present efforts with a hypothetical situation in which all those who used television sets had to become qualified as electronic engineers. For all this, the plan is almost certain to be approved at the extraordinary general meeting at the end of March.

The simple reason for this is the remarkable way in which the membership of the society has increased since the plan was announced. Although the reference books list the membership of the society as 3,000, it has now reached a figure of 18,000, 10,000 of whom have joined since the new plans for the society became public knowledge. Although the secretary of the society, Mr J. G. Mackarness, sees this as evidence of general approval for the society's plans, others see it as a rush to get into the society before the examinations are established. Existing associate or ordinary members will be exempt from the examinations if they have more than seven years experience—if not, they will have to serve an apprenticeship as subscribing members until the seven years are up, when they will become members without examination. Existing student members will only have to take examinations if they are under twenty-one on May 1 this year.

In fact, the society had a problem in pitching the terms at exactly the right level. If all existing members had to take examinations—as properly they should if the society is to become as respectable as the engineering institutions—then new applicants would have been discouraged. The society admits that its reserves are "tiny", and hence needed to increase its subscription income substantially. This has been done most successfully, but possibly at the cost of losing credibility as a learned society. Moreover, subscription income will increase substantially again soon, for the rate for subscription is to double from £5 a year to £10 for members—a figure "very much in line with other professional bodies" according to Mr Mackarness, particularly as publications of the society are sent free to members. When the decision is taken at the end of March, only a fraction of the membership will be able to be present, but the rest—including those who have joined in the last year—will be able to vote by proxy.

Too Many Institutions

Undeterred by the refusal of the Institution of Heating and Ventilating Engineers to contemplate a merger with his institution, Mr H. G. Conway, president of the Institution of Mechanical Engineers, had another try on February 16. He was invited to speak at the annual dinner of another engineering institution, the Institution of Plant Engineers, and made use of the occasion to offer the plant engineers a merger with his own institution. Mr Conway was anxious not to be cast in the role of an empire builder—"My own institution has no territorial ambitions or illusions of grandeur. We presidents come and go. . . . We speak of our profession as our conscience may dictate. Some of us feel that if a few of our institutions could get together we would have benefited the engineering profession as a whole".

The terms offered for the merger are generous, and very similar to those turned down by the Institution of Heating and Ventilating Engineers (see *Nature*, **217**, 498; 1968). The plant engineers would form a divisional section with a good deal of autonomy. They could elect their own president and council, hold their own meetings and publish a journal. The alternatives, Mr Conway suggested, were either to apply for status as a chartered engineering body—which he thought unlikely to succeed, as there was a general view that the present proliferation of institutions is wrong—or become a technician body and aspire to chartered technician status.

The Institution of Plant Engineers is taking the offer quietly. It says only that it has "taken note" of Mr Conway's suggestion, which will be "carefully considered by the council of the institution", and a further statement will be made in due course. The mechanical engineers will be hoping that this time their efforts to act as a reorganization corporation for the engineering industry come off. Others, scanning the list of engineering institutions, can easily see some candidates for mergers. The Institution of Railway Signal Engineers and the Institution of Locomotive Engineers look obvious bedfellows. And it is hard to see why the Institution of Mining Engineers, which looks after coal and iron ore mining, should be a separate body from the Institution of Mining and Metallurgy, which covers mining other than coal, and metallurgy other than iron. The most improbable institution of all is the Institution of Polish Engineers in Great Britain, established in 1940. Surprisingly, it still has 500 members.

Long Short Story

The appointment at last of a new chairman to Short Brothers and Harland, the Belfast aircraft company, marks the end of an episode which has won few friends for the Ministry of Technology. Some two-thirds of the company is owned by the British Government, which therefore has an important say in its operation. In June last year, readers of the *Daily Express* and the then chairman, Mr C. E. Wrangham, in that order, discovered that Mr Wrangham was to be asked to resign his job. It seems possible that the Minister, Mr Benn, had not even met Mr Wrangham.

Air Vice-Marshal E. M. F. Grundy.

Mr Benn has not yet given the board of Short Brothers or the House of Commons a reason for his decision. In reply to six questions tabled on the matter on July 11, 1967, Mr Benn referred in the House of Commons to the substantial loss incurred by Shorts on the Belfast air freighter before Mr Wrangham's chairmanship, and the need for a reorganization to regain profitability. "This reorganization, which has already begun, can, I believe, best be carried through under the leadership of a new chairman." The ground for Mr Benn's belief, it has been suggested, was the Government's desire to see the company diversify itself out of the aircraft industry. Was it Mr Wrangham's fault that he was too successful in keeping Shorts in it? The company's troubles began in 1960 when it accepted a government order for ten Belfast aircraft in the belief that further orders would follow. None did, and the consequent loss of some £15 million made the company heavily dependent on government funds. Since that time Shorts has developed the Skyvan, a small two-engined freighter, and has built up a thriving export market for its Seacat guided missile. Substantial hopes are also placed on Blowpipe, a radar controlled guided weapon against low-flying aircraft which can be carried and operated by one man. In addition, the company has contracts for the wings of the Fokker 28 and 228 jet airliners, the engine pods for the Rolls-Royce RB 203 engines and the wing tips of the Phantom. In 1967 the company won the Queen's Award to industry.

It may be a measure of disenchantment in British industry with the Ministry's handling of the matter that it has taken until now to find a new chairman for the company. He is Air Marshal Sir Edouard Grundy, who has been managing director of Royston Instruments since 1966. Royston Instruments, which manufactured the Midas flight recorder, was a member of the Royston Group of Companies, now in liquidation. Sir Edouard has had a distinguished career in the Royal Air Force and was Controller of Guided Weapons and Electronics in the Ministry of Aviation from 1962 to 1966. His experience of the RAF, government and industry should stand him in good stead if he is to keep Shorts in business and retain Mr Benn's favour.

Beyond the Classroom

How can any institution in America now be in education and not in politics? The answer of the Dean of Harvard's Graduate School of Education is that it cannot. His report for the academic year of 1966–67 is a chilling document. The American faith in the public—that is, tax-supported and tuitionless—school is no longer justified. Urban schools are failing the "deprived" today, as suburban schools failed the "academically talented" fifteen years ago. And the Federal Government's entrance into educational reform—marked by an initial infusion of $1·2 billion in 1966—has not justified the hopes held out for it. The critics—white and Negro—are right to be disillusioned.

Even the enthusiasm which greeted the entrance of large corporations such as IBM and Time Inc. into the business of selling textbooks and making teaching machines seems to have been misplaced, according to Dr Theodore Sizer, dean of the school. Dr Sizer maintains that many of these companies have been rudely shocked. The school market is scattered and requires a large sales force; local school boards have little money for experimentation and teachers prefer to buy what is familiar. And "the gadgetry of computers and teaching machines and the rest has yielded interesting, but financially intolerable, results". Malaise has hit the education profession itself. State commissioners of education seem ready to wrest control away from the Office of Education in Washington; the private philanthropic foundations and the National Science Foundation give hints of stopping their outpourings of money into educational reforms. And the teachers themselves, underpaid—their average salary in 1966–67 was $6,820—are not only having success at becoming trade unionists but are being truculent as well. The state of American education is uneasy indeed.

Dr Sizer could almost be accused of exaggerating the darkness to accentuate the light of his own school at Harvard. It has faced the challenge pretty squarely, by accepting as a fact the proposition that much of education, and perhaps the best of it, takes place outside the classroom. The candidate for the doctorate in education can no longer expect to be trained to be a polished performer behind the desk; he must learn to be something of the street-corner social worker.

This change of direction emerged from a project called the Center for Research and Development on Educational Differences. Sustained by federal research money, various inquiries "of a scientific nature" were made into what influences determine a child's capacity to learn from formal education. One study looked into the mental abilities of six-year-olds and into the contrasting patterns possessed by children from different social classes and ethnic groups around Boston. In another project, researchers worked with Negro boys from ten to fourteen years old in one of Boston's most solidly black slums. For slightly older students, courses were offered in a variety of settings for real or potential school drop-outs, and were successful in making the boys more interested in academic achievement. The result of these and a number of other experiments was the abandonment by Harvard's Graduate School of Education of the traditional idea of its purpose—the study and improvement of instruction in the classroom—for the radical belief that education's educators must be expressly concerned with what children do and can learn outside school.

Although the interests of the school range widely—from improving the design of Boston's school buildings to improving the teaching of physics, from Boston to Minnesota and Nigeria—its primary concern has been what it offers to the postgraduate students who come to Cambridge, Massachusetts, to take higher degrees in education. The new philosophy affects them directly. One of their programmes, instead of concentrating on teaching academic subjects in classrooms, has turned training teachers into tutors, asking them to spend time with children as individuals and to follow no set curriculum. Another, the training of primary school teachers, has asked them to study a few subjects well, rather than the usual small helping of everything; this, it is hoped, will encourage more men to become teachers of the younger age group.

These steps toward revolution will be intensified in the present academic year (although the Educational Differences Center has had to be reorganized because of educational differences with the Federal Office of Education). But the aim in the future will be the same as Dean Sizer described it for the year just concluded: "to prevent the national spirit of malaise from turning in our particular community to one of negative cynicism. Schools are failing, yes; but this does not mean that they will or should always fail".

Student Participation

THE bold recommendations that students at the London School of Economics should be granted membership of its three main governing bodies create a situation unparalleled in any university or institution in Britain or, indeed, in the world. These recommendations appear in the first report on the machinery of government of the school, signed by eighteen members of a committee chaired by Lord Bridges.

The need for changes in the way in which LSE is governed has become increasingly obvious over the past few years, as a result of the growth of the school and the complexity of its activities. This need was first seen in August 1966 when Sir Sydney Caine presented specific proposals in a paper circulated to the Court of Governors and to the academic bodies in the school. On February 2, 1967, the Court of Governors agreed to establish a joint committee consisting of members of court and the academic body, and five student members were added to this committee.

The main object of student participation, the report says, is to secure student co-operation, thereby strengthening the unity of the school. It may also help to make accurate information on the school's activities available to all students. There are two ways in which students' participation could be achieved: by "consultation" which would need to be official, involving the establishment of joint student–staff consultative committees which would meet regularly to consider in advance matters on the agenda; the alternative scheme would involve the election or nomination of appropriately qualified students to serve as members of committees or other bodies. Of these two, the committee decided that "membership" would be most advantageous.

Many of the recommendations follow the general proposals of Sir Sydney Caine's memorandum. The report recommends that there should be about sixty members of court, including twelve members of academic staff and four to eight student members. A council should be established to replace the existing Standing Committee as the central budgetary authority of the school, and it is proposed that three members of the council should be students. The committee recommends that a senate be established to have executive powers in academic matters, and that it should include five student members. Students will not be allowed, however, to become members of the Academic Board—the forum through which all teachers would discuss and be asked to endorse important issues of policy. In addition, students would be members of some fourteen sub-committees of the senate and council, but staff appointments and promotions would be made by committees other than those on which students are members.

A minority report presented by two student members, Mr Dick Atkinson and Mr David Adelstein, lists a number of criticisms of the majority report. In the introductory comment, it is suggested that this report has limited itself to "managerially specified problems" and ignored "contemporary educational matters". In addition to challenging the assumptions and limitations of the report, Mr Atkinson and Mr Adelstein suggest a number of alternative proposals. For example, they consider that the court should comprise forty representatives of "society", including persons in trade unions, the theatre, the professions, peers of the realm and others. Furthermore, they suggest that the court should be assisted in its functions by electing a committee consisting of three governors, three staff and three students, all of whom should be members of court.

Academic Awards

CONTINUING success is reported by the Council for National Academic Awards (CNAA) in its third annual report covering the period up to September 1967. Initial misgivings about the success of the council are not confirmed by figures of the student enrolments for 1966 to 1967—26 per cent more students enrolled for courses leading to the council's first degrees in September 1967, compared with the number in 1966. The 1967 figure is 167 per cent more than that for 1965. The total number of students attending CNAA courses this session is 10,687, compared with 7,109 at the same time last year.

During 1966–67 the council approved fifty-four courses out of 136 proposed by colleges. Of the courses approved, forty-seven were in science and technology (50 per cent of the number considered) and seven in arts and social studies (17 per cent of the number considered). There are now 173 courses recognized by the council (as at September 1967) covering subjects ranging from aeronautical engineering, agricultural engineering, to textile technology and urban and regional planning. Most are sandwich courses—77 per cent of the courses in science and technology are of this type. One new course, of particular interest to some, is a part-time day and evening course in mathematics at Hatfield College of Technology which is designed especially for mature students.

A useful feature of the CNAA courses is that some are in fields where there are few or no courses in universities; degrees can now be obtained, for example, in building economics, estate management, food science, and photographic technology. The council also reports that it is considering librarianship and architecture as suitable fields for CNAA degrees. Since the report was written it is understood that a degree in information science has been approved by the council. It is to be offered by the Department of Librarianship at Newcastle College of Commerce as from September 1968. This course is designed primarily to prepare students to work in governmental and industrial information departments.

It is easy to forget that the council also has the power to award higher degrees. During 1966–67, five courses leading to the council's M.Sc. degree were approved, bringing the total number of CNAA postgraduate courses to seven—fifty-three students are enrolled on these. Two of the courses offered are in corrosion science and engineering and in experimental nuclear physics.

There is a growing number of registrations for the council's research degrees, particularly from candidates in research organizations. The flexibility of the council's arrangements, and the absence of a residential requirement, seem to be proving attractive to many candidates not eligible for higher degrees awarded by universities. The council's Ph.D. degree or M.C.T. (Membership of the College of Technologists) was awarded to twelve students during 1966–67. There are now 317 candidates registered for research degrees.

The council reports continuing good relations with the universities. Many organizations and professional bodies now give CNAA degrees the same recognition as they give to university degrees; for example, the Civil Service in its recruitment policies, and the Burnham committees in their acceptance of graduate qualifications for salary purposes.

It is inevitable that the council's activities are inseparable from the development of the polytechnics. In this connexion the council is continuing its efforts for new types of course, and broader courses combining more than one discipline—courses covering science and technology and social studies, which might be attractive to students who have read arts subjects in the sixth form at school.

Visiting Fellows

The Senior Visiting Fellowship scheme of the Science Research Council is undergoing spring cleaning, and the revised regulations are likely to be published in the near future. As it stands at the moment, "the council will make grants to applicants of acknowledged standing to invite senior scientists of distinction in other research schools in this country or abroad to the investigator's institution as senior visiting fellows for a period not exceeding one year to give full-time advice and assistance to the investigator in the field in which the visitors are eminent". The fellowships were designed to bring expert outsiders into individual research projects when specific advice was required, and are therefore included in the research grant scheme. Despite criticism on this score, and suggestions that the visiting fellows should be treated in the same way as research fellows, it is unlikely that the new regulations will alter the grouping of the schemes.

People like Dr N. Kurti of Oxford are enthusiastic about the possibilities of such a scheme, but regret that it has not received more publicity. Another criticism has been raised over the question of pay, because, as the regulations state, "if contributions to salary or expenses are being made by a senior visiting fellow's parent organization or other body, the charges to the grant will be reduced accordingly". This is thought to be an unfortunate arrangement, particularly for American visitors whose salary is paid on a nine-monthly basis but is considered to cover a year.

Until now, applications for senior visiting fellowships have had to be made on the same complicated forms as those for research grants. But here the criticism seems to have had some effect and the tidying up of the scheme will probably result in a shorter form being produced. It is thought unlikely that the improved scheme will be put before the council itself, which indicates that there is little hope for major changes. Mr L. S. Smith of the SRC has already stated that there will be no increase in the daily allowance for senior visiting fellows. During the year 1965–66, the last year for which figures are available, the council spent £42,645 on fifty senior visiting fellowships—a reduction of more than £13,000 compared with the year before.

Changes at Cold Spring Harbor

The Cold Spring Harbor Laboratory for Quantitative Biology, famous for its annual symposia, its summer courses and for its year-round research in genetics, has recently been strengthened by the appointment of Dr J. D. Watson as director from February 1 of this year. Dr Watson seems to have been persuaded to take on the job, while remaining as Professor of Biochemistry and Molecular Biology at Harvard, by his wish to see that the laboratory's dramatic improvement from a state of financial and physical bankruptcy would not come to a halt now that Dr John Cairns, the current director, had chosen to concentrate once again on research. Dr Cairns, who before his appointment to Cold Spring Harbor had made some elegant autoradiographical studies of DNA duplication, found on his arrival in Cold Spring Harbor an institution entirely without money, some $80,000 in debt and with physical plant requiring at least $1,000,000 for restoration to a first-class condition. Over the past five years, Cairns has worked heroically; now much of the laboratory has a strikingly pleasant appearance, and the cash reserves are up to $125,000. But after five years of this, Cairns naturally wishes to return to his scientific career.

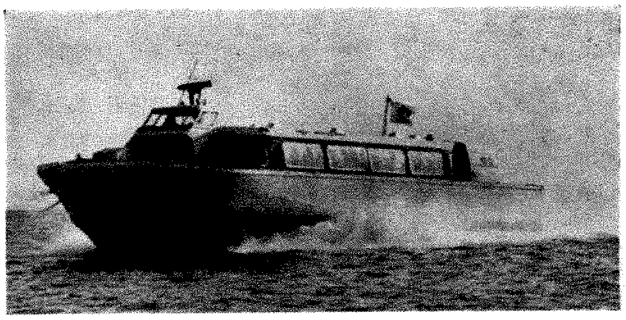
Dr J. D. Watson

The appointment of Dr Watson, already closely connected with the laboratory as a member of the Board of Trustees, and a frequent summer visitor since 1948, should allow a smooth administrative transition. He and Dr Cairns have worked closely together over the past few years, and one of Dr Watson's first aims as director will be to find an endowment for the secure backing of Dr Cairns's position as a staff member. Other immediate objectives will be to find capital funds for a new library to house Cold Spring Harbor's extensive collection of genetic literature, additional summer housing for an enlarged summer course programme and a new laboratory building for expansion of research into the area of molecular biology of higher cells, in particular the multiplication of viruses capable of causing tumours.

Another Hovercraft

Hovermarine, a small firm based in Southampton, has now produced its first hovercraft, the HM 2, on licence from Hovercraft Development, Ltd., which handles the patents for the National Research Development Corporation. The design is particularly interesting; the hovercraft has submerged sidewalls, so that in motion it looks much like a conventional boat (see picture).

The makers suggest that this sort of craft will be best suited to use in estuaries or rivers, where very large seas are not often experienced. At a speed of 28 knots, the craft will comfortably accommodate seas of up to 3 ft. in height; in more severe conditions, the company says, the maximum speed attainable will depend on the state of the sea and the amount of discomfort which the passengers are prepared to accept. When hovering, as in the picture, the sidewalls of the craft act as twin keels, giving the craft good directional stability. Shrewdly, Hovermarine says that this will make it possible for operators to pay realistic wage rates to the helmsmen, instead of having to employ skilled pilots. The craft will carry sixty passengers, or sixty-five at full stretch, and costs about £70,000.



The Hovermarine HM 2 on trials.

In contrast with other hovercraft designs, the Hovermarine HM 2 should be reasonably quiet in operation. It is driven by twin submerged marine screws and not by aircraft-type propellers. Motive power is supplied by two Cummins lightweight diesel engines, and the hover power is supplied by another Cummins engine. The hull is made of glass fibre-reinforced plastics, and the craft can be used from conventional jetties and quays. Hovermarine's own calculations, unsupported as yet by any statistics from operators, show that, at a load factor of 50 per cent and an average speed of 30 knots, operators could make a profit if fares were charged at the rate of 6*d*. a mile. Fares of 9*d*. a mile would produce an annual profit of £25,000 on the same assumptions, if the craft were depreciated to 10 per cent residual value in 10 years.

Research on Foreign Languages

In spite of two British applications to join the European Economic Community, British people remain conspicuously slow at learning foreign languages. There are, however, some signs of impending change, according to the first report of the Committee on Research and Development in Foreign Languages (HMSO, 5*s*.), which was set up in 1964 under the sponsorship of the Nuffield Foundation, the University Grants Committee, the British Council and what is now the Confederation of British Industry to co-ordinate research and development and to spend a little money—mostly other people's. The chairman is Dr L. Farrer-Brown, a former director of the Nuffield Foundation.

The committee's first experiment was with the teaching of French in primary schools. It has been helped particularly by a pilot scheme begun by the Nuffield Foundation in 1963 which is intended to discover whether a modern language can become a normal part of the primary school curriculum. The report says quite frankly that there are many questions yet to be answered—what is the best starting age ? How much time should be devoted to foreign languages at these tender ages ? Are specialists necessary or can ordinary class teachers manage ? What is the effect of teaching French on the general performance of the pupils in primary schools ? The answers to some of these questions may emerge when the National

Foundation for Educational Research has finished its programme of evaluation.

Teaching languages at ordinary level (up to 16) has risen considerably during the past twenty years: the number of pupils offering Russian, for example, has increased nearly 600-fold. If the first modern language can be started in primary schools, it is hoped that more pupils will take a second modern language in secondary schools. Emphasis is now being laid on teaching the spoken language.

The growth of French teaching in primary schools has also led to a conspicuous growth of courses in colleges of education, and a handbook prepared in 1966 showed that French could be studied in 81 colleges. In recent years, language courses have also increased in further education, and it seems that some of the most successful courses have been those provided in direct response to an approach from a firm or firms in the area. Several universities now provide for the study and teaching of languages in new ways: for example, the report points out that in some universities the study and teaching of languages are linked with the study of linguistics, either within a unified centre or in a separate department.

New IBP Chairman

Dr W. Franklin Blair, professor of zoology at the University of Texas, has been appointed chairman of the United States National Committee for the International Biological Programme (IBP). He succeeds Dr Roger Revelle, director of the Center for Population Studies at Harvard University. Dr Revelle was appointed chairman at the beginning of the three-year planning period for the IBP in 1964. Now that the IBP has moved into its five-year operational stage, Dr Revelle will act in an advisory capacity only, because of his commitments at Harvard and the National Academy of Sciences. Dr Revelle was recently appointed chairman of the academy's Science Organization Development Board and now acts, in effect, as Deputy Foreign Secretary of the Academy.

Dr Blair has been associated with the United States IBP programme from its beginning. One of his positions has been as co-chairman of the Subcommittee on Environmental Physiology. He has also organized the IBP integrated research programme on Convergent and Divergent Evolution. His main interest is vertebrate biology, particularly population genetics and the ecology and speciation of mammals, reptiles and amphibians.

Drugs in School

Drug dependence in young people under eighteen years of age in the City of Westminster has recently been investigated by a social worker under Dr J. H. Briscoe-Smith, medical officer of health for the area. The investigation, started in February 1966, was prompted because it was felt that the Health Department should have as much information as possible on the extent of the problem in the Westminster area, particularly as Soho lies within the city's bounds.

Much of the report seems to state the obvious, but it does at least prepare the ground for more extensive research. The aims of the survey were to estimate the size of the problem, whether gaps exist in the provision at present made and to see how local authorities might help. Of the fifteen head teachers of secondary schools interviewed, nine had experienced problems with pill taking by children in their schools. While about half the teachers felt that many of the young people in the fourth and fifth forms experimented with pills at some time, the others considered that this was a pattern pursued by only a small minority. It does seem, however, that the problem is more serious in schools in the "old" City of Westminster. It is also known that there are a number of young heroin addicts around Victoria Station and a number of boys who were known to be taking pills at school in the Westminster area are now heroin addicts.

Of nine youth clubs investigated, six of the larger ones reported a drug problem although it was only a slight one in three of them. Between April 1965 and April 1967, 74 young people were convicted at the Westminster Juvenile Court on charges of possessing drugs, but of these only eight were residents in the area. As the report emphasizes, this is complicated by young people passing in and out of the area, which is in the centre of London.

On the whole, head teachers were against lectures on prevention because they felt that this might draw attention to drug taking and thereby create a challenge for young people. Instead, they thought that lectures for parents might be a better idea. Most schools felt that teachers would welcome more practical information on the matter and a book is to be published later this year containing coloured photographs of pills to enable teachers to recognize them. Four Rotary clubs in the Westminster area are hoping to set up an advice centre for drug addicts, and the Men's Social Services Branch of the Salvation Army is providing a hostel for young boys on probation for drug charges. By setting up an information service, it is suggested in the report that this might assist other workers in the field such as general practitioners and probation officers. A social work service offered by the Health Department to help people with problems of drug dependency is also suggested.

On the Nature Trail

Owners of land in Britain with areas of natural history interest should read *Nature Trails*, a handbook produced by the Nature Conservancy (distributed by Frederick Warne, Ltd., 5*s*.). This gives advice on the setting up of nature trails—the routes along which visitors can walk, with information provided by signs, posters, pamphlets and sometimes guides.

The first nature trail was set up in 1961, many more were instituted at the time of National Nature Week in 1963, and there are now about ninety of them. Some have been set up on national nature reserves by the Nature Conservancy; the Forestry Commission has some and others have been opened by county naturalist trusts and by private organizations and individuals. (A list of more than eighty nature trails can be obtained for 2*s*. from the Council for Nature.)

Where a good guide is available—and the handbook points out that a bad guide is worse than no guide at all—the tour can be varied according to ecological changes or the interests of the visitors. On the whole, a shortage of manpower makes guided trails rare; more common are the trails for which a pamphlet describes points which refer to numbered stakes found at intervals along the route, which is marked out by arrows and signs There may be displays giving further information about the important features of the environment At Gibraltar Point Local Nature Reserve, in Lincolnshire, there is an information centre where voluntary wardens answer questions at the weekends

At Alvecote Pools the National Coal Board has leased a disused Methodist chapel to the West Midlands Trust for Nature, and this has been equipped as a day centre, supervised by a committee of local teachers It is used regularly by about twenty-five schools in the area Nature trails are clearly very valuable as a supplement to biology lessons The handbook points out that, although the trails are chiefly a teaching aid for younger children, they can help to illustrate and clarify examination syllabuses such as advanced level botany Plant identification and taxonomy can certainly be made more real, but whether they can be taught painlessly, as the handbook claims, is less certain

A Catalogue of Earthquakes

The International Seismological Centre in Edinburgh is beginning to fulfil what it sees as its main function, the publication of a regional catalogue of earthquakes The centre, formed in 1963, last year issued its first regional catalogue, which listed earthquakes for the first six months of 1964 The catalogue is based on readings from a global network of 612 stations, and lists nearly 5,000 events thought to be earthquakes, arranged according to the geographical region in which they occurred. In addition, the catalogue contains details of 186 known or probable explosions, data which can be useful for the calibration of seismographs

Computer processing of the raw data is essential for cataloguing work of this kind, but computer programmes are remarkably prone to teething troubles, and these seem to be the main reason for the 3½ year delay in publication Some time lag is inevitable, it seems, because of the inaccessibility of many earthquake recording stations, and the time the data take to reach Edinburgh Because the catalogue is intended to be a final record, replacing preliminary results published only months after the events, data from remote stations, some in the Antarctic, are included

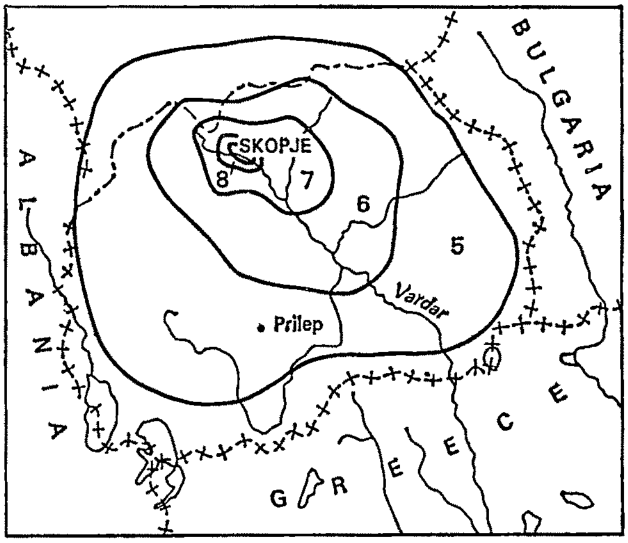
The director of the centre, Dr P. L Willmore, is hoping to cut down the delay in publication to two years or eighteen months, now that the initial difficulties have been ironed out Once the influx of data is under control and the catalogue brought up to date, the ISC expects to find time to analyse records from before 1964 and to produce catalogues of earthquakes occurring in the 1950s

The most rapid publication of preliminary seismic observations and determinations of the epicentre of earthquakes is the province of the United States Coast and Geodetic Survey These reports are especially useful to relief expeditions dispatched to assist at earthquake disasters where local lines of communication have been destroyed In such cases, the times and locations at which shocks occurred can be invaluable

On the other hand, catalogues like that produced by the International Seismological Centre find their main application in long-term statistical investigations of earthquakes Designers of large-scale structures which are especially prone to earthquakes—dams and tall office blocks, for example—like to know how susceptible the area they are building in is to earthquakes Here the regional catalogue of earthquakes will help.

Skopje Earthquake

After the earthquake in Skopje on July 26, 1963, the government of Yugoslavia decided to investigate the causes and effects of the earthquake damage, so that the city could be rebuilt with better resistance to the stresses of any future earthquake As well as calling in Yugoslavian scientists and engineers, the government sent a request to UNESCO for experts to advise them The findings of these four investigators have now been published (UNESCO, 51*s*)



Distribution of observed intensities for earthquake on July 26, 1963 (compiled by D Hadzievski and A Zátopek)

Mr J Despeyroux, chief engineer of SOCOTEC in Paris, reported on building problems and Dr N N Ambrayseys of Imperial College produced an engineering seismology study of the earthquake It was concluded that there would be no point in moving the city completely, but if new building developments are spread out around the city the risk will be minimized In replanning the old city wide streets can be included to give good access to the centre Special building codes for earthquake resistant structures are advisable, and controlled quality of structural materials, about which builders should be informed, would increase the safety of permanent buildings Thirty per cent of the most important buildings in the city were left intact or were not irreparably damaged, those which were properly designed and constructed being the best survivors Regulation of the River Vardar is important because it affects the seismic quality of the ground Earthquake engineering and seismology are different, although related, subjects, and the former is neglected Dr Ambrayseys suggests that a school should be set up in Skopje to train engineers and architects to postgraduate level in earthquake engineering, geotectonics and seismology It would be the first of its kind in Europe

Dr A Zátopek from Czechoslovakia, an expert in seismology, assembled and analysed macro- and microseismic data with the conclusion that the epicentral intensity was IX MCS and the focal depth 5 km at Skopje, the epicentre In all, the shaken area covered 105 km^2 The earthquake was described in general terms as a superficial or shallow tectonic, composed of several shocks, with typical small block structure mechanism within the epicentral area Dr Zátopek recommended that there should be detailed studies of the seismology of Yugoslavia

The final part of the report was produced by Dr A A Sorsky of the University of Moscow, who studied the geological and tectonic factors of the 1963 earthquake His conclusions include the fact that Skopje is on a seismologically dangerous area, and earthquakes are liable to occur there again Seismological and geological data are being collected by Yugoslav scientists so that a microregionalization map can be compiled, from which can be worked out the areas of most movement and consequently most seismological danger Provided building sites are chosen according to this map, Dr Sorsky believes that building should continue on the present site of the city

Keeping Physicists Aware

Comparatively recently there has emerged a new type of indexing journal, aimed at alerting readers quickly to titles of papers written in their respective fields In their simplest form, current awareness journals, as they are called, consist of a collection of facsimile reproductions of contents tables of individual journals The weekly list, *Current Contents*, published by the Institute for Scientific Information, Philadelphia, since 1958, is a well known example of a journal of this type The Polish Academy of Sciences produces a similar key to the Polish literature A more sophisticated example of a current title index is provided by the semi-monthly *Chemical Titles*, a product of the Chemical Abstracts service, which is a computer produced keyword index

In January 1966, the Institution of Electrical Engineers (IEE), in association with the American Institute of Physics (AIP), inaugurated a new current awareness journal called *Current Papers in Physics* to be used in conjunction with *Physics Abstracts* produced by the same institutions The IEE also publishes two other current awareness journals, *Current Papers on Control* and *Current Papers in Electrotechnology*, and two other abstract journals, *Control Abstracts* and *Electrical and Electronics Abstracts*—together the journals are known as INSPEC (Information Service in Physics, Electrotechnology and Control)

It was decided, before *Current Papers in Physics* (*CPP*) began publication, that it would be sensible to assess the need for this new journal and its usefulness During its first year of publication (1966) a joint study was therefore undertaken in Britain by the Aslib Research Department for the IEE and in the United States by the then Documentation Research Project staff of AIP The first report of this study has recently been published (*Results of Questionnaire on Current Awareness Methods used by Physicists prior to Publication of "Current Papers in Physics"*) Panels of physicists were set up in Britain (268 physicists) and the United States (682 physicists) Before *CPP* appeared, the panel members received the first of a series of three tests This initial questionnaire covered panel composition, methods used by physicists to keep up to date before *CPP*, and expectations of a current awareness journal in the field of physics Results of this questionnaire are given in this report Two later questionnaires covered physicists' comments on *CPP* and suggested improvements, and a record of the actual use made of the publication and the order in which sections were scanned Reports of these studies are to be published later

The results of the first survey show that current awareness journals were not much used by panel members before *CPP* appeared In general, the use tended to follow the pattern of the country of publication The journals published in the UK—*British Technology Index* and *Current Papers for the Professional Electrical and Electronics Engineer* (now *Current Papers in Electrotechnology*) were more used in the UK than in the US, and the reverse was true for the US physicists in terms of *Chemical Titles*, *Current Contents · Physical Sciences*, for example. Physicists co-operating in the study seemed to rely mainly on scanning current issues of journals as a means of keeping up to date before *CPP* It was thought to be the best method by 36 per cent of the British physicists, while 37 per cent of United States physicists ranked this method in first place The physicists indicated, however, that they would welcome a publication that would relieve them of the chore of actually scanning journals, provided that "nothing relevant" was missed There were various constructive suggestions about the requirements of a current awareness journal, and also comments about available abstract journals The IEE must be satisfied with the discovery that *Physics Abstracts* was the most widely used publication in the field, in both Britain and the United States. As the report puts it, "the heavy usage of this existing IEE publication in physics augurs well for the success of a complementary current awareness journal from the same publishers" The next two reports may well show if this is indeed the case. At the moment the IEE appears to be satisfied with the sale of *CPP* in Britain and in the United States

Fish Farming

Fish farming has been shown to be practicable, but the "rate at which the prospects are converted into reality will depend critically on the research effort we are able to mount" This was the conclusion of Dr H A Cole, Director of Fishery Research at Lowestoft, who addressed the Royal Society of Arts last week on the artificial cultivation of sea and shell fish and described the research which must be done before a successful intensive rearing system can be built There must be "systematic optimization of the environmental factors present in the system followed by scientific breeding to provide animals highly adapted to culture conditions"

The depletion of fish supplies accessible to British fishermen has led to the investigation of alternative sources, and to the realization that artificial cultivation can lead to advantages in processing and marketing, with possibilities for export Plaice was one of the first fish to be investigated, 15 years ago, and much has been learned from rearing young plaice in tanks and ponds from eggs collected at sea The best conditions of temperature, salinity, light, water exchange and quantity of food have been investigated for plaice As a result 400,000 were produced in 1965 until they were large enough to take to the bottom of the sea as adult flat-fish In the future, however, it is likely that sole will be cultivated more widely than plaice, as it is more valuable, the young grow readily and the adults adapt to tank life and can be induced to spawn This fish favours a warm temperature and is therefore suitable for cultivation in the heated sea water which is put out by power stations Dr Cole suggested that within ten years sole production at these power station sites will be in full swing, and turbot and brill are likely candidates for the same treatment Some shellfish also need warm water and can be grown in these warm water wastes

Oysters of the *Ostrea edulis* variety were once common in estuaries around Britain, but successive pests and floods have reduced the breeding stock to such an extent that any recovery of the oyster industry will depend on successful artificial rearing Fifty years of slow but steady progress in artificial breeding at Conway led to commercial scale techniques being used four years ago and a commercial hatchery now being built in South Devon is likely to produce a steady supply of young oysters New Zealand and Japanese oysters are being investigated, as are American clams The latter can be planted on the shore at the age of 1–1½ years, but must be protected from crabs by plastic mesh until they grow to an inch in diameter. After four years they are large enough to be marketable

The English prawn is another suitable species for artificial cultivation, despite its cannibalistic tendencies, and a habit for producing eggs rather than getting fat As with all other artificially reared fish, the feeding problem for shrimps has yet to be completely solved The brine shrimp *Artemia* is a useful food source for many kinds of fish, but attempts are being made to produce a protein based artificial food Waste fish flesh and poultry offal may provide the basis of the substance, which must be soft Attempts are also being made to find a palatable fish which is herbivorous Grey mullets and the freshwater *Tilapia* are two possibilities, but as yet there is little demand for these varieties

Parliament in Britain

Nationalized Industry Research

The programmes of the National Coal Board provide for estimated expenditure on research and development in the current financial year of £5 million The Gas Council and Boards will spend £5,650,000, and the Electricity Council and Boards £10,650,000 The National Coal Board in 1966 employed 356 qualified staff on research and development, and the Gas Council and Boards 340, in the current year the Electricity Council and Boards employed about 900 The figures were given by Lord Winterbottom concluding a debate for the Government on February 14

Atomic Energy Authority

Dr J Bray, Joint Parliamentary Secretary, Ministry of Technology, stated that the Atomic Energy Authority aimed to achieve cuts of £3 5 million in 1968–69 in the cost of their civil research and development programme principally by some reduction in capital expenditure and by some run down of manpower The balance of the cut would be met by limiting extramural research and development contracts The effect of the cut of £6 million in 1969–70 was still being studied by the authority (Written answer, February 12)

Scientific Officers

The Minister of Technology, Mr A Wedgwood Benn, gave the average age on January 1, 1968, of officers in each grade of the scientific officer class employed in the Ministry's Research and Development Establishments as chief scientific officer, 52, deputy chief scientific officer, 50, senior principal scientific officer, 47, principal scientific officer, 45, senior scientific officer, 33, and scientific officer, 26 The Minister of Defence for Administration, Mr G W Reynolds, gave the corresponding figures for scientific officers in the Ministry of Defence's establishments 54, 45, 48, 46, 36, 26 (Written answers, February 12)

Chemical Defence

The Minister of Defence for Equipment, Mr R Mason, stated that the work of the Chemical Defence Experimental Establishment was directed towards research on defence against chemical warfare, but some of the work of the establishment had found applications in medical science Such benefits included the investigation of the mode of action of several toxic compounds leading to the development of measures for treating intoxication, the development of an antidote to poisoning by organophosphorus insecticides (a service providing advice and first-aid treatment had been maintained since 1960), the development of aids to therapy, such as an automatic injection device and a portable resuscitator which is used by fire brigades and other rescue services, the investigation of the structure of the skin and the processes of skin penetration, and the investigation of the nature of the alveolar lining of the lung Mr Mason later gave the number of qualified engineers and scientists working in defence research and development establishments on December 31, 1967, as 2,838 compared with 2,612 on December 31, 1964 (Written answers, February 13 and 14)

Protein from Leaves

Mrs Shirley Williams, Minister of State at the Department of Education and Science, stated that the Rothamsted Experimental Station is carrying out research on the extraction of protein from the leaves of crop plants Figures for the extractability of leaf (including tree-leaf) protein from many parts of the world were now being published These were not strictly comparable because of differences in the techniques of grinding and pressing Measurements of the extractability of protein from local crops were an essential prelude to any attempt to make leaf protein for use as a food in regions of protein deficiency Unexpected differences had been found in the nutritional value of protein preparations The effects of variations in the technique of precipitation, filtering and processing or drying the protein would be studied and the National Institute for Research in Dairying and the Rowett Research Institute would co-operate in measuring the nutritional value of some of these products (Written answer, February 13)

NEWS AND VIEWS

Accelerators for the Seventies?

HIGH-ENERGY physicists are almost as dismayed as the governments which support them by the high costs of making particle accelerators, which is a part of the reason why there is at present great interest in the kinds of accelerators called collective ion accelerators On the face of things there is at least a possibility that this new technique may eventually lead to the design of simpler and even cheaper particle accelerators To be sure, there is not the slightest prospect that collective ion accelerators will allow European governments to shelve the awkward question of whether or not to contribute towards the 300 GeV accelerator that Cern is anxious to build At this stage, indeed, nobody can be sure that schemes like these can ever justify the enthusiasm which they have stimulated in many places By all accounts, however, the symposium on the subject at Berkeley the week before last showed that these developments are too valuable to be ignored

The new proposals turn on the way in which, in the presence of a sufficiently strong magnetic field, it is possible to embed large numbers of protons in an organized cloud which consists of a still larger number of electrons Doing this is simple enough, given the availability of some of the techniques which have come into fashion in plasma physics A toroidal ring of 10^{13} electrons with linear dimensions of the order of a metre can be loaded with protons simply by the intervention of hydrogen gas It seems usually to be assumed that there will be one proton for every hundred electrons or so The large ring can then be contracted into a more manageable one a few centimetres across, and the result is an electrified object, a charge to mass ratio which is for practical purposes the same as that of an electron—with the understanding that the mass is the relativistic mass of the electrons moving within the torus, possibly with an energy of 20 MeV or so The result is that, when an electron lump like this is stripped of its electrons—which is, by all accounts, all too easy to accomplish—the protons which are left have individual energies which are greater than those which would have been acquired by electrons in the same circumstances in the ratio of the mass of the proton to that of the relativistic electrons within the lump—a factor of forty or thereabouts

The first practical suggestion for using these electrified objects as fodder for a linear accelerator seems to have been made by the Russian group at Dubna, which described some of its ideas at a conference of techniques for particle accelerators at the Massachusetts Institute of Technology last September The notion seems to have been taken up with great enthusiasm, particularly at Berkeley, still then mourning the loss of its scheme for a 200 GeV proton synchrotron to Weston in Illinois The recent symposium there was preceded by a working party which spent two weeks on detailed discussion of possible realizations of the principle, and which seems to have confirmed the mood of watchful optimism which prevailed before Thus it does appear that it should be possible to accelerate electron lumps along the axis of a linear accelerator in such a way as to give the protons which they contain about 1 MeV of energy for each centimetre length, which is a handsome promise On the other side of the balance sheet, however, are several snags The meeting at Berkeley has, for example, drawn attention to the very large amounts of power that would be needed to keep an accelerating machine in operation There are also potential problems in the maintenance of a magnetic field along the axis of the accelerator And potential users of machines like this are quick to point out that pulses of fast particles which last for a nanosecond or thereabouts would not bring much joy to those among the conventional machine builders who spend much of their time seeking ways of flattening the much longer pulses of particles which are now available from accelerating machines so as to make more efficient use of them Clearly there is a great deal to be done before anybody will be able to face any government with a request for money

How Immortal are Amoebae?

from our Microbiology Correspondent

ONE of the most intriguing questions posed to biologists concerns the life span of cells and how this defined period of viability is controlled Data are accumulating on the differential life spans of cells in animal bodies and, in a few instances, this conclusion is being corroborated by studies of animal cell cultures Hayflick (*Exp Cell Res*, **37**, 614, 1965), for example, showed that human foetal lung cells had a life span equivalent to a doubling potential of 50 ± 10, whereas cell lines derived from adult lung had a significantly lower doubling potential The presence of a latent virus, mycoplasma or the nature of the growth medium did not account for this finite life-time

For a number of years, Danielli has been investigating the life span phenomenon in *Amoeba* Clones of *A proteus* and *A discoides* were reported to have apparent immortality when cultured with an unlimited supply of *Tetrahymena* as food organism, but after a period of culture on maintenance diet a defined life span was induced Further analysis of the "immortal" and "spanned" states in *A proteus* has been directed towards elucidating the control of this switch phenomenon (Muggleton and Danielli, *Exp Cell Res*, **49**, 116, 1968) and transplantation experiments have allowed some assessment of the roles of the nucleus and cytoplasm Muggleton and Danielli found earlier that spanned amoebae exhibited two behavioural patterns following division In the first, type *A*, stemline growth occurred in which one product of binary fission showed early mortality while the second daughter cell underwent disparate division again This sequence continued for a finite period and was followed by death of both daughter cells In contrast, division of type *B* cells produced two viable daughters which resulted in exponential growth until such time as all cells of the clone died When an immortal or normal nucleus was implanted into spanned cytoplasm, all the amoebae were spanned and, moreover, all were converted to type *B* behaviour regardless of whether the recipient cytoplasm was type *A* or *B* Transfer of a spanned nucleus to a normal cytoplasm, however, produced type *A* spanned amoebae One conclusion here is that a spanned nucleus normally confers stemline behaviour on its descendants, while a spanned cytoplasm normally induces an exponential growth pattern Small injections of spanned cytoplasm into normal amoebae also induced a finite life span in the recipient and a similar effect followed injections of cytoplasmic supernatant from spanned amoebae So far, conversions have been in one direction only, and Muggleton and Danielli have been unable to convert amoebae from the spanned state to the immortal state Finer analysis of this system is an exciting prospect In particular, the bimodal behaviour of spanned clones is indicative of a complex "spanning" phenomenon Cell life span itself may be under the control of a higher order mechanism, with which we are not yet familiar, or may be the resultant of an accumulation of errors in DNA replication which inactivates part of the genome This latter may be analogous to the "fault-load" hypothesis of Szilard or Hayflick's proposed "multi-hit, multi-target" phenomenon

Flow of Blood

from a Correspondent

A SYMPOSIUM on Haemorheology and the Microcirculation was held at the Royal College of Surgeons on February 10, under the joint sponsorship of the British Society of Rheology and the British Microcirculation Society

Undoubtedly the main contribution was in the field of the biophysics of blood flow in the smallest vessels The mathematics involved was usually simplified for the benefit of the members of the audience who were principally concerned with practical applications to clinical diseases, but the solution of the equations involved often required the use of a computer M J Lighthill provided an analysis of the behaviour of a deformable pellet, such as a blood cell, which has a resting maximum diameter just too large for it to pass through a small tube, such as a capillary, without deformation He predicted the existence of a thick film in which there must be a substantial leak-back from one "bolus" to another If the velocity is reduced, such a film becomes very thin so that the red cell's motion temporarily "sticks" or "seizes up". This type of behaviour may often be observed in the smallest capillaries in the living circulation, but it does not account for the deformations of the cells that are recorded by high speed photography S R Montgomery described the behaviour of large simple rigid models of the shape of resting red cells and showed that their motion is essentially unstable unless "pores" are made in their centre corresponding to about one-tenth of their leading surface area A mathematical analysis of pulsating flow was provided by J H Gerrard in the case of flow driven by simple pistons In these circumstances the axial velocity is less than that nearer the wall of the tube E W J Mardles examined the behaviour of simple liquids containing a meniscus, flowing at very low shear rates in narrow channels, and showed that the resistance of this meniscus must be very great if the channel is less than 10 microns in diameter Although this is the mean diameter of the larger capillaries, it is not yet certain how these conclusions, which might apply to the plasma, would also apply to the blood cells It has been suggested that fibrinogen in the plasma is responsible for giving whole blood its non-Newtonian characteristics, but D N Walder, J P A Weaver and A Evans, using a cone-and-plate viscometer, showed that the wide range of variations in fibrinogen level that normally occurs in patients with different diseases does not seem to have any physiological effect except perhaps at very low shear rates, discussion on this was controversial The effect of cryoglobulins in human plasma was discussed by R B Whittington and J Harkness, and R Pringle showed the results of interesting observations in patients with Raynaud's disease who had abnormal blood viscosities and flow cessation pressures The latter phenomenon was treated in a different manner by C G Caro, T H Foley and M F. Sudlow, who provided interesting data on the effect of altering the transmural pressure in normal human subjects The elastic behaviour of the wall of "living" blood vessels was considered by D J Patel and D L Fry J L Corbett and S H Milton showed that dye studies on the vascularity of experimental skin flaps in animals demonstrated considerable species variability, and B A Warren and M C Davey illustrated the ultrastructure of platelets and fibrin by means of electron photomicrographs

The symposium as a whole served to bring together workers in previously unrelated disciplines and it is hoped that the resulting introductions will foster closer relationships between these people Physicists, hydraulic engineers and rheologists would learn much of the problems of microcirculation if they were able to see living tissues and high speed photographs of blood cells in motion, and the more clinical research workers have much to learn of the methods and parameters needed before the solution of those problems is likely to yield to mathematical analysis

Pituitary Hormone Actions

from our Medical Biochemistry Correspondent

ALTHOUGH the pituitary gland is often described as the "conductor of the endocrine orchestra" because of the way in which pituitary hormones have stimulatory effects on many other endocrine glands, the way in which pituitary hormones act on their target tissues is not completely clear Three recent papers have shown that some pituitary hormones seem to have direct effects on some enzymes concerned with carbohydrate and fatty acid metabolism These effects might be responsible for their action on the metabolism of distant tissues

Bornstein and co-workers (*Biochim Biophys Acta*, **156**, 31, 1968) have isolated from anterior pituitary extracts two polypeptide fractions which they have named Ac-P and In-P Ac-P accelerates fatty acid synthesis from acetate in liver slices In-P inhibits fatty acid synthesis in liver slices and homogenates and the soluble fraction after separation of the homogenate particles In-P has also a strong inhibitory effect on glyceraldehyde 3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase, but does not affect hexokinase, phosphofructokinase, aldolase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, malic enzyme or aspartate aminotransferase

The same group has also shown that preparations of growth hormone will also inhibit glyceraldehyde 3-phosphate dehydrogenase, but only if the hormone has been treated with acid and then returned to neutral solution (*Biochim Biophys Acta*, **156**, 38, 1968) They were able to separate two polypeptide fractions from this acid-treated growth hormone The fraction called In-G inhibited glyceraldehyde 3-phosphate dehydrogenase and also decreased the incorporation of acetate into fatty acids in liver slices The other fraction, Ac-G, was able to prevent the inhibition caused by In-G without itself having any effect on the enzyme activity The two polypeptides appeared to be similar to, if not identical with, the two polypeptides extracted from the anterior pituitary Ac-G seems to reverse the dehydrogenase inhibition produced by In-P Because the action of these two peptides is in many ways antagonistic, growth hormone may have different effects on the metabolism of different tissues, depending on the tissue *p*H and possibly other factors

In the same journal, Butcher and Serif (*Biochim Biophys Acta*, **156**, 59, 1968) present evidence that another pituitary hormone, thyroid stimulating hormone (TSH), affects the carbohydrate-degrading enzyme phosphorylase Other workers have not been able to find phosphorylase in thyroid, but Butcher and Serif, using a new sensitive assay, have shown that thyroid does contain phosphorylase and that TSH changes the ratio of inactive phosphorylase *B* to the active phosphorylated form—phosphorylase *A* Within one minute after the addition of TSH, the proportion of phosphorylase *A* had increased substantially Since compounds related to cyclic adenosine 3′,5′-monophosphate produced the same effect, TSH may increase the cyclic adenosine 3′,5′-monophosphate and increase phosphorylase *A* indirectly. Phosphorylase is regarded as a key enzyme in the control of carbohydrate metabolism in other tissues, so that this effect of TSH may be important in thyroid stimulation Pituitary hormones may alter the metabolism of target tissues by these effects of key enzymes

Exchange of Ribosomal Sub-units

from our Cell Biology Correspondent

ARE the 30*S* and 50*S* sub-units of *Escherichia coli* ribosomes permanently associated *in vivo* in a 70*S* ribosome, or do they undergo exchange ? The elegant experiments which Kaempfer, Meselson and Raskas have just reported (*J Mol Biol*, **31**, 277, 1968) firmly establish that there is sub-unit exchange in *E coli* during protein synthesis Last year (see *Nature*, **216**, 853, 1967) Mangiarotti and Schlessinger reached the same conclusion from somewhat less compelling evidence

Kaempfer *et al* have analysed, on sucrose and caesium chloride density gradients, the density distribution of ribosomes extracted from *E coli* cells which had been uniformly labelled with heavy isotopes (deuterium and ^{15}N) and then transferred to a medium containing only light isotopes Following the classic Meselson and Stahl experiment, they argued that if sub-unit exchange occurs, then, on transfer to a light medium, heavy ribosomes should be progressively replaced by two species of ribosomes with hybrid densities One species of hybrid would have a heavy 50*S* sub-unit and a newly synthesized light 30*S* sub-unit and the other hybrid a light 50*S* and heavy 30*S* sub-unit This interpretation is only valid, of course, if the ribosomal sub-units labelled with heavy isotopes are stable and remain heavy when the cells are grown in a light medium

In fact, as predicted, both hybrid species are formed during growth in the light medium Furthermore, virtually all the ribosomes undergo at least one cycle of sub-unit exchange during a cell generation Kaempfer *et al* have found no evidence for ribosomes which were incapable of dissociation nor for the accumulation of sub-units that were unable to cycle through ribosomes Their experiments also show that ribosomal sub-units are stable, heavy 50*S* sub-units maintain their original density during growth in the light medium Less than 3 per cent of their protein is exchanged in 1 3 generations (about 1 h)

Ribosomal proteins are apparently themselves also stable during cell growth Schlief has recently reported (*Mol Gen Genetics*, **100**, 252, 1967) that the rate of degradation of ribosomal protein to free amino-acid cannot be greater than 0 7 per cent per hour

The simplest explanation of sub-unit exchange in growing cells is that it is a necessary step in protein synthesis The current interpretation is that a round of translation 30*S* and 50*S* sub-units from an intracellular pool join to form a ribosome and at the end of translation they dissociate and return to the pools (see *Nature*, **216**, 638, 1967, and **217**, 508, 1968) A search for evidence of sub-unit exchange in eucells is needed now

Carboxypeptidase

from our Molecular Biology Correspondent

THE successful determination of a protein structure at high resolution is still an event to be celebrated The structure of the proteolytic enzyme, carboxypeptidase *A*, at a resolution of 2 0 Å has been described by Lipscomb and his associates (Reeke *et al*, *Proc US Nat Acad Sci*, **58**, 2220, 1967) In the absence of extensive data on the sequence, many of the side chains cannot be uniquely identified, but the course of the backbone is unambiguous

The essential structural features include four α-helical regions making up some 30 per cent of the chain in all, and two sections of the distorted α-helix also seen in lysozyme are present The model bears out once more the ubiquity of the β-structure, for 20 per cent of the backbone has the form of a pleated sheet containing four pairs of parallel and three of antiparallel chains The active site, as elsewhere, is located in a cavity, and includes the zinc atom, which is present in the native enzyme and is required for activity Three side chains are thought to function as ligands for the zinc, but only one—a histidine residue—has been identified

The most interesting feature of this work concerns the introduction of a substrate, glycyltyrosine The electron density map of the complex at 2 8 Å reveals a number of aspects of the interaction, and in particular—by marked contrast, for example, with ribonuclease—shows that the entry of the substrate provokes appreciable local changes in disposition of side chains. There is no evidence of specific interactions between any groups in the enzyme and the C-terminal side chain of the substrate, which fits easily into the cavity. However, the terminal carboxylate group is brought into contact with an arginine side chain, which moves 2 Å in the process. It is tentatively suggested, moreover, that the oxygen atom of the substrate peptide bond may become a ligand for the zinc atom. It was previously observed by Vallee and co-workers that certain tyrosine residues seem to be involved in enzymatic activity, and indeed it is found that one tyrosine moves a distance of no less than 14 Å when the substrate is introduced so as to bring up the phenolic hydroxyl group close to the substrate peptide bond. The motion occurs in a non-helical part of the chain, and evidently involves a considerable disturbance of the backbone conformation as well as rotation about side chain carbon–carbon bonds. A carboxylate group also seems to form part of the active site.

The details so far available are consistent with chemical data: whether a more precise delineation of the interaction between the active centre and the substrate will immediately suggest a catalytic mechanism remains to be seen. There are grounds for optimism, despite the remarkable failure of known protein structures so far to reveal the mechanism of their function unequivocably.

Observation of a Rapidly Pulsating Radio Source

by

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Unusual signals from pulsating radio sources have been recorded at the Mullard Radio Astronomy Observatory. The radiation seems to come from local objects within the galaxy, and may be associated with oscillations of white dwarf or neutron stars.

IN July 1967, a large radio telescope operating at a frequency of 81.5 MHz was brought into use at the Mullard Radio Astronomy Observatory. This instrument was designed to investigate the angular structure of compact radio sources by observing the scintillation caused by the irregular structure of the interplanetary medium[1]. The initial survey includes the whole sky in the declination range $-08° < \delta < 44°$ and this area is scanned once a week. A large fraction of the sky is thus under regular surveillance. Soon after the instrument was brought into operation it was noticed that signals which appeared at first to be weak sporadic interference were repeatedly observed at a fixed declination and right ascension; this result showed that the source could not be terrestrial in origin.

Systematic investigations were started in November and high speed records showed that the signals, when present, consisted of a series of pulses each lasting ~ 0.3 s and with a repetition period of about 1.337 s which was soon found to be maintained with extreme accuracy. Further observations have shown that the true period is constant to better than 1 part in 10^7 although there is a systematic variation which can be ascribed to the orbital motion of the Earth. The impulsive nature of the recorded signals is caused by the periodic passage of a signal of descending frequency through the 1 MHz pass band of the receiver.

The remarkable nature of these signals at first suggested an origin in terms of man-made transmissions which might arise from deep space probes, planetary radar or the reflexion of terrestrial signals from the Moon. None of these interpretations can, however, be accepted because the absence of any parallax shows that the source lies far outside the solar system. A preliminary search for further pulsating sources has already revealed the presence of three others having remarkably similar properties which suggests that this type of source may be relatively common at a low flux density. A tentative explanation of these unusual sources in terms of the stable oscillations of white dwarf or neutron stars is proposed.

Position and Flux Density

The aerial consists of a rectangular array containing 2,048 full-wave dipoles arranged in sixteen rows of 128 elements. Each row is 470 m long in an E–W direction and the N–S extent of the array is 45 m. Phase-scanning is employed to direct the reception pattern in declination and four receivers are used so that four different declinations may be observed simultaneously. Phase-switching receivers are employed and the two halves of the aerial are combined as an E–W interferometer. Each row of dipole elements is backed by a tilted reflecting screen so that maximum sensitivity is obtained at a declination of approximately $+30°$, the overall sensitivity being reduced by more than one-half when the beam is scanned to declinations above $+90°$ and below $-5°$. The beamwidth of the array to half intensity is about $\pm\frac{1}{2}°$ in right ascension and $\pm 3°$ in declination; the phasing arrangement is designed to produce beams at roughly 3° intervals in declination. The receivers have a bandwidth of 1 MHz centred at a frequency of 81.5 MHz and routine recordings are made with a time constant of 0.1 s; the r.m.s. noise fluctuations correspond to a flux density of 0.5×10^{-26} W m^{-2} Hz^{-1}. For detailed studies of the pulsating source a time constant of 0.05 s was usually employed and the signals were displayed on a multi-channel 'Rapidgrap' pen recorder with a time constant of 0.03 s. Accur timing of the pulses was achieved by recording ser pips derived from the *MSF* Rugby time transmissic

A record obtained when the pulsating source w

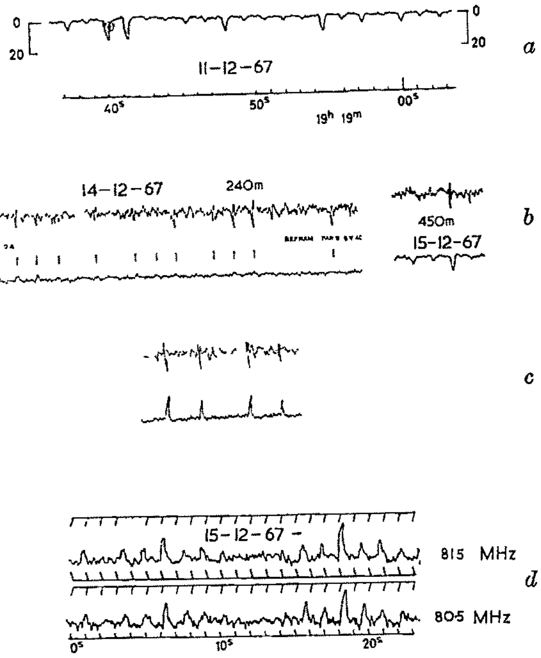

Fig 1 *a*, A record of the pulsating radio source in strong signal conditions (receiver time constant 0 1 s) Full scale deflexion corresponds to 20×10^{-26} W m^{-2} Hz^{-1} *b*, Upper trace records obtained with additional paths (240 m and 450 m) in one side of the interferometer Lower trace normal interferometer records (The pulses are small for $l=240$ m because they occurred near a null in the interference pattern, this modifies the phase but not the amplitude of the oscillatory response on the upper trace) *c*, Simulated pulses obtained using a signal generator *d*, Simultaneous reception of pulses using identical receivers tuned to different frequencies Pulses at the lower frequency are delayed by about 0 2 s

usually strong is shown in Fig. 1*a* This clearly displays the regular periodicity and also the characteristic irregular variation of pulse amplitude. On this occasion the largest pulses approached a peak flux density (averaged over the 1 MHz pass band) of 20×10^{-26} W m^{-2} Hz^{-1}, although the mean flux density integrated over one minute only amounted to approximately $1\ 0 \times 10^{-26}$ W m^{-2} Hz^{-1} On a more typical occasion the integrated flux density would be several times smaller than this value It is therefore not surprising that the source has not been detected in the past, for the integrated flux density falls well below the limit of previous surveys at metre wavelengths

The position of the source in right ascension is readily obtained from an accurate measurement of the "crossover" points of the interference pattern on those occasions when the pulses were strong throughout an interval embracing such a point The collimation error of the instrument was determined from a similar measurement on the neighbouring source 3*C* 409 which transits about 52 min later On the routine recordings which first revealed the source the reading accuracy was only ±10 s and the earliest record suitable for position measurement was obtained on August 13, 1967. This and all subsequent measurements agree within the error limits. The position in declination is not so well determined and relies on the relative amplitudes of the signals obtained when the reception pattern is centred on declinations of 20°, 23° and 26°. Combining the measurements yields a position

$$\alpha_{1950} = 19\text{h}\ 19\text{m}\ 38\text{s} \pm 3\text{s}$$

$$\delta_{1950} = 22^\circ\ 00' \pm 30'$$

As discussed here, the measurement of the Doppler shift in the observed frequency of the pulses due to the Earth's orbital motion provides an alternative estimate of the declination Observations throughout one year should yield an accuracy of ±1′ The value currently attained from observations during December–January is $\delta = 21^\circ\ 58' \pm 30'$, a figure consistent with the previous measurement

Time Variations

It was mentioned earlier that the signals vary considerably in strength from day to day and, typically, they are only present for about 1 min, which may occur quite randomly within the 4 min interval permitted by the reception pattern In addition, as shown in Fig 1*a*, the pulse amplitude may vary considerably on a time-scale of seconds. The pulse to pulse variations may possibly be explained in terms of interplanetary scintillation[1], but this cannot account for the minute to minute variation of mean pulse amplitude Continuous observations over periods of 30 min have been made by tracking the source with an E -W phased array in a 470 m × 20 m reflector normally used for a lunar occultation programme The peak pulse amplitude averaged over ten successive pulses for a period of 30 min is shown in Fig 2*a* This plot suggests the possibility of periodicities of a few minutes duration, but a correlation analysis yields no significant result If the signals were linearly polarized, Faraday rotation in the ionosphere might cause the random variations, but the form of the curve does not seem compatible with this mechanism The day to day variations since the source was first detected are shown in Fig. 2*b*. In this analysis the daily value plotted is the peak flux density of the greatest pulse Again the variation from day to day is irregular and no systematic changes are clearly evident, although there is a suggestion that the source was significantly weaker during October to November. It therefore appears that, despite the regular occurrence of the pulses, the magnitude of the power emitted exhibits variations over long and short periods

Instantaneous Bandwidth and Frequency Drift

Two different experiments have shown that the pulses are caused by a narrow-band signal of descending frequency sweeping through the 1 MHz band of the receiver. In the first, two identical receivers were used, tuned to frequencies of 80 5 MHz and 81 5 MHz Fig 1*d*, which illustrates a record made with this system, shows that the lower frequency pulses are delayed by about 0 2 s This corresponds to a frequency drift of ~ -5 MHz s^{-1}. In the second method a time delay was introduced into the signals reaching the receiver from one-half of the aerial by incorporating an extra cable of known length l This cable introduces a phase shift proportional to frequency so that, for a signal the coherence length of which exceeds l, the output of the receiver will oscillate with period

$$t_0 = \frac{c}{l}\left(\frac{d\nu}{dt}\right)^{-1}$$

where $d\nu/dt$ is the rate of change of signal frequency Records obtained with $l=240$ m and 450 m are shown in Fig 1*b* together with a simultaneous record of the pulses derived from a separate phase-switching receiver operating with equal cables in the usual fashion Also shown, in Fig. 1*c*, is a simulated record obtained with exactly the same arrangement but using a signal generator, instead of the source, to provide the swept frequency For observation with $l > 450$ m the periodic oscillations were slowed down to a low frequency by an additional phase shifting device in order to prevent severe attenuation of the output signal by the time constant of the receiver The rate of change of signal frequency has been deduced from the additional phase shift required and is $d\nu/dt = -4\ 9 \pm 0\ 5$ MHz s^{-1}. The direction of the frequency drift can be obtained from the phase of the oscillation on the record and is found to be from high to low frequency in agreement with the first result

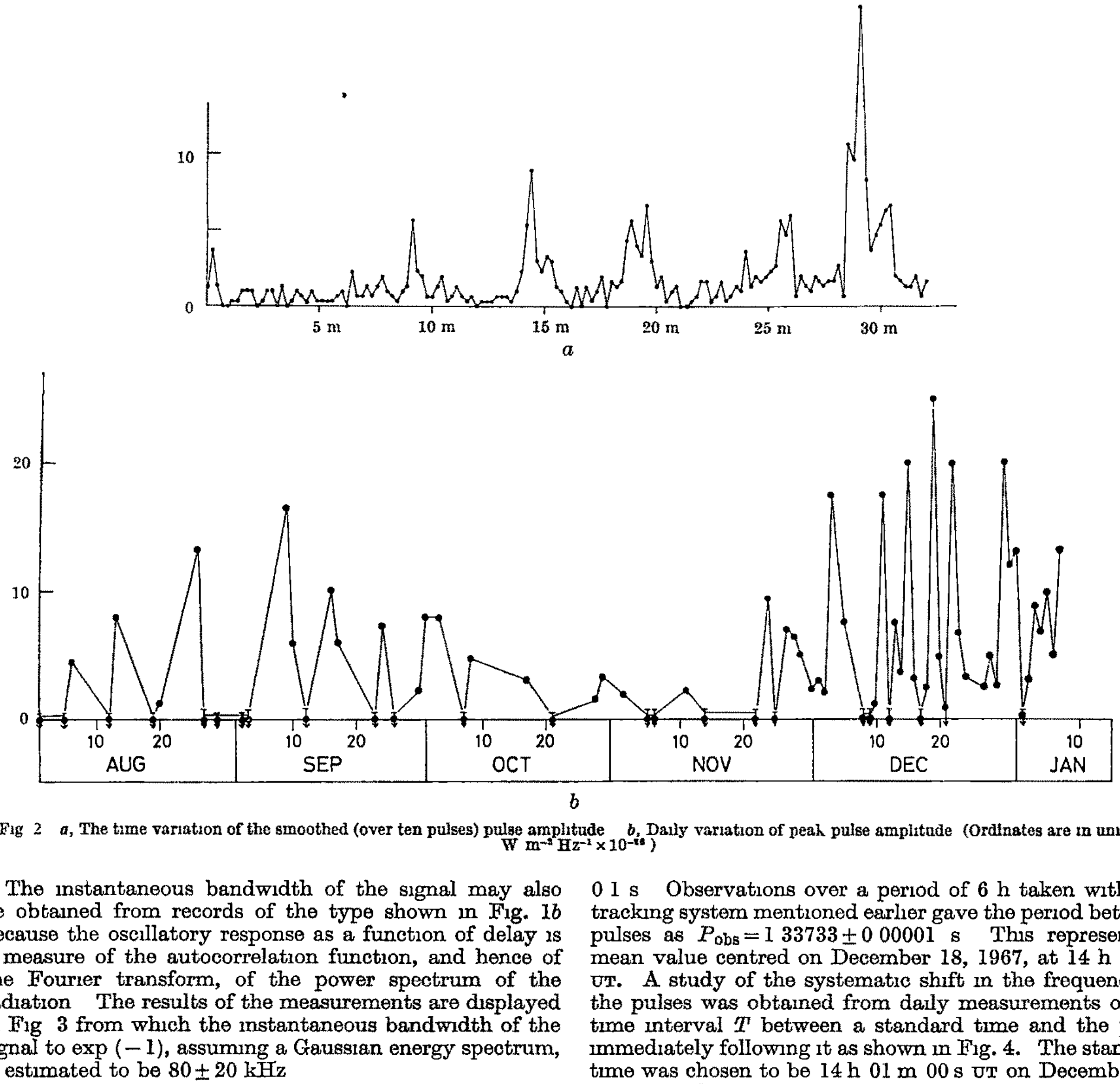

Fig 2 *a*, The time variation of the smoothed (over ten pulses) pulse amplitude *b*, Daily variation of peak pulse amplitude (Ordinates are in units of W m^{-2} Hz^{-1} $\times 10^{-26}$)

The instantaneous bandwidth of the signal may also be obtained from records of the type shown in Fig. 1*b* because the oscillatory response as a function of delay is a measure of the autocorrelation function, and hence of the Fourier transform, of the power spectrum of the radiation The results of the measurements are displayed in Fig 3 from which the instantaneous bandwidth of the signal to exp (-1), assuming a Gaussian energy spectrum, is estimated to be 80 ± 20 kHz

Pulse Recurrence Frequency and Doppler Shift

By displaying the pulses and time pips from *MSF* Rugby on the same record the leading edge of a pulse of reasonable size may be timed to an accuracy of about 0 1 s Observations over a period of 6 h taken with the tracking system mentioned earlier gave the period between pulses as $P_{\mathrm{obs}} = 1\ 33733 \pm 0\ 00001$ s This represents a mean value centred on December 18, 1967, at 14 h 18 m UT. A study of the systematic shift in the frequency of the pulses was obtained from daily measurements of the time interval T between a standard time and the pulse immediately following it as shown in Fig. 4. The standard time was chosen to be 14 h 01 m 00 s UT on December 11 (corresponding to the centre of the reception pattern) and subsequent standard times were at intervals of 23 h 56 m 04 s (approximately one sidereal day) A plot of the variation of T from day to day is shown in Fig 4 A constant pulse recurrence frequency would show a linear increase or decrease in T if care was taken to add or subtract one period where necessary The observations, however, show a marked curvature in the sense of a steadily increasing frequency. If we assume a Doppler shift due to the Earth alone, then the number of pulses received per day is given by

$$N = N_0 \left(1 + \frac{v}{c} \cos \varphi \sin \frac{2\pi n}{366\ 25}\right)$$

where N_0 is the number of pulses emitted per day at the source, v the orbital velocity of the Earth, φ the ecliptic latitude of the source and n an arbitrary day number obtained by putting $n = 0$ on January 17, 1968, when the Earth has zero velocity along the line of sight to the source This relation is approximate since it assumes a circular orbit for the Earth and the origin $n = 0$ is not exact, but it serves to show that the increase of N observed can be explained by the Earth's motion alone within the accuracy currently attainable. For this purpose it is convenient to estimate the values of n for which $\delta T/\delta n = 0$, corresponding to an exactly integral value of N. These occur at $n_1 = 15\ 8 \pm 0{\cdot}1$ and $n_2 = 28\ 7 \pm 0\ 1$, and since N

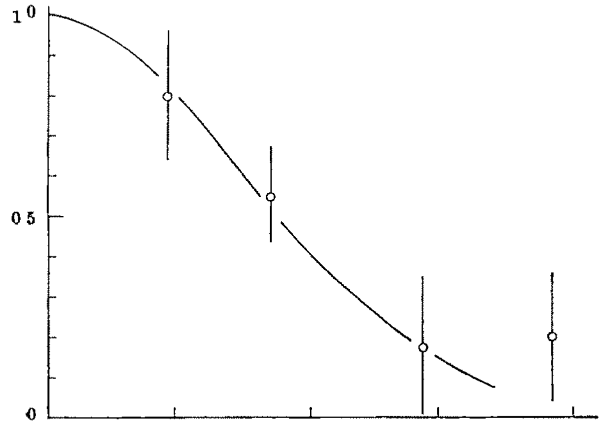

Fig 3 The response as a function of added path in one side of the interferometer

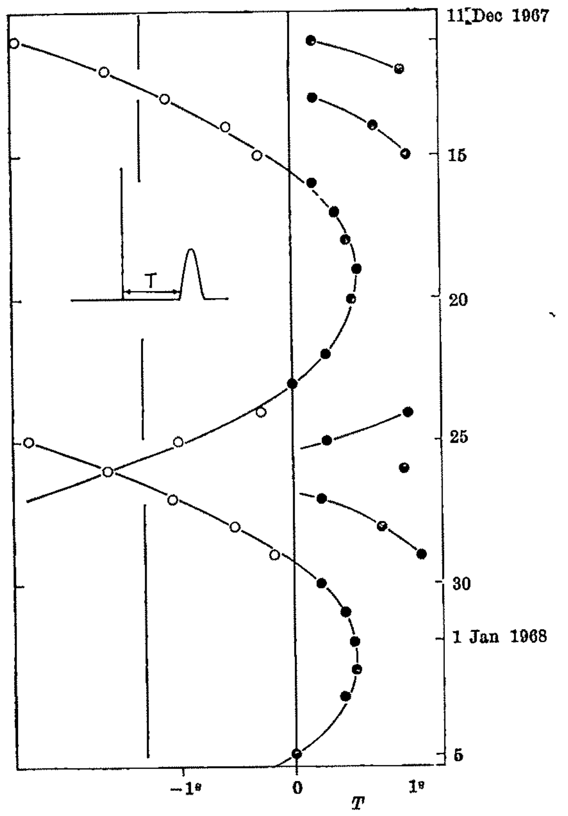

Fig. 4. The day to day variation of pulse arrival time

is increased by exactly one pulse between these dates we have

$$1=\frac{N_0 v}{c}\cos\varphi\left[\sin\frac{2\pi n_2}{366\cdot25}-\sin\frac{2\pi n_1}{366\cdot25}\right]$$

This yields $\varphi=43°\ 36'\pm30'$ which corresponds to a declination of $21°\ 58'\pm30'$, a value consistent with the declination obtained directly. The true periodicity of the source, making allowance for the Doppler shift and using the integral condition to refine the calculation, is then

$$P_0=1\cdot3372795\pm0\cdot0000020\text{ s}$$

By continuing observations of the time of occurrence of the pulses for a year it should be possible to establish the constancy of N_0 to about 1 part in 3×10^8. If N_0 is indeed constant, then the declination of the source may be estimated to an accuracy of $\pm1'$, this result will not be affected by ionospheric refraction.

It is also interesting to note the possibility of detecting a variable Doppler shift caused by the motion of the source itself. Such an effect might arise if the source formed one component of a binary system, or if the signals were associated with a planet in orbit about some parent star. For the present, the systematic increase of N is regular to about 1 part in 2×10^7 so that there is no evidence for an additional orbital motion comparable with that of the Earth.

The Nature of the Radio Source

The lack of any parallax greater than about 2′ places the source at a distance exceeding 10^3 A.U. The energy emitted by the source during a single pulse, integrated over 1 MHz at 81·5 MHz, therefore reaches a value which must exceed 10^{17} erg if the source radiates isotropically. It is also possible to derive an upper limit to the physical dimension of the source. The small instantaneous bandwidth of the signal (80 kHz) and the rate of sweep ($-4\cdot9$ MHz s^{-1}) show that the duration of the emission at any given frequency does not exceed 0·016 s. The source size therefore cannot exceed $4\cdot8\times10^3$ km.

An upper limit to the distance of the source may be derived from the observed rate of frequency sweep since impulsive radiation, whatever its origin, will be dispersed during its passage through the ionized hydrogen in interstellar space. For a uniform plasma the frequency drift caused by dispersion is given by

$$\frac{d\nu}{dt}=-\frac{c}{L}\frac{\nu^3}{\nu_p^2}$$

where L is the path and ν_p the plasma frequency. Assuming a mean density of 0·2 electron cm^{-3} the observed frequency drift ($-4\cdot9$ MHz s^{-1}) corresponds to $L\sim65$ parsec. Some frequency dispersion may, of course, arise in the source itself, in this case the dispersion in the interstellar medium must be smaller so that the value of L is an upper limit. While the interstellar electron density in the vicinity of the Sun is not well known, this result is important in showing that the pulsating radio sources so far detected must be local objects on a galactic distance scale.

The positional accuracy so far obtained does not permit any serious attempt at optical identification. The search area, which lies close to the galactic plane, includes two twelfth magnitude stars and a large number of weaker objects. In the absence of further data, only the most tentative suggestion to account for these remarkable sources can be made.

The most significant feature to be accounted for is the extreme regularity of the pulses. This suggests an origin in terms of the pulsation of an entire star, rather than some more localized disturbance in a stellar atmosphere. In this connexion it is interesting to note that it has already been suggested[2,3] that the radial pulsation of neutron stars may play an important part in the history of supernovae and supernova remnants.

A discussion of the normal modes of radial pulsation of compact stars has recently been given by Meltzer and Thorne[4], who calculated the periods for stars with central densities in the range 10^5 to 10^{19} g cm^{-3}. Fig. 4 of their paper indicates two possibilities which might account for the observed periods of the order 1 s. At a density of 10^7 g cm^{-3}, corresponding to a white dwarf star, the fundamental mode reaches a minimum period of about 8 s, at a slightly higher density the period increases again as the system tends towards gravitational collapse to a neutron star. While the fundamental period is not small enough to account for the observations the higher order modes have periods of the correct order of magnitude. If this model is adopted it is difficult to understand why the fundamental period is not dominant, such a period would have readily been detected in the present observations and its absence cannot be ascribed to observational effects. The alternative possibility occurs at a density of 10^{13} g cm^{-3}, corresponding to a neutron star, at this density the fundamental has a period of about 1 s, while for densities in excess of 10^{13} g cm^{-3} the period rapidly decreases to about 10^{-3} s.

If the radiation is to be associated with the radial pulsation of a white dwarf or neutron star there seem to be several mechanisms which could account for the radio emission. It has been suggested that radial pulsation would generate hydromagnetic shock fronts at the stellar surface which might be accompanied by bursts of X-rays and energetic electrons[2,3]. The radiation might then be likened to radio bursts from a solar flare occurring over the entire star during each cycle of the oscillation. Such a model would be in fair agreement with the upper limit of $\sim5\times10^3$ km for the dimension of the source, which compares with the mean value of 9×10^3 km quoted for white dwarf stars by Greenstein[5]. The energy requirement for this model may be roughly estimated by noting that the total energy emitted in a 1 MHz band by a type III solar burst would produce a radio flux of the right order if the source were at a distance of $\sim10^3$ A.U. If it is assumed that the radio energy may be related to the total flare energy ($\sim 10^{32}$ erg)[6] in the same manner as for a solar flare and supposing that each pulse corresponds to one flare, the required energy would be $\sim 10^{39}$ erg yr^{-1}, at a distance of 65 pc the corresponding value would be $\sim 10^{47}$ erg yr^{-1} It has been estimated that a neutron star may contain $\sim 10^{51}$ erg in vibrational modes so the energy requirement does not appear unreasonable, although other damping mechanisms are likely to be important when considering the lifetime of the source[4]

The swept frequency characteristic of the radiation is reminiscent of type II and type III solar bursts, but it seems unlikely that it is caused in the same way For a white dwarf or neutron star the scale height of any atmosphere is small and a travelling disturbance would be expected to produce a much faster frequency drift than is actually observed As has been mentioned, a more likely possibility is that the impulsive radiation suffers dispersion during its passage through the interstellar medium

More observational evidence is clearly needed in order to gain a better understanding of this strange new class of radio source If the suggested origin of the radiation is confirmed further study may be expected to throw valuable light on the behaviour of compact stars and also on the properties of matter at high density

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[1] Hewish, A , Scott, P F , and Wills, D , *Nature*, **203**, 1214 (1964)

[2] Cameron, A G W , *Nature*, **205**, 787 (1965)

[3] Finzi, A , *Phys Rev Lett* , **15**, 599 (1965)

[4] Meltzer, D W , and Thorne, K S , *Ap J* , **145**, 514 (1966)

[5] Greenstein, J L , in *Handbuch der Physik, L* , 161 (1958)

[6] Fichtel, C E , and McDonald, F B , in *Annual Review of Astronomy and Astrophysics*, **5**, 351 (1967)

Action Potentials induced in Biomolecular Lipid Membranes

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Alamethicin, a cyclopeptide, can induce action potentials in biomolecular lipid membranes.

ALAMETHICIN, a cyclopeptide antibiotic from *Trichoderma viride* containing the amino-acids $(GluN)_2$, $(Glu)_1$, $(Pro)_2$, $(Gly)_1$, $(Ala)_2$, (Methyl $Ala)_8$, $(Val)_2$ and $(Leu)_1$ (ref 1), develops a cationic conductance in experimental bilayers which can be controlled by a factor of 10^4 either chemically or by changing the membrane potential using applied voltages or ionic gradients When it is brought together with an ionic gradient and a few μg/ml of protamine, polylysine or spermine in the aqueous phase, the membranes develop characteristics of negative slope resistance (Fig 1), delayed rectification (Fig 2), bistable changes of EMF (Fig 3*A*) and single or rhythmic action potentials (Fig 3*B* and *C*) These changes resemble those occurring in excitable algae or those produced in bilayers by an unidentified proteinaceous material obtained from *Enterobacter cloacae* called excitability inducing material (EIM)[2,3]. As shown in Fig 4, alamethicin structurally resembles other macrocyclic compounds like the depsipeptides and macrolids which develop extreme K^+/Na^+ selective conductances of an ohmic nature in experimental bilayers[4] Alamethicin, however, is a pure peptide containing functional side groups

The electrokinetic and chemical data suggest that six or more alamethicin molecules form either carriers or tubular channels through which ions flow across the membrane and that assembly and dissembly of these aggregates by the voltage or chemical factors regulate the conductance The kinetic and chemical characteristics of alamethicin apparently fulfil the criteria of an allosteric protein specifically designed to form complexes of ionic co-ordination within lipid membranes We discuss the possibility that related compounds could generate active ion transport and act as high energy intermediates in oxidative phosphorylation

Bilayer membranes separating two aqueous phases were made by published methods[2,3,5] Alamethicin acts on membranes made from mixed brain lipids and tocopherol, lecithin and decane, sphingomyelin and tocopherol[2,3] or oxidized cholesterol and octane[6], although the sensitivity to alamethicin and to protamine varies from one lipid type to another Best results were obtained with membranes made from oxidized cholesterol (1 per cent), dodecyl acid phosphate (0 25 per cent), cholesterol (0 42 per cent in octane dodecane, 1 3[6]) and with membranes made from purified lecithin (10 per cent in decane–squalene, 2 1) In the presence of adsorbates the life of the membrane lasts from a few minutes to several hours according to the type of lipid Alamethicin can be introduced either by dissolving it in the bulk lipid solution or by adding it to the aqueous phase after bilayer formation

Fig 1 presents three steady state current–voltage, I(V), curves in the presence of 10^{-7} g/ml of alamethicin on one side of the membrane With 0 1 M sodium chloride on both sides, the membrane shows strong and more or less symmetrical rectification (Fig 1*A*) The conductance decreases 10^4 times between zero and 100 mV and attains a maximum change of *e*-fold/5 mV An ionic gradient generates a cationic resting potential of 10–30 mV/decade without intracationic selectivity. This acts like an applied potential to drive the system into its high conductance state although it does not deliver a net current As shown in Fig 1*B*, the resting potential shifts the I(V) curve along the voltage axis, raises the resting conductance and, for analytical reasons given elsewhere[3], transforms the rectification characteristic in one quadrant into a characteristic of negative slope resistance If protamine is added to either aqueous phase under zero ion gradient there is a similar effect as shown in Fig 1*C* The resting conductance is increased and a negative resistance arises in the absence of a resting potential This is the first of three distinct effects produced by protamine, histone or spermine acting as secondary adsorbates Because this particular effect resembles that of a resting potential it may result from a change in the phase boundary potential accompanying adsorption of basic proteins or polyamines

The voltage–time phenomena generated by constant current pulses in the three sets of conditions just described are given in Fig 2 If the system rests in its low conductance state corresponding to Fig 1*A*, applied voltages raise the conductance This usually occurs slowly with respect to the time constant of the membrane so that the positive coupling between the conductance and the potential produces a delayed voltage restoring effect shown in Fig 2*A* If the system rests in its high conductance state in the conditions of Fig 1*B* or *C*, then applied voltages of that sign which drive it back into the low conductance state develop the regenerative transition shown in Fig 2*B* because the two variables are now negatively coupled But voltages of opposite sign which raise the conductance again develop the delayed rectification process It has already been shown that EIM can generate these same phenomena and change its resting conductance state under the influence of either a resting potential or chemical agents[2,3] It should be noted, however, that, in contrast to alamethicin, EIM remains intrinsically in the high conductance state

The second effect of protamine, histone or spermine is to introduce an alternative resting potential level of

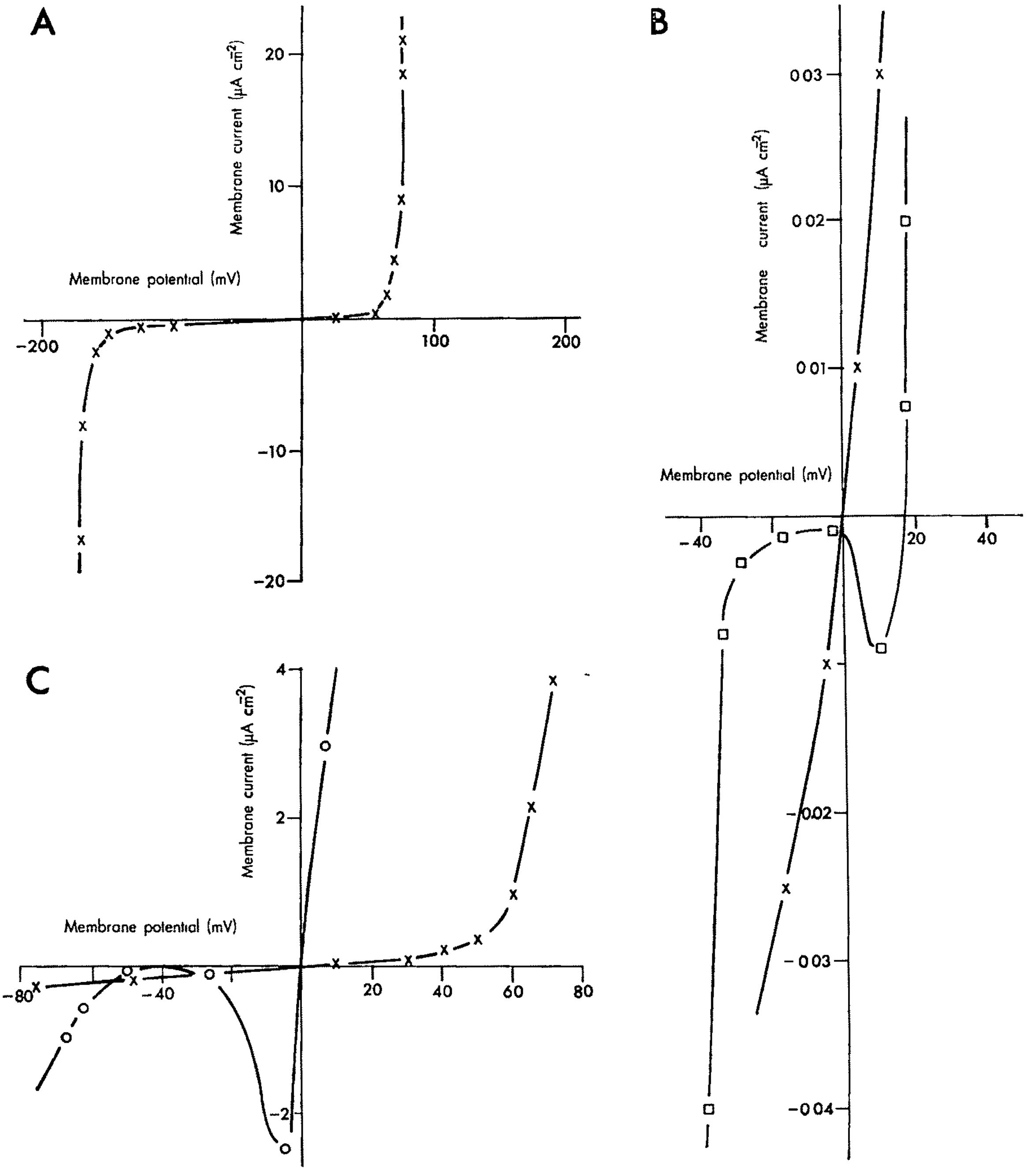


Fig 1 Current–voltage curves from different bimolecular lipid membranes in the presence of 10^{-7} g cm^{-3} alamethicin in the inside aqueous compartment and 5 mM/l histidine Cl, *p*H 7 0, in both sides in all cases Outward current is plotted as positive and the potential is that of the inside compartment minus that of the outside *A*, 0 1 M sodium chloride on both sides Membrane solution 2 5 per cent purified sphingomyelin in tocopherol chloroform methanol, 5 3 2 The asymmetry of the rectification characteristic is because alamethicin was added to one side only *B*, (squares) Membrane solution same as upper left but 0 05 M sodium chloride outside only There is a resting potential at the point of zero current caused bv the ionic concentration gradient The ion gradient also develops a negative resistance The upper left curve (crosses) is reproduced for comparison *C*, (circles) 0 1 M sodium chloride both sides and 10^{-4} g cm^{-3} protamine sulphate inside Membrane solution 4 per cent oxidized cholesterol in decane Protamine converts the rectification into a negative resistance characteristic The curve of Fig *A* (crosses) is reproduced on these expanded ordinates for comparison

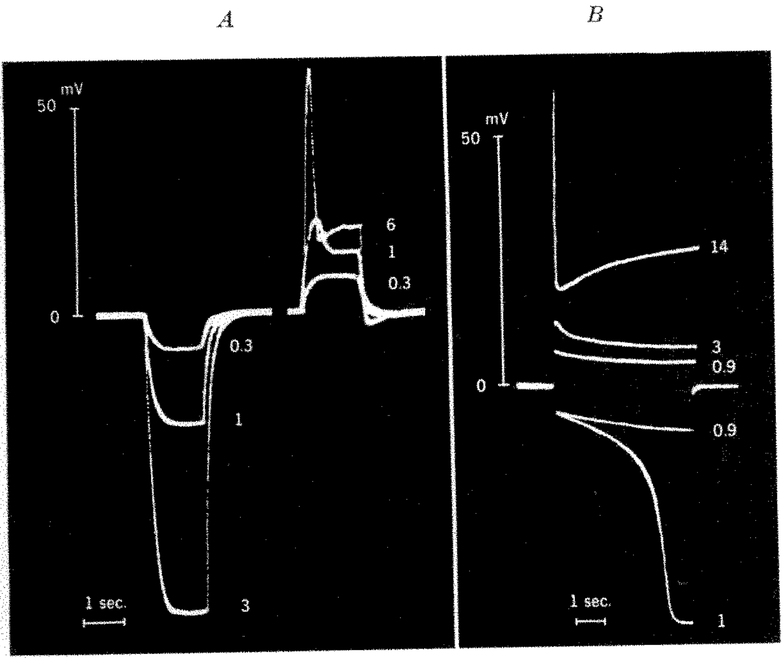


Fig. 2. Recorded membrane potentials in response to applied constant rectangular currents in the presence of 10^{-7} g cm^{-3} alamethicin in the inside compartment and 5 mM/l. histidine Cl, *p*H 7·0, in both sides in all cases. Upward deflexions denote inside positive. Numbers indicate the current density in μA cm^{-2}. *A*, 0·1 M sodium chloride in both compartments, 10^{-5} g cm^{-3} protamine inside. Membrane solution: 5 per cent total white matter lipids plus 3 per cent cholesterol in 3 : 4 : 2, tocopherol : chloroform : methanol. If there had been no protamine, the rectification would have been more nearly instantaneous and the potentials would show less initial upward transient. *B*, Same as above but using 10^{-4} g cm^{-3} protamine and a membrane solution of 4 per cent oxidized cholesterol in decane. Increasing the protamine concentration develops the regenerative non-linear response to inward currents which correspond to the negative resistance characteristic in the I(V) curve of Fig. 1*C* (circles).

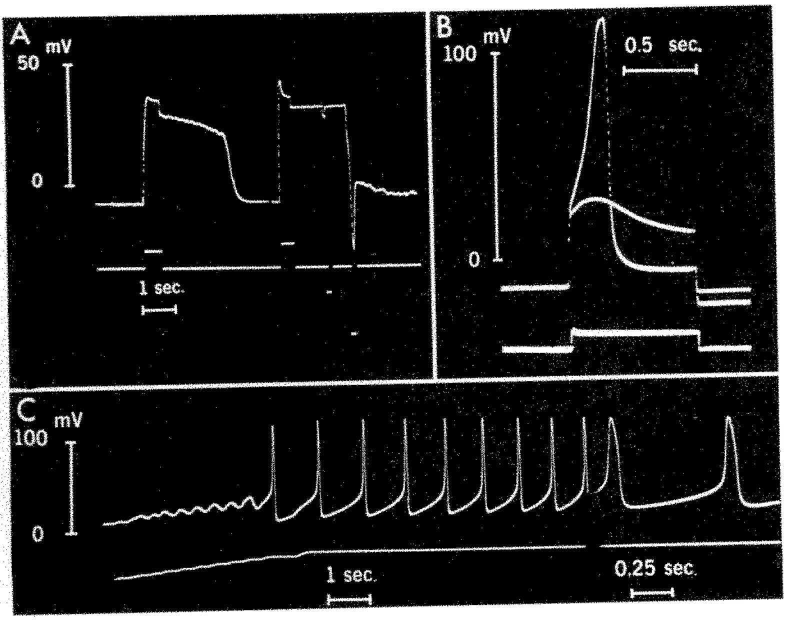


Fig. 3. Conditions as in Fig. 2*B* but 40 mM/l. potassium chloride inside and none outside. The lower traces show the applied currents. *A*, Bistability caused by a slight excess of protamine. The system was monostable initially as seen in the response to the first pulse, but then it became intrinsically bistable. Because there is no steady applied polarization the response results from an EMF change. The resistance is lower in the more anion permeable state which is the uppermost level. *B*, Subthreshold response and monostable action potential during applied depolarizing pulses. *C*, Rhythmic firing begins as the depolarizing current is increased to a critical level. Note expanded time scale on the right. These action potentials and those in *B* are largely resistive in type, the resistance increasing during the spike. They occur at depolarization levels greater than those initiating the EMF changes in the upper left figure and always require sustained applied voltages for their development. Lecithin–decane–squalene membranes, in the same conditions, develop self-regenerative single or rhythmic action potentials which can be initiated by brief currents or appear in the absence of any applied currents. Their amplitude is usually 30 to 60 mV but their wave form and frequency are identical to those in records *B* and *C*.

anionic sign. If, in the presence of an ionic gradient, the concentration of protamine is carefully titrated against alamethicin, the membrane potential becomes bistable and switches reversibly through zero from the original cationic level to the new level under the stimulus of brief applied pulses (Fig. 3*A*). The anionic level is more effectively developed by arginine-rich than lysine-rich basic proteins and by membranes containing sphingomyelin rather than lecithin. Although any combination will work with alamethicin, only the first member of each pair is effective with EIM[3].

In membranes composed of oxidized cholesterol and dodecyl acid phosphate "resistive action potentials" occur as shown in Fig. 3*B*. They resemble those occurring in frog skin[7] and in bilayers exposed to EIM in certain conditions[3]. They can enter the rhythmic state under maintained polarization (Fig. 3*C*) when the chemical parameters are properly adjusted. They are not self-regenerative and do not make use of the EMF change just described but seem to involve a secondary increase of the membrane resistance induced by protamine. This new gating characteristic constitutes the third effect of basic proteins.

In membranes composed of selected types of commercial purified lecithin containing squalene and decane, it is possible to adjust the ionic gradient and the ratio of alamethicin to protamine, so that the bistable flip-flop kinetics shown in Fig. 3*A* become monostable and generate either spontaneous rhythmic firing in the absence of applied voltages, or single action potential responses following short shock stimulation. These self-regenerative action potentials have all the kinetic properties of cellular action potentials and are similar to those already described in sphingomyelin membranes containing EIM and protamine[2,3]. Their time course and frequency are like those shown in Fig. 3*B* and *C* except that their amplitude is about 30–60 mV.

Fig. 5 shows that the maximum change of the steady state conductance varies exponentially with the sixth power of the potential (*e*-fold/5 mV) for a wide range of alamethicin concentrations. This is the same value reported for the potassium permeability system in nerve[8]. In addition, Fig. 6*A* shows that the steady state conductance also varies as the sixth power of both the alamethicin concentration and the ion concentration. Furthermore, this relation has been found to be independent of the voltage. These findings indicate that the molecularity of the reaction underlying the development of the conductance is hexamolecular with respect to both alamethicin and ions. It is also hexamolecular both in the resting state and when it is voltage induced.

The apparent kinetic order of the reaction is also six. As in nerve[8], the membrane current and conductance undergo a sigmoid rise with time following an instantaneous displacement of the potential by voltage clamp methods. The time constant of the response is voltage dependent and larger for the conductance increase than the decrease. The maximal rate of change de-

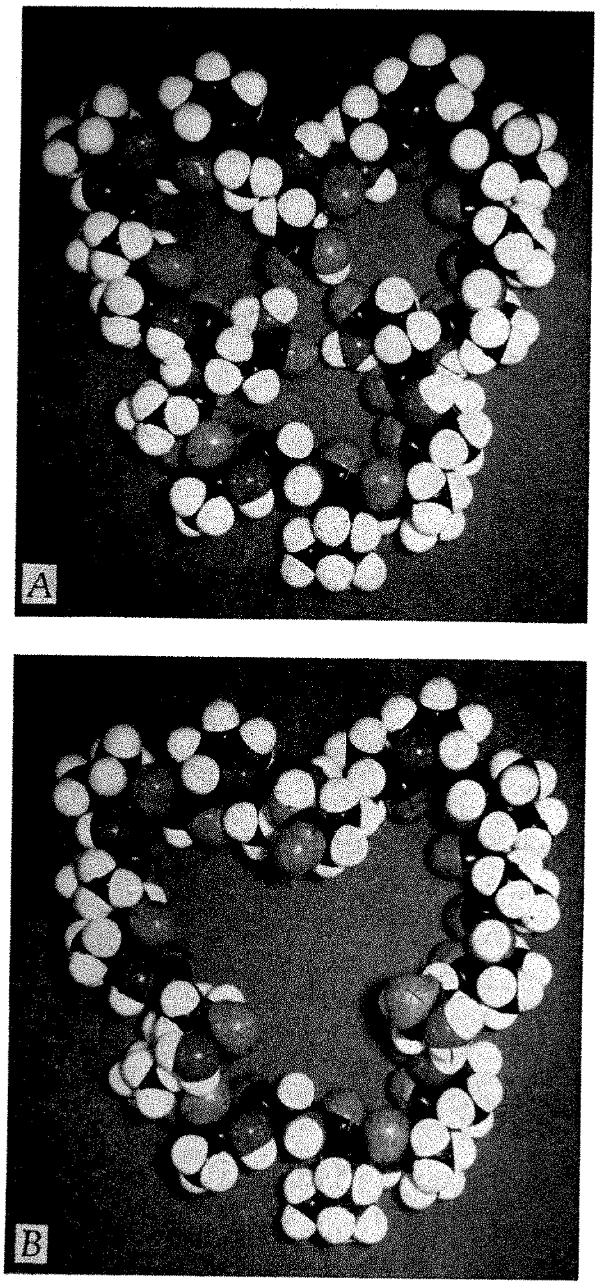


Fig. 4. Two hypothetical molecular configurations of alamethicin: *A*, By rotating the carboxyl and amide groups out of the plane of the ring, an open ring configuration is attained which might conduct by acting either as a carrier or as part of a channel. In addition to the peptide bonds, the three functional side groups could contribute to the formation of a hexamer of tubular or other shape via intermolecular hydrogen or ionic bonding. *B*, Intramolecular chelate. The carboxyl group of the glutamic acid side chain is arranged to form internal hydrogen bonds or possibly a metal ion chelate with the two glutamine residues. This may be the monomeric non-conducting form. The electrokinetic data suggest that the field regulates the conductance by moving monomeric co-ordination complexes of counterion and alamethicin which are positively charged into the hydrocarbon region of the bilayer where they form a hexameric channel unit. The ion with a partial hydration shell is thought to be sequestered within the ring.

pends strongly on the lipid type, ionic strength, the temperature (Q_{10} 3–4), and the presence of protamine. When these parameters are held constant, then the maximum rate is found to increase with the sixth power of the alamethicin concentration as shown in Fig. 6*B*. Hence the reaction is kinetically of the sixth order with respect to this parameter.

Voltage clamp studies have also shown that, if an applied voltage is instantaneously reversed in sign, the reversed membrane current undergoes a marked decline before building up again to its starting level showing that the conducting system exists in some well defined polarized state across the membrane.

We may begin the evaluation of these findings with a consideration of the origin of the ion selective conductances. It has been proposed[4] that macrocyclic compounds develop a cationic conductance by providing a dominating proton acceptor function in the form of carbonyl oxygen atoms. These atoms project toward the centre of the ring to form an adjustable co-ordination complex with either the naked or hydrated cation by means of multiple hydrogen bonds or dipole interactions. This orientation simultaneously sequesters the ion while placing the hydrocarbon side chains along the ring exterior to permit membrane solubilization of the entire charges complex by van der Waals interaction with the lipid hydrocarbon chains. Similar co-ordination complexes might also be formed by non-covalent ring closures as in the case of the gramicidins[4,9] or perhaps even by an open helix.

In this view, the poor intracationic selectivity of alamethicin would result from a larger ring diameter (13 Å inner diameter) compared with valinomycin (7 Å), for example. Basic proteins and spermine may invert the sign of this conductance, and therefore the sign of the resting potential in an ionic gradient, by increasing the ratio of proton donors and positive charges to proton acceptors and negative charges in the conducting system. Because protamine and sphingomyelin are essentially chemical analogues of histones and lecithin containing two extra proton donor groups in each case, protamine would be expected to be more effective in converting the ionic selectivity as is observed experimentally.

When the titration of protamine against alamethicin is not carried to completion, two distinct conducting populations with different selectivities could arise, provided the effect involves specific chemical complex formation and not a diffuse effect on the Donnan potential.

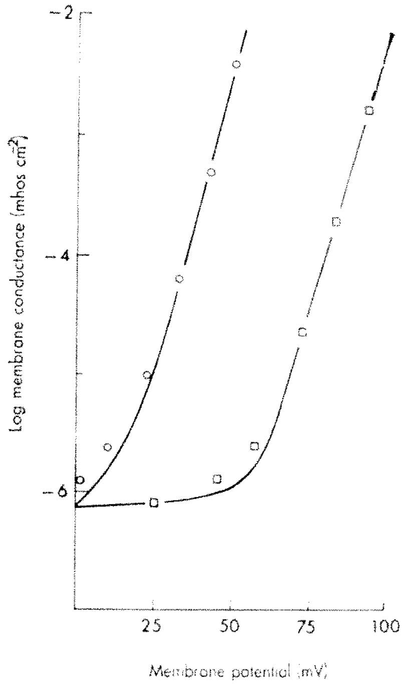


Fig. 5. The relation between membrane conductance and applied potential at two different concentrations of alamethicin in the aqueous phase (circles 5×10^{-8} g/ml., squares 10^{-6} g/ml.) membrane lipid composition as in Fig. 1*A*. Ionic concentrations in both aqueous compartments, 0·1 M sodium chloride. The points are experimental; the curves were calculated from equations (3), (4) and (6) using the following values for the constants which were adjusted for best fit: $G_s = 8 \times 10^{-7}$ mhos cm^{-2}, $G_{Al} = 10^{-11}$ mhos cm^{-2}, Al = 1 M/l. and 0·2 M/l., $Me^+ =$ 0·1 M/l., $K_{1,2} = 0{\cdot}1$, $E_3 = 2$ kcal/mol, $E_4 = -2$ kcal/mol, $K_{5,6} = 4 \times 10^{11}$, $n = 6$. The thickness of the hydrocarbon layer was assumed to be 50 Å.

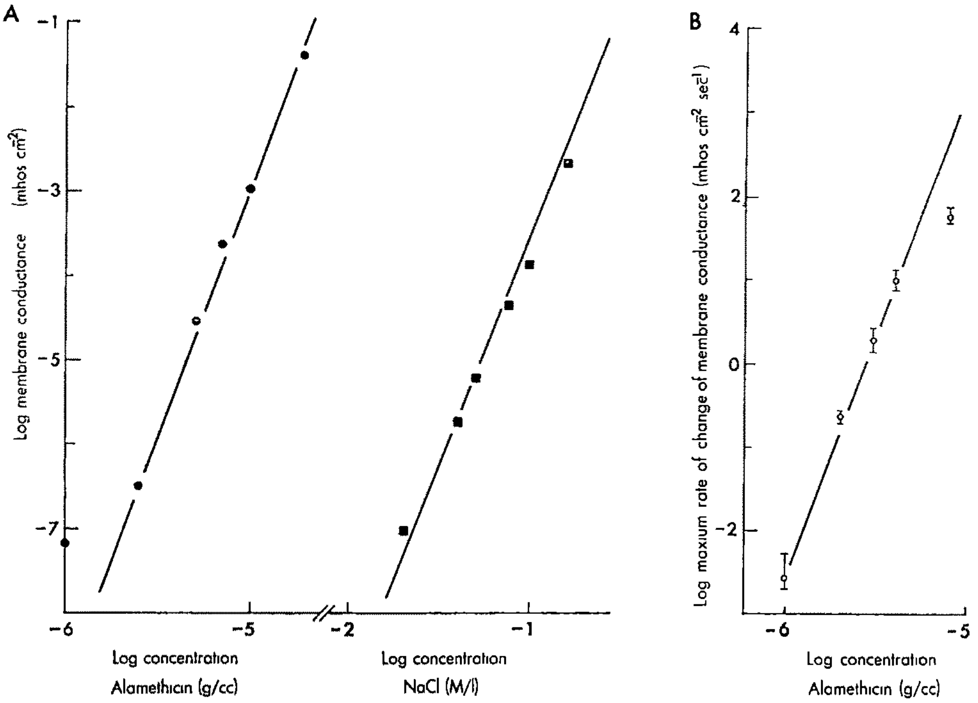


Fig 6 *A*, (Left) Relation between membrane conductance (I/V) and concentration of alamethicin in the aqueous phase at constant ion concentration (0 1 M sodium chloride) and constant voltage (60 mM) (Right) Relation between membrane conductance and concentration of sodium chloride in the aqueous phase, at constant alamethicin concentration (10^{-5} g/ml), and constant voltage (60 mV) All concentrations are identical in both aqueous compartments Membrane lipid composition as in Fig 1*A* The straight lines have slopes to the sixth power which are independent of membrane lipid composition and of the voltage as long as the conductances induced by alamethicin are much larger than the intrinsic membrane conductance These curves were each obtained from a single membrane *B*, Maximum rate of change of membrane conductance after a sudden displacement of the membrane potential by 100 mV (voltage clamp), as a function of the concentration of alamethicin Membrane in 0 1 M sodium chloride, both sides, membrane solution as in Fig 1*B*, temperature, 35° C The slope of the straight line again indicates a sixth power function Each point is an average of ten values obtained from ten different membranes The bars give the total scatter

This is the simplest interpretation for the origin of the bistability, and if it is assumed that the gating mechanism of the pure alamethicin system remains intact within the alamethicin–protamine complex then all the conditions are fulfilled for developing action potentials within the framework of the Hodgkin and Huxley theory[8] namely, that two gated conducting systems with appropriate time constants and different ionic selectivities act as parallel EMFS and variable resistive loads on each other This is the likeliest interpretation for the origin of the experimental action potentials

Although the nature of the underlying gating mechanism has not been resolved, some preliminary models can be considered A simple monovalent carrier mechanism can be excluded as inconsistent with the highly non-linear co-operative phenomena shown by alamethicin and EIM, and carriers composed of six or more alamethicin molecules having molecular weights of 11,000 or larger would be rather bulky items in terms of membrane dimensions Moreover, if these aggregates form complexes with compounds like protamine or polylysine having molecular weights up to 50,000, it seems doubtful whether they could retain the essential mobility of a carrier In addition, it has been pointed out[8] that in certain conditions carriers should develop measurable currents because of their own transient movements None has been found[8] For these reasons carrier mechanisms are not favoured at this time although their existence would not change the validity of the mathematical model or critical aspects of the discussion to follow

As an alternative, alamethicin might cause a reversible micellization of the bilayer structure Co-operative interactions between micelles to form channel domains or clusters could account for the non-linear effects Although channel domains may exist, however, it is at present considered more likely that the primary gating event results from the self-aggregation of the cyclopeptide component As discussed later, the properties of alamethicin resemble allosteric proteins which characteristically undergo polymerization in the presence of extraneous molecules and alamethicin exhibits a strong tendency to aggregate in both aqueous and non-aqueous solution It has also been found that most of the electrokinetic effects of alamethicin, except protamine interaction, can be induced in bilayers by amphophilic ion precipitants such as dipicrylamine which develop strong attractive forces between the organic moiety[10] accounting for their unique effects in aqueous solutions Furthermore, if the cyclic compounds do form ionic co-ordination complexes, we must assume that they and not lipid polar groups provide the immediate conduction sites for ions across the bilayer Finally, the invariance of the principal electrokinetic phenomena despite wide lipid variation gives additional support to this interpretation

We therefore tentatively suggest that through self-aggregation, or a change in aggregation state, alamethicin provides a series of sites bridging the bilayer, whatever lipid rearrangements may secondarily accompany such an effect

The enniatins and valinomycin, which develop large conductances but no gating effects, lack the functional side groups of alamethicin and, as depsipeptides, also lack every other nitrogen atom of the peptidic proton donor The enniatins, in fact, contain none because the remaining nitrogen groups are blocked by *N*-methylation Consequently, these compounds, as compared with the homodetic peptide, alamethicin, can form no more than half the number of peptide hydrogen bonds between rings of the type which stabilize the α-helix of linear proteins It is therefore conceivable that within the constraints imposed by the anisotropic hydrocarbon chains of the bilayer the additional intermolecular hydrogen bonds of alamethicin and the development of aggregates of tubular form resembling phage tail cores

At present the simplest interpretation of the fact that the kinetic order and molecularity of the reaction are both hexameric would be that the potential varies the concentration of alamethicin monomers and metal ions in the hydrocarbon region to form hexameric conducting units Because the conductance varies as the sixth power of the ionic strength even when extrapolated to zero voltage the conducting configuration is probably stabilized in part by ionic or ion dipole interactions as well as hydrogen ion bonding. Within this formulation EIM and alamethicin would differ only in having free energies of opposite sign for the monomer–hexamer transition, the former normally resting in the hexameric form, the latter in the monomeric form

The oligomers may exist in several alternative bond states which could account for the polarized nature of the conducting system If the hydrogen bonds were polarized from donor to acceptor, as they are in α-helices, they would lie in a particular direction across the membrane and a reversal of the field might cause a reversal of bond polarization which could explain the observed decrease in transient conductance This could occur with the existing set of channels or involve a complete dissembly of one set and reassembly of another set from the opposite side of the membrane In addition, or as an alternative, the side groups might be similarly polarized or exist in a number of inter- or intra-molecular (chelate) bond configurations (see Fig 4), or the rings may be stacked with their peptide bonds lying in the same or alternating senses around the rings, or the atoms of adjacent rings could be located in several alternative registers

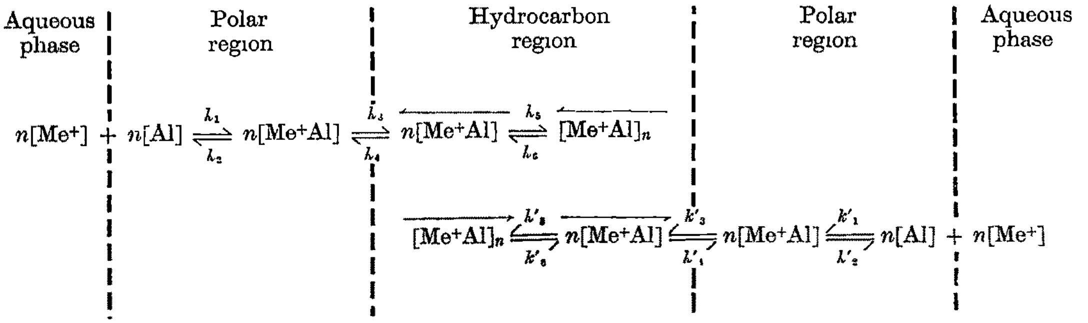
The idea of reversible bridge formation by an aggregate of six ions under the control of the membrane potential has already been suggested by Hodgkin and Huxley as one possible basis for action potentials in nerve[8] If we agree with them and make the following three assumptions, we can account quantitatively for the chief features of the chemical and electrokinetic findings

(1) In the absence of a large phase boundary, resting or applied membrane potential, the alamethicin molecules (Al) are primarily absorbed as monomers or possibly aggregates of a non-conducting type to the two polar regions of the bilayer In addition to the counterion of the carboxyl group a fraction of the rings carry an extra cation (Me$^+$) sequestered by the peptide carbonyls of the ring giving the total co-ordination complex a net positive charge. Only this fraction forms conducting oligomers This accounts for the fact that a positive voltage on the side of the membrane to which alamethicin has been added induces a larger increase in conductance than a negative voltage and that the conductance is a function of the ionic concentration to the sixth power

(2) An applied or resting potential, or an increase of the phase boundary potential caused by changing absorbates, ions or lipid, drives the monomers [Me$^+$Al] from the positive side into the hydrocarbon region where they aggregate as hexameric tubular channels, carriers or other aggregate form of a conducting nature (Me$^+$Al)$_n$ Because of these complex dynamics the distinction between carrier and channel concepts becomes blurred

(3) The intra-oligomeric bonds are polarized in one direction or the other normal to the plane of the membrane according to the sign of the potential The polarization sign, indicated by an arrow in equation (1), also dictates the direction of assembly and dissembly of the oligomer No transfer of monomers takes place from one polar region to the other, presumably because the oligomers are formed by sequential apposition of polarized monomers from only one side or because secondary lipid re-arrangements block this movement

These assumptions can be represented by the following simplified reaction scheme


Aqueous phase
Polar region
Hydrocarbon region
Polar region
Aqueous phase
n[Me+] + n[Al] ⇌(k1, k2) n[Me+Al] ⇌(k3, k4) n[Me+Al] ⇌(k5, k6) [Me+Al]n
[Me+Al]n ⇌(k'5, k'6) n[Me+Al] ⇌(k'3, k'4) n[Me+Al] ⇌(k'1, k'2) n[Al] + n[Me+]

(1)

The concentration of positively charged alamethicin monomers located in the polar region at zero potential [Me$^+$Al] is related to the concentration of metal ions [Me$^+$] in the aqueous phase and alamethicin [Al] in the polar region by

$$K_{12}\,[\mathrm{Me}^+] + [\mathrm{Al}] = [\mathrm{Me}^+\mathrm{Al}] \qquad (2)$$

where $K_{12} = k_1/k_2$ is the dissociation constant of the metal ion–alamethicin complex For simplicity, equation (1) neglects the distribution constant for [Me$^+$] between aqueous and polar regions as well as the dissociation and polymerization side reactions which may go on in each of the three regions

The fraction, p, of the charged monomers, [Me$^+$Al], driven from the polar region into the hydrocarbon region by the potential, V, is given by

$$p = k_3/(k_3 + k_4) \qquad (3)$$

in which k_3, k_4 are the rate constants for the transition into and out of the hydrocarbon region and are of the form

$$k_3,\ k_4 = A \exp E_{3,4} \pm z\ 11\ 5\ V/RT \qquad (4)$$

where E_3, E_4 are the activation energies in kcal/mol for the forward and backward transitions, z is the valence of the monomer, A is the collisional frequency constant and V is the membrane potential The concentration of oligomers, or channels, is then

$$[\mathrm{Me}^+\mathrm{Al}]_n = ([\mathrm{Me}^+\mathrm{Al}]p)^n K_{5\,6} \qquad (5)$$

where $K_{5\,6} = k_5/k_6$ is the equilibrium constant for the formation of channels from the monomer The membrane conductance, G_m, as a function of the potential, the concentration of alamethicin in the aqueous phase and the concentration of monovalent cations in the aqueous phase are then given by

$$G_m = G_0 + G_{\mathrm{Al}}\,K_{5\,6}\,([\mathrm{Me}^+]\,[\mathrm{Al}]\,K_{1\,2}\,p)^n \qquad (6)$$

where G_0 is the intrinsic conductance of the bilayer in the absence of alamethicin and G_{Al} is the conductance of the individual alamethicin oligomer.

With most lipids, $k_4 \gg k_3$, so that at low concentrations of alamethicin the resting conductance is equal to G_0 because there are only a few conducting oligomers. In this case it is sufficient to deal with one side of the reaction scheme only At higher concentrations, oligomers of both polarization signs appear at zero potential and those of one sign will be dissembled by the voltage while those of the opposite sign are simultaneously assembled The experiments indicate that $n = 6$ in all conditions examined so far, including variations of lipid composition, temperature and pH, and that $k_1 \ll k_2$ so that excess Me$^+$ can still regulate the concentration of [Me$^+$Al]

Some examples are calculated and compared with the experimental data in Fig 5 At low voltages the experimental values deviate from those predicted by equation (6), perhaps because the profile of the surface potential which determines the resting conductance is different from the potential profile caused by the applied voltage

The model assumes a causal relation between the high order of the conductance versus concentration and that of the conductance versus voltage functions This relation could be fortuitous and the channel closure could occur without disaggregation, for example, as a result of the statistical operation of six series gates formed by the glutamic residues blocking the ring opening through chelate formation But, in this event, additional and rather unlikely hypotheses would have to be introduced to account for the fact that the rates of gate operation also depend on the sixth power of the concentration of alamethicin Moreover, the state of low conductance approaches the intrinsic value of the conductance of the bilayer itself and, while this condition is easily met by total channel dissembly, in its absence effective ring blockage would be required

Within the framework of this model alamethicin resembles an allosteric protein in which the observed co-operative and modifier-substrate effects result from configurational changes which involved oligomeric systems Protamine and possibly the potential may act as allosteric modifiers of the ionic (substrate) conductance by controlling the monomer–oligomer transition, a typical ligand effect in regulatory enzymes This effect of protamine represents a type of chemical control over the ionic gating mechanism which might be elaborated into a mechanism for actively pumping ions against an electrochemical gradient Compounds containing high energy phosphate are common ligands of allosteric systems and in appropriate conditions a single phosphorylation reaction might induce the bond reversal or other oligomeric re-arrangement discussed earlier Because of the bond constraints in such systems perturbation of a terminal bond in a linear oligomer could sweep over the entire structure causing the vectorial ejection of 5 to 10 ions against an ion gradient

In fact, three lines of experimental evidence indicate a close relation between ion gates and ion pumps First, both are ion handling systems showing the same ion discriminating capabilities[11] known to occur only with macrocyclic compounds, and both seem to manipulate an unusually large number of ions (5 to 10) per elementary cycle[12]. Second, as with alamethicin in bilayers, ion pumps show homotropic ionic co-operative effects, heterotropic modifier-substrate interactions and large entropy changes characteristic of allosteric systems[13,14] Finally, preliminary data suggest some chemical similarities Ion pumps, phosphorylation reactions and the membrane properties of alamethicin or EIM are modified by chlorpromazine and promethazine[3,13,15], basic proteins[3,16], atabrine and octyl guanidine[3,17], all at concentrations of a few μg/ml Finally, ion pumps may be composed of five[18] or six[19] molecular sub-units The sub-units of both may have molecular weights of a few thousand and are exopeptidase insensitive[19], implying again that both contain ring compounds or blocked terminal groups as in gramicidin

An allosteric gate-pump mechanism for regulating ion movements across bimolecular lipid membranes may provide a system for carrying out cellular energy transformations because it has been shown in cells that electron transport, oxidative phosphorylation and ion movements are closely coupled[20] High energy electrons in the redox system, high energy phosphate bonds in ATP and the electrochemical energy of a transmembrane ion gradient can be taken as three equivalent energy forms which are interconverted in a symmetrical manner by the gate-pump system Six reversible energy transformations would then be predicted and the evidence suggests that all six have been observed. Thus either high energy electrons[21] or ATP[11] can generate an ion gradient, or an ion gradient can reduce the cytochromes[22] or form ATP[23] and the cytochromes can generate ATP[17] or ATP can reduce the cytochromes[24] The energy per ion varies with the electrochemical gradient but is usually 2 to 10 times smaller than the chemical energy units, that is to say, ATP or redox step The system, however, maintains a high efficiency by transporting five or more ions per unit of chemical energy This unusual stoichiometry requires a special molecular machinery to couple the disparate energies efficiently The ion handling and polarization properties of alamethicin suggest that a cyclopeptide hexamer sequestering six ions and capable of cycling between conducting and non-conducting states of polarized configuration could supply the necessary coupling machinery It would be necessary only for the same configurational cycle to be induced by each of the three energy forms Because of the mutual bond constraints in the oligomer a change induced in the bond state of one monomer, for example, by phosphorylation or a valence change of Fe^{++} to Fe^{+++}, could drive the entire oligomer into an alternative configuration of higher energy through a series of consecutive reactions across the membrane Conversely, because the bond energy in each monomer is a function of all the others, such a system may provide a way to lead off the configurational energy of the entire oligomer through one of its monomers to form a high energy phosphate bond These distributive characteristics would be particularly suitable for coupling reactions operating at different levels of unit energy as discussed earlier Conformational states[25] of cyclopeptide oligomers may therefore satisfy the requirements for the unknown metabolic intermediates[26] often designated as X, Y and $\sim X$, $\sim Y$ If so, then excitability phenomena, active transport processes and the key steps in the utilization of respiratory and radiation energy, that is, oxidative and photosynthetic phosphorylation, would all result from related modes of operation of a common mechanism located in cell membranes

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[1] Meyer, C E , and Reusser, F , *Experientia*, **23**, 85 (1967)

[2] Mueller, P , and Rudin, D O , *Nature*, **213**, 603 (1967)

[3] Mueller, P , and Rudin, D O , *J Theoret Biol* (in the press).

[4] Mueller, P , and Rudin, D O , *Biochem Biophys Res Commun* , **26**, 398 (1967)

[5] Mueller, P , Rudin, D O , Tien, H T , and Wescott, W C , *J Phys Chem* , **67**, 534 (1963)

[6] Tien, H T , Carbone, S , and Dawidowicz, E A , *Nature*, **212**, 718 (1966)

[7] Finkelstein, A , *J Gen Physiol* , **47**, 545 (1964)

[8] Hodgkin, A L , and Huxley, A F , *J Physiol* , **117**, 500 (1952)

[9] Lardy, H A , Graven, S H , and Estrada-O S , *Fed Proc* , **26**, 1355 (1967)

[10] Kielland, J , *J Amer Chem Soc* , **61**, 2285 (1939)

[11] Skou, J C , *Physiol Rev* , **45**, 596 (1965)

[12] Sen, A K , and Post, R L , *J Biochem* , **239**, 345 (1964) Rossi, C S , Bielawski, J , Carafoli, E , and Lehninger, A L , *Biochem Biophys Res Commun* , **22**, 206 (1966)

[13] Squires, R F , *Biochem Biophys Res Commun* , **19**, 27 (1965).

[14] Jardetsky, O , *Nature*, **211**, 969 (1966) Robinson, J D , *Biochemistry*, **6**, 3250 (1967)

[15] Judah, J D , Rees, K R , and Dawkins, M J R , *Nature*, **183**, 821 (1959)

[16] McIlwain, H , *Biochem J* , **90**, 442 (1964) McIlwain, H , *Biochem J* , **73**, 514 (1959) Schwartz, A , Bachelard, H S , and McIlwain, H , *Biochem J* , **84**, 626 (1962)

[17] Pullman, M E , and Schatz, G , *Ann Rev Biochem* , **36**, 539 (1967)

[18] Abrams, A , and Baron, C , *Biochemistry*, **6**, 225 (1967)

[19] Bader, H , Sen, A K , and Post, R L , *Biochim Biophys Acta*, **118**, 106 (1966)

[20] Chance, B , Lee, C P , and Mela, L , *Fed Proc* , **26**, 1341 (1967) Mitchell, P , *Fed Proc* , **26**, 1370 (1967) Jagendorf, A T , *Fed Proc* , **26**, 1361 (1967)

[21] Conway, E J , *Nature*, **198**, 760 (1963)

[22] Mayne, B C , and Clayton, R K , *Proc US Nat Acad Sci* , **55**, 494 (1966)

[23] Garrahan, P J , and Glynn, I M , *J Physiol* , **192**, 237 (1967) Jagendorf, A T , and Uribe E , *Proc US Nat Acad Sci* , **55**, 170 (1966)

[24] Ernster, W , and Lee, C P , *Ann Rev Biochem* , **33**, 729 (1964)

[25] Boyer, P D , Bieber, L L , Mitchell, R A , and Szabolcsi, G , *J Biol Chem* , **241**, 5384 (1967)

[26] Slater, E C , in *Regulation of Metabolic Processes in Mitochondria* (edit by Tager, J M , *et al*), 166 (Elsevier, New York, 1966)

RNA in Mouse Cells exposed to Different Antigens

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The behaviour of peritoneal cells in producing different species of RNA after exposure to different antigens is consistent with the idea that the potential to produce antibodies of varying specificity is inherited

THE finding that plasma cell tumours secrete a homogeneous protein, thought to be identical to a normal immunoglobulin produced in excess, has made it possible to determine the amino-acid sequence of the light chain sub-unit of the whole molecule[1-3]. Sequence analysis of light chains isolated from mice bearing several types of tumours reveals that approximately half of the molecule has a constant structural identity and that there are variations in sequence throughout most of the other half[4] At least three hypotheses have been proposed to explain the molecular mechanism by which immunoglobulins arise *in vivo* (*a*) Half of a single gene for antibody synthesis undergoes mutation during somatic development[5-7], (*b*) a single species of messenger RNA is misread during translation[8], and (*c*) the potential to produce antibodies of varying specificity is inherited in multiple genes, and antigens activate those genes appropriate for the synthesis of specific immunoglobulins[9]

In an attempt to test these hypotheses, we first reported[10] that new species of RNA† appeared in the mouse spleen after inoculation with sheep red blood cells (SRBC) The presence of this RNA was detected *in vitro* by hybridization with mouse DNA In further experiments, we sought to determine whether the formation of new species of RNA by the mouse was a non-specific response to immunization, or whether separate and distinct RNAs appeared after immunization with separate and distinct antigens

Peritoneal exudate cells (p e cells) were obtained from B_6AF_1 mice (Jackson Laboratories, Bar Harbor, Maine) or *CD*-1 mice (Charles River, Wilmington, Mass) Each of approximately 100 mice was injected intraperitoneally with 3-thioglycollate broth (Difco) Three days later the mice were killed and the peritoneal cavity of each mouse was rinsed with 5 ml of Eagle's medium (Grand Island Biologicals, without added serum) containing 100 u of penicillin, 50 μg of streptomycin and 0 5 u of heparin/ml The cells were centrifuged gently at 4° C and resuspended in fresh medium with the addition of 0 01 mCi/ml of tritiated uridine (Schwarz Bioresearch) Antigen was added‡, as indicated in the legends to the figures, and the cells were incubated in medium for 1 h at 37° C in a water bath equipped with a shaking device

After incubation the cells were centrifuged and the RNA was extracted from the pellet by the warm phenol method[11] DNA was extracted[12] from the spleens and thymus glands of B_6AF_1 mice and after heat denaturation was immobilized on membrane filters ('Millipore')[13] Hybridization occurred when the labelled RNA dissolved in 2 5 ml of 2 × SSC (SSC is 0 15 M sodium chloride and 0 015 M sodium citrate) was incubated with a filter containing DNA for 20 h at 65° C After incubation the filters were washed by passing 50 ml of 2 × SSC in one direction and then 50 ml of fresh 2 × SSC in the other direction The filters were then treated with 25 μg of RNase (Worthington, boiled before use) in 2 5 ml of 2 × SSC to remove non-specifically bound RNA[13] and then washed again. Scintillator (PPO, POPOP, toluene) was added to the dried filters and the remaining radioactivity was determined in a Packard 'Tri-Carb' liquid scintillation counter Peritoneal cells were separated into two populations—those which adhered to glass (more than 99 per cent macrophages) and those which did not adhere to glass (more than 95 per cent lymphocytes) The entire population was suspended in Eagle's medium and incubated in glass bottles for 3 h At the end of the incubation period, the cells which did not adhere to the glass were removed by decantation The cells which adhered were washed gently with fresh medium. Antigen was incubated with the adhering population for 1 h at 37° C, after which RNA was extracted as described previously

The cells which failed to adhere to glass were centrifuged gently and resuspended in fresh medium The population was then incubated at 37° C for 1 h with antigen before RNA was extracted

In studies involving molecular hybridization a saturation plateau results when increasing amounts of labelled RNA are added to a fixed amount of DNA in conditions which allow the formation of RNA DNA hybrids. This plateau is reached when all the available sites for hybridization in DNA are occupied with complementary species of RNA The number of counts which remain complexed with the DNA after removal of non-hybridized RNA is an index of the number of different molecular species of RNA which are present in the preparation It is independent of the specific activity of the preparation of RNA used for hybridization studies (A representative member of each species of RNA hybridizes to its complementary site, and so other members of that species are washed away The specific activity of each molecular species may differ, so that the specific activity of the whole preparation may vary for a wide range without affecting the c p m which remain with the filter after incubation with saturating amounts of labelled RNA)

The basis of an important control, involving cells incubated without added antigen, is the ability of unlabelled RNA to compete with labelled RNA for sites of hybridization This capacity for competition between two RNAs is an indication of the number of molecular species of RNA in common in the two preparations

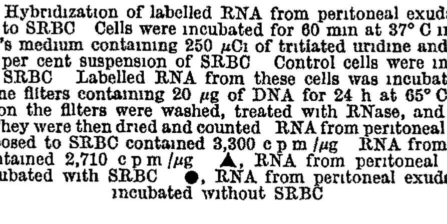

Fig 1 Hybridization of labelled RNA from peritoneal exudate cells exposed to SRBC Cells were incubated for 60 min at 37° C in 25 ml of Eagle's medium containing 250 μCi of tritiated uridine and 0 3 ml of a 10 per cent suspension of SRBC Control cells were incubated without SRBC Labelled RNA from these cells was incubated with membrane filters containing 20 μg of DNA for 24 h at 65° C After incubation the filters were washed, treated with RNase, and washed again They were then dried and counted RNA from peritoneal exudate cells exposed to SRBC contained 3,300 c p m /μg RNA from control cells contained 2,710 c p m /μg ▲, RNA from peritoneal exudate cells incubated with SRBC ●, RNA from peritoneal exudate cells incubated without SRBC

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† We realize that some species of RNA may be present in amounts too small to be detected by molecular hybridizations and that "new" species may represent an increase in such species The term "new" is used for convenience only

‡ In general terms, a foreign material becomes an antigen only if it stimulates the formation of antibody In these experiments, we did not determine whether the cells exposed to "antigen" would eventually form antibody, hence the term "antigen" is used for convenience

Similarly, the relative capacity of two unlabelled RNAs to compete with one preparation of labelled RNA is an index of the number of molecular species in common between the two unlabelled preparations.

When filters containing mouse DNA were incubated with increasing amounts of RNA, a plateau was reached which was higher when the RNA was isolated from peritoneal exudate cells that were exposed to SRBC (Fig 1) This suggested that peritoneal exudate cells incubated with SRBC formed species of RNA that were not actively synthesized by control cells Experiments were carried out involving competition between labelled RNA from cells exposed to SRBC and unlabelled RNA from cells incubated with or without added sheep cells The results (Fig 2) indicate that unlabelled RNA from stimulated cells was a more efficient inhibitor than was RNA from unstimulated control cells

To determine whether different species of RNA formed after peritoneal exudate cells were exposed to different antigens, we carried out a series of reciprocal competition experiments between labelled and unlabelled RNAs from cells exposed to one antigen and labelled and unlabelled RNAs from cells exposed to a second antigen

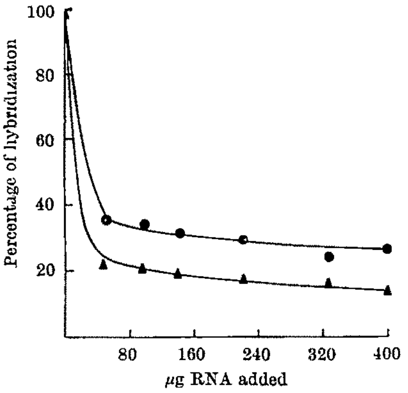

Fig 2 Competition for sites of hybridization by unlabelled RNA from either SRBC-exposed or control peritoneal exudate cells Tritiated RNA (40 μg) from peritoneal exudate cells exposed to SRBC was incubated as described with membrane filters containing 20 μg of DNA and varying amounts of unlabelled RNA from either control cells or cells exposed to SRBC After incubation the filters were treated as described previously ▲, Unlabelled RNA from peritoneal exudate cells incubated with SRBC ●, Unlabelled RNA from peritoneal exudate cells incubated without SRBC

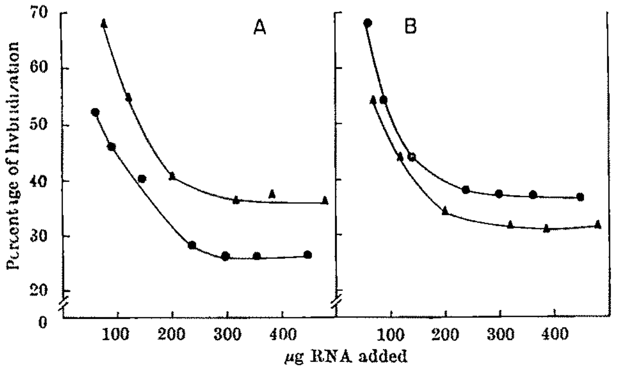

Fig 3 Competition for sites of hybridization by unlabelled RNA from either SRBC or CRBC-exposed cells *A*, Tritiated RNA (40 μg) from peritoneal exudate cells exposed to SRBC was incubated with filters containing 20 μg of DNA and varying amounts of unlabelled RNA from cells exposed to either SRBC or CRBC *B*, Other filters were incubated with 40 μg of labelled RNA from peritoneal exudate cells exposed to CRBC and varying amounts of unlabelled RNA from the same pools of either SRBC or CRBC-exposed cells ▲, Unlabelled RNA from peritoneal exudate cells incubated with CRBC ●, Unlabelled RNA from peritoneal exudate cells incubated with SRBC

Pools of labelled or unlabelled RNA were prepared from peritoneal exudate cells exposed for 1 h *in vitro* to either SRBC or chicken red blood cells (CRBC) Filters containing mouse DNA were incubated with labelled RNA prepared from cells exposed to SRBC and with increasing amounts of unlabelled RNA from cells exposed to either SRBC or CRBC Other filters were incubated with labelled RNA isolated from cells exposed to CRBC and increasing amounts of unlabelled RNA from the same pools obtained from cells exposed to either SRBC or CRBC The results (Fig 3) indicate that unlabelled RNA from specifically stimulated cells was a more efficient competitor for sites of hybridization than was unlabelled RNA from non-specifically stimulated cells. Similar results were found with RNAs from SRBC and cells stimulated with *E coli* (Fig 4)

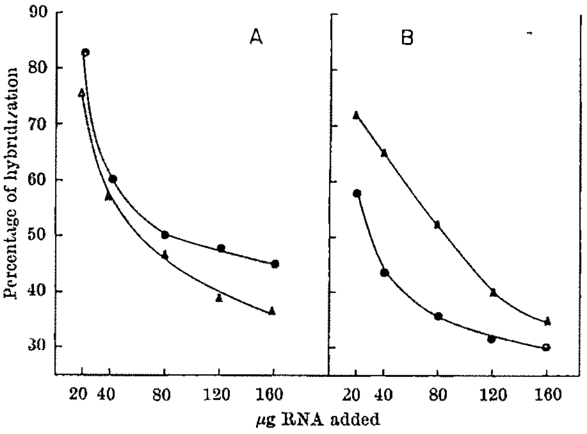

Fig 4 Competition for sites of hybridization by unlabelled RNA from cells exposed to either SRBC or *E coli* *A*, Tritiated RNA (50 μg) from peritoneal exudate cells exposed to SRBC was incubated with filters containing 20 μg of DNA and varying amounts of unlabelled RNA from cells exposed to either SRBC or *E coli* *B*, Other filters were incubated with 50 μg of labelled RNA from peritoneal exudate cells exposed to *E coli* and varying amounts of unlabelled RNA from the same pools of cells exposed to either SRBC or *E coli* ▲, Unlabelled RNA from peritoneal exudate cells incubated with SRBC ●, Unlabelled RNA from peritoneal exudate cells incubated with *E coli*

An additional control experiment with cells incubated without antigen was performed Filters containing mouse DNA were incubated with a fixed amount of unlabelled RNA from cells exposed to either SRBC or CRBC and increasing amounts of labelled RNA from cells exposed to SRBC. The results (Fig 5) indicate that unlabelled RNA from specifically stimulated cells was a more effective competitor for sites of hybridization than was unlabelled RNA from non-specifically stimulated cells The fact that unlabelled RNA from cells exposed to CRBC also competed suggests that many of the species of RNA in the two preparations were the same

These findings strongly suggest that some similar and some distinct species of RNA appeared after peritoneal exudate cells had been exposed to different antigens

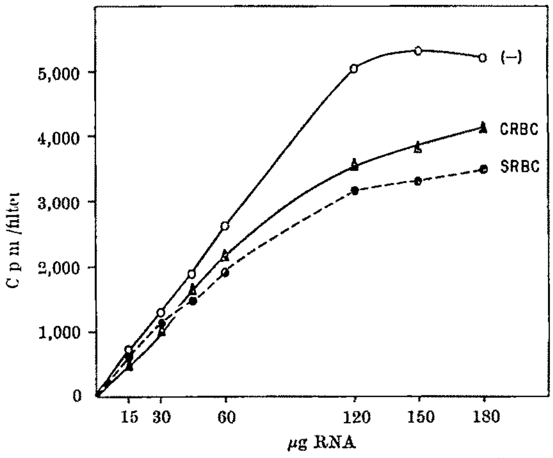

Fig 5 Competition for sites of hybridization by unlabelled RNA from cells exposed to either SRBC or CRBC Unlabelled RNA (120 μg) from cells exposed to either SRBC or CRBC was incubated for 24 h at 65° C with filters containing 20 μg of DNA Control filters were incubated without added RNA After incubation the filters were washed and varying amounts of labelled RNA from peritoneal exudate cells exposed to SRBC was added The filters were then incubated for an additional 24 h and washed Labelled RNA contained 12,600 c p m /μg

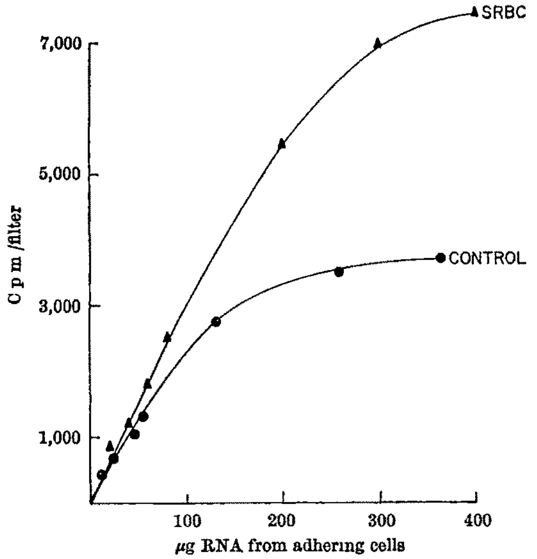

Fig 6 Hybridization of labelled RNA from peritoneal exudate cells which adhered to glass Cells were exposed to SRBC for 60 min in the presence of tritiated uridine After incubation the cells were washed and RNA extracted Control cells were incubated without SRBC. Varying amounts of labelled RNA were added to filters containing 40 µg of DNA ▲, RNA from cells exposed to SRBC, 10,450 c p m /µg ●, RNA from control cells, 6,300 c p m /µg

Peritoneal exudate cells were separated into populations which adhered or failed to adhere to glass Labelled or unlabelled RNA was extracted from both kinds of cells after incubation with antigen. Reciprocal competition experiments were performed between RNAs from cells exposed to either SRBC or CRBC. Similar experiments were performed between RNAs from cells exposed to SRBC or *E coli*

Cells which adhered to glass as well as cells which failed to adhere to glass synthesized new species of RNA exposure to antigen (Figs 6 and 7) Different species of RNA, however, were not found when the adhering cells were incubated with different antigens (Table 1) Also, different species of RNA were not found after adhering cells had been exposed to either SRBC or *E coli* On the contrary, different species of RNA were detected after the non-adhering cells had been exposed to either SRBC or CRBC (Fig 8). Similar results were obtained when non-adhering cells were exposed to either SRBC or *E. coli*

The role of these antigen-stimulated species of RNA in the immune response remains to be determined They were detected in cells which did not adhere to glass (consisting predominantly of lymphocytes) One of the principal functions of lymphocytes is the synthesis and release of antibody. Thus the species of RNA formed in these cells may play an essential part in antibody formation Cells which adhered to glass, consisting of more than 99 per cent macrophages, synthesized new but not antigen-specific species of RNA.

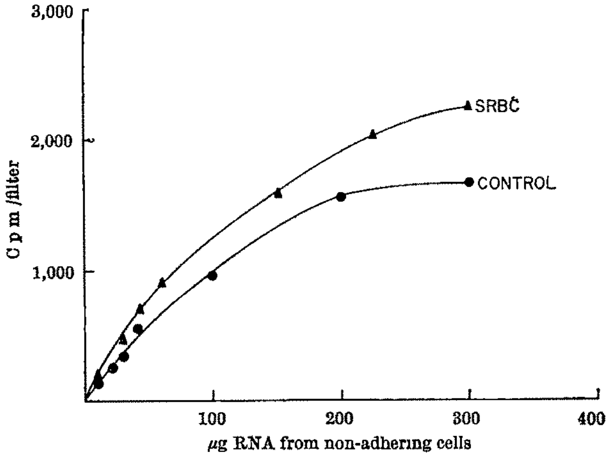

Fig 7 Hybridization of labelled RNA from peritoneal exudate cells which did not adhere to glass Cells were exposed to SRBC for 60 min in the presence of tritiated uridine After incubation RNA was extracted, and hybridization was performed as described ▲, RNA from cells exposed to SRBC, 7,100 c p m /µg ●, RNA from control cells, 6,100 c p m /µg

Table 1 HYBRIDIZATION OF RNA FROM CELLS WHICH ADHERED TO GLASS IN THE PRESENCE OF COMPETING RNA

Labelled RNA from cells exposed to	Experiment	Percentage hybridization with competing RNA from cells exposed to SRBC	CRBC
SRBC	1	35	36
(*A*)	2	44	42
	3	32	32
CRBC	1	26	27
(*B*)	2	45	46
	3	37	36

Each experiment was performed as a competition for sites of hybridization between labelled and unlabelled RNAs from the cells after incubation with antigen (*A*) Competition between labelled RNA from adhering cells exposed to SRBC and unlabelled RNA from adhering cells exposed to either SRBC or CRBC. (*B*) Competition between labelled RNA from adhering cells exposed to CRBC and unlabelled RNA from either of the same two pools used in (*A*)

Peritoneal exudate cells from *CD*–1 mice were incubated in Eagle's medium for 3 h at 37° C in glass bottles After incubation the non-adhering cells were removed and the adhering cells were incubated for an additional hour with either SRBC or CRBC Labelled RNA from such cells was prepared in a similar manner except that 0 1 mCi/ml of tritiated uridine was added to the incubation medium The results of three separate experiments are given Labelled RNA (20 µg) and at least 180 µg of unlabelled RNA were used in each experiment The filters contained 20 µg of DNA

Gottlieb *et al*[14] reported that immunogenic RNA from macrophages which formed a complex with phage was not unique for a given antigen This RNA was thought to be present in the cell before the addition of phage antigen Our findings—namely, that the adhering population formed new, but not different, species of RNA—are consistent with this report It is reasonable to suppose that the new species we detected are devoted to other cell functions, such as the formation of degradative enzymes Others have examined the immunogenicity of RNA-antigen complexes Askonas and Rhodes[16] and Friedman *et al*[17] concluded that a trace of the original antigen was present in the RNA fractions active in stimulating an immune response *in vitro* Youmans and Youmans[18] reported that RNA extracts of tubercle bacilli stimulated immunity when administered to normal mice before challenge

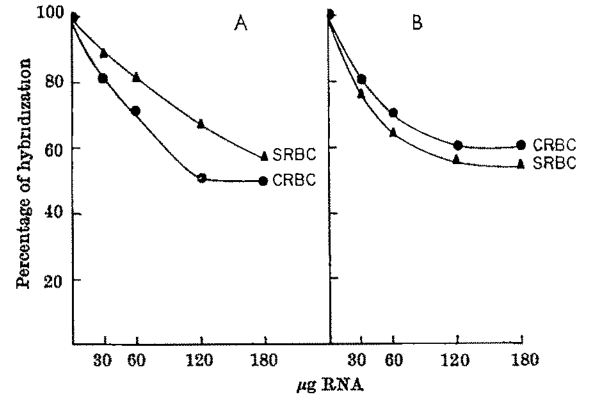

Fig 8 Reciprocal competition between unlabelled RNAs from peritoneal cells which did not adhere to glass Cells from the suspension used in the experiment reported in Fig 7 were incubated for 1 h with either SRBC or CRBC as described *A*, Labelled RNA (30 µg) from non-adhering cells exposed to CRBC was incubated with membrane filters containing 10 g of DNA from the spleens of mice and increasing amounts of unlabelled RNA from cells exposed to either SRBC or CRBC *B* Labelled RNA (30 µg) from non-adhering cells exposed to SRBC was incubated with membrane filters containing 10 g of DNA from the same preparation used in (*A*) and increasing amounts of unlabelled RNA from the same pools used in (*A*)

Reports from several laboratories indicate that RNA with properties of messenger converts otherwise normal cells to specialized function. Adler *et al*[19] found that RNA from rabbit peritoneal cells of *A4*/*A4* allotype exposed *in vitro* to antigen converted non-immune cells from rabbits of *A5*/*A5* allotype to form an antibody which was inactivated by anti-*A4* antiserum. Segal *et al*[20] and Mansour and Niu[21] reported that uterine tissue from immature rats was converted to a form which looked like tissue that was stimulated by oestrogen after it was exposed to RNA from mature rat uteri Fujii and Villee[22] found similar results with RNA from androgen-stimulated tissues. In both laboratories the preparations were thought to be free of residual hormone. Cohen *et al.*[23] reported that RNA from spleens of immunized mice converted a small proportion of non-immune cells to a form producing antibody The activity of the RNA extracts was unaffected by proteases and amylases Thus it may be that two types of intercellular stimulatory effects occur an effect based on RNA which is associated with antigen, and an effect based on information containing RNA free of antigen

On the basis of these experiments, we can make two additional suggestions on the origin of antigen-stimulated RNAs (1) The proportion of peritoneal lymphocytes which responded *in vitro* to antigen was probably large It is unlikely that sufficient unique RNA to have been detected would have arisen from the few stem cells which responded to antigen (2) The information for the synthesis of these RNAs was present in a large proportion of normal mouse DNA The DNA used in these experiments was obtained from non-immune mice These results are most consistent with the hypothesis that the potential to produce antibodies of varying specificity is inherited and not acquired through mutational processes thought to occur throughout somatic development Further evidence for this point rests on the demonstration that these unique species of RNAs are messengers for the synthesis of immunoglobulins

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[1] Mannick, M , and Kunkel, H E , *J Exp Med* ,116, 859 (1962)
[2] Putnam, F W , Easley, C W , and Melling, J W , *Biochim Biophys Acta* 78, 231 (1963)
[3] Hilschman, N , and Craig, L C , *Proc US Nat Acad Sci* , 53, 1403 (1965)
[4] Gray, W R , Dreyer, W J , and Hood, L , *Science*, 155, 767 (1967)
[5] Burnet, F M , *The Clonal Selection Theory of Acquired Immunity* (Vanderbilt University Press, 1959)
[6] Lederberg, J , *Science*, 129, 1649 (1959)
[7] Smithies, O , *Science*, 149, 151 (1965)
[8] Bennett, J C Hood, L E , Dreyer, W. J , and Potter, M , *J Mol Biol* , 12, 81 (1965)
[9] Hood, L E , Gray, W R , and Dreyer, W J , *Proc US Nat Acad. Sci.*, 55, 826 (1966)
[10] Cohen, E P , *Proc US Nat Acad Sci* , 57, 673 (1967)
[11] Scherrer, K , and Darnell, J E , *Biochem Biophys Res Comm* , 7, 486 (1962)
[12] Marmur, J , *J Mol Biol* , 3, 208 (1961)
[13] Spiegelman, S , and Yankofsky, J A , in *Evolving Genes and Proteins* (edit by Vogel, H J , and Bryson, V) (Academic Press, 1965)
[14] Gottlieb, A A , Glisin, V R , and Doty, P , *Proc US Nat Acad Sci* , 57, 1849 (1967)
[15] Ritossa, F M , and Atwood, K C , *Proc US Nat Acad Sci* , 56, 496 (1966).
[16] Askonas, B A , and Rhodes, J M , *Nature*, 205, 470 (1965)
[17] Friedman, H P , Stavitsky, A B , and Solomon, J M , *Science*, 149, 1106 (1965)
[18] Youmans, A S , and Youmans, G P , *J Bact* , 91, 2139 (1966) Ibid , 91, 2146 (1966)
[19] Adler, F L , Fishman, M and Dray S , *J Immunol* , 97, 554 (1966)
[20] Segal, S J , Davidson, O W , and Wada, K , *Proc US Nat Acad Sci* , 54, 782 (1965)
[21] Mansour, A M , and Niu, M C , *Proc US Nat Acad Sci* , 53, 764 (1965).
[22] Fujii, T , and Villee, C , *Proc US Nat Acad Sci* , 57, 1468 (1967)
[23] Cohen, E P , Newcomb, F W , and Crosby, L K , *J Immunol* , 95, 583 (1965)

Toxicity of Pyrrolizidine Alkaloids

by

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A new class of metabolite, with a pyrrole-like structure, has been demonstrated in the tissues of animals poisoned by pyrrolizidine alkaloids. There is some correlation between the degree of hepatotoxicity and the amount of "pyrrole" found in the liver Evidence has been found of the types of reactions such metabolites might undergo with tissue constituents.

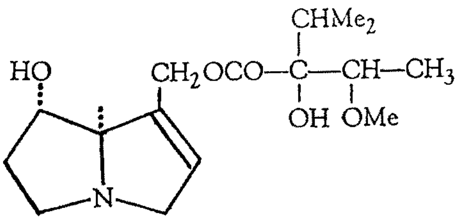
CERTAIN pyrrolizidine alkaloids, such as heliotrine (I), lasiocarpine (II) and retrorsine (III), have long been known to cause chronic liver poisoning in animals, and the pathology has been well described[1,2] Progressive liver damage has been induced experimentally in rats by single doses of certain alkaloids[3,4]

Usually the most hepatotoxic alkaloids are cyclic diesters, like retrorsine (III) and seneciomine (IV) Higher doses of lasiocarpine (II) and still more of heliotrine (I) are needed to give similar effects, while supinine (V) is even less hepatotoxic[2] Some alkaloids, especially fulvine (VI) and monocrotaline (VII), frequently cause lung as well as liver lesions[4-6]

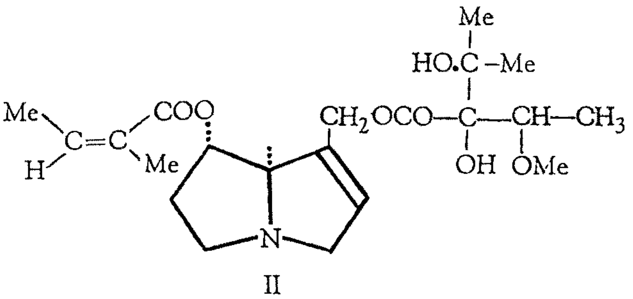
In contrast to the pathological studies, very little progress has been made in elucidating the mechanism by which these alkaloids exert their toxic effects A requirement for hepatotoxicity seems, *inter alia*, to be the allylic ester function[7] Thus rosmarinine (VIII), platyphylline (IX), and the amino-alcohol retronecine (X), among other alkaloids, are not hepatotoxic Culvenor, Dann and Dick[8] have suggested that the "toxic reaction" with cell constituents is an alkylation, based on the known tendency of the allylic ester group to undergo alkyl–oxygen fission

These alkaloids, however, are not notable as alkylating agents, and their chemical reactivity is usually of a low order, being limited to hydrolysis of the ester linkages and to readily reversible *N*-oxidation. The *N*-oxides display a toxicity which is similar to, but sometimes weaker than, that of the corresponding bases Neither the alkaloids nor their *N*-oxides respond in normal conditions to tests for alkylating agents, such as those employing 4(*p*-nitrobenzyl)pyridine[9] or 4-picoline[10] Nevertheless, it is hard to see how the alkaloids could exert their toxic effects without undergoing reaction with cell constituents. The chemically unreactive hepatocarcinogen dimethylnitrosamine (XI), the early effects of which on the liver are very similar to those caused by the pyrrolizidine alkaloids[11], is known to be metabolized to a reactive alkylating species which methylates proteins and nucleic acids *in vivo*[12] It is tempting to suppose that those pyrrolizidine alkaloids which damage the liver are also metabolized in that organ to a highly reactive intermediate. Some of this metabolite might in certain conditions be transported to the lungs or beyond This has been suggested[5] as an explanation for the lung lesions caused by fulvine

We have found evidence of metabolites from certain pyrrolizidine alkaloids which can be demonstrated in liver and other tissues after the alkaloids themselves have ceased to be detectable This evidence, and the nature and type of reactivity of such metabolites, is discussed here.



I



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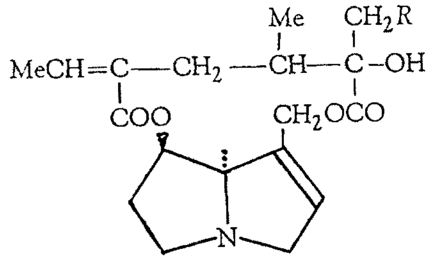
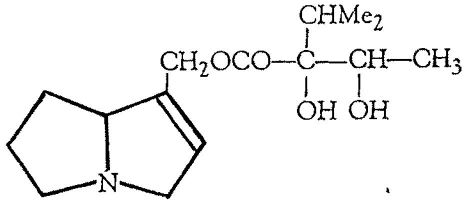
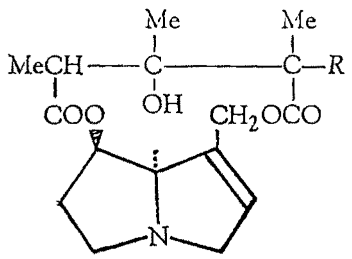


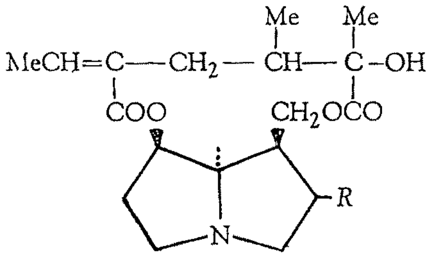
III R=OH
IV R=H



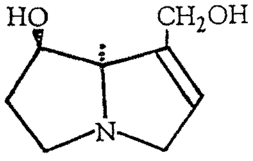
V



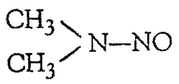
VI R=H
VII R=OH



VIII R=OH
IX R=H



X



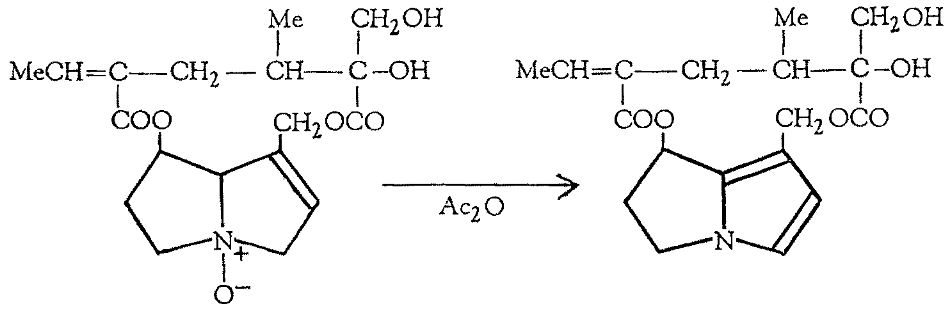
$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>N\text{—}NO$$

XI

A procedure for the spectrophotometric determination of unsaturated pyrrolizidine alkaloids, recently described[13], involves dehydrogenation of the alkaloids—by treating their *N*-oxides with acetic anhydride—to pyrrole-like derivatives which give colours with a modified Ehrlich reagent (4-dimethylaminobenzaldehyde). An example is the conversion of retrorsine *N*-oxide (XII) to (XIII). Derivatives like (XIII) will for convenience be termed "chemical pyrroles". Usually, "chemical pyrroles" are only formed from alkaloids which have unsaturation in the basic moiety, that is, a 3-pyrroline ring. This structural feature is also present in all the markedly hepatotoxic alkaloids. It has now been found that similar (but not necessarily identical) derivatives are formed *in vivo* by metabolism of pyrrolizidine alkaloids. These "metabolic pyrroles" are detectable in the urine and tissues of rats dosed with pyrrolizidine alkaloids by the formation of mauve or magenta colours after warming with Ehrlich reagent. Such colours are not given by the alkaloids themselves or by their *N*-oxides.

Pyrroles in Urine

While measuring excretion of bases and *N*-oxides in the urine of rats which had been injected intraperitoneally with pyrrolizidine alkaloids using the new estimation



XII XIII

procedure[13], it was found that the urine gave a magenta colour when treated with Ehrlich reagent, and it was established that this was caused by metabolites of the alkaloids (my unpublished work). The alkaloids and their N-oxides did not undergo chemical decomposition to pyrrole derivatives when incubated for several days with rat urine Similar pyrroles were not found in the urine of rats the livers of which had been damaged by single doses of dimethylnitrosamine (given intraperitoneally) or by intravenous beryllium sulphate The "metabolic pyrroles" did not give reactions characteristic of urobilinogen or porphobilinogen[14]

Table 1 RECOVERY OF ALKALOID AND METABOLITES FROM URINE OF 6 × 150 G MALE RATS GIVEN RETRORSINE, 60 MG/KG, INTRAPERITONEALLY

Time after dosing (h)	Percentage recovered Alkaloid	N-oxide	"Pyrroles"
0–3 5	8 6	10 8	4 1
3 5–7	0 9	1 2	3 0
7–24	1 1	1 3	5 3
24–48	0 1	0 2	1 8
Totals	10 7	13 5	14 2

Results are percentage of dose given, calculated as alkaloid

With all the pyrrolizidine alkaloids tested, excretion of unchanged alkaloids and of N-oxides after intraperitoneal injection was rapid and had almost finished within 24 h Excretion of "metabolic pyrroles" varied with the alkaloid, but they usually reached a maximum a little later than the alkaloids and N-oxides Typical figures for retrorsine are given in Table 1 The amount of "metabolic pyrroles" excreted is of the same order as the amounts of unchanged alkaloids and of N-oxide "Metabolic pyrroles" excreted in the urine of animals given other alkaloids are listed in Table 2 Like the "chemical pyrroles", they formed red derivatives in the presence of strong acids

That the "metabolic pyrroles" were not the same as the "chemical pyrroles" derived from the same alkaloids was evident from differences in the absorption spectra of the colours formed with Ehrlich reagent The curves were always similar, but they were displaced in wavelength by a few millimicrons (Fig 1) "Metabolic pyrroles" from hepatotoxic alkaloids always showed a shift to higher wavelength compared with the "chemical pyrroles", whereas the non-hepatotoxic rosmarinine showed a slight drop in wavelength Similar wavelength shifts were observed with "metabolic pyrroles" formed *in vitro* (below)

Pyrroles in Organs *in vivo*

When rats were dosed, orally or intraperitoneally, with retrorsine (III), then killed after various times, it was possible to detect "metabolic pyrroles" in several organs, but especially in the liver The simplest way was to chop the tissues into small pieces, rinse them in water and in ethanol, homogenize them in absolute ethanol and centrifuge the homogenate The alcoholic liquor gave practically no Ehrlich reaction The residue was shaken with a modified Ehrlich reagent containing more acid than the usual reagent to overcome the buffering action of the tissues, then heated for 1 or 2 min at 80°–95° C (The reagent contained 3 g of 4-dimethylaminobenzaldehyde in 60 ml of absolute ethanol and 40 ml of 14 per cent methanolic boron trifluoride) The reagent was decanted and replaced with clean ethanol The presence of pyrroles was shown by a mauve colour in the suspended solids and often in the decanted reagent In these conditions, the liver of a rat given 30–100 mg/kg of retrorsine, and killed 0 5–12 h later, was stained mauve, whereas a control liver (untreated animal) was colourless The colour was detectable 5 min after dosing, reached a maximum probably between 2 and 5 h, and decreased until it was barely detectable at 45 h (Fig 2).

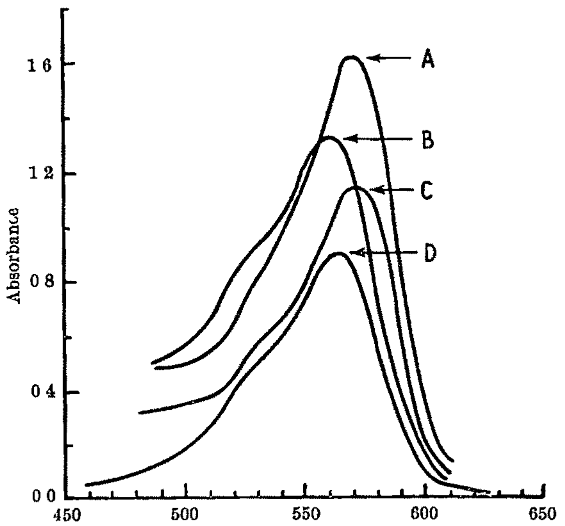


Fig 1 Absorption spectra of colours from Ehrlich reagent and "pyrrole" derivatives *A*, "Metabolic pyrrole" from lasiocarpine, *B*, "metabolic pyrrole" from rosmarinine, *C*, "metabolic pyrrole" from retrorsine, *D*, "chemical pyrrole" from retrorsine The "metabolic pyrroles" were from rat urine samples

Another method of demonstrating the colour in liver was to make thin slices, fix them in alcohol and heat them for a few minutes in the Ehrlich reagent, which was then replaced with clear alcohol This gave strongly coloured slices from retrorsine-treated animals compared with almost colourless controls The colour was also visible in sections prepared in the freezing microtome, but the intensity was not sufficient for it to be used as a histochemical stain

The metabolite in liver responsible for the Ehrlich reaction was demonstrable after the fresh tissue had been stored in a deep-freeze for at least 2 weeks, and also in ethanol-fixed slices or in ethanolic homogenates after a similar time That it was firmly bound to tissue constituents was shown by the failure either of ethanol or of isotonic aqueous media to extract it from liver to any great extent

An attempt to determine the distribution of "metabolic pyrroles" in subcellular liver fractions was made as follows A male rat was given retrorsine (140 mg/kg),

Table 2 "METABOLIC PYRROLES" FROM A NUMBER OF PYRROLIZIDINE ALKALOIDS, DETECTED IN RAT URINE AND LIVER *in vivo* AND IN LIVER SLICES *in vitro*

100–120 g male rats used

Alkaloid	Rough relative hepatotoxicity	"Pyrroles" in urine Dose of alkaloid given (mg/kg)	Percentage of dose excreted as "pyrroles" in 24 h	"Pyrroles" in liver *in vivo* Dose of alkaloid given (mg/kg)	Mauve colour (2–5 h)	"Pyrroles" in liver slices *in vitro* In slices	In medium (based on absorbances at 570 mμ)
Retrorsine (III)	+ + + + + +	60	12 4	50	+ + +	+ + + +	3 8
Retrorsine N-oxide (XII)	+ + +	60	3 6	60	+	+ + +	2 1
Monocrotaline (VII)	+ + + +	60	6 7	60	+ +	+	1 3
Senecionine (IV)	+ + + + +	60	7 1	50	+ + +		
Lasiocarpine (II)	+ + + +	60	8 5	100	+ + +	+ + + + +	6 3
Heliotrine (I)	+ +	150	2 1	250	+	+	1 0
Supinine (V)	+	150	1 2	250	+	+	1 2
Rosmarinine (VIII)	0	60	8 3	200	0	+ + +	17
Retronecine (X)	0	200	0 2	250	0	0	0

This table is explained in the text

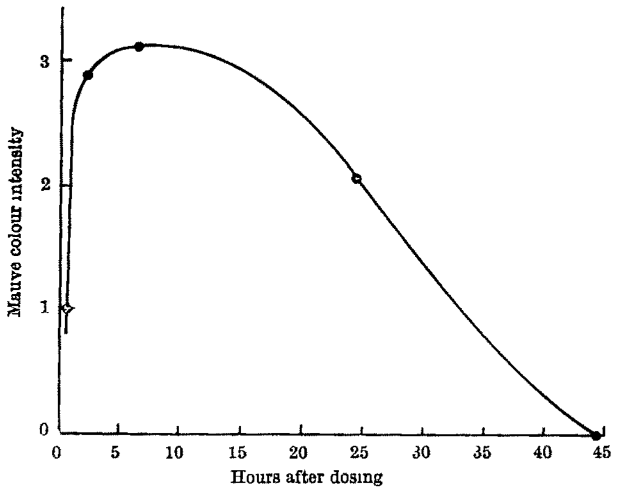


Fig 2 Build-up of Ehrlich-positive material in liver of rats injected with retrorsine (30 mg/kg) and killed after various times. Based on visual estimates of colour intensity.

intraperitoneally, and killed 1 h later. The liver was homogenized and fractionated in ice cold 0 3 M sucrose[15] Each solid fraction was treated with absolute ethanol and centrifuged, and the residues were heated with the modified Ehrlich reagent Mauve colours were observed in all the solids and in the reagent liquors from the debris (including nuclei) and microsomal fractions (λ_{max} 570 mμ) The solid debris and microsomal fractions were stained more strongly than the mitochondria or soluble proteins The ethanol supernatants gave no Ehrlich reactions

Other pyrrolizidine alkaloids also gave rise to "metabolic pyrroles" in the liver, as shown in Table 2 Some correlation between the strengths of colour and the hepatotoxic potency of the alkaloids is apparent in most cases Livers of rats treated with comparable doses of dimethylnitrosamine showed no colour with Ehrlich reagent

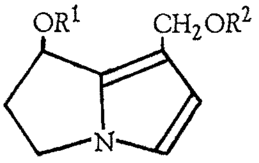
"Metabolic pyrroles" were sometimes detected in other organs The lung was the principal organ affected The colour, though much weaker than that in the liver, sometimes persisted after the liver colour was no longer apparent The staining of the heart, spleen and kidneys (but not the small intestine), 4 h after a large dose of monocrotaline, showed that the metabolite could be retained in these, too, in small amounts It may be pointed out that renal lesions have been found in rats given monocrotaline[6], while slight changes in the spleens and possibly hearts of rats were found after fulvine poisoning[5]

Formation of "Metabolic Pyrroles" *in vitro*

The staining of livers taken from animals dosed with pyrrolizidine alkaloids could be reproduced *in vitro*, using liver slices from normal rats Slices (about 0 25 g) were shaken at 37° C in 2 ml of Krebs Ringer phosphate[16] containing glucose (0 3 per cent w/v), but no added co-factors, in an oxygen atmosphere The alkaloids (500 μg) were added to the medium After 3–5 h the medium was decanted and the slices were washed with alcohol and heated with the modified Ehrlich reagent in the way already described, and colour changes were observed The aqueous media were diluted with alcohol, centrifuged, and the supernatants heated with Ehrlich reagent In most cases, colours similar to those of the slices were observed, and rough quantitative comparisons could be made (Table 2) Pyrroles were not formed by portions of lung tissue in similar conditions

"Chemical Pyrrole" Derivatives

The analytical method for determining unsaturated pyrrolizidine alkaloids[13] involves dehydrogenation of the alkaloids by heating their *N*-oxides with acetic anhydride This was extended to a preparative scale with modified conditions. Retrorsine *N*-oxide was treated at 50° C with acetic anhydride for 1–2 min, then with excess methanol containing sodium carbonate for 5 min Among the products was a petroleum-soluble fraction which was shown by chemical, chromatographic, infrared, nuclear magnetic resonance and mass-spectrographic evidence (which will be described elsewhere) to be a mixture (i) of (XIV), (XV) and (XVI) Aqueous alkaline hydrolysis of (i) converted (XIV) to (XV), leaving a mixture (ii) containing (XV) and (XVI) Both the mixtures (i) and (ii) gave strong Ehrlich reactions and decomposed instantly in acids to give red polymers



XIV $R^1 = CH_3$, $R^2 = CH_3CO$
or $R^2 = CH_3$, $R^1 = CH_3CO$

XV $R^1 = CH_3$, $R^2 = H$
or $R^2 = CH_3$, $R^1 = H$

XVI $R^1 = R^2 = CH_3$

The toxicities to rats of mixtures (i) and (ii) are summarized in Table 3 It is apparent that (i) is a powerful toxin, acting directly on the tissues with which it comes into contact Because (ii) is less toxic than (i), it seems that the principal toxic component is (XIV) The non-toxicity of these mixtures when given orally is understandable, for probably the compounds are rapidly decomposed on contact with stomach acid

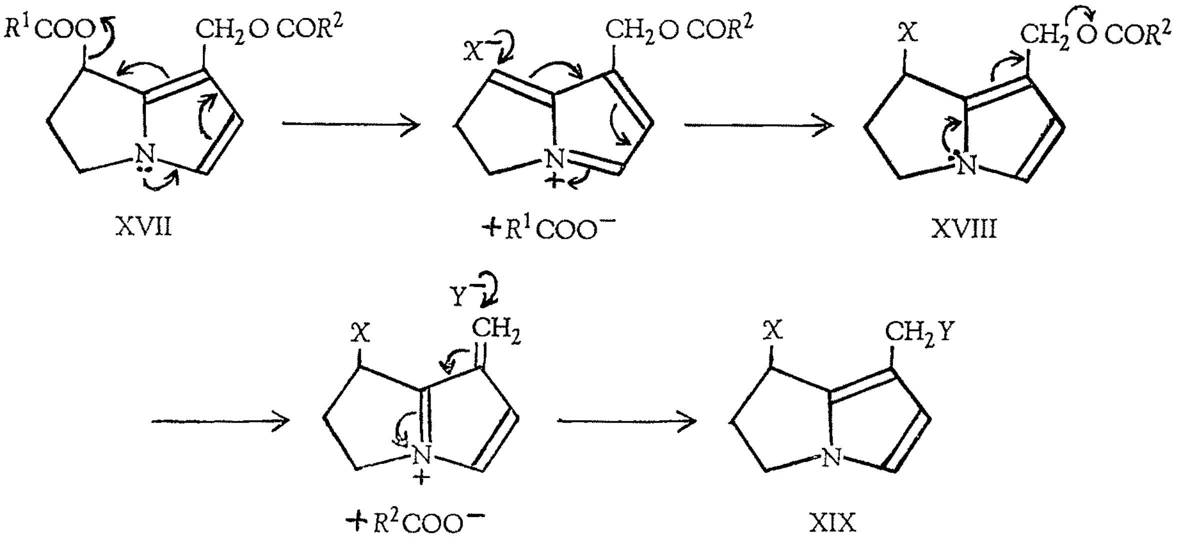
Table 3 TOXIC EFFECTS OF SINGLE DOSES OF SEMI-SYNTHETIC "PYRROLE" MIXTURES IN MALE RATS

	Route of administration and dose range		
Mixture	Oral 40–80 mg/kg	Intraperitoneal 25–60 mg/kg	Intravenous 20–40 mg/kg
(i)	No action	Severe local irritation. Peritonitis	Lung damage at 4–5 weeks typical of that caused by monocrotaline and fulvine
(ii)	—	—	No action

The course of poisoning and the striking pleural effusions seen at post-mortem in rats given a single intravenous dose of (i) are of particular significance, because they closely resemble those seen in rats 4–5 weeks after a single dose of monocrotaline or fulvine[5] The pathology will be described in detail elsewhere (unpublished results of Barnes, Butler and Mattocks)

Implications of the Results

The results summarized in Table 2 show that certain metabolites formed *in vivo* from a number of pyrrolizidine alkaloids have the following properties (*a*) They give magenta colours with Ehrlich reagent (*b*) In their reactions with acids and in the spectra of the Ehrlich derivatives they are similar to, but not identical with, the "chemical pyrroles" which can be prepared by dehydrogenating the same alkaloids (*c*) The "metabolic pyrroles" are partly excreted in urine, but some are also bound strongly to the tissues of the liver and to a decreasing



XVII $+R^1COO^-$ XVIII

$+R^2COO^-$ XIX

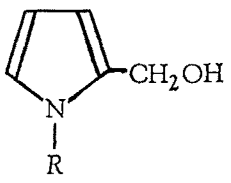
extent, the lung and other organs, for 48 h or more after being formed (*d*) With the exception of rosmarinine (which is discussed later), there is a rough correlation between the hepatotoxicity of the alkaloids[*] and the amounts of pyrroles to which they give rise *in vivo*

The following hypothesis is consistent with these results The alkaloids themselves are not hepatotoxic A proportion of the alkaloid (depending on its structure) is metabolized in the liver (by a process amounting to dehydrogenation) to a pyrrole-like derivative which has one or more highly reactive (electrophilic) centres in addition to its pyrrole-like properties This metabolite reacts rapidly with certain nucleophilic tissue constituents, both soluble and insoluble, forming "bound pyrroles" in which the high reactivity is lost but the pyrrole function remains Soluble "bound pyrroles" are either eliminated in urine or possibly adsorbed on local macromolecular sites A proportion of the reactive metabolite may be carried in the bloodstream to the lungs or beyond, to react and form "bound pyrroles" in these organs

Thus the metabolites detected in the urine and in the tissues of animals poisoned with pyrrolizidine alkaloids are soluble and insoluble "bound pyrroles", respectively

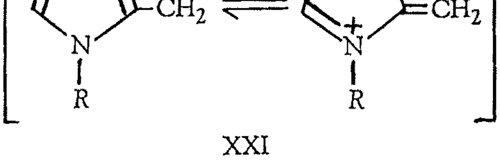
That the "metabolic pyrroles" consist of covalent compounds of alkaloid metabolites with various tissue constituents is not yet proved, but there is good circumstantial evidence The present results can be compared with the early experiments of Miller and Miller[17], who found that metabolites of the carcinogen *p*-dimethylaminoazobenzene were bound tightly to cellular constituents in the liver

Clues to the chemical nature of the reactive metabolite, and of any "toxic reaction" which it might undergo with tissue constituents, are provided by experiments with "chemical pyrrole" derivatives The cytotoxicity of compounds of type (XIV) has particular significance for the hypothesis that pyrrole-like derivatives are responsible for pyrrolizidine alkaloid toxicity Of special importance is the observation that a single dose of the mixture (i) (XIV+XV+XVI), administered intravenously, can cause a delayed lesion in the chest of the same kind as that produced by a single larger dose of fulvine or monocrotaline given by mouth A severe irritant effect is evident after intraperitoneal administration, and this may indicate that the pyrrole derivative does not survive long enough to reach the liver by the blood stream What might be the local effect if only a small amount of a similar compound were to be released metabolically within the liver cell can be appreciated



XX R=H or CH_3

The mode of preparation of the mixture (i) indicates the ease with which methanol and acetate (CH_3COO^-) can react with (probably) (XIII), with loss of the original acid moiety of the alkaloid The ester groups in retrorsine (III) are relatively resistant to hydrolysis by acyl–oxygen fission In contrast, the ester linkages in (XIII) can be broken by unimolecular alkyl–oxygen fission, and thus rendered highly reactive, being unaffected by the steric hindrance associated with the S_N2 reaction It is emphasized that both the ester groups of a diester alkaloid can be thus activated when the 3-pyrroline ring is converted to a pyrrole ring This is illustrated by the reaction of the nucleophiles X^- and Y^- with the pyrrole derivative of a diester alkaloid (XVII) to form (XVIII) and then (XIX) (not necessarily in that order) In (XIX), both X^- and Y^- have been "alkylated" by the pyrrole moiety, which can thus be regarded as a bidentate alkylating agent The special properties attributed to (XVII) are paralleled by the behaviour of 2-hydroxymethyl pyrroles (XX) Silverstein *et al*[18,19] found it impossible to prepare derivatives of these alcohols, owing to their ability to form resonance-stabilized cations (XXI) (ref 20) Also comparable are the α-hydroxy amines (pseudo-bases)[21] (XXII), in which the hydroxy group is unusually labile for similar reasons, and can exchange with alkoxy groups in mild conditions In fact, the hydroxymethyl pyrrole derivatives can be regarded as conjugated pseudo-bases



XXI

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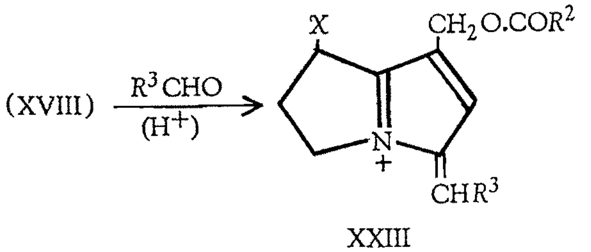
[*] Designations of hepatotoxicity are necessarily crude, being derived from results from various sources Short-term LD_{50} figures, where available, are not necessarily a reliable guide to chronic hepatotoxicity[7], so designations of the latter are rather based on comparisons of the doses of different alkaloids usually stated to be needed to produce typical long-term effects in rats

$$R_2N.CH_2OH \underset{HOH(H^+)}{\overset{R^1OH}{\rightleftharpoons}} R_2N.CH_2OR^1$$

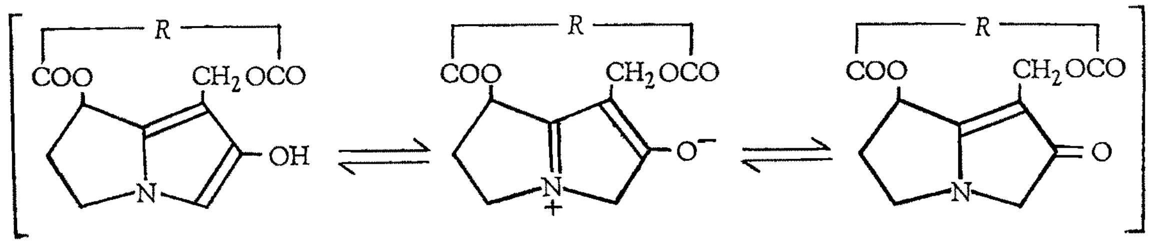
XXII

the esters of which, as exemplified by the "chemical pyrroles", are accordingly highly reactive Seen in this light, it is possible that, in certain conditions, "alkylations" caused by such pyrroles may be reversible. (This again might suggest possibilities for interference with biochemical systems)

Another unusual feature of this system is the further possibility of reaction of electrophilic groups at the α-position of the pyrrole ring This is a normal reaction of pyrroles, and the Ehrlich reaction is an example. Thus (XVIII) could react with an aldehyde to give (XXIII) Side effects of this would be to stabilize the alkylation bond to *X*, and also to de-activate the alkylating ability of the remaining ester group, as well as to prevent the subsequent detection of the derivative with Ehrlich reagent



XXIII



XXIV

There is direct evidence that the "chemical pyrroles" can act as alkylating agents The mixture (i), mentioned earlier, and also (XIII) and similar derivatives (but not the parent alkaloids or *N*-oxides), give positive reactions as alkylating agents using 4(*p*-nitrobenzyl)-pyridine[9] or 4-picoline[10]. In addition, preliminary experiments have shown that (XIII) reacts with bases such as *cyclo*hexylamine, pyrrolidine and guanosine to form compounds which give coloured Ehrlich derivatives the absorption maxima of which are higher than that of the starting material, and similar to the "metabolic pyrroles" Thus the elevated λ_{max} of "metabolic pyrroles" compared with the corresponding "chemical pyrroles" could be the result of similar alkylation-type reactions *in vivo*

The alkaloid rosmarinine (VIII) presents an unusual case Although the concentration of "metabolic pyrroles" found in urine is usually an indication of the amount of reactive metabolite initially formed in the liver, it is the "bound pyrroles" in liver and other tissues which are more likely to be of importance in accounting for the toxicity of the alkaloids Thus, although rosmarinine gives rise to large amounts of urinary "pyrroles", no "bound pyrroles" are found in the liver *in vivo*, and this could account for the lack of hepatotoxicity of this alkaloid (The "bound pyrroles" found in liver slices treated with rosmarinine *in vitro* (Table 2) may result from differences in metabolism between the two systems, or to the lack of a flushing blood flow in the slices.) Furthermore, the pyrroles from rosmarinine differ from those from other alkaloids, such as the related senecionine (IV), as shown by the differences in the absorption maxima of their Ehrlich derivatives This might be due to the retention of the β-hydroxy group in the pyrrole ring, giving rise to tautomeric forms (XXIV) and thus considerably reducing the alkylating power of the ester groups

The gradual disappearance of "bound pyrroles" from tissues may be accounted for in several ways Adsorbed pyrroles might be slowly eluted Covalently bound pyrroles might be removed, either by a reversal of the alkylation reaction or through breakdown of the tissue constituent Alternatively, the pyrrole function might be metabolically removed, leaving a smaller fragment attached to the tissue constituent, or it may react with an electrophilic group, as mentioned earlier Thus, because no colour reaction can be obtained in liver tissue after, say, 48 h, this does not necessarily mean that no fragment of the alkaloid molecule remains The Ehrlich reaction is an insensitive test for trace amounts, and final residues may in any case be Ehrlich-negative

Emphasis has been placed on pyrroles, because these can be detected easily Evidence connecting these with alkaloid hepatotoxicity, though strong, is, however, circumstantial the phenomena occur at the same time More work will be needed to further establish the connexion The possibility of other, undetected metabolites must not be overlooked

I thank Dr J. M Barnes for valuable discussions, Dr W H Butler for help with the pathology, Dr Francesco De Matteis for carrying out tests for naturally occurring pyrroles, Dr L Keefer, Mr James Loo and Dr W. Lijinsky (Chicago) for help with NMR and mass spectra, B A J Alexander and C R Kennedy for technical assistance, and B W Street for the liver fractionation

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[1] Davidson, J , *J Pathol Bact* , **40**, 285 (1935)
[2] Bull, L B , and Dick, A T , *J Pathol Bact* , **78**, 483 (1959)
[3] Schoental, R , and Magee, P N , *J Pathol Bact* , **74**, 305 (1957)
[4] Schoental, R , and Magee, P N , *J Pathol Bact* , **78**, 471 (1959)
[5] Barnes, J M , Magee, P N , and Schoental, R , *J Pathol Bact* , **88**, 521 (1964)
[6] Hayashi, Y , and Lalich, J J , *Proc Soc Exp Biol and Med* , **124**, 392 (1967)
[7] Schoental, R , *Nature*, **179**, 361 (1957)
[8] Culvenor, C C J , Dann, A T , and Dick, A T , *Nature*, **195**, 570 (1962)
[9] Epstein, J , Rosenthal, R W , and Ess, R J , *Anal Chem* , **27**, 1435 (1955)
[10] Bender, D F , Sawicki, E , and Wilson, R N , *Analyst*, **90**, 630 (1965).
[11] Magee, P N , *Proc Roy Soc Med* , **59**, 751 (1966)
[12] Magee, P N , and Farber, E , *Biochem J* , **83**, 114 (1962)
[13] Mattocks, A R , *Anal Chem* , **39**, 443 (1967)
[14] Schwartz, S , Berg, M H , Bossenmaier, I , and Dinsmore, H , *Methods of Biochemical Analysis*, **8**, 221 (Interscience, New York, 1960)
[15] Witschi, H P , and Aldridge, W N , *Biochem J* (in the press)
[16] Umbreit, W W , Burris, R H , and Stauffer, J F , *Manometric Techniques*, 149 (Burgess Publ Co , Minneapolis, 1957)
[17] Miller, E C , and Miller, J A , *Cancer Res* , **7**, 468 (1947)
[18] Silverstein, R M , Ryskiewicz, E E , and Chaikin, S W , *J Amer Chem Soc* , **76**, 4485 (1954)
[19] Ryskiewicz, E E , and Silverstein, R M , *J Amer Chem Soc* , **76**, 5802 (1954)
[20] Baltazzi, E , and Krimen, L I , *Chem Revs* , **63**, 525 (1963)
[21] Robinson, G M , and Robinson, R , *J Chem Soc* , 532 (1923)

LETTERS TO THE EDITOR

PLANETARY SCIENCE

Occurrence of Olivine in a Type I Carbonaceous Meteorite

A REPORT of the discovery of olivine in the Orgueil carbonaceous meteorite[1] aroused considerable scepticism[2,3]. The identification was based on selected area electron diffraction patterns obtained from two mineral fragments observed during an electron microscope examination of a powdered meteorite specimen. Because, with the electron microscope in use at that time, it was only possible to examine particles in the orientation which they had arbitrarily taken up on the specimen support film, each diffraction pattern gave only limited information about the unit cell parameters and space group of any individual unknown mineral. The possibility of using such restricted data to identify mineral species rests on the fact that many minerals develop pronounced and specific faces, either by growth or cleavage, so that different fragments of the same mineral tend to lie in a common orientation, and thus to exhibit the same type of single crystal diffraction pattern in the electron microscope. The match between patterns from terrestrial olivine samples and those from olivine in Orgueil was good, but it was clearly desirable to put the identification on a firmer basis, if possible, by obtaining for a single mineral particle the full complement of unit cell parameters and the space group.

A Philips *EM*-300 electron microscope equipped with a goniometer stage made it possible to take diffraction patterns over a wide range of crystal orientations and thus to re-create virtually the entire reciprocal lattice of an unknown mineral. The Philips goniometer stage permits tilt through ±60° and rotation through 360°. The rotation axis coincides with the centre of the specimen

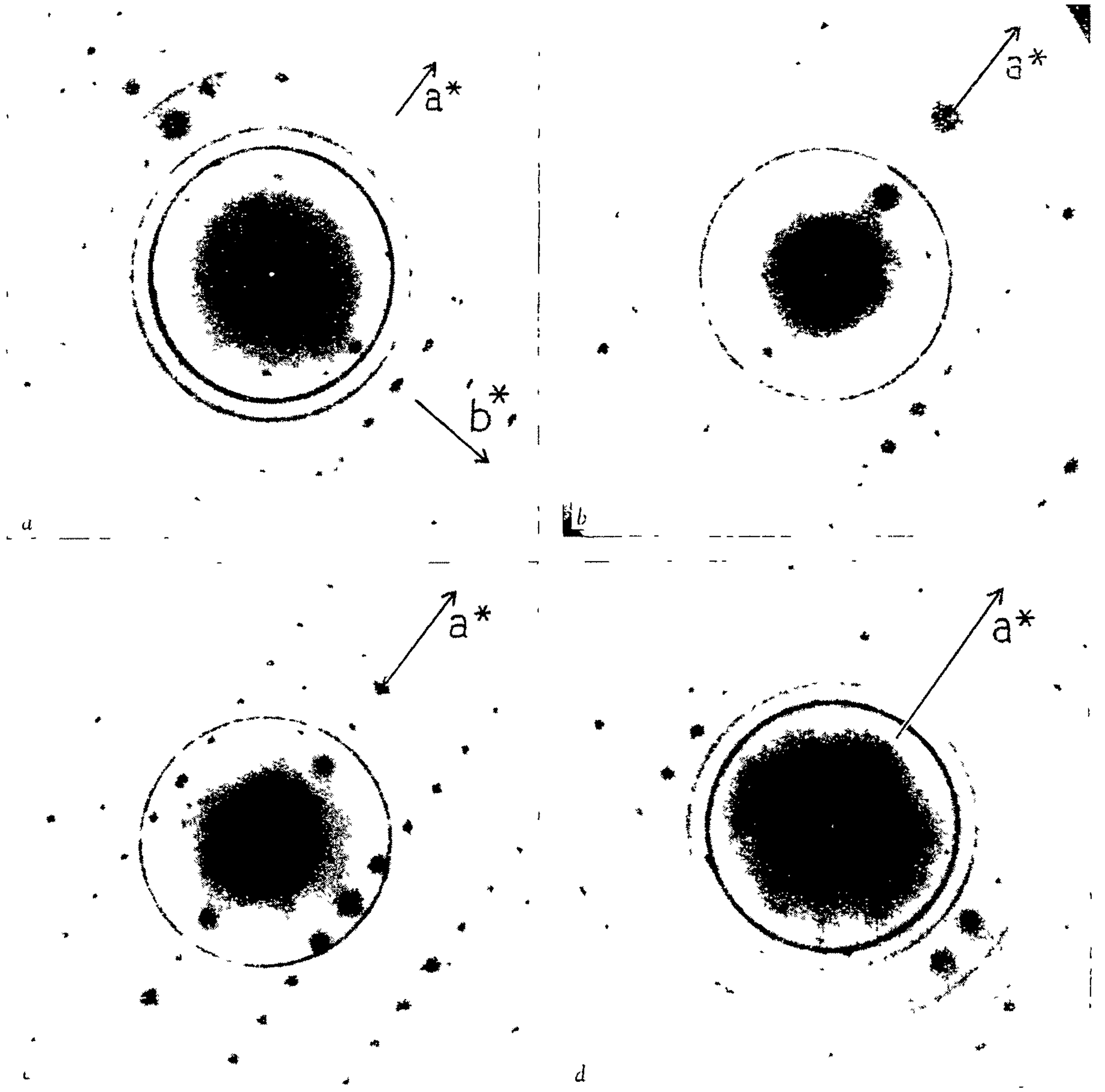

Fig 1. Selected area electron diffraction patterns taken at different orientations of an olivine crystal from the Orgueil meteorite. The tilt axis common to all four patterns coincides with the a^* axis of the reciprocal lattice. Angles of tilt relative to the normal specimen plane are *a*, −25°, *b*, 0°, *c*, +12°, and *d*, +23°. The innermost powder ring is the 2.338 Å reflexion of the aluminium standard.

grid, but an important advantage of this goniometer stage is that the tilt axis can be adjusted to intercept the electron optic axis for any position on the grid Thus the crystal remains stationary in the field of view during changes in tilt orientation, so that it is possible to observe the diffraction pattern directly in the microscope while the orientation is changing Both tilt and rotation are motor-driven. For this study, the anti-contamination device was in operation, for photographing patterns over a full range of orientations can result in the crystal being irradiated for up to 1 h during which considerable contamination, with consequent deterioration of the diffraction pattern, would occur The blades of the anti-contamination device restricted the range of tilt angles from ±60° to ±45° This was not, however, a real disadvantage, for, at the extreme angles of tilt, the path length for electrons through a crystal, which is commonly flattened in the direction perpendicular to the microscope grid, became so great that the beam was almost entirely absorbed

During a recent examination of a sample of the Orgueil meteorite a series of patterns was obtained from an irregularly shaped fragment approximately 2μ in diameter Four of these patterns are shown in Fig 1 The single crystal reflexions are produced by the unknown mineral and are superimposed on a powder pattern caused by the aluminium film used both to support the specimen and as a built-in diffraction standard for determining the scale of the unknown patterns By measuring the patterns it was possible to construct reciprocal lattice sections parallel to the electron beam All reflexions can be fitted to an orthorhombic network corresponding to a unit cell with parameters of 4 69 ± 0 10 Å, 6 04 ± 0 01 Å and 10 33 ± 0 03 Å. Following the conventions of Donnay[4] these are designated as the *c*, *a* and *b* axes, respectively.

The next stage in the identification is the elucidation of space group data from systematic absences among the reflexions These are listed in Table 1

Table 1 CONDITIONS FOR APPEARANCE OF REFLEXIONS

Reflexion	Condition	Conclusion
hkl	No conditions	Primitive cell
$hk0$	$k=2n$	*b*, Glide plane parallel to (001)
$h0l$	Not observed	
$0kl$	No conditions	
$h00$	$h=2n$*	Two-fold screw axis along <100>

* Reflexions, visible on some patterns, with $h \neq 2n$ are caused by double diffraction brought about by dynamical interactions

The possibility must be considered that the condition observed for $h00$ reflexions is a special manifestation of a more general condition and two such conditions are given in Table 2

Table 2 MORE GENERAL CONDITIONS WHICH COULD LEAD TO OBSERVED $h00$ CONDITION

Reflexion	Condition	Conclusion
$h0l$	$h=2n$	*a*, Glide plane parallel to (010)
$h0l$	$h+1=2n$	*n*, Glide plane parallel to (010)

Space groups which fulfil these observational requirements are $P2_1ab$, $P2_1nb$, $Pmab$ and $Pmnb$ The space group of olivine is $Pmnb$ The values of the unit cell parameters correspond to an olivine with 40–45 per cent fayalite which falls near the top of the olivine compositions observed in type II carbonaceous meteorites[5,6] It should be pointed out that olivine has never been observed as a contaminant in this laboratory

The significance of olivine in a type I carbonaceous meteorite is that it is necessary to postulate a high temperature (that is above about 500° C) stage for at least a small fraction of the meteorite and this seems to be incompatible with suggestions that such a meteorite is identical to primitive, unaltered cosmic material[7–9] It is concluded that type I meteorites resemble those of type II in that they are largely mechanical mixtures of minerals with different thermal histories[5,6,10]. This mixing must have occurred after the aqueous reaction stage necessary to explain certain other mineralogical features of the carbonaceous meteorites[1–3,11]

I thank Professor J D Bernal for advice and encouragement and Dr G F Claringbull for the specimen of Orgueil

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[1] Kerridge, J F, *Ann NY Acad Sci*, 119, 41 (1964)
[2] Bostrom, K, and Fredriksson, K, *NASA Contract Rep —CR—*69097 (1966)
[3] Nagy, B, *Geologiska Föreningens i Stockholm Forhandlingar*, 88, 235 (1966)
[4] Donnay, J D H, Donnay, G, Cox, E G, Kennard, O, and King, M V, *Crystal Data*, second ed (Amer Crystallographic Assoc, 1963)
[5] Fredriksson, K, and Keil, K, *Meteoritics*, 2, 201 (1964)
[6] Wood, J A, *Geochim Cosmochim Acta*, 31, 2095 (1967)
[7] Ringwood, A E, *Geochim Cosmochim Acta*, 15, 257 (1959)
[8] Mason, B, *J Geophys Res*, 65, 2965 (1960)
[9] Greenland, L, *J Geophys Res*, 68, 6507 (1963)
[10] Anders, E, *Space Sci Rev*, 3, 583 (1964)
[11] DuFresne, E R, and Anders, E, *Geochim Cosmochim Acta*, 26, 1085 (1962)

Composition of the Optically Active Fraction of a Texas Petroleum

Hills and Whitehead have investigated the optically active components of Nigerian, Libyan, Kuwaitian and Iranian crude oils[1,2] The enriched optically active fractions from these sources have been found to contain pentacyclic triterpanes and, furthermore, they seem to be made up essentially of the same components, although in varying proportions We have found that these generalizations also seem to be true for the optically active material from a Texas petroleum

Louis[3,4] has isolated an optically active saturated solid hydrocarbon mixture (purest sample $\alpha_D = +36°$) from a

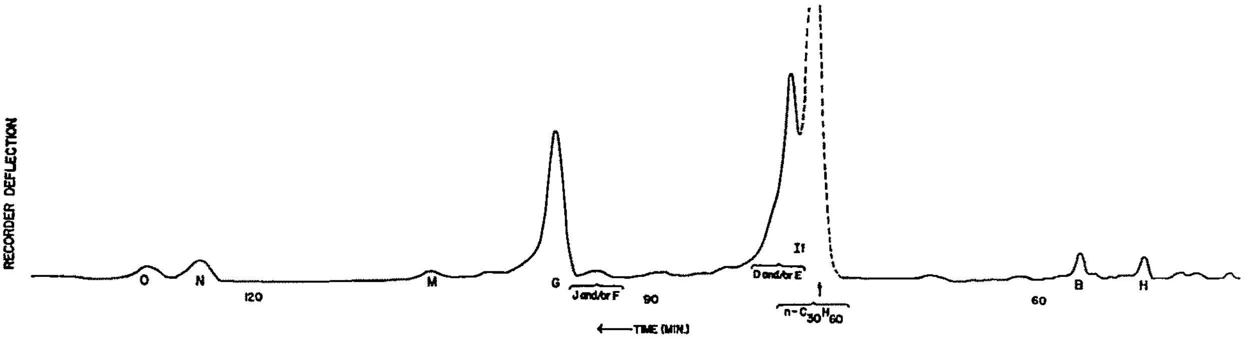

Fig 1 Part of a chromatogram of the optically active fraction isolated from the Mirando crude oil with added n-$C_{30}H_{62}$ 148 ft glass capillary of 0 01 in inner diameter, coated with *SE* 52 silicon gum Carrier gas hydrogen at 250° C Flame ionization detector Solvent peak (cyclohexane, not shown) appears at 2 min 11 s after injection

fraction (boiling range 162°–191° C at 0 01 mm) of the crude oil from Mirando A sample of this material was chromatographed on a capillary column in the same conditions as the optically active fraction originating from the Nigerian oil[1] (Fig 1, and ref 1, Fig 5), the stationary phase used was, however, *SE* 52 instead of *SE* 30 silicon gum

By co-injection of a mixture of the two optically active materials (on a glass column 148 ft × 0 01 in internal diameter, coated with *SE* 52, *t*, 250° C) it was shown that the Nigerian fraction caused peak enhancement of the chief components marked[1] *H*, *B*, *D* (and/or *E*, and possibly *I*), *J* (and/or *F*), *G*, *M*, *N* and *O* (Fig 1)

The mass spectra of the material, using ionization potentials of 15 and 70 eV, are given in Fig 2 Comparison of the peak intensities in the two conditions showed, notably, that the two most prominent ion masses, that is, 398 and 412 m/e, are molecular ions These peaks correspond to pentacyclic hydrocarbons of the formula $C_{29}H_{50}$ and $C_{30}H_{52}$, respectively It is further seen that the fragment at 191 m/e is of relatively high intensity particularly at 70 eV This fragment is considered to be characteristic of triterpanes[5] and is found, for example, in the following components of the Nigerian fraction[1] *E* and *F* (prominent masses at 413 and 191 m/e), and *D* (prominent masses at 398 and 191 m/e)

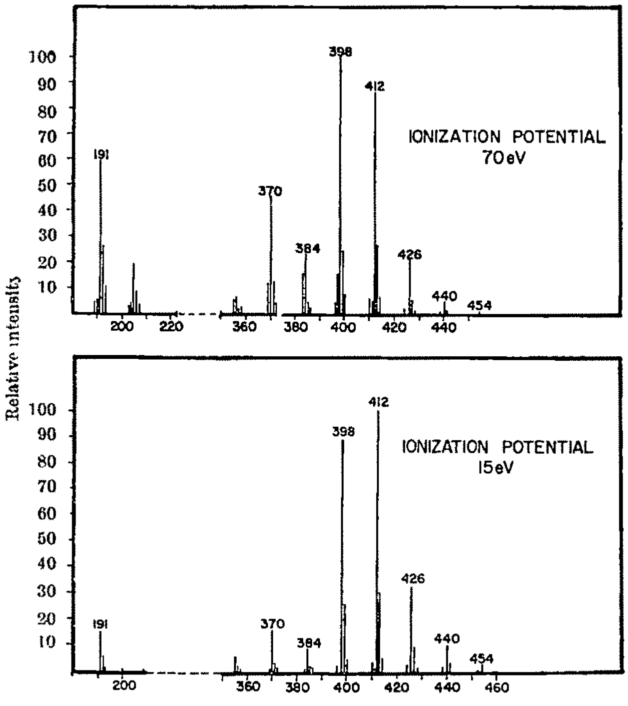

Fig 2 Mass spectra of the optically active fraction isolated from the Mirando crude oil, measured with an ionization potential of 15 and 70 eV, respectively

The large degree of coincidence of the gas chromatographic and mass-spectral data is also exhibited by the nuclear magnetic resonance spectra. The ratio of the methine plus methylene protons to the methyl protons has been found to be equal to about one for the Mirando sample

This evidence suggests that the optically active fractions from the Texas petroleum, and those from the African and Middle Eastern crude oils examined previously, may have, qualitatively, a largely similar composition The proportion of the various components seems, however, to vary In particular, *J* and *F* are found only in small amounts in the Mirando sample investigated Similar observations were made for the fractions originating from Libya and the Middle East, as compared with the Nigerian petroleum[1] It should, however, be remembered that the method of isolation used by Louis[3,4] differed from that of Hills and Whitehead[1]. Further work is needed to determine the extent to which the quantitative differences observed are analytically significant.

We thank E V Whitehead for carrying out the gas–liquid chromatographic analysis of our samples and for comparing the results with those reported in ref 1.

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[1] Hills, I R , and Whitehead, E V , *Nature*, **209**, 977 (1966)
[2] Hills, I R , and Whitehead, E V , *Proc Third Intern Meeting on Geochem* , London, September, 1966 (in the press)
[3] Louis, M , *CR Acad Sci* , **256**, 5610 (1963)
[4] Louis, M , *Rev Inst Franç Pétrole*, **19**, 277 (1964)
[5] Budzikiewicz, H , Wilson, J. M , and Djerassi, K , *J Amer Chem Soc* , **85**, 3688 (1963)

Composite Minor Intrusions in South Eastern Snowdonia

Composite minor intrusions were first recognized in North Wales by Beavon[1], who described a series of sills between 5 and 300 ft thick which are concordantly emplaced among lower Caradocian Glanrafon Slates outcropping on the eastern limb of the Arddu syncline (Fig 1) The sills have features which characterize them as composite minor intrusions as defined by Bailey and McCallien[2].

Mapping in the area to the north west of Blaenau Ffestiniog (Fig 1) has shown that the intrusions Beavon described are part of a more extensive suite which outcrop from the Glaslyn estuary to the neighbourhood of the Crimea Pass, between Blaenau Ffestiniog and Dolwyddelan They are emplaced among a great plexus of dolerite sheets which surround the mid-Caradocian volcanic ring structure of south-central Snowdonia[3,4]

The composite intrusions of the north eastern part of the area differ substantially from those described by Beavon and from most composite sheets of the British Tertiary province and elsewhere First, the basic margins of the sills vary in thickness between 0 and 150 ft even within a single intrusion, whereas the basic margins of the Tertiary composite intrusions are usually thin (2–6 ft) and remarkably constant even where there are local irregularities in the form of the intrusion[5]

Second, the acid components of the two largest sills leave their basic envelopes, by lateral failure of the basic margins in the case of the Allt Fawr sill and by transgression of the acid core of the Llyn Coch sill to form a dyke in the overlying rocks (Fig 1)

These intrusions afford a unique opportunity to examine the behaviour of the acid magma both within and outside its envelope of basic rocks Their basic margins are usually identical to the augite dolerites which are well documented throughout North Wales[6–9] The acid cores are made up from fine grained, equigranular granodiorite, or flow banded and spherulitic quartz latite which are both chemically and mineralogically similar to massive quartz latite intrusions which invade the Moelwyn Volcanic Series, about 1,000 ft stratigraphically below the composite sills[10] The acid member is not chilled against the dolerite, although the acid rocks contain sporadic xenocrysts of plagioclase like the feldspar in the basic margins The dolerite, however, often possesses a fine grained margin where it is in contact with the acid rock This marginal rock is riddled with secondary quartz, feldspars are highly decomposed and original pyroxene is converted to chlorite and acicular amphibole Its appearance suggests metamorphism by the acid member rather than chilling in the manner envisaged by Walker and Skelhorn[11] Lower contacts between the acid member and the dolerite are straight but thin and regularly spaced acid veins with matching contacts which penetrate downwards into the basic rock along incipient joints Small, subangular xenoliths of dolerite are aligned parallel with the contacts in the lower few feet of the acid member of the sills At upper internal contacts, the dolerite frequently lobes downwards into the acid core Sometimes the lobes are completely detached and form xenoliths with crenulate margins In the Llyn Coch sill flow, banding in the acid core sweeps upwards towards the dolerite roof from opposite directions, diverges and then runs parallel with the contact Where the flow bands sweep upwards and bifurcate, the dolerite sags into the negative area between them At the point of bifurcation the number of vesicles in the acid rock rapidly diminishes, suggesting that the discharge of volatiles at such points may have contributed to the upward flow of heat energy responsible for the local reomorphism of the overlying dolerite

Where the acid cores of the large Allt Fawr and Llyn Coch sills leave their basic envelopes, there are rapid changes in texture, grain size and in the style of contact metamorphism adjacent to the intrusions Where the core of the Allt Fawr sill lies within its dolerite envelope, it is made up from fine grained, equigranular granodiorite As the envelope thins, and finally disappears out, the acid core becomes finer grained and passes into flow banded and spherulitic quartz latite The Llyn Coch sill, emplaced at a higher stratigraphical level, has a core of flow banded quartz latite Where the acid member leaves its envelope and transgresses into the overlying sediments the rock becomes nodular The size and frequency of the nodules increase rapidly away from the dolerite envelope Blake *et al*[5] have suggested that in mixed acid/basic intrusions the presence of the basic magma increases the mobility of the acid magma They infer that the increased mobility is brought about by heat exchange between the hotter basic and the cooler acid magma The behaviour of the acid components in these composite sills supports this suggestion in that there was a marked increase in the viscosity of the acid magma where it left its warm dolerite envelope and where crystallization of the acid component was apparently more rapid A consideration of metamorphism adjacent to the composite intrusions suggests that the retention of volatiles also played an important part in increasing the mobility of the magma within the basic envelope Adjacent to the dolerite envelopes of the composite intrusions, metamorphism of the country rocks is similar to that associated with ordinary dolerite intrusions in the same area The shaly sediments are baked and have resisted cleavage Minutely spotted adinole is developed around the thick basic margins of the Llyn Coch sill and above the upper dolerite of the Allt Fawr sill Where the acid components of the sills leave their basic envelopes the style and extent of metamorphism change rapidly The sediments above the acid member of the Allt Fawr sill are heavily spotted with chloritic pseudomorphs after cordierite for 150 ft above the intrusion The style of metamorphism reflects only that component of the sills which is in contact with the country rocks The more extensive metamorphic aureole above the cooler acid

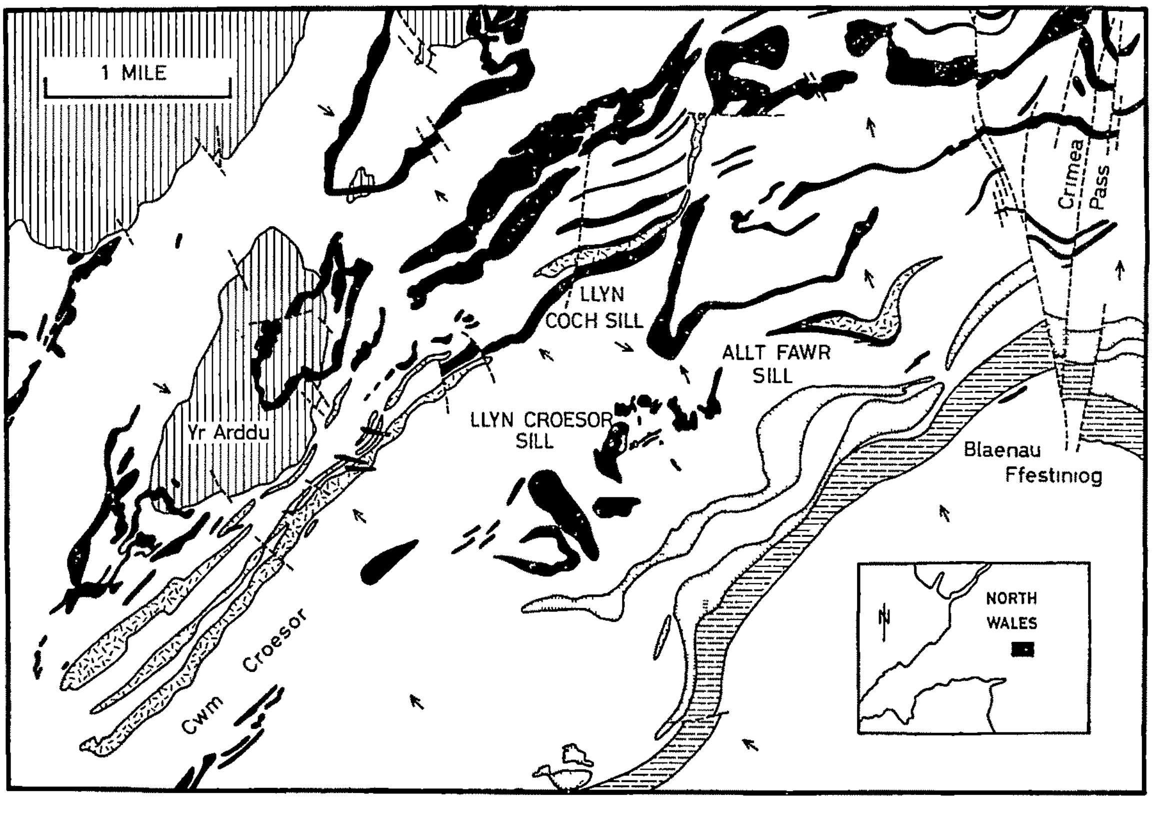

Fig 1 Simplified geological map to show the location of composite minor intrusions in south eastern Snowdonia Composite minor intrusions, dashes, dolerite, black, quartz latite, dots Cambrian, unornamented, Lower Ordovician, broken lines, Glanrafon Series, unornamented, Snowdon Volcanic Series, vertical lines Arrows indicate dip of bedding, faults, broken lines Inset map, location of area

member of the Allt Fawr sill suggests that volatile streaming into the surrounding sediments played an important part in metamorphism and that even a thin basic envelope prevented volatiles escaping from the acid member It seems likely that the retention of volatiles was important in maintaining higher mobility of the acid magma within its basic envelope

Blake *et al*[5] have suggested that in the formation of mixed acid/basic intrusions, acid magma mobilized at depth by rising basic magma rapidly prevents the continued rise of basic material Thus the basic margins of composite intrusions tend to be uniformally thin The unusual thickness of the basic margins of these intrusions can be explained by assuming that mobilization of the acid member took place more slowly so that correspondingly more basic material rose to a high level. There is some indication of a time lag between the arrival of basic and acid components in that incipient joints formed in the lower basic margins before the acid magma was emplaced The crenulate basic xenoliths in the upper parts of the acid cores of the sills can be shown to have been derived from the upper dolerite margins The occurrence of such xenoliths has been cited as evidence of the simultaneous liquidity of acid and basic magma[5,11] The contrasting behaviour of the dolerite at the upper and lower internal contacts in the Snowdonian intrusions suggests that the basic magma had become sufficiently viscous to fracture before the acid magma arrived Upward flow of heat energy and probably discharge of volatiles at the acid/basic interface caused partial reomorphism of the dolerite, giving rise to lobe structures and in extreme cases to detached xenoliths within the acid hosts

The composite sills of south eastern Snowdonia demonstrate conclusively that acid and basic magmas existed simultaneously in the Caradocian volcanic province of North Wales The basic component of the sills is identical in every way to the dolerites with which they are spatially associated This suite of dolerites has been interpreted as belonging to the mid-Caradocian (Snowdon Volcanic Series) igneous cycle[3,4], and it seems likely that the composite minor intrusions belong to the same phase of activity The acid cores of the intrusions, however, have much closer affinities with the sodic quartz latite intrusions associated with the lower Caradocian Moelwyn Volcanic Series than with the dominantly potassic rocks of the Snowdon Volcanic Series What is more significant is that the composite intrusions have much the same lateral extent as the quartz latites which outcrop about 1,000 ft below them It is possible that basic magma, rising through the already emplaced quartz latite sills, remelted portions of them and that the reconstituted acid magma rose to higher levels and was emplaced among the dolerites Elsewhere, away from the quartz latite sills, the basic magma was emplaced as a suite of simple dolerites

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[1] Beavon, R V , *Quart J Geol Soc* , **119**, 479 (1963)

[2] Bailey, E B , and McCallien, W J , *Liverpool Manchester Geol J* , **1**, 466 (1956)

[3] Rast, N , in Proc Symp Aberystwyth, *The Pre-Cambrian and Lower Palaeozoic Rocks of Wales* (in the press)

[4] Bromley, A V , in Proc Symp Aberystwyth, *The Pre-Cambrian and Lower Palaeozoic Rocks of Wales* (in the press)

[5] Blake, D H , Elwell, R W D , Gibson, I L , Skelhorn, R R , and Walker G P L , *Quart J Geol Soc* 121, 31 (1965)

[6] Williams, H , *Liverpool Geol Soc Proc* , **13**, 166 (1922)

[7] Williams, H , *Quart J Geol Soc* , **83**, 346 (1927)

[8] Fearnsides, W D , and Davies, W , *Quart J Geol Soc* , **99**, 247 (1944)

[9] Shackleton, R M , *Liverpool Manchester Geol J* , **2**, 216 (1959)

[10] Bromley, A V , *Liverpool Manchester Geol J* , **4**, 247 (1965)

[11] Walker, G P L , and Skelhorn, R R , *Earth-Sci Rev* , **2**, 93 (1966)

PHYSICS

Radial Distribution Functions from Small Packings of Spheres

Scott[1] has derived a radial distribution function from a packing of about 1,000 spheres by measuring the Cartesian co-ordinates of each sphere and calculating the individual distribution functions of each of a group of 25 spheres known to be in the centre of the packing These 25 separate distribution functions were averaged to give the final result Clearly the edge of the packing interferes and limits both the maximum radial distance to which the distribution function can be determined and the number of centres that can be found sufficiently far from this edge An improvement would be to use all the spheres in the packing as centres and to correct the distribution function obtained for the effect of the edge A maximum separation equal to the diameter of the packing would be obtainable together with better statistics in the histogram Alternatively, results similar to those of Scott[1] could be obtained from packings of half the linear size containing only about 125 spheres.

If it is assumed that the packing is spherical in shape, with radius $R_{\max}$, that it is homogeneous and of density ρ_0 centres per unit volume, and if the radial distance of the distribution function is r, then all spheres within a distance of $(R_{\max} - r)$ from the centre of the packing will have complete shells of neighbours within the radius r and so no correction need be applied. The spheres between $(R_{\max} - r)$ and $R_{\max}$ are the ones that do not have complete shells of radius r and a correction factor (F) has to be applied for these This factor will be the ratio of the number of pairs that should be observed to the number that actually are observed and will be a function of $(r/R_{\max})$ given values of r and $R_{\max}$

For an infinite packing the number of sphere centres in an elemental shell between r and $(r + \delta r)$ from a central sphere is

$$4\pi r^2 \rho(r) \delta r$$

where $\rho(r)$ is the radial distribution function For a centre at a distance R from the centre of a packing of limited size the apparent value of $\rho(r)$ will be less than the true

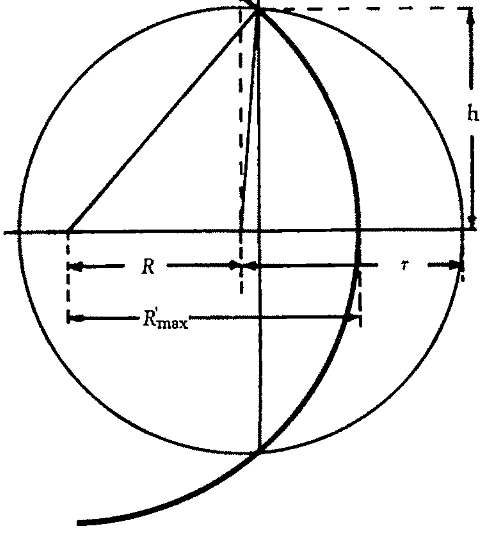

Fig 1 A section through a spherical packing of radius $R_{\max}$ The centre at a distance R from the packing centre only has a partial shell of neighbours at a distance r

value because part of the shell will lie outside the packing (Fig 1) The fraction will be the ratio of the area of the cap of the sphere, radius r, inside sphere radius $R_{\max}$, to the total area of the sphere radius r Thus the apparent density from the centre at R is

$$\rho_{A,R}(r) = \rho(r) \quad \frac{(r+x)}{2r}$$

where

$$x = \frac{R_{\max}^2 - R^2 - r^2}{2R}$$

The number of centres in an elemental shell, thickness δR and at a distance R from the centre of the packing to which $\rho_{A,R}(r)$ pertains, is

$$4\pi R^2 \, \delta R \, \rho_0$$

Thus the apparent density $\rho_A(r)$ taken over the whole packing will be

$$\rho_A(r) = \int_0^{R_{\max}} \frac{(\text{number of centres}) \, \rho_{A,R}(r)}{\text{total number of centres}}$$

$$= \frac{\int_0^{(R_{\max}-r)} 4\pi R^2 \, \rho_0 \rho(r) \, \mathrm{d}R + \int_{(R_{\max}-r)}^{R_{\max}} 4\pi R^2 \, \rho_0 \rho(r) \, \frac{(r+x)}{2r} \, \mathrm{d}R}{\int_0^{R_{\max}} 4\pi R^2 \, \rho_0 \, \mathrm{d}R}$$

Because $\rho_A(r)$ is the apparent density observed for the packing of limited size, the correction factor (F) by which the experimental density has to be multiplied to give the true distribution function is

$$F = \frac{\rho(r)}{\rho_A(r)}$$

Integrating $\rho_A(r)$ and substituting $y = \left(\frac{r}{R_{\max}}\right)$ gives

$$F = \frac{16}{(y-2)^2(y+4)}$$

When $r \geqslant R_{\max}$, there can be no core of centres in the packing with a complete shell of neighbours Consequently a term is lost in $\rho_A(r)$ which becomes

$$\rho_A(r) = \frac{\int_{(r-R_{\max})}^{R_{\max}} 4\pi R^2 \, \rho_0 \rho(r) \, \frac{(r+x)}{2r} \, \mathrm{d}R}{\int_0^{R_{\max}} 4\pi R^2 \, \rho_0 \, \mathrm{d}R}$$

and

$$x = \frac{R^2 + r^2 - R_{\max}^2}{2R}$$

Integration gives the same correction factor as before

$$F = \frac{16}{(y-2)^2(y+4)}$$

When $y = 0$ there is no edge effect and $F = 1$ When $y = 1$, $F = 3\ 2$ When $y = 2$ the pair separation equals the diameter of the packing and $F = \infty$.

The co-ordinates of Scott[1], corrected by Bernal and with a few additional minor corrections, were analysed in this way The packing density was calculated for spherical samples of increasing radius cut from the packing (Fig. 2) The actual volume of solids within this radius was determined by calculating the volume of the spheres totally within the radius and adding the inner sphere segments of those spheres intersecting the radius The edge of the packing was determined by the radial distance at which this packing density began to decrease rapidly

A spherical sample, radius 15 0 mm and containing 536 spheres, was taken from the packing and the separation of every pair of spheres determined The uncorrected radial distribution function was determined for intervals of 0 05 sphere diameters. The correction factor was then evaluated and applied for each of these intervals and the result reduced to the average value for one sphere by division by 268 and normalized by division by $4\pi r^2 \delta r$ where r was the radial distance and δr the interval width in diameters (0 05 in this case).

The results are shown in Fig 3 and they reinforce the observations of Scott[1]. The structure of the peaks can, however, be more clearly seen. The peak at 1 0 diameters is very sharp as would be expected for hard spheres but the peak between 1 7 and 2 0 is curiously square The other peaks slowly merge into the continuum at about 5 5 diameters

Fig. 4 shows the structure in the important region between 1 and 1 5 diameters The number of neighbours within a radial distance is plotted against distance At 1·1 diameters this is in agreement with Scott[1], who found 9 3 ± 0 8 neighbours There are, however, only about 5 actual contacts but the gradient is very steep in this

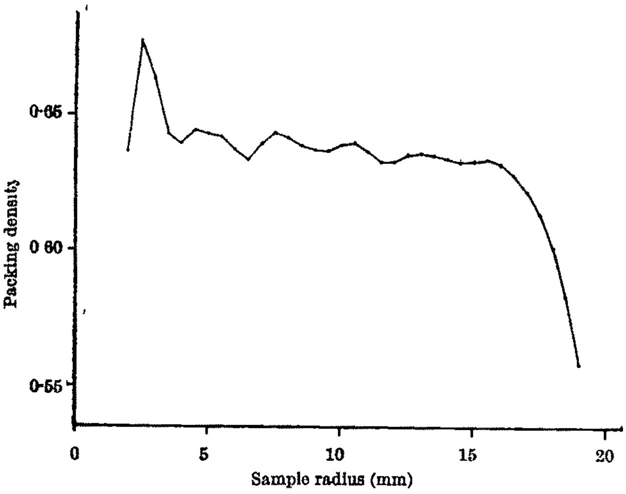

Fig 2 The packing density of spherical samples of increasing radius cut from the packing

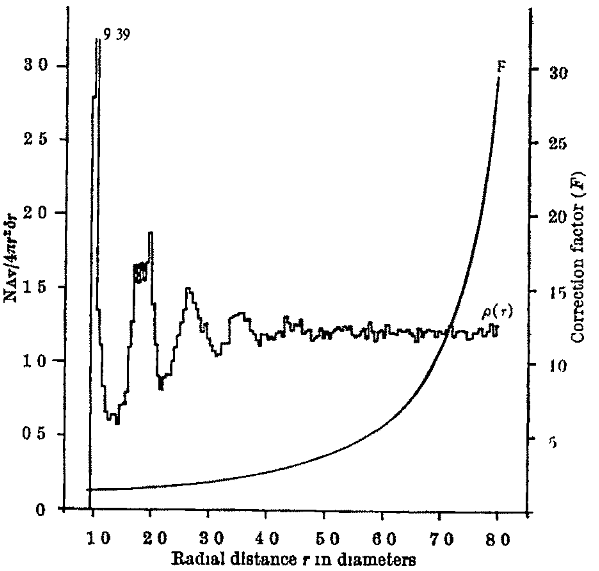

Fig 3 The radial distribution function, ρ(r), showing also the values of the correction function (F)

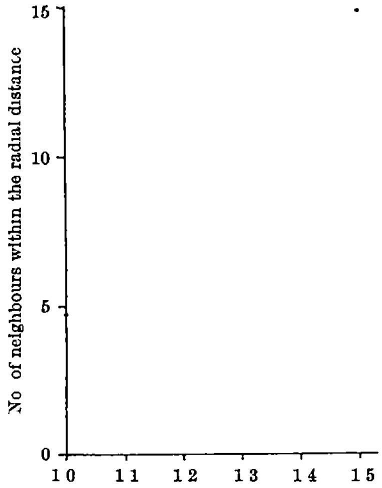

Fig 4 The cumulative near neighbour distribution function The average number of neighbours within a radial distance (r) of the central sphere is shown

region and there are nearly 7 neighbours within 1 02 diameters

I thank Mr and Mrs A C Wright for their helpful discussions. I also thank Professor G D Scott for a copy of the original co-ordinates of his packing and Professor J D Bernal for the few corrections to these co-ordinates

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[1] Scott, G D , *Nature*, 194, 956 (1962)

Rational Geometry for measuring Elastic and Viscoelastic Parameters

Recent developments in rubber elasticity theory have led to the recognition that measurements of elastic properties of gels and rubbers near the gel point are of special value[1-3] The need to develop new methods of measurement has been foreshadowed[2]. We describe here a method under trial for materials in the range 10^3–10^5 dyne cm^{-2} (but which could be extended well beyond this range), the importance of which stems from its rational basis

Elasticity investigations depend on measuring deformation in response to an applied force The geometry of the area over which the force is applied to the specimen is important, for it will enter the interpretation of experimental data through the boundary conditions for the differential equations which are relevant. Even in the domain of routine uniaxial tensile modulus measurements, international committees have given much thought to the best geometry of the clamps which transmit the force (see ref 4)

From both the experimental and theoretical viewpoints, perhaps the simplest testing geometry is that of a rigid sphere embedded in a large reservoir of the material under examination For materials which adhere to the spherical surface, it is possible to obtain complete theoretical solutions for the relation between the displacement of the sphere ξ and the externally applied force F. Under static conditions the relation for an incompressible material is

$$F = 6\pi\mu a\xi \qquad (1)$$

where μ is the shear modulus and a the radius of the sphere. Thus for materials which exhibit the appropriate tactile properties, measurement of F and ξ leads directly to a value of μ

Preliminary results for a nickel sphere (radius $a = 0\ 0080$ cm) embedded in a gelatine gel are shown in Fig. 1 The sphere was at distance $> 50a$ from the surface of the gel An electromagnet capable of generating up to 1,000 gauss at the sphere was used to exert forces of order 1 dyne on the sphere These forces were calibrated in terms of the "pick-up distance" of the free nickel sphere versus its distance from the tip of the electromagnet (At the pick-up distance the magnetic force just exceeds the known weight of the sphere) Displacements of the embedded sphere were measured with a microscope (magnification × 400), they were reproducible for several hours and reversible within experimental error Fig 1 shows linear elastic behaviour up to the maximum displacement of about 0 7a Young's modulus calculated from the slope is $1\ 30 \times 10^4$ dyne/cm^2 (using equation (1) and assuming Poisson's ratio to be 1/2) As a check, a value of $2\ 1 \times 10^4$ was obtained on a sample of the same gel by the surface indentation method[5] The agreement is thought to be satisfactory because the surface method is much less accurate and liable to give high values of Young's modulus owing to loss of plasticizing action by evaporation of water

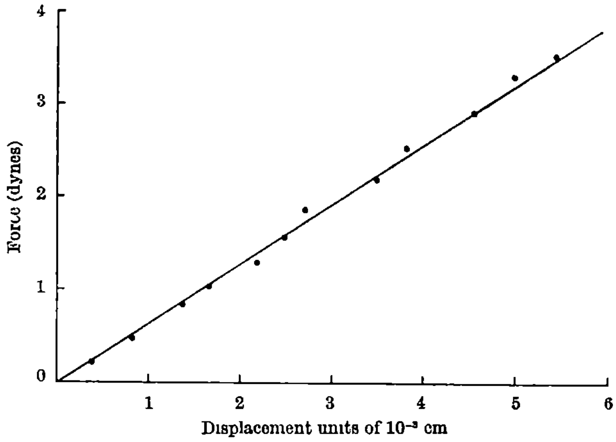

Fig 1

One of us[6] has generalized the result (1) to the case of time dependent displacement $\xi(t)$ The principal result is that for an elastic solid, (1) is replaced by

$$F = 6\pi\mu a\xi + 6\pi a^2(\mu\rho)^{1/2}\dot{\xi} - \tfrac{1}{2}M\ddot{\xi} \qquad (2)$$

where ρ is the density and $M = 4\pi a^3\rho/3$ is the mass of material displaced by the sphere. In turn, equation (2) is readily generalized to the viscoelastic case[6], thus permitting analysis of dynamic experiments in terms of viscoelastic parameters

One of us (J A L) thanks the Plastics Institute education fund for a grant

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[1] Dobson, G R , and Gordon, M , *J Chem Phys* , 43, 705 (1965).
[2] Gordon, M , *Plaste und Kautschuk*, 12, 103 (1965)
[3] Strella, S , and Bibeau, A A , *J Makromol Chem* , 1, 417 (1966).
[4] *Tension Testing of Vulcanized Rubber*, ASTM, D 412–62T.
[5] Waters, N E , *Brit J Appl Phys* , 16, 557 (1965)
[6] Hunter, S C , *J Edin Math Soc* (in the press)

Tensile Strength of Granular Materials

In several communications[1-4] on the tensile strength of granular materials bound by liquids, authors have tried to confirm or improve the equation of Rumpf[5]. At the Institute of Mechanical Process Engineering of the University of Karlsruhe, where the first measurements of the tensile strength of moist agglomerates started in 1957, investigations have recently led to a complete theoretical and experimental description of the tensile strength relationship in the whole moisture range.

Considering previous communications, it should first be pointed out that the liquid in moist or "green" agglomerates can be distributed in three characteristic ways, which depend on the amount of liquid present and the porosity of the agglomerate. These are (*a*) the "pendular state" with only liquid bridges between the individual particles which make up the agglomerate; (*b*) the "capillary state" in which all pores within the pellet are filled completely with the liquid which forms concave menisci at the pore ends, and (*c*) a transition range where both liquid bridges and capillaries filled with liquid are present. The binding mechanisms are different for all three ranges.

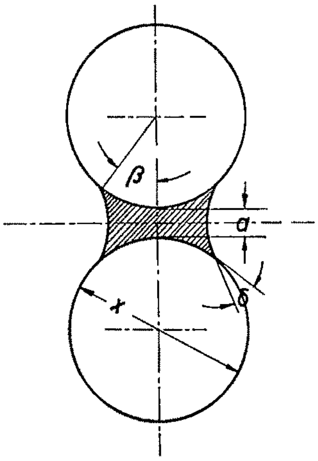

Fig 1 Liquid bridge between two spherical particles of equal size (model)

Theoretically, equations for the cases (*a*) and (*b*) can be derived. Equation (1) is the relationship derived statistically by Rumpf[5]

$$\sigma_{tg} = \frac{9}{8} \cdot \frac{1-\varepsilon}{\varepsilon} \cdot \frac{H(x)}{x^2} \tag{1a}$$

where σ_{tg} is the tensile strength of granular materials which is produced by binding forces, $H(x)$, of any physical nature at the co-ordination points between the individual particles within the agglomerates, ε is the porosity of the pellet and x is the mean size of the particles forming the agglomerate. For a liquid bridge between two special particles of equal size x with a distance a at the co-ordination point (Fig 1), the binding force is[6]

$$H(x) = \alpha \quad x \quad FH$$

where α is the surface tension and $FH = FH(\beta, \delta, a/x)$ is the complicated function of the half centriangle β, the contact angle δ and the dimensionless length ratio a/x. Thus σ_{tb}, the tensile strength of agglomerates in the bridge or "pendular" state, is

$$\sigma_{tb} = \frac{9}{8} \; \frac{1-\varepsilon}{\varepsilon} \; \frac{\alpha}{x} \; FH \tag{1b}$$

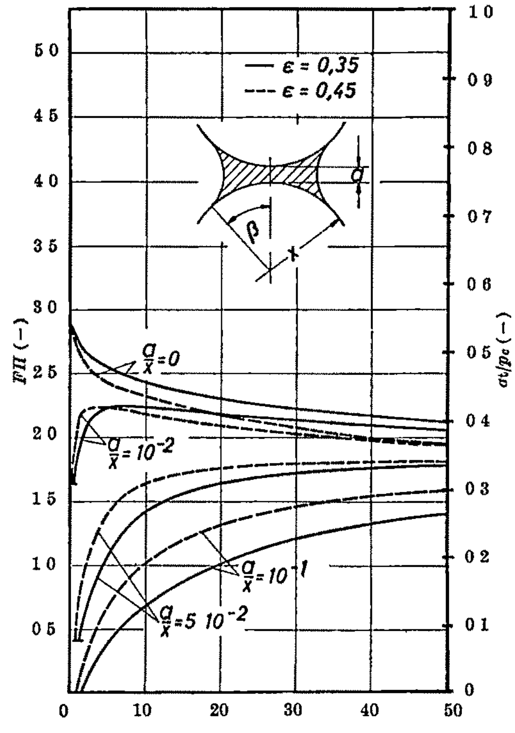

Fig 2 Dimensionless adhesion force, *FH*, as a function of the percentage liquid saturation, *S*. Parameters are the length ratio, *a/x*, and the porosity, *ε*

Fig 2 shows the dimensionless adhesion force, *FH*, plotted against the percentage saturation, *S*, the relation between the pore volume occupied by the liquid and the total pore volume of the pellet[6]. Different porosities, ε, and distances, a, give different curves. Because in practice no absolutely smooth particles are found, the case $a = 0$ seldom occurs in a real agglomerate. In most cases, the situation at the co-ordination points is very probably similar to that shown in Fig 3. This explains the discrepancy between the Haines–Fisher equation, which was derived assuming $a = 0$ and experimental results[4]. Recent measurements of tensile strengths of granular materials in the "pendular state" are in good agreement with the theoretical curves shown in Fig 2, if an experimentally estimated length ratio, a/x, is introduced (result to be published).

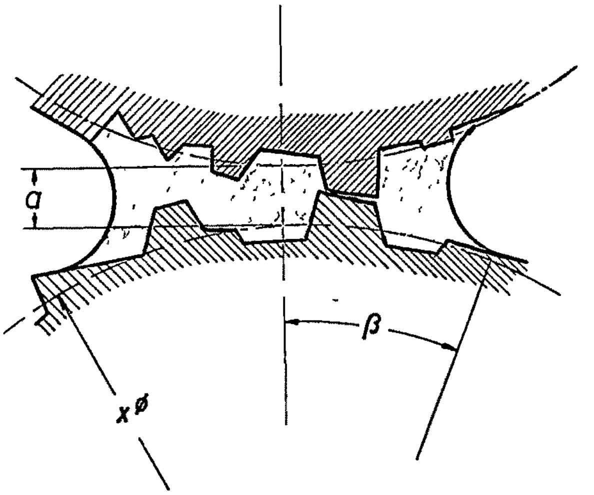

Fig. 3 Schematic representation of a liquid bridge at the co-ordination point of two real particles

Equation (2) is the relationship for the capillary pressure in granular materials, because the tensile strength of agglomerates in the "capillary state", σ_{tc}, is approximately equal to the capillary pressure[5] According to Rumpf[5] a conventional equation for the capillary pressure, p_c, in granular materials is

$$\sigma_{tc} \sim p_c = \text{const} \; \frac{\alpha}{x} \; \frac{1-\varepsilon}{\varepsilon} \; f(\delta) \qquad (2a)$$

where $f(\delta)$ is a contact angle function which is, by definition, unity for liquids wetting the solid completely ($\delta = 0°$) The constant has values between 6 and 8.

Using certain assumptions, the capillary pressure in a liquid bridge between two spherical particles of equal size can also be used to characterize the capillary pressure in granular materials (result to be published) This equation reads

$$\sigma_{tc} \sim p_{cb} = \frac{\alpha}{x} \; FPC \qquad (2b)$$

where $FPC = FPC(\beta, \delta, a/x)$ is a complicated function of the half centriangle, β, the contact angle, δ, and the dimensionless length ratio, a/x (ref 6).

The tensile strength of moist granular materials can be described in the complete moisture range by assuming that the relationship between the tensile strength, σ_t, and the percentage liquid saturation, S, in the transition range (c) increases linearly from a point S^*—the critical percentage liquid saturation where liquid bridges in the aggregate begin to touch each other—to $S = 100$ per cent Below S^* the tensile strength is described by equation (1a)

Defining a tensile strength characteristic, FZ,

$$FZ = \sigma_t \; \frac{x}{\alpha} \qquad (3)$$

and a capillary pressure characteristic, FPK,

$$FPK = p_c \cdot \frac{x}{\alpha} \qquad (4)$$

the set of equations for the tensile strength of moist granular materials gives

$$FZ = \frac{9}{8} \; \frac{1-\varepsilon}{\varepsilon} \; FH = FZ_b \qquad \text{for } 0 \leq S < S^* \qquad (5a)$$

$$FZ = FZ_b^* + \frac{S - S^*}{1 - S^*} (FPK - FZ_b^*) \text{ for } S^* < S < 100 \quad (5b)$$

with

$$FZ = \frac{9}{8} \; \frac{1-\varepsilon}{\varepsilon} \; FH(\beta^*, \quad) = FZ_b^* \text{ for } S = S^* \qquad (5c)$$

and

$$FZ = FPK \qquad \text{for } S = 100 \qquad (5d)$$

Equations (5) represent, under the assumptions made previously, the most general solution of the problem The characteristics, FZ and FPK, can incorporate any equation for the tensile strength, σ_t, and the capillary pressure, p_c Fig 4 shows, for example, some tensile strength curves which were calculated using equations (1b), (2a) and (2b) and plotted in a dimensionless form over the percentage liquid saturation, S While below the critical percentage liquid saturation, S^*, the curves are unequivocal for a given set of ε and a/x values, they differ considerably in the transition range The dashed lines in Fig 4 represent the curves calculated according to equation (2a), where

$$FPK = \text{const} \; \frac{1-\varepsilon}{\varepsilon} \cdot f(\delta), \; \text{const} = 6 \text{ and } f(\delta) = 1$$

and the full lines have been calculated using equation (2b), with

$$FPK = FPC, \; \beta = 28° \text{ and } \delta = 0°$$

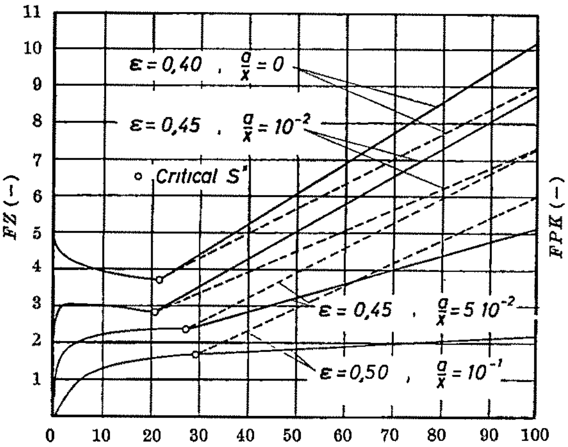

Fig 4 Theoretical tensile strength curves of moist granular materials Parameters are the length ratio, a/x, and the porosity, ε Both equation (2a) (– – – –) and equation (2b) (——) have been used for the calculation of FPK

The critical percentage liquid saturation has been estimated using the rules of spherical trigonometry

Experimental results confirmed the trend of the curves in Fig 4 Above the critical percentage liquid saturation a better approximation could be obtained by using equation (2b) (solid lines in Fig 4)

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[1] Smalley, V , and Smalley, I J , *Nature*, **202**, 168 (1964)
[2] Morgan, B B , *Nature*, **205**, 36 (1965)
[3] Carr, J F , *Nature*, **213**, 1158 (1967)
[4] Douglas, A G , *Nature*, **215**, 952 (1967)
[5] Rumpf, H , in *Agglomeration* (edit by Knepper, W A), 379 (Interscience Publishers, 1962)
[6] Pietsch, W , and Rumpf, H , *Chem -Ing -Technik* , **39**, 885 (1967)

THE SOLID STATE

Crystal Structures of some Protactinium Bromides

THERE has recently been considerable interest in the crystal chemistry of the f-electron transition elements The often unusual co-ordination arrangements found around these rather large metal atoms may in some cases be connected with f-orbital participation in the bonding Protactinium is of particular interest because of the proximity of the 5f and 6d energy levels, but few detailed structural studies of protactinium compounds have yet been reported

We briefly report here the results of three crystal structure determinations in which protactinium has been found to show co-ordination numbers of six, seven and eight Co-ordination numbers nine[1] and ten[2] for this element are also known

Protactinium pentabromide has been found to be dimorphic, and the crystal structure of one form—designated β—has been determined Work is proceeding on the α-form β-$PaBr_5$, obtained by sublimation at 400°–410° C *in vacuo*, crystallizes in the monoclinic system (unit cell $a = 8\ 385$ Å, $b = 11{\cdot}205$ Å, $c = 8\ 950$ Å, $\beta = 91{\cdot}1°$; space group $P2_1/n$) and is virtually isostructural

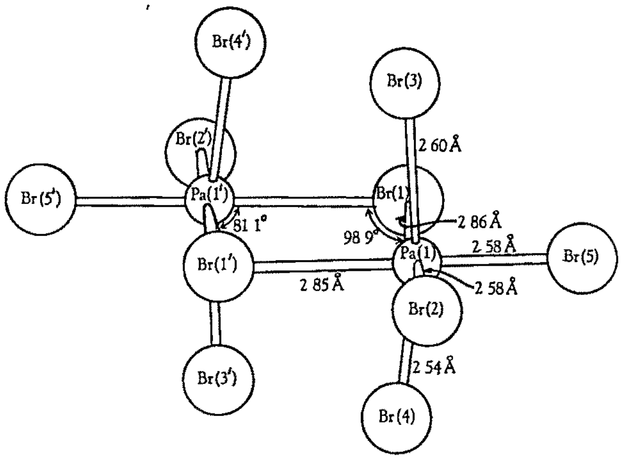

Fig 1 The dimeric Pa_2Br_{10} unit

with uranium pentachloride[3]. The bromine bridge is symmetrical, however, whereas UCl_5 has an asymmetric chlorine bridge. The *bis*-octahedral molecular unit (Fig 1) is obviously closely related to that of niobium (and tantalum) pentachloride[4] The structure has been refined by three-dimensional least-squares methods to a final residual of 9·05 per cent for 281 visually estimated single crystal reflexions The single-bond covalent radius of protactinium (V) derived from this six co-ordinate β-$PaBr_5$ structure is 1·44 ± 0·02 Å, which can be compared with the mean single bond values of 1·45 ± 0·02 and 1·52 ± 0·02 Å for the seven co-ordinate pentachloride, $PaCl_5$, and nine co-ordinate heptafluorocomplex, K_2PaF_7, respectively

Protactinium (V) oxytribromide also crystallizes in the monoclinic system (unit cell $a = 16{\cdot}911$, $b = 3{\cdot}871$, $c = 9{\cdot}334$ Å, $\beta = 113{\cdot}67°$, space group $C2$) The presence of static disorder in the best crystal available has complicated the structure determination, but due allowance for 22 per cent disorder in a full-matrix least squares refinement scheme has enabled us to attain a final residual of 11·8 per cent for 259 observed reflexions. $PaOBr_3$ has a double chain structure (Fig 2) in which each protactinium atom is seven co-ordinate The chains are bound together by oxygen and bromine bridging, each oxygen being co-ordinated to three protactinium atoms. The protactinium co-ordination sphere is a distorted pentagonal bipyramid and tends towards the C_2 arrangement of Claxton and Benson[7] A similar chain structure has been reported[8] for the non-stoichiometric uranyl complex $Cs_{0{\cdot}9}UO_3Cl_{0{\cdot}9}$ The $PaOBr_3$ structure is markedly different from that of $NbOCl_3$ (ref. 9) in which Nb—O—Nb—O chains are cross-linked by bridging chlorine atoms Full structural information is not available for either $NbOBr_3$ or $TaOBr_3$, but recent[10] infrared results suggest that the former has a similar type of oxygen bridge to that found for $NbOCl_3$, this niobium-oxygen stretching vibration occurs at 775 cm^{-1} in $NbOBr_3$ compared with 770 cm^{-1} for $NbOCl_3$ The protactinium-oxygen stretching vibrations, on the other hand, are observed at 515m, 476w, 364s and 303w cm^{-1}, and these lower values are presumably a consequence of the increased co-ordination number of the oxygen atoms

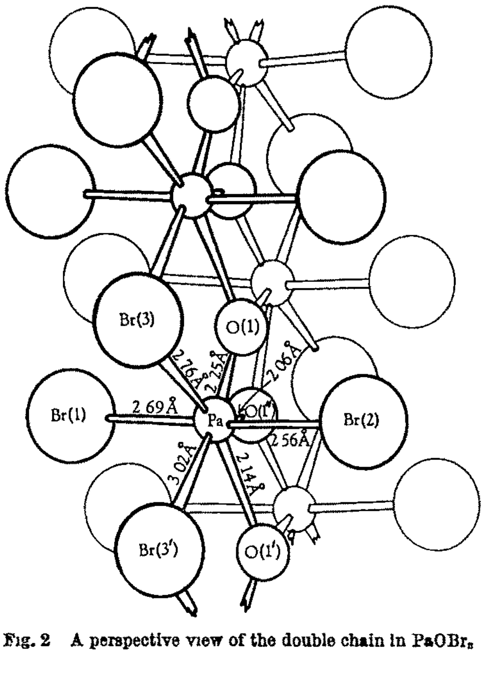

Fig. 2 A perspective view of the double chain in $PaOBr_3$

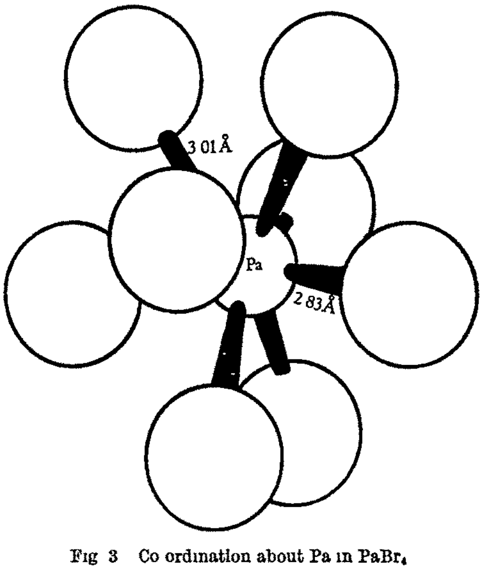

Fig 3 Co ordination about Pa in $PaBr_4$

Protactinium tetrabromide has been shown to be isostructural with thorium tetrachloride[11] It crystallizes in the tetragonal system (unit cell $a = 8{\cdot}824$ Å, $c = 7{\cdot}957$ Å, space group $I4_1/amd$). Intensity data were collected by the powder diffraction method, but because of difficulties of absorption correction, refinement has so far proceeded only to $R = 20$ per cent for forty-four observed reflexions The structure (Fig 3) is, however, confirmed and positional parameters are adequately determined The protactinium (IV) is co-ordinated by eight bromines in the form of a dodecahedron

Full experimental details of all three analyses will be submitted for publication in due course.

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[1] Brown, D , Kettle, S F A , and Smith, A J , *J Chem Soc* (*A*), 688 (1967)
[2] Zachariasen, W H , *Acta Cryst* , 5, 19 (1952)
[3] Smith, G S , Johnson, Q , and Elson, R E , *Acta Cryst* , 22, 300 (1967)
[4] Zalkin, A , and Sands, D E , *Acta Cryst* , 11, 615 (1958)
[5] Dodge, R P , Smith, G S , Johnson, Q , and Elson, R E , *Acta Cryst* 22 85 (1967)
[6] Brown, D , and Jones, P J , *J Chem Soc* (*A*), 262 (1966)
[7] Claxton, T A , and Benson, G C , *Canad J Chem* , 44, 157 (1966)
[8] Allpress, J G , and Wadsley, A D , *Acta Cryst* , 17, 41 (1964)
[9] Sands D E , Zalkin, A , and Elson, R E , *Acta Cryst* , 12, 21 (1959)
[10] Brničević, N , and Djordjević, C , *J Less Comm Metals*, 13, 470 (1967)
[11] Mooney, R C L , *Acta Cryst* , 2, 189 (1949)

Contact Angles for Thin Lipid Films and the Determination of London–van der Waals Forces

From the theory of thin liquid films it is evident that the tension of the two-sided film should be less than twice the tension of the bulk liquid surfaces with which the film is in equilibrium. One would therefore expect there to be a contact angle between the thin film and the surfaces of the adjacent bulk liquid. This was recently pointed out by Mysels, Huisman and Razouk, who have also confirmed the existence and measured the magnitude of this contact angle for "black" aqueous soap films in air[1].

We now report some measurements of the contact angle between optically black hydrocarbon films in aqueous solution and the equilibrium bulk hydrocarbon liquid. The formation of films of this type has already been described[2,3], as have been the measurement of the film thickness and composition[4–9]. The contact angle in hydrocarbon/water systems is not readily measurable by the method of Mysels *et al.*, and we have used an interference method instead. The films were formed in a 1 mm hole in a horizontal block of 'Fluon' by an injection method similar to that of Vreeman (personal communication). When the formation of the black film was complete, vertical illumination by monochromatic light produced Newton's rings in the meniscus immediately adjacent to the edges of the black film. It is possible, in principle, to deduce the contact angle from measurements of the spacing of these rings. Indeed, Sheludko[10] has recently mentioned this approach and has referred to some unpublished results. This procedure, however, involves a difficult extrapolation and the following procedure was found to be an improvement.

The thinning of the film was controlled in such a way that when the formation of the black film was complete, a lens of bulk liquid remained as an island in the black film. Under monochromatic light, such a lens appears as shown in Fig. 1. This particular lens was 83μ in diameter but a wide range of sizes may be obtained. The lenses are evidently in equilibrium with the surrounding black film and remain constant in size and appearance for at least 30 min, by which time they have usually drifted to the edge and coalesced with the meniscus. Apart from the very small influence of gravity, we should expect the lenses to be symmetrical about a horizontal plane through the centre of the film and to consist of two spherical segments. Calculations from the orders and radii of the interference rings show that this is very accurately so. From the radius of the lens one can calculate the contact angle. From measurements of a number of lenses, no significant dependence of contact angle on the size of the lens was found.

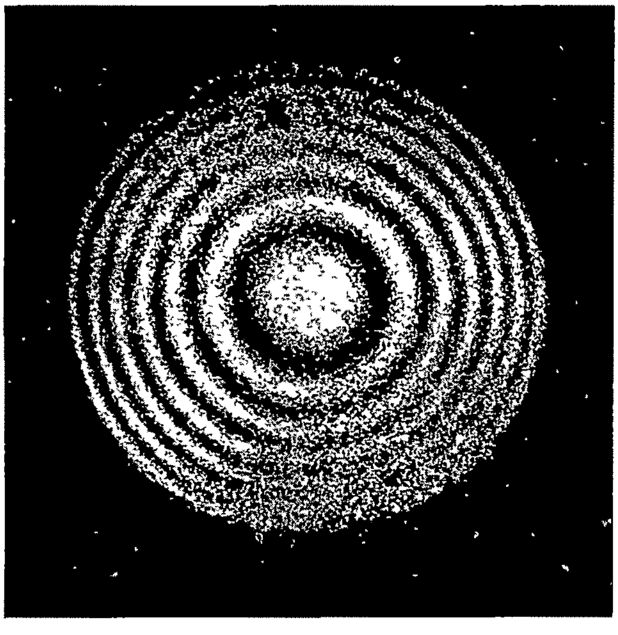

Fig. 1. A lens of diameter 83μ in an optically black lipid film as seen under vertically reflected monochromatic light

For films formed from a solution of pure glycerol mono-oleate in *n*-decane, the contact angle in an aqueous phase of distilled water for several lenses was calculated to be 1° 52′. The interfacial tension of the bulk media at 20° C was 3.72 dyne/cm so that the difference ($\Delta\gamma$) between this and one half of the film tension was 0.00197 dyne/cm.

A knowledge of this interfacial tension difference is important for at least two reasons. First, it enables the film tension to be determined from the bulk interface tension without having to bulge the film—a procedure which is often impossible owing to the breakage of the film[9,11]. Knowing the film tension, we can then apply the Gibbs equation to the determination of adsorption in the thin film[9,12]. Second, the interfacial tension difference for hydrocarbon films has an especially close relationship to the London–van der Waals forces for hydrocarbon/water systems. To a very close approximation, the free energy change on formation of 1 cm² of equilibrium thin film from thick film is, in the present systems, numerically equal to twice the difference in interfacial tension between the thin and thick film—in other words, to 2(−0.00197) or −0.00394 ergs/cm². This free energy change is made up of terms arising from the London–van der Waals, electrostatic (double layer), and steric forces acting in the film. The electrostatic term can be calculated to an order of magnitude from conductivity data together with an assumption of the value of the double layer potential and seems to be negligible. The steric forces are responsible for stabilizing the films, and the available evidence suggests that these arise from the steric interference of the alkyl chains of the surface active molecules (the glycerol mono-oleate in this case)[6]. These forces are evidently negligible until the opposing chains of the two monolayers meet each other, and then increase very rapidly with further decrease in film thickness. This point can be argued from theoretical considerations and from the fact that the thickness of the hydrocarbon part of the equilibrium film is very close to twice the length of the extended oleate chains[6]. By contrast, the London–van der Waals potential energy is, in the present systems, believed to be a much weaker function of the film thickness. The depth of the free energy minimum (ΔF) for 1 cm² of thin film (relative to that for a film of large thickness) should consequently be determined almost entirely by the London–van der Waals forces. We may therefore write[13]

$$\Delta F \simeq 2\Delta\gamma \simeq -\frac{A}{12\pi\delta^2} \times \text{retardation correction}$$

where A is the Hamaker–London–van der Waals constant and δ is the film thickness. For the present films, the thickness of the hydrocarbon core has been given as 47 Å (ref. 6) and the combined thickness of the polar group layers is estimated from models as approximately 8–10 Å. If we take δ as 56 Å and estimate the retardation correction from the data of Overbeek[14], we find that $A = 5.6 \times 10^{-14}$ erg.

From the nature of the assumptions involved, this value should be considerably more precise than previously published values for liquids. A more thorough appreciation of the validity of the assumptions is, however, needed, and this should be gained by the examination of further systems. It is interesting to compare this value with those obtained from flocculation data for lipid/water systems which depend on the validity of an electrical double layer model Thus for their arachidic acid sols Ottewill and Wilkins[15] found a value of the order of 10^{-14} erg and for aqueous suspensions of solid and liquid paraffinic hydrocarbon Srivastava and Haydon[16,17] found approximately 10^{-13} erg

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[1] Mysels, K J, Huisman, H F, and Razouk, R I, *J Phys Chem*, **70**, 1339 (1966)
[2] Mueller, P, Rudin, D O, Tien, H T, and Wescott, W C, *J Phys Chem*, **67**, 534 (1963)
[3] van der Berg, H J, *J Mol Biol*, **12**, 290 (1965)
[4] Hanai, T, Haydon, D A, and Taylor, J, *Proc Roy Soc*, A, **281**, 377 (1964)
Hanai, T, Haydon, D A, and Taylor, J, *J Theor Biol*, **9**, 278 (1965)
[6] Taylor, J, and Haydon, D A, *Disc Faraday Soc*, No 42, 51 (1966)
[7] Huang, C, and Thompson, T E, *J Mol Biol*, **13**, 183 (1965)
[8] Henn, F A, Decker, G L, Greenawalt, J W, and Thompson, T E, *J Mol Biol*, **24**, 51 (1967)
[9] Cook, G M W, Redwood, W R, Taylor, A R, and Haydon, D A, *Kolloid-Z* (in press)
[10] Sheludko, A, *Adv Colloid Interface Sci*, **1**, 391 (1967)
[11] Tien, H T, *J Phys Chem*, **71**, 3395 (1967)
[12] Deryaguin, B V, Martynov, G A, and Gutop, Yu V, *Kolloid Zh*, **27**, 29 (1965)
[13] Verwey, E J W, and Overbeek, J Th G, *Theory of the Stability of Lyophobic Colloids* (Elsevier, Amsterdam, 1948)
[14] Overbeek, J Th G, in *Colloid Science*, 1 (edit by Kruyt, H R), 269 (Elsevier, Amsterdam, 1952)
[15] Ottewill, R H, and Wilkins, D J, *Trans Faraday Soc*, **58**, 608 (1962)
[16] Srivastava, S N, and Haydon, D A, *Trans Faraday Soc*, **60**, 971 (1964)
[17] Srivastava, S N, and Haydon, D A, *Proc IV Int Congress on Surface Active Substances*, **2**, 1221 (1964)

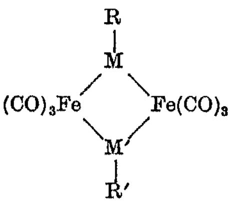
CHEMISTRY

Ligands containing Fluoroaromatic Groups

THE relatively high electronegativity of the pentafluorophenyl group, C_6F_5, is expected to alter the donor characteristics of ligand molecules We wish to report initial results of studies with three types of fluoroaromatic ligands

Pentafluorophenyl cyanide, C_6F_5CN, reacts partially with tungsten hexacarbonyl in petrol ether solvent, when the mixture is irradiated with ultraviolet light Two yellow compounds were separated by column chromatography under a nitrogen atmosphere, but in neither case could analytical samples be obtained, because all attempts at crystallization (under nitrogen) failed because of a facile decomposition The infrared spectra (in the region close to 2,000 cm^{-1}) of the solutions from the column were almost identical to those of $RCNW(CO)_5$ and $(RCN)_2W(CO)_4$ (R, alkyl) Clearly the pentafluorophenyl group is strongly affecting the stability of these complexes *bis*(Pentafluorophenyl)sulphide and -selenide do not react with either chromium hexacarbonyl or triiron dodecacarbonyl when heated under reflux in 80/100 petrol ether, and palladium dichloride forms no complex with pentafluorophenyl cyanide after 5 days at 110° C in a sealed tube

Iron pentacarbonyl and triiron dodecacarbonyl react with $S_2(C_6F_5)_2$, $Se_2(C_6F_5)_2$ (ref 1), $Te_2(C_6F_5)_2$* and C_6F_5SH to give air-stable compounds of the type



(I) M, M′=S, R, R′=C_6H_5
(II) M, M′=S, R=C_6F_5, R′=C_6H_5
(III) M, M′=S, R, R′=C_6F_5 (ref 2)
(IV) M, M′=Se, R, R′=C_6F_5
(V) M=S, M′=Se, R, R′=C_6F_5
(VI) M, M′=Te, R, R′=C_6F_5

^{19}F and ^1H nuclear magnetic resonance spectra of these dimers indicate the presence of isomers, but we have, so far, been unable to separate them by chromatographic techniques A comparison of the stabilities of I and III indicated little difference between them A competitive reaction carried out using triiron dodecacarbonyl (1 mole) and a mixture of $S_2(C_6H_5)_2$ and $S_2(C_6F_5)_2$ (1 mole each) gave the expected three products (I, II and III) which were readily separated by preparative scale thin-layer chromatography on kieselguhr using petrol ether as eluent The yields of products were very close to the ratio 1 2 1 (I II III) expected if the bridging ligands were little influenced by the group R, a ready interchange of ligands was also observed if either I or III was treated with S_2R_2 It is interesting to note that the melting-decomposition points of I, II and III were all 140°–143° C, mixed melting points of any two were some 20°–30° C lower.

The thermal decomposition of III in a sealed tube at 160° C gave carbon monoxide and decafluorobiphenyl as the only isolable products Attempts to remove the sulphur ligand from the iron by heating III with mercury failed, decafluorobiphenyl again being formed, this is in contrast to the free ligand $S_2(C_6F_5)_2$ [and $Se_2(C_6F_5)_2$] which reacts with both mercury and germanium on heating

$$S_2(C_6F_5)_2 + Hg \xrightarrow{180°} \underset{\text{m p } 188°\text{–}190° \text{ C}}{Hg(SC_6F_5)_2} \xrightarrow[200° \text{ C}]{Cu} Hg(C_6F_5)_2$$

$$Se_2(C_6F_5)_2 + Hg \xrightarrow{180°} \underset{\text{m p } 171°\text{–}173° \text{ C}}{Hg(SeC_6F_5)_2} \xrightarrow[200° \text{ C}]{Cu} Hg(C_6F_5)_2$$

$$S_2(C_6F_5)_2 + Ge \xrightarrow{\quad} \underset{\text{m p } 143°\text{–}145° \text{ C}}{Ge(SC_6F_5)_4}$$

Mixed aromatic/fluoroaromatic acetylenes, $\varphi_F C\equiv C\varphi$, react with cobalt carbonyl in a manner similar to diphenyl acetylene[3], the fluoroaromatic group having little influence on the reaction Typical compounds synthesized are

$(4\text{-}C_6F_5C_6F_4C\equiv CC_6H_5)Co_2(CO)_6$, m p 140° C (decomp)
$(4\text{-}ClC_6F_4C\equiv CC_6H_5)(Co_2(CO)_6$, dark red oil
$(4\text{-}C_6H_5C\equiv CC_6F_4C\equiv CC_6H_5)Co_2(CO)_6$, dark red oil

In the latter case, where two acetylene groups are present, it is possible to isolate a rather unstable solid (decomposes without melting at 156° C) which has two cobalt atoms attached to each acetylenic bond

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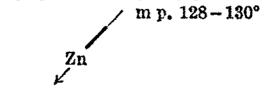

* Synthesized by $TeCl_4 + C_6F_5HgCl \longrightarrow Cl_3TeC_6F_5$ m p. 128–130° $\xrightarrow{Zn}$ $Te_2(C_6F_5)_2$ m p 42–43°

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[1] Cohen, S C, Reddy, M L N, and Massey, A G, *J Organometallic Chem* (in the press)
[2] Beck, W, Stretter, K H, Tadros, S, and Schwarzhans, K E, *Ber*, **100**, 3944 (1936)
[3] Sly, W G, *J Amer Chem Soc*, **81**, 18 (1959)

RADIOBIOLOGY

Failure of Chinese Hamster Cells to repair Sub-lethal Damage when X-irradiated in the Plateau Phase of Growth

THE ability of exponentially growing Chinese hamster cells to repair sub-lethal damage induced by X-irradiation was shown by Elkind and Sutton[1] These workers showed that a dose of X-rays killed cells more efficiently when administered in one acute irradiation than when given in two fractions separated by a time interval The survival curve for the latter (with time between the two doses as variable) showed an early rise which was interpreted by Elkind as repair, the cyclic fluctuations following repair being a result of cell-cycle associated kinetics[2]

Repair of sub-lethal damage has since been observed in many cell systems and under a variety of experimental conditions In fact, one of the interesting features of this phenomenon is that it can take place in situations in which many other cell processes are inhibited low temperatures[3], in conditions where the cells are hypoxic[4], and in the presence of agents inhibiting DNA and protein synthesis (although RNA synthesis may be required[3]) All these experiments were performed with cells which had been actively growing It now seems, however, that cells in crowded culture conditions progressively lose their repair capacity even in the absence of specific inhibitors

Chinese hamster cells (strain *HA*-2) were plated on plastic Petri dishes The medium (Eagle's minimal essential medium, supplemented with 15 per cent foetal calf serum) was changed daily In such conditions, the cultures formed dense monolayers with about 10^7 cells on a 60 mm dish This cell number did not increase if the medium was exchanged more frequently Many properties of these cultures as well as detailed experimental procedures are reported elsewhere[5,6]

Fig 1 shows survival curves of X-irradiated cells (technical factors 120 kV, 9 mA, 67 rads/min, 1 mm Al filtration) The ability to form colonies was used as the criterion of survival The slopes of the linear portions of the two curves do not differ significantly, but the extrapolation number ($\bar{n}$) of the survival curves obtained from cells irradiated in plateau phase is significantly lower

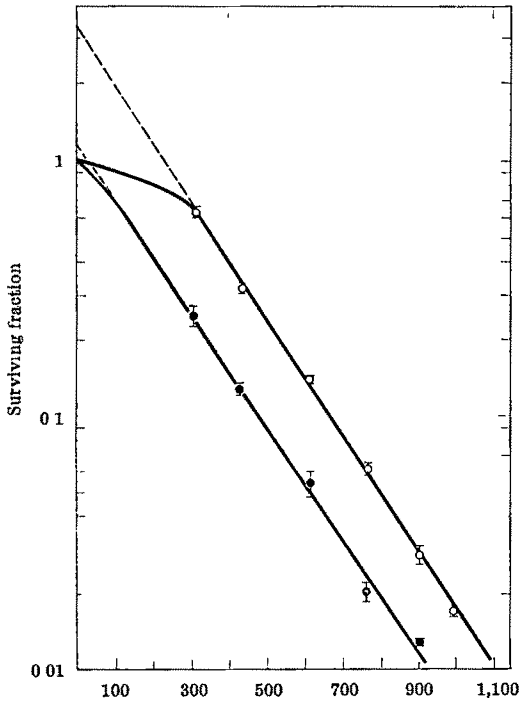

Fig 1 X-ray survival curves of Chinese hamster cells (*HA*-2) ○, Cells from cultures irradiated in exponential period, $D_0 = 142\ 3 \pm 4\ 3$ rads, $\bar{n} = 2\ 8 \pm 0\ 2$ ●, Cells from cultures irradiated in plateau period, $D_0 = 136\ 4 \pm 0\ 68$ rads, $\bar{n} = 1\ 2 \pm 0\ 4$ Vertical bars ±1 *S E*

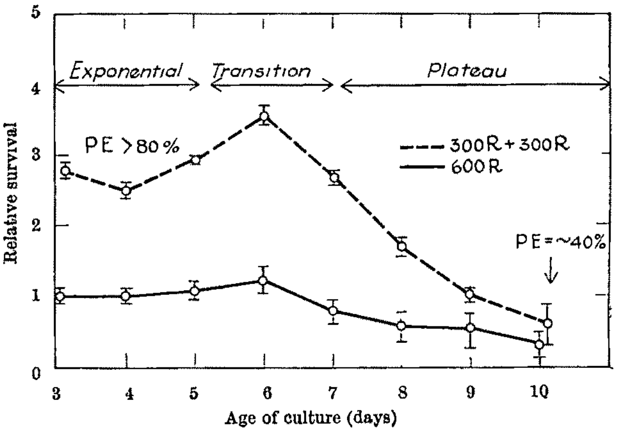

Fig 2 Repair ability in various growth phases Replicate dishes were seeded with equal number of cells on day 0 Medium was changed daily beginning with day 3 Cells were irradiated daily on and after day 3 Fractionated doses (○ - - - ○) were separated by 3 h In parallel, a growth curve was constructed to determine growth periods of the cultures The periods are defined operationally with respect to cell doubling time exponential (doubling time constant), transition (doubling time increased with increasing age of culture), plateau (doubling time long, not measurable) Vertical bars ±1 *S E*

The magnitude of the extrapolation number has been related to the amount of sub-lethal damage a cell can repair[1], the reduction in $\bar{n}$ during the plateau phase should therefore be accompanied by a loss of ability to repair damage This is indeed the case and the progressive reduction of repair with age of the culture is indicated in Fig 2 The lower curve represents survival of cells from replicate cultures irradiated with 600 rads at different stages of growth of the cultures Survival values were normalized to unity during exponential growth The reduction in level of absolute survival with culture age is a reflexion of the reduction in $\bar{n}$ The upper curve was obtained from cultures similarly treated but irradiated with a split dose (2 × 300 rads) separated by 3 hours During exponential growth, fractionation resulted in a threefold increase in survival The effect of fractionation decreased with culture age until the two curves became indistinguishable

In order to rule out the possibility that repair occurred more slowly in plateau phase cells, or that the repair effects were counteracted by kinetic factors peculiar to the 3 h interval, a split dose-response curve was obtained from cells which had been in plateau phase for 4 days (Fig 3) The lower curve in Fig 3 does not show the early repair-associated rise, and the cyclic fluctuations seen in the split dose response from exponentially growing cells (upper curve) are absent As has been said earlier, these survival fluctuations are associated with cell cycle progression Most of the plateau phase cells are in a *G*-1 part of the cell cycle[5] and therefore no cell cycle progression effects are observed

At the present time no explanation can be offered as to why this progressive loss of repair ability occurs when these cells are in plateau phase The phenomenon does not correlate with changes in DNA, RNA or protein synthesis[5] The only other quantity that we have measured which does change progressively is viability For example, in one experiment, plating efficiency dropped almost linearly from 80 per cent to 40 per cent during 4 days of plateau phase If the cells were sub-cultured and re-seeded in less crowded surroundings, both repair capability of the surviving cells and their plating efficiency were restored within 12 hours

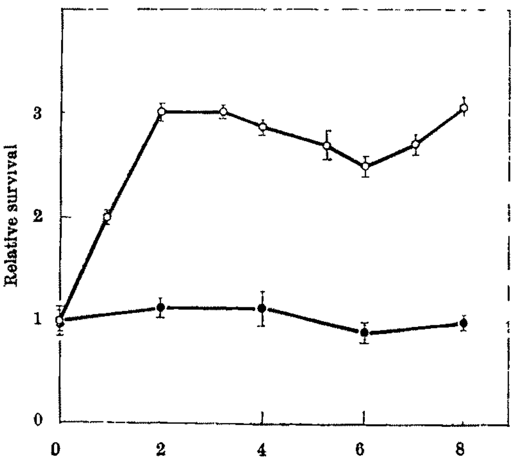

Fig 3 Two-fraction response of Chinese hamster cells ○, Cells in exponential period (day 5 of Fig 2) ●, Cells in plateau period (day 10 of Fig 2) All cells were irradiated at time 0 with 300 rads without sub-culturing and dishes were returned to incubator An additional 300 rads was then given at various times from 0 h to 8 h Sub-culturing to test for survival was done approximately 30 min following second irradiation Vertical bars ±1 *S E.*

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[1] Elkind, M M, and Sutton, H, *Radiat. Res.*, 13, 556 (1960)
[2] Elkind, M M, Sutton, H., and Moses, W. B, *J. Cell Comp. Physiol*, 58, suppl 1, 113 (1961).
[3] Elkind, M M, Alescio, T, Swain, R W, Moses, W B, and Sutton, H, *Nature*, 202, 1190 (1964)
[4] Elkind, M M, Whitmore, G F, and Alescio, T, *Science*, 143, 1454 (1964)
[5] Hahn, G M., Stewart, J R., Yang, S-J., and Parker, V, *Exp. Cell Res* (in the press)
[6] Stewart, J R, Hahn, G. M., Parker, V., and Bagshaw, M A, *Exp Cell Res* (in the press)

Radial Diffusion and Power Function Retention of Alkaline Earth Radioisotopes in Adult Bone

THE retention of alkaline earth radioisotopes in skeletal systems can be approximated by the power function[1]

$$R = \varepsilon^{b} (t + \varepsilon)^{-b} \tag{1}$$

where b is a fraction between 0 0 and 0 5, t is the time after intravenous injection and ε is a short time which for adult dog and man is the order of 1 day A physiological explanation for this form of retention function is now presented which may be applicable to normal adult bone

Suppose that at times which are long compared with ε the principal effect on tracer retention is the transfer of tracer ions between the blood and existing volumes of bone, the diffuse component[2], rather than the formation or resorption of bone This supposition seems to hold for adult dogs[3,4] and rabbits[5] The diffuse component has also been observed in man, but whether it has a dominant effect on retention has not been established

To reach the volume of existing bone the tracer must pass through canaliculi, longitudinal flow of tracer along canaliculi occurs in the liquid phase and so is relatively rapid According to this new explanation, there is, then, a diffusion radially outwards from each canaliculus, this is very slow because it takes place in the essentially solid calcified matrix Retention of tracer in this process is long because there is a large mass of mineral with which to exchange Furthermore, retention is enhanced by the geometry of the cylinder, because once an ion has left the cylinder axis it is improbable that it will return by a random path The retention problem is therefore similar to that for the diffusion of an instantaneous source of tracer from the axis of a cylinder

Consider a long cylinder with a very small canal running along the cylinder axis If a diffusible tracer is distributed uniformly along the canal at time zero, the amount of tracer remaining in the canal as a function of time can be calculated by solving the diffusion equation in cylindrical co-ordinates

$$\frac{\partial C}{\partial t} = D\left[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}\right] \tag{2}$$

where C is the concentration of tracer as a function of time t at any radius r and D is the coefficient of diffusion[6] For the initial conditions given here, the solution of equation (2) for the concentration of tracer at $r = 0$ is

$$C = \frac{q'}{4\pi D t} \tag{3}$$

where q' is the initial quantity of tracer per unit length deposited along the axis at $t = 0$ (ref 6) The quantity of activity remaining in the canal depends exactly on the power function t^{-1} If the canal is assumed to have a non-zero diameter, C remains finite at time zero The difference between t^{-1} and the functions $t^{-1\ 2}$ to $t^{-1\ 5}$ observed for blood specific activities[1] at times that are long compared with ε can be explained as the effect of excretion

The effect of excretion on equation (2) can be taken into account by introducing a small drain on the axial canal after instantaneous labelling This analysis was performed on the 'PACE' analogue computer using an eighteen compartment series model The differential equations for the model reduced to equation (2) as the number of compartments was made large Furthermore, when the drain was set equal to zero, the analogue com-

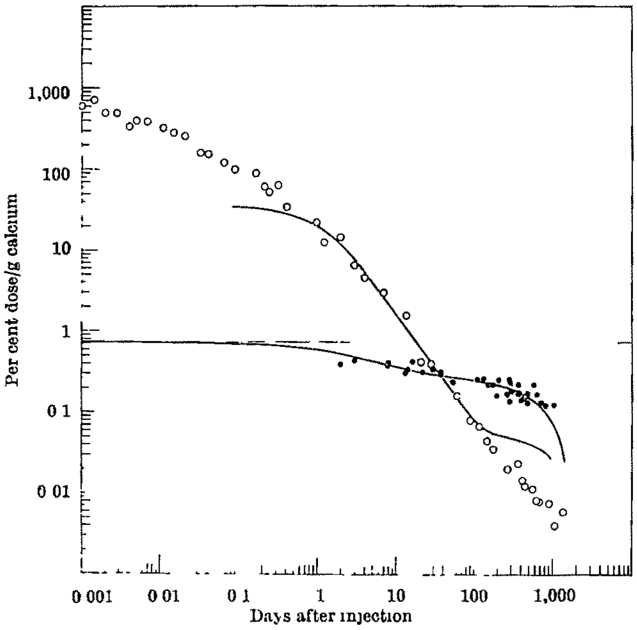

Fig 1 Data for radium-226 in the plasma (○) and in the body (●) of beagles injected at 1 3 years of age[7] The curves (——) show the corresponding analogue computer results for the specific activity of canalicular fluid and of the body as a whole, considered as a large number of canaliculi together with their surrounding territories C = 135 g calcium, 10 mg/100 ml

puter yielded a t^{-1} dependence for axial activity as in expression (3) This provided a check on the model. When the drain was increased, a series of curves for axial activity against time was produced which could be well approximated by a set of power function expressions varying continuously from $t^{-1\ 0}$ to $t^{-1.5}$. We assume that the specific activities of blood and canalicular fluid are equal at times which are long compared with ε Because the specific activity of blood is proportional to the time derivative of retention[1], the corresponding retention curve can be approximated by t^{-b} with b between 0 0 and 0 5 When the drain was adjusted to a value consistent with the endogenous excretion rate for radium-226 in dogs, the model gave a good fit to the data of Van Dilla *et al.*[7] over three decades of time as shown in Fig 1

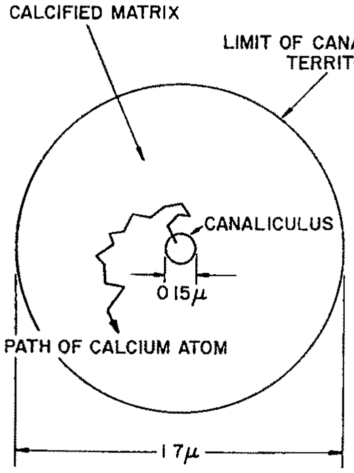

Fig 2 Radial diffusion of an atom of calcium or other alkaline earth from a canaliculus into the surrounding territory of calcified matrix The dimensions are from Baud's[8] electron microscope measurements of mouse bone

We picture this radial diffusion as involving a penetration of alkaline earth ions through a complex material consisting of collagen, ground substance, amorphous component and embedded bone crystals (Fig 2) We have taken the diameter (0 15μ) and average spacing (1 7μ) of canaliculi from electron microscope measurements of mouse bone[8] The data for calcium-45, strontium-90 and radium-226 in adult dogs[1] can be fitted with an effective coefficient of diffusion of the order of 10^{-16} cm^2/s At this rate, it would take almost a year for an ion to diffuse from a canaliculus to the limit of canalicular territory The theory therefore represents a marked departure from the common hypothesis that the typical bone crystal is bathed by extracellular fluid which is in fairly rapid exchange with the blood

It may be possible to test this theory of slow radial diffusion by carefully timed electron microscope autoradiography

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[1] Marshall, J H., *J. Theoret Biol* , 6, 386 (1964)
[2] Marshall, J H , Rowland, R E , and Jowsey, J , *Radiat Res* ,10, 258 (1959)
[3] Rowland, R E , *Radiat Res* , 15, 126 (1961)
[4] Jowsey, J , Lafferty, W , and Rabinowitz, J , *J Bone and Joint Surgery*, 47A, 359 (1965)
[5] Lloyd, E., *Calcified Tissues*, 381 (University of Liège, 1965)
[6] Barrer, R M , *Diffusion In and Through Solids*, 32, 46 (Cambridge University Press, New York, 1951)
[7] Van Dilla, M A , Stover, B J , Floyd, R L , Atherton, D R , and Taysum D H , *Radiat Res* , 8, 417 (1958)
[8] Baud, C. A., *Acta Anat* , 51, 209 (1962)

Effect of Ecdysone on the Puparium Formation in Irradiated *Ephestia kühniella* Z.

COMPLETE inhibition of metamorphosis and pupa formation is observed in some insect species when they are irradiated with 20 kr. and more of γ and X-rays[1]. Puparium formation is delayed in *Ephestia kuhniella* by doses of 20–30 kr [2-4]

The absence of pupation might be explained by radiation damage to the hypodermis nuclei and by the inability of DNA to give any new genetic information (under the influence of the pupation hormone ecdysone)[4] It may also be caused by a disturbance of neurosecretory cell activity and the absence of ecdysone in the body of the caterpillar To find the true reason for the absence of pupation we have studied puparium formation in irradiated larvae after the injection of ecdysone.

Larvae of *Ephestia kuhniella* Z , 7 and 2 days before pupation, were given 30 kr. of gamma rays. Another group was irradiated with 150 kr 2 days before they were due to pupate Ecdysone, isolated by the method of Butenandt and Karlson from pupae of *Bombyx mori*[5], was injected into the irradiated larvae just at the time of puparium formation. High concentrations of ecdysone were used because of the low sensitivity of *Ephestia* to this hormone, 0 5–1 0 mg (in 0 005–0 01 ml) was injected. The activity of the preparation of ecdysone was determined by the method of Karlson[6]; 48 h after injection fourteen abdomens showed 64 per cent pupation After being tested in this way ecdysone was injected into irradiated larvae Water was injected into controls

Features of pupation were shortening of the body into a triangular shape, induration and the appearance of dark-brown pigment in the cuticle.

Table 1. EFFECT OF ECDYSONE ON PUPARIUM FORMATION IN IRRADIATED *Ephestia kühniella* Z.

				Pupation				
Preparation injected, weight	Dose (kr)	Time after irradiation	No. of larvae	Slight	Active	None	Total No of pupated animals	Pupation (per cent)
Water								
0 005 ml	30	48 h	10	0	0	10	0	0
0 01 ml	30	48 h	10	0	0	10	0	0
Ecdysone preparation								
0 5 mg	30	48 h	10	2	2	6	4	40
1 mg	30	48 h	9	5	1	3	6	66
0 5 mg	30	7 days	4	0	2	2	2	50
1 mg	30	7 days	4	1	1	2	2	50
1 mg	150	48 h	29	4	0	25	4	13

The results in Table 1 show that the injection of an ecdysone preparation induced the formation of the puparium in irradiated insects. Injection of water had no effect, and so the activation of genes by ecdysone and the specific synthesis of *m*RNA and pupation enzymes take place after the injection of an ecdysone preparation to irradiated larvae, as observed in normal conditions under the effect of endogenic ecdysone[7]

Thus the absence of puparium formation in the irradiated larvae (given 20–30 kr) results not from a disturbance of the DNA of the hypodermis but from the absence of ecdysone Absence of ecdysone, in its turn, is evidently the result of irradiation damage to neurosecretory cells The macromolecular system necessary for puparium enzyme synthesis was rather radioresistant The disturbances were observed only under the effect of 100–150 kr of irradiation

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[1] Whiting, A R, *Anat Rec*, **108**, 609 (1950)
[2] Cornwell, P B, Crook, Z J, and Bull, J O, *Nature*, **179**, 670 (1957)
[3] Erdman, N E, *Amer Midland Naturalist*, **69**, No 1, 34 (1963)
[4] Kuzin, A M, and Yusifov, N I, *Radiobiologia* (in Russian), **7**, 1, 3 (1967)
[5] Butenandt, A, and Karlson, P, *Z fur Naturforschung*, **9b**, 6, 389 (1954)
[6] Karlson, P, *Vitamins and Hormones*, **14**, 227 (1956)
[7] Karlson, P, in *Perspectives in Biological Medicine*, **6**, 203 (1963)

MICROBIOLOGY

High Titre T Antigen with Minimal Amounts of Structural Antigen in Adenovirus-infected Cells treated with Hydroxyurea

Virus-specific non-structural antigens (T antigens) are produced early in the lytic cycle of adenovirus infection and are also found in cells transformed by adenovirus[1–3] Synthesis of T antigen, unlike that of the structural antigen, is not dependent on DNA synthesis, for example, fluorodeoxyuridine (FUdR) effectively inhibits the synthesis of viral antigen (V) without markedly suppressing the production of T antigen[4,5] Our studies have been designed to produce large quantities of T antigen "free" from viral structural antigen by manipulation of the lytic interaction of adenoviruses in *KB* cells

In the *KB* line at present used in this laboratory (originally obtained from Dr H S Ginsburg), FUdR markedly reduces V titres, but the residual V titres are still too high for a serologically useful T antigen preparation, similarly, cytosine arabinoside at 100 µg/ml of medium, even freshly added at 12 h intervals, does not alter the production of V antigen Other approaches such as the use of virus particles partially inactivated by ultraviolet light and the incubation of infected cultures at sub-optimal temperatures[6] have also been found effective in separating the synthesis of T and V antigens These procedures however, require careful control of a variety of experimental variables and for this reason a more reliable method is desirable

Hydroxyurea, an inhibitor of DNA synthesis, interferes with duplication of some DNA viruses[7–9] Its present use seems to provide an effective method of fulfilling the objective stated here

Washed *KB* monolayers (approximately 4×10^6 cells) in milk dilution bottles were exposed for 1 h at 37° C to various multiplicities of adenovirus type 7 ($10^{8\ 3}$ $TCID_{50}$/ ml in human embryonic kidney cells) A range of concentrations of hydroxyurea (Nutritional Biochemicals Corporation, Cleveland, Ohio) was prepared in Eagle's basal medium supplemented with 5 per cent foetal bovine serum, 2 per cent glutamine and antibiotics Following removal of unabsorbed virus, 20 ml of medium containing hydroxyurea was added to the experimental cultures which were subsequently incubated at 37° C

After 24–72 h, the cells were scraped into the medium with a rubber policeman and were centrifuged at 2,000 r p m for 10 min at 4° C After decanting the supernatant fluid, the cells were washed in balanced salt solution and re-pelleted The last traces of fluid were removed and the cell packs frozen at −70° C until tested by the microtitre complement-fixation technique[2,10]

Table 1 EFFECT OF HYDROXYUREA ON FORMATION OF T AND VIRAL STRUCTURAL ANTIGEN (V) IN *KB* CELLS INFECTED WITH ADENOVIRUS 7

Input multiplicity ($TCID_{50}$) adenovirus 7	mM HU	Time (h) 24 T	V	48 T	V	72 T	V
125	5	4	< 2	16	< 2	ND	ND
	4	4	< 2	16	< 2	ND	ND
	0	16	16	≥16	≥16	ND	ND
50	5	8	< 2	4	< 2	16	4
	4	8	< 2	8	< 2	32	4
	3	8	< 2	8	< 2	16	4
	0	16	16	32	≥128	64	≥128
25	5	8	< 2	8	< 2	16	2
	4	4	< 2	8	< 2	32	< 2
	3	4	< 2	8	< 2	32	2
	0	8	8	64	≥1,024	256	≥4,096
5 0	5	2	< 2	2	< 2	8	< 2
	4	< 2	< 2	2	< 2	8	< 2
	3	< 2	< 2	2	< 2	8	< 2
	2	< 2	< 2	4	< 2	32	< 2
	0	< 2	8	≥16	≥32	32	4,096
0 5	4	< 2	< 2	< 2	< 2	4	< 2
	3	< 2	< 2	< 2	< 2	8	< 2
	2	< 2	< 2	< 2	< 2	16	< 2
	0	< 2	4	2	≥16	16	≥1,024
0	5	< 2	< 2	< 2	< 2	< 2	< 2
0	4	< 2	< 2	< 2	< 2	< 2	< 2
0	3	< 2	< 2	< 2	< 2	< 2	< 2
0	0	< 2	< 2	< 2	< 2	< 2	< 2

An example of the results of several experiments (Table 1) shows that a high input of virus in the presence of appropriate concentrations of hydroxyurea will result in high titres of T antigen with minimal or no formation of detectable adenovirus group-specific (hexon, V) antigen Haemagglutination studies indicate that type-specific (penton) synthesis is also effectively suppressed by hydroxyurea At lower virus inputs, similar results are obtained particularly with lower concentrations of hydroxyurea and later times of cell collection Note the high titre of T antigen in the absence of detectable V antigen obtained at 3 days with a viral input of 0 5 and 2 mM hydroxyurea (Table 1) The reduced titre of T antigen produced with some concentrations of hydroxyurea probably reflects the suppressive effects of this drug. at those concentrations, on RNA and protein synthesis[8]

Preliminary experiments with adenovirus types 2 (Table 2) and 12 in *KB* cells suggest that similar results may be expected for all human adenoviruses and perhaps even infra-human types The use of hydroxyurea provides a relatively simple method for the production of large amounts of T antigen (free of significant viral structural antigen) for purification and functional studies In a recent experiment, a 40 per cent suspension of pooled cell pack from multiple cultures (25 ml) gave a complement fixation

Table 2 EFFECT OF HYDROXYUREA ON T AND VIRAL STRUCTURAL ANTIGEN (V) FORMATION IN *KB* CELLS INFECTED WITH ADENOVIRUS 2

Input multiplicity ($TCID_{50}$) adenovirus 2	mM HU	Time (h) 24 T*	V	48 T	V	72 T	V
50	5	ND	ND	16	< 2	32	2
	4	ND	ND	32	2	ND	ND
	3	ND	ND	32	2	ND	ND
	0	ND	ND	≥ 64	≥16	ND	ND
25	5	16	< 2	32	2	32	2
	4	32	< 2	32	2	32	2
	3	16	< 2	16	2	64	4
	0	256	≥2,048	128	≥4,096	128	≥4,096
5 0	5	16	< 2	32	< 2	32	< 2
	4	16	< 2	64	< 2	64	2
	3	16	< 2	32	2	64	4
	0	128	1,024	128	≥4,096	128	≥4,096
0 5	5	8	< 2	8	< 2	8	< 2
	4	8	< 2	16	< 2	16	< 2
	3	16	< 2	8	< 2	16	2
	0	16	64	64	≥4,096	64	2,048
0	5	< 2	< 2	< 2	< 2	< 2	< 2
0	0	< 2	< 2	< 2	< 2	< 2	< 2

Washed *KB* monolayers were exposed to varying multiplicities of adenovirus types 7 and 2 for 1 h at 37° C After washing, various concentrations of hydroxyurea dissolved in 5 per cent BME medium were added Cell packs were collected after 24–72 h and tested for complement fixation antigen using four units of homologous antiserum Titres are expressed as complement fixation reciprocals

mM/HU, Millimoles of hydroxyurea

ND, Not done

* T antigen with type 2 was measured using serum from hamsters hyperimmunized with adenovirus–*SV*40 hybrid tumour extracts In these experiments with Ad 2, *SV*40, T and V antigen controls were negative

titre for adenovirus type 7 T antigen of 1 : 128 with a V titre of < 1 : 2; a similar experiment with adenovirus type 2 yielded a T antigen titre of ≥ 1 : 128 with a V titre of 1 : 4.

Such preparations will be useful in sero-epidemiological studies evaluating the role of adenoviruses in human cancer.

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[1] Hoggan, M. D., Rowe, W. P., Black, P. H., and Huebner, R. J., *Proc. US Nat. Acad. Sci.*, **53**, 12 (1965).

[2] Huebner, R. J., Rowe, W. P., Turner, H. C., and Lane, W. T., *Proc. US Nat. Acad. Sci.*, **50**, 379 (1963).

[3] Pope, J. H., and Rowe, W. P., *J. Exp. Med.*, **120**, 577 (1964).

[4] Gilead, Z., and Ginsberg, H. S., *J. Bacteriol.*, **90**, 120 (1965).

[5] Lewis, jun., A. M., Wiese, W. H., and Rowe, W. P., *Proc. US Nat. Acad. Sci.*, **57**, 622 (1967).

[6] Gilden, R. V., Kern, J., Lee, Y. K., and Huebner, R. J., *Proc. Soc. Exp. Biol. and Med.* (in the press).

[7] Rosenkranz, H. S., and Levy, J. A., *Biochim. Biophys. Acta*, **95**, 181 (1965).

[8] Rosenkranz, H. S., Garro, A. J., Levy, J. A., and Carr, H. S., *Biochim. Biophys. Acta*, **114**, 501 (1966).

[9] Rosenkranz, H. S., Rose, H. M., Morgan, C., and Hsu, K. C., *Virology*, **28**, 510 (1966).

[10] Sever, J. L., *J. Immunol.*, **88**, 320 (1962).

In vitro Characteristics of a Poxvirus isolated from Rhesus Monkeys

PRIMATES are susceptible to poxviruses of the variola–vaccinia–monkeypox group and to Yaba poxvirus. When an epidemic of skin lesions with the clinical characteristics of vaccinia appeared in rhesus monkeys (*Macaca mulatta*), Japanese macaques (*Macaca fuscata*) and animal personnel in our colony, monkeypox was suspected. Electron micrographs of biopsied skin lesions showed particles resembling poxvirus[1,2]. The agent, subsequently isolated in tissue cultures, is, however, morphologically and serologically distinct from the vaccinia group, and the histopathology is totally different from that of Yaba virus. We therefore think that this agent, temporarily called '1211', is a new unclassified poxvirus which is infectious for primates and may be the same as that recently reported from Texas[2].

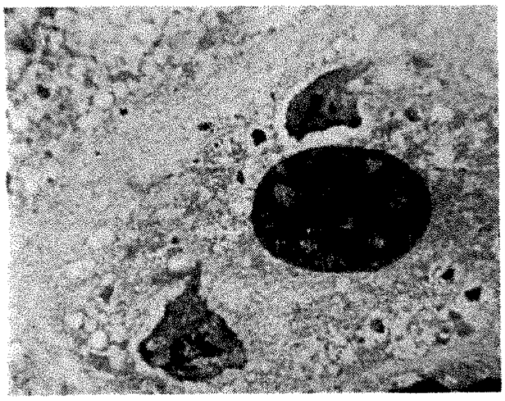

Fig. 1. '1211' virus in human amnion cell culture 7 days after infection. (× c. 465.)

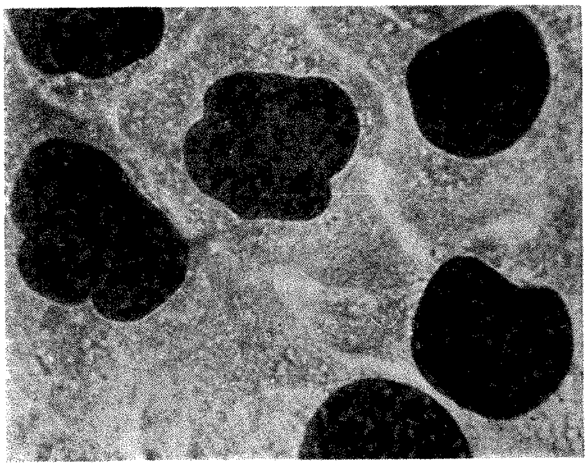

Fig. 2. Uninfected human amnion cell culture after 7 days. (× c. 465.)

An extract of a homogenized skin lesion did not produce pocks on chick chorioallantoic membrane and no cytopathic effects were observed in HeLa cells, chick embryo cells or rabbit kidney cells. Cytopathic changes were produced in cultures of *BS-C*-1 (ref. 3), human amnion, rhesus amnion and rhesus epidermal cells after incubation for more than 10 days. The time of appearance of these changes shortened to 4–5 days after repeated passage. After eight passages, sonicated cells and culture medium were scarified into rhesus monkey skin and caused lesions clinically and histologically identical with the original disease.

The *BS-C*-1 cells are suitable for plaque assay under agar, although plaques cannot be seen for 4–6 days, are counted after 8–10 days and remain less than 1·0 mm in diameter. Cells and medium sonicated at the time of maximum cytopathic change yield 10^5–10^6 plaque forming units (PFU)/ml.; the virus can be concentrated and purified in sucrose density gradients[4] and thus produce a titre of 10^7–10^8 PFU/ml.

Primary human amnion cells stained with Giemsa show numerous cytoplasmic inclusions 5 days after infection with '1211' virus and nuclear vacuoles after 6 days (Figs. 1 and 2). The cytoplasmic inclusions probably contain virus; in electron micrographs of biopsied skin lesions, similar inclusions are seen to contain virus particles and these are found only in the cytoplasm. The significance of the nuclear vacuoles is unknown; they have been noted in cell cultures infected with sheeppox[5], myxoma[6] and swinepox[7], but not with Yaba[8] or monkeypox[9].

Results of preliminary serological comparisons by neutralization and complement fixation are shown in Tables 1 and 2. Hyperimmune rabbit sera were collected after serial injection with virus that had been grown in cell culture, purified by gradient centrifugation and inoculated at 10^7 PFU per injection for '1211' virus and 10^9 PFU for vaccinia and monkeypox. Sufficient quanti-

Table 1. SEROLOGICAL COMPARISON BY NEUTRALIZATION

	Serum titres*			
Virus	Monkeypox	Vaccinia	'1211'	Yaba†
Monkeypox	6,400	3,200	< 10	ND
Vaccinia	1,600	3,200	ND	ND
'1211'	< 10	< 10	800	100

ND, Not done.

* Titres are expressed as the reciprocal of the serum dilution/0·2 ml. showing 50 per cent plaque reduction of 100–200 PFU/0·2 ml.

† Yaba serum, supplied by Dr D. S. Yohn, is monkey convalescent serum. All others are rabbit hyperimmune sera.

Table 2. SEROLOGICAL COMPARISON BY COMPLEMENT FIXATION

Antigen	Serum titres* Monkeypox	Vaccinia	'1211'	Yaba
Monkeypox	128	32	< 2†	< 4†
Vaccinia	128	128	< 4	< 4
'1211'	< 2	< 2	64	8

* Titres are expressed as the reciprocal of the serum dilution showing 4+ fixation of complement with 4–8 U of antigen using the microtitre technique.
† < 2 or < 4: lowest dilution tested.

ties of other poxviruses are not yet available for preparation of sera.

The morphological differences in cell cultures and lack of serological cross-reaction clearly set '1211' apart from the variola–vaccina–monkeypox sub-group of poxviruses. Cross-reactivity with Yaba virus is apparent, but quantitation of this relationship awaits volume growth and reliable plaque assay of this virus in our laboratory.

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[1] McNulty, W. P., Lobitz, W. C., Hu, F., Maruffo, C. A., and Hall, A. S., *Arch. Dermatol.* (in the press).
[2] Casey, H. W., Woodruff, J. M., and Butcher, W. I., *Amer. J. Pathol.*, **51**, 431 (1967).
[3] Hopps, H. E., Bernheim, B. C., Nisalik, A., Tjio, J. H., and Smadel, J. E., *J. Immunol.*, **91**, 416 (1963).
[4] Joklik, W. K., *Virology*, **18**, 9 (1962).
[5] Plowright, W., and Ferris, R. D., *Brit. J. Exp. Pathol.*, **39**, 424 (1958).
[6] Chaproniere, D. M., *Virology*, **2**, 599 (1956).
[7] Kasza, L., Bohl, E. H., and Jones, D. O., *Amer. J. Vet. Res.*, **21**, 269 (1960).
[8] Yohn, D. S., Grace, J. T., and Haendiges, W. V., *Nature*, **202**, 881 (1964).
[9] Prier, J. E., Sauer, R. M., Malsberger, R. C., and Sillaman, J. M., *Amer. J. Vet. Res.*, **21**, 381 (1960).

Combined Lethal Effect of Zinc Chloride and Gamma Irradiation on *Bacillus megaterium*

IT has been known for several years that zinc chloride, like the salts of other heavy metals, has a lethal effect on bacteria[1–5]. Inactivation of micro-organisms by irradiation has also been extensively studied[6–10], and yet the effect of the combination of these two inactivating agents has received little attention.

The addition of radioactive zinc-65 to cultures of *B. megaterium* in the exponential phase of growth has been shown to be inhibitory, while non-radioactive zinc, in the same concentration, had little or no effect[11]. In the experiments described here, zinc, combined with gamma irradiation, had a synergistic inhibitory effect on the ability of *B. megaterium* to form colonies.

Cultures of *B. megaterium* (strain Elstre) were maintained in the logarithmic phase of growth for 18 h at 37° C in nutrient broth (Difco), until the optical density of the cultures was about 0·150. From this culture, 0·5 ml. of bacterial suspension was taken and diluted in 9·5 ml. of nutrient broth, and irradiated at room temperature with gamma rays from a 500 Ci cobalt-60 source. The tubes containing culture were randomly placed in a circular device which rotated around the source. The dose rate was approximately 400 rads/min given for 5, 15 or 60 min, corresponding to 2,000, 6,000 and 24,000 rads, respectively.

Zinc chloride was added to bacterial suspensions, prepared and diluted in the same way, to give concentrations of 6×10^{-6} or 12×10^{-6} M in the medium. Bacteria were left in the samples containing zinc chloride for 45, 60 or 120 min.

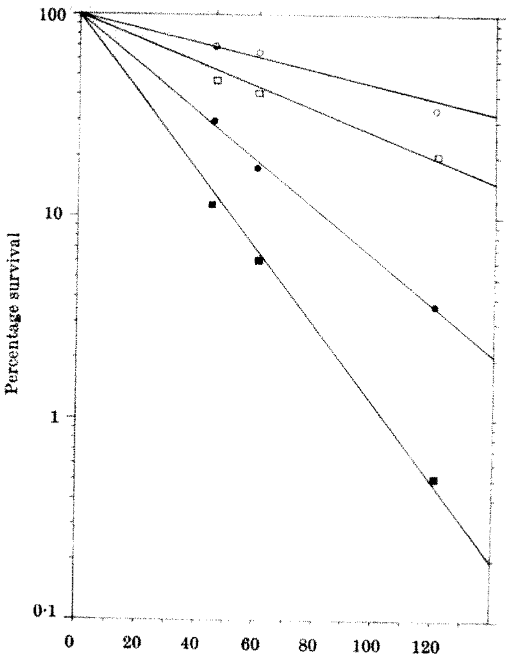

Fig. 1. Inactivation of *Bacillus megaterium* after exposure to two different concentrations of zinc chloride, for various times, and subsequent irradiation. ○, 6×10^{-6} M zinc chloride; ●, with irradiation; □, 12×10^{-6} M zinc chloride; ■, with irradiation.

Another series of tubes, with exposures similar to those of zinc chloride, were also irradiated as described. Surface plate counts were made on Difco nutrient agar before and after irradiation and the colonies were counted after incubation at 35° C for 48 h.

Survival curves of samples containing various concentrations of zinc chloride, of samples irradiated at different doses, and of samples containing zinc which were then irradiated, are shown in Figs. 1 and 2. The effect of zinc chloride and gamma irradiation on the survival of the micro-organisms is also shown in Table 1.

The chemical agent remained in contact with the cells for quite a long time and was moderately toxic to them. By increasing the time of exposure, as well as the concentration of the chemical, the toxicity was increased. Thus exposure to zinc chloride alone for 45, 60 or 120 min at a concentration of 6×10^{-6} M decreases the percentage of survivals to 70, 66 or 34 per cent, respectively. At a stronger concentration (12×10^{-6}) and with the same exposures, the percentage survivals decreased to 45, 40 or 20 per cent, respectively. Gamma irradiation alone causes a significant decrease of survivals and here also the reduction is directly related to the dose. Approximately 28, 45 and 85 per cent of the bacilli lost the ability to form new colonies when irradiated with 2,000, 6,000 and 24,000 rads, respectively. For comparison, the percentages of survivals here are 72, 55 and 15 per cent, respectively.

Table 1. SURVIVAL PERCENTAGE OF *Bacillus megaterium* AFTER ZINC TREATMENT, GAMMA IRRADIATION AND COMBINATION OF EXPOSURES TO ZINC AND GAMMA RAYS

Time (min)	Zinc chloride		Zinc chloride + gamma irradiation		Gamma irradiation 400 rads/min	Dose (rads)
	6×10^{-6} M	12×10^{-6} M	6×10^{-6} M	12×10^{-6} M		
0	100	100	100	100	100	0
45	70 ± 6·1	45 ± 5·8	30 ± 4·9	11 ± 2·8	72 ± 3·6	2,000
60	66 ± 5·9	40 ± 5·7	17 ± 2·1	6 ± 1·5	55 ± 3·2	6,000
120	34 ± 3·6	20 ± 3·6	3·7 ± 0·9	0·5 ± 1·6	15 ± 1·8	24,000

When zinc treatment and gamma irradiation are combined, the decrease of survivals is more important. With zinc chloride at a weak concentration and after irradiation with 2,000, 6,000 and 24,000 rads, the percentage survivals

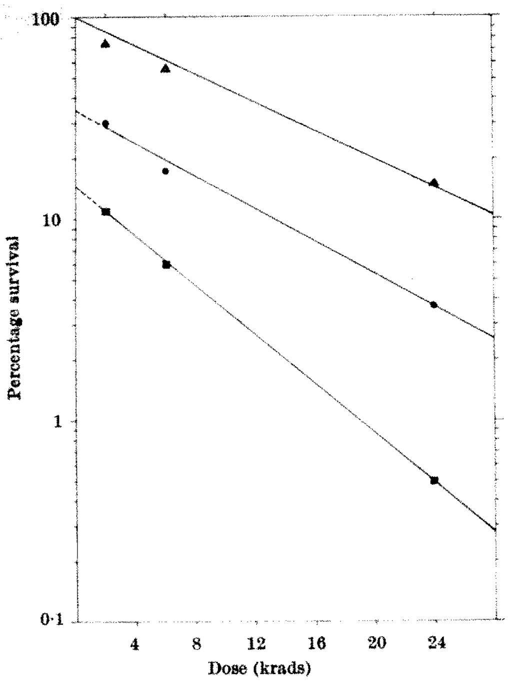

Fig. 2. Effect of gamma irradiation at various doses alone or with two different concentrations of zinc chloride, on survival of *B. megaterium*. ▲, Irradiation (400 rads/min); ●, irradiation and zinc chloride (6×10^{-6} M); ■, irradiation and zinc chloride (12×10^{-6} M).

are 30, 17 or 3·7, respectively. With the stronger concentration of zinc and the same irradiation doses the percentage of survivals diminishes to 11, 6 or 0·5, respectively. This clearly demonstrates a synergistic effect of the metal ions and the radiation. Even by supposing that the two agents reach different targets in the cells, the expected total decrease of survivals after the combined action is much lower than the observed one.

Metal salts, in concentrations which are not toxic, are known to increase the resistance to ionizing radiations. It has been suggested and partly supported by experimental evidence that ions of those metals as well as more complex redox systems increase the radioresistance by donating or receiving electrons. They thus facilitate the neutralization of active molecules which have gained or lost electrons during the ionization process[12]. Heavy metals in appropriate concentration, however, exert a deleterious effect on bacteria by combining with sulphydryl groups which are essential for the metabolism of the cell[1-3]. Reagents that combine with sulphydryls have been shown to increase radiation sensitivity, at least in some micro-organisms[13,14].

The synergistic effect of radiation and zinc shown by the experiments reported here could be attributed to similar phenomena. In other experiments, to be published later, the same synergistic effect was observed on the growth rate curve of bacteria. Thus the combination of the two inactivating agents seems to affect both cell growth and cell multiplication.

The mechanism of the synergistic phenomena cannot yet be explained, but zinc probably sensitizes the bacteria to the lethal action of the gamma rays. This apparent sensitization might also be caused by a combination of toxic injuries, induced by the salt and by the irradiation.

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[1] Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity*, 1, 119 (1957).
[2] Oginsky, E. L., and Umbreit, W. W., *An Introduction to Bacterial Physiology*, 95, 117 (1959).
[3] Jawetz, E., Melnick, J. L., and Adelberg, E. A., *Review of Medical Microbiology*, 81 (1966).
[4] Hochster, R. M., and Quastel, J. H., *Metabolic Inhibitors*, 2, 311 (1963).
[5] Witham, I. J., *Nature*, **197**, 1116 (1963).
[6] Zelle, M. R., and Hollaender, A., *Radiation Biology*, 2, 365 (1955).
[7] Kelner, A., Bellamy, W. D., Stapleton, G. E., and Zelle, M. R., *Bact. Rev.*, **19**, 22 (1955).
[8] Kimball, R. F., *Ann. Rev. Microbiol.*, **11**, 199 (1957).
[9] Bhattacharjee, S. B., *Radiat. Res.*, **14**, 50 (1961).
[10] Gunter, S. E., and Kohn, H. T., *J. Bact.*, **71**, 571 (1956).
[11] Kiortsis, M., thesis, Univ. Athens (1965).
[12] Butler, J. A. V., and Robins, A. B., *Nature*, **193**, 673 (1962).
[13] Bridges, B. A., *Nature*, **188**, 415 (1960).
[14] Lee, J. S., Anderson, A. W., and Elliker, P. R., *Radiat. Res.*, **19**, 593 (1963).

IMMUNOLOGY

DNA Synthesis in the Development of Antibody-forming Cells during the Early Stages of the Immune Response

THERE is considerable evidence that antibody-forming cells arise by mitotic division after injection of antigen, and that they continue to divide and produce antibody while differentiating (refs. 1–3 and unpublished work of N. K. Jerne, A. Nordin, C. Henry, H. Funi and A. Koros). According to this view, the many antibody-forming cells present at the peak of an immune response are derived by extensive proliferation from a relatively small number of precursors. It has been suggested, however, that plaque-forming cells which release 19*S* haemolytic antibody may develop from precursors without division[4-6]. If this is true it follows that lymphoid tissue must contain a large number of cells capable of being directly "switched on" for synthesis of a specific antibody. Experiments designed to test this point are described here.

CBA mice, 8–14 weeks old and of either sex, were injected intravenously with 2×10^8 sheep red cells. At the same time, they received an intraperitoneal injection of ^{3}H-thymidine (Radiochemical Centre, Amersham), 2 µCi/g of body weight, specific activity 5,000 mCi/mmole. Injections of ^{3}H-thymidine were given at 12 h intervals (Table 1) until the mice were killed, 2–4 h after the last injection. Four non-immunized mice received five similar doses of ^{3}H-thymidine for a period of 2 days before killing.

Table 1. PROPORTION OF LABELLED PLAQUE-FORMING CELLS IN THE SPLEEN OF MICE AT DIFFERENT TIMES AFTER IMMUNIZATION

Time after injection of antigen (h)	No. of mice used	Mean PFC/10^6 spleen cells	No. of PFC examined Labelled	Total
0	4	2	1	12
26–28	5	8	7	18
40	2	34	13	13
52	1	60	16	16
76	1	300	10	10
98–100	2	4,470	34	34

PFC, plaque-forming cells.

Single cell suspensions were made from the spleens of killed mice, and cells producing haemolytic plaques were identified, picked out and smeared individually using techniques described fully elsewhere[7,8]. Briefly, monolayers of spleen cells and target sheep erythrocytes were incubated in droplets of Eisen's medium[9] (with 10 per cent of foetal calf serum and 5 per cent guinea-pig complement) under paraffin oil. After incubation for 15–30 min, plaques developed in the monolayers; a single antibody-forming cell could be picked out from the centre of many of these with a micropipette. Where antibody-forming cells were rare, as in non-immunized animals, a two step procedure was necessary[8]: lymphoid cells were first incubated at relatively high concentration in the droplets, then all the cells from the centre of the plaque were removed, diluted and reincubated with more erythrocytes and complement in a second monolayer. The active cell could usually be identified after this second incubation. Plaque-forming cells were smeared in a very thin stream of serum blown from the micropipette and their position on the slide was marked by ringing with a diamond objective. Slides were then coated with Kodak *NTB*-2 emulsion and exposed for 19–22 days, after which the emulsion was developed and the cells were lightly stained with Giemsa.

Table 1 records that from 40–100 h after injection of antigen all plaque-forming cells were labelled (usually with more than thirty grains). These cells were mostly basophilic mononuclears of various sizes, with a few plasma cells[8]. By contrast, in the small sample of "background" plaque-formers from non-immunized mice, all but one were unlabelled, indicating that these cells or their precursors divide infrequently. The one labelled cell was a typical small lymphocyte. The others were also small cells (all of average diameter < 11μ), although usually more basophilic than typical small lymphocytes, with a nucleus occupying most of the apparent area of the smeared cells[8]. One was a plasma cell. At 26–28 h after antigen had been injected, the population was mixed by both morphological and labelling criteria. All eleven unlabelled cells were less than 12μ in average diameter, while the seven labelled cells were mostly larger (diameters were 10, 12, 12, 13, 17, 19, 21μ). The emergence of these labelled cells was presumably provoked by antigen. The unlabelled cells at this time may well have been background plaque-formers present before antigenic stimulation, although the data do not exclude the possibility that some of these may have begun to produce antibody only after antigenic stimulation and without dividing.

These results show clearly that after a mouse is immunized with sheep erythrocytes the great majority of IgM-producing cells synthesize DNA before releasing antibody. Sterzl[4] has reported experiments very similar in design to ours, except that ^{14}C-thymidine was used instead of ^{3}H-thymidine. In his mice, only 50 per cent of plaque-forming cells were labelled at 4 days after antigen. This low figure may be a result of differences in the technique used to detect labelled cells. Eidinger and Pross[6] have concluded that the very rapid increase in number of 19*S* plaque-forming cells which occurs in some tissues during a primary immune response can be explained only by recruitment of new cells into antibody production. This may well be partly true; many antigen-responsive cells are probably derived from a circulating pool in the blood[10]. Nevertheless, our observation that all plaque-forming cells were labelled at days 2, 3 or 4 after antigen shows that some DNA synthesis, and almost certainly at least one division, occurs between recruitment of a cell and antibody production. Tannenberg[5] concluded that the plaque-forming cells in his experiments did not undergo enough mitotic divisions to account for the observed increase in the number of antibody-forming cells. His assumption that cells which have been supplied constantly with ^{3}H-thymidine should incorporate much more label after several mitotic cycles than after only one or two, is difficult to accept on theoretical grounds. If a cell incorporates an amount x of tritiated thymidine into its DNA during one period of synthesis, it then loses $x/2$ at the subsequent mitotic division. After the next *S* phase, the total label in the cell becomes $x+x/2$, of which half is again lost in mitosis, and so on. The maximum amount incorporated cannot exceed $2x$. This means that the difference in grain count between cells after 2 and 10 mitotic cycles could be, at most, 25 per cent. (This calculation assumes even distribution of the labelled material throughout the pool of thymidine in the animal, which Tannenberg probably achieved by taking the precaution of continuously infusing his animals.) His results did show that most plaque-forming cells 4 days after antigen do contain label, although grain counts tended to be lower than ours. The lower counts may have been caused by the presence of a thin layer of agar over the cells which would have absorbed some of the emitted β-rays.

In conclusion, while we cannot exclude the possibility that a very small proportion of cells produce antibody without first synthesizing DNA, our results support the view that most IgM-plaque-forming cells arise by mitotic division.

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[1] Baney, R. N., Vazquez, J. J., and Dixon, F. J., *Proc. Soc. Exp. Biol. and Med.*, **109**, 1 (1962).

[2] Nossal, G. J. V., and Mäkelä, O., *J. Exp. Med.*, **115**, 209 (1962).

[3] Balfour, B. M., Cooper, E. H., and Alpen, E. L., *Immunology*, **8**, 230 (1965).

[4] Sterzl, J., Vesely, J., Jilek, M., and Mandel, L., in *Molecular and Cellular Basis of Antibody Formation* (edit. by Sterzl, J.), 463 (Academic Press, New York, 1965).

[5] Tannenberg, W. J. K., *Nature*, **214**, 293 (1967).

[6] Eidinger, D., and Pross, H. F., *J. Exp. Med.*, **126**, 15 (1967).

[7] Cunningham, A. J., Smith, J. B., and Mercer, E. H., *J. Exp. Med.*, **124**, 701 (1966).

[8] Cunningham, A. J., *Austral. J. Exp. Biol. Med. Sci.* (in the press).

[9] Kern, M., and Eisen, H. N., *J. Exp. Med.*, **110**, 207 (1959).

[10] Ford, W. L., Gowans, J. L., and McCullagh, P. J., in *The Thymus*, Ciba Found. Symp. (edit. by Wolstenholme, G. E. W., and Porter, R.), 58 (J. and A. Churchill, Ltd., London, 1966).

CYTOLOGY

Sensitivity of Micrurgically Removed Chromosomal Spindle Fibres to Enzyme Disruption

THE physio-chemical nature of chromosomes and chromosomal spindle fibres isolated by micrurgy is being studied by enzyme disruption of these structures, recorded by phase contrast cinematography.

Using a modification of the technique of McClendon[1], chromosomes and chromosomal spindle fibres can be removed micrurgically from human cells *in vitro* by an incision in the cytoplasmic membrane[2]. Groups of chromosomes along lengths of chromosomal spindle fibres can then be separated by teasing them with microneedles. Alternatively, one microneedle can be used to stabilize a mitotic cell while the other microneedle is inserted to pull out lengths of spindle with attached chromosomes[3] (Fig. 1).

With either approach, the isolated chromosomal spindle fibres show considerable elasticity when the surrounding solution is Hanks' BSS, Earle's BSS or Eagle's basic medium with or without the addition of serum. In Hanks' BSS, the spindle can be stretched over distances greater than 300μ before breakage occurs (Fig. 2*a*). Spindle fibres stretched up to 300μ may remain taut when relaxed to distances of about 100μ, showing their great elasticity (Fig. 2*b*).

When the surrounding Hanks' solution contains RNase 5× crystallized or pepsin 3× crystallized (Nutritional Biochemical Company) in concentrations of 2·5 mg/ml. the spindle can be stretched over 300μ (Fig. 2*c*), but on relaxation it does not return to less than 100μ. Instead, the relaxing spindle fibres form coiled configurations (Fig. 2*d*) indicating loss of elasticity and suggesting deformation of structure at the macromolecular level.

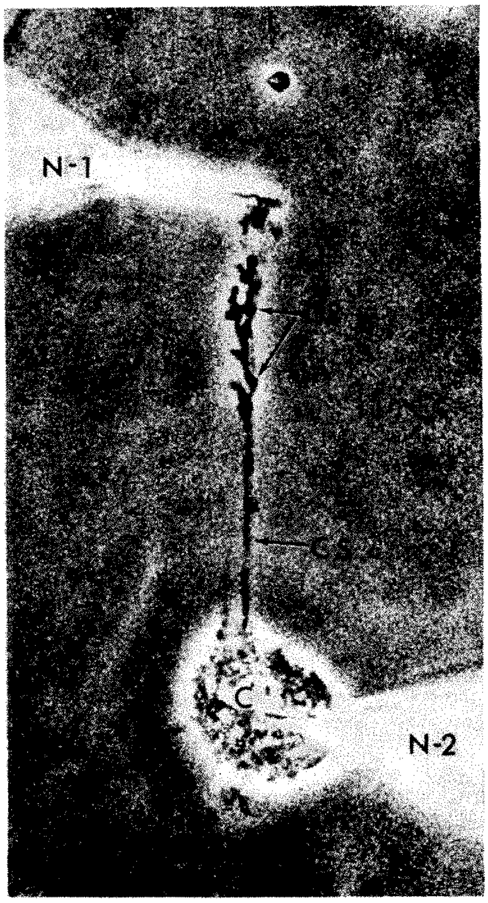

Fig. 1. Microneedle 1 (N-1) pulls chromosomal spindle fibres (CS) with attached chromosomes (CR) from cell (C) which is stabilized by microneedle 2 (N-2).

Spindle fibres isolated into surrounding Hanks' BSS and attached to the coverslip while stretched can be followed by cinematography, while the surrounding solution is changed to Hanks' BSS with 0·5 mg/ml. of DNase added. Within a few seconds of the change of solutions, the spindle fibres are seen to break and to become slightly less dense under phase contrast microscopy. Teasing with a microneedle shows that all stretchability is lost and no elastic recoil remains. When spindles are pulled out of cells into the DNase, they rarely survive long enough to stretch more than 50–100μ before breaking (Fig. 2*e*). (The fact that the spindle fibres do survive the first few seconds of isolation indicates that the cytoplasmic membrane is not significantly permeable to DNase during the 1–2 h periods of observation.) When the entire mitotic apparatus is isolated into this DNase solution, efforts to tease the chromosomes apart give the impression that the chromosomes are no longer connected by spindle fibres.

These studies indicate that the principal sources of strength and stretchability of chromosomal spindle fibres are susceptible to destruction by DNase but survive treatment with RNase and pepsin. Elastic recoil of these spindle fibres, however, seems to be reduced by the action of RNase and by pepsin.

Electron micrographic studies have shown mitotic spindle fibres (isolated by the method described here) as four groups of fibres attached to the kinomere in both mouse and human metaphase chromosomes[4]. These four groups of fibres appear to be each about 600 Å thick and each seems to be made up of smaller fibres which are bound into a fascicle by a yet smaller very dense spiralling fibre less than 100 Å thick[5] (Fig. 3). Of the fibres seen, only the dense spiralling fibre seems to be small enough to be a DNA strand. The larger fibres would then presumably be protein.

In electron micrographic studies, small dense fibres less than 100 Å thick, in sectioned chromosomes, were found to be sensitive to DNase[6]. In light microscopic studies of well stained Feulgen preparations such as those by Lima-de-Faria[7] (especially Figs. 40 and 41) some Feulgen positive material seems to taper off from the chromosome as if it were following the spindle. The studies described here suggest that not only does DNA

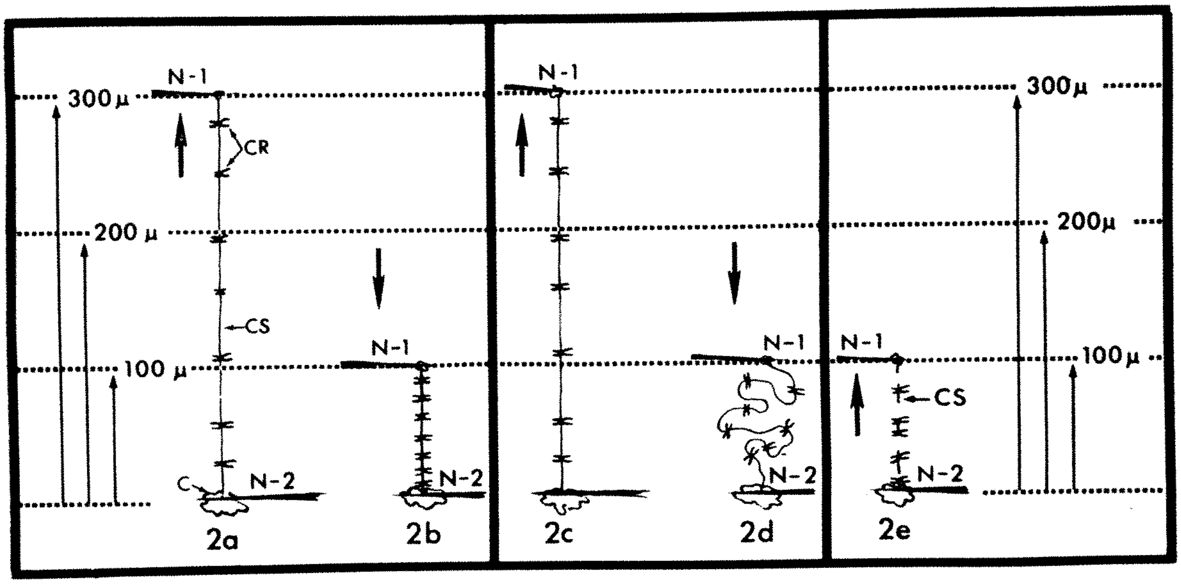

Fig. 2. A schematic representation of the studies described in the text. (*a*) Microneedle 1 (N-1) stretches chromosomal spindle fibres (CS) a distance of 300μ from the cell (C) which is stabilized by microneedle 2 (N-2). (*b*) When microneedle 1 (N-1) returns to within 100μ of microneedle 2 (N-2) the chromosomal spindle fibres remain taut as is characteristic for such fibres isolated into Hanks' BSS, Earle's BSS or Eagle's basic media. (*c*) Chromosomal spindle fibres (CS) can be stretched for a distance of 300μ in solutions containing RNase or pepsin (2·5 mg/ml.). (*d*) Chromosomal spindle fibres which have been stretched to 300μ in solutions of RNase or pepsin fail to remain taut when relaxed to 100μ. Instead they remain somewhat stretched and tend to coil as if macromolecular structures had been deformed. (*e*) Attempts to stretch chromosomal spindle fibres (CS) in solutions containing 0·5 mg/ml. of DNase fail because the fibres become disrupted and break within a few seconds of removal from the cell and exposure to the enzyme.

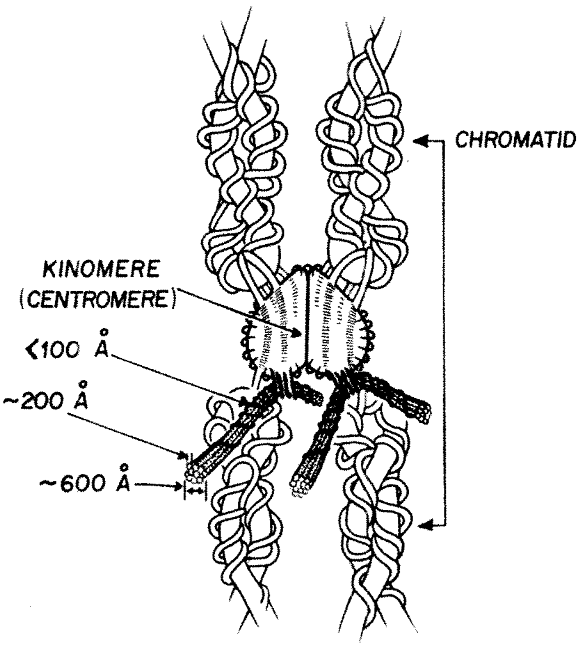

Fig. 3. A schematic representation of chromosomal spindle fibres as fascicles about 600 Å thick composed of fibrils about 200 Å thick surrounded by one or more dense spiralling fibre(s) less than 100 Å thick as they near the kinomere. The dense spiralling fibres are small enough to be DNA, and such fibres may be responsible for the structural integrity of the chromosomal spindle fascicles. (This drawing is not to scale.)

accompany the chromosomal spindle fascicles, but that DNA provides the chief source of structural integrity for these fascicles.

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[1] McClendon, J. F., *Biol. Bull.*, **12**, 141 (1906).
[2] Hoskins, G. C., *Nature*, **207**, 1215 (1965).
[3] Hoskins, G. C., *Proc. Twenty-fifth Anniversary Meeting Electron Microsc. Soc. Amer.*, Chicago, 128 (1967).
[4] Hoskins, G. C., *Proc. Third Intern. Cong. Human Genetics*, Chicago (1966).
[5] Hoskins, G. C., and Hoskins, B. B., *Proc. Twenty-fifth Anniversary Meeting Electron Microsc. Soc. Amer.*, Chicago, 190 (1967).
[6] Dales, S., *Exp. Cell Res.*, **19**, 577 (1960).
[7] Lima-de-Faria, A., *Intern. Rev. Cytol.* (edit. by Bourne, G. H., and Danielli, J. F.), **7**, 123.

Apparent HeLa Cell Contamination of Human Heteroploid Cell Lines

INTERSPECIFIC cell culture contamination has been detected several times by karyotypic and immunological procedures[1–3]. These same measurements are of little value as detectors of intraspecific contamination, but polymorphic variants detectable at the cell culture level can be very useful for this purpose. A survey of twenty heteroploid human cell lines for glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase (PGM) electrophoretic polymorphisms revealed that all had the same G6PD and PGM phenotypes. These results were interpreted as reflecting widespread contamination of cultures by the first established human cell line HeLa. These findings were presented at the Second Decennial Review Conference on Cell Tissue and Organ Culture[4] and have since been extended to include other American Type Culture Collection lines. Further evidence on the stability of these markers in various conditions has been obtained, and in view of the widespread use of cell lines in a variety of investigations it seems important to make these findings readily accessible to other workers.

Table 1. ESTABLISHED HETEROPLOID HUMAN CELL LINES ANALYSED IN THIS STUDY

Original designation	ATCC No. (if listed)	Supply for this study
HeLa	*CCL* 2	ATCC, RL (6 lines assayed)
Detroit 6	*CCL* 3	ATCC, C
Minnesota *EE*	*CCL* 4	ATCC, RL
Embryonic lung	*CCL* 5	ATCC
Intestine 407	*CCL* 6	ATCC, C
Chang liver	*CCL* 13	ATCC, RL
KB	*CCL* 17	ATCC, C
Detroit 98	*CCL* 18	ATCC
NCTC 2544	*CCL* 19	ATCC
AV$_3$	*CCL* 21	ATCC
He p-2	*CCL* 23	ATCC, RL
J-111	*CCL* 24	ATCC, C
WISH	*CCL* 25	ATCC, RL
Giradi heart	*CCL* 27	ATCC
Heart	—	RL
Leucocytes	—	RL
Leucocytes	—	RL
Embryonic	—	RL
Prostate	—	RL
CMP	—	RL

RL, Research laboratory; C, commercial source; ATCC, American Type Culture Collection.

There are two principal G6PD (sex linked) electrophoretic variants, the A (fast) and B (slow) types. The A type has been found only in Negroes, its frequency in the American Negro male population being about 30 per cent. There are three common PGM (autosomal) electrophoretic variants, 1, 1–2 and 2, with frequencies of approximately 65, 20 and 15 per cent in both Negro and Caucasian population. All the human heteroploid cell lines so far examined (Table 1) were found to have the G6PD A band and were PGM type 1. I have not ascertained the racial origin of all the lines examined; it is known, however, that at least some were thought to have been derived from Caucasians (*KB*, *WISH*, Prostate, *CMP*) and at least one (HeLa) is from a Negro. The absence of the A band in Caucasians excludes a statistically based sampling explanation of these results; however, even if all the lines are assumed to be of Negro origin the probability of all being G6PD A and PGM 1 by chance sampling is absurdly low (with values of 0·3 for A and 0·65 for PGM 1, the probability is 6×10^{-15}).

Two explanations for these observations may be considered: (1) G6PD or PGM expression or both are modified in established cell lines such that only the G6PD A and PGM 1 types are found; or (2) these lines are all or nearly all HeLa cell contaminants (HeLa being the first established cell line and derived from a Negro). To explore the first argument, a number of experiments were carried out to see if modification of G6PD and/or PGM expression was possible. Because heteroploid established cell lines are often associated with tumorous tissues, a number of tumours were studied to see whether the G6PD or PGM types of the tumour or both deviated from that of normal tissue in the same person. Tumours from more than 100 individuals have now been surveyed (leukaemias, fibromas, carcinomas of various tissues, trichoepithelioma and sarcomas); and in all cases the tumour phenotype was identical to the normal tissue phenotype. In the case of individuals heterozygous for G6PD (AB), tumours may show only the A or B phenotype; however, this is because of the cell mosaic nature of such heterozygotes and the possibility of single phenotype tumours developing through single cell origin or selection[5]. The tumour cases studied included all PGM types but only hemizygous and homozygous G6PD individuals. Several normal cell strains have been initiated from individuals of known G6PD and PGM genotypes and in all cases the phenotype of the culture throughout its life history *in vitro* has remained identical to that of the donor. G6PD and PGM phenotypes of normal cultures have been examined in a variety of culture conditions including PPLO contamination, *SV*40 infection, X-irradiation, 5-bromo-deoxyuridine incorporation and treatment with corticosteroids; in no case was the G6PD or PGM phenotype modified. Finally, two long term near diploid cell lines derived from tumours and maintained in culture for more than 3 years were both G6PD B. All evidence seems to point to the stability of the G6PD and PGM phenotypes both *in vivo* and *in vitro*.

The simplest and most likely explanation of these findings remains contamination from HeLa cells. Compatible with this argument is the derivation of the HeLa line from a Negro patient and the fact that it was the first established human cell line. If we assume that most, if not all, the heteroploid human cell lines are HeLa cell derivatives, then some important questions are raised regarding investigations with these cells. In cases where the investigator's requirement was for any human cell line, the problem of whether the line was HeLa, *KB* or Detroit does not seem important. In fact, differences between such lines for certain characters—virus sensitivity, for example—are perfectly compatible with a common origin of the lines, because *in vitro* developed variations within clones are well known in these systems. In those cases where the investigator has assumed a specific tissue origin of the cell line (liver or lymphocytes, for example), however, the work is of dubious value.

With the continued expansion of cell culture technology, it is almost certain that both interspecific and intraspecific contamination will continue to occur. The electrophoretic variants mentioned here are not only useful as intraspecific tags, but are even more powerful in species identification. The PGM pattern alone is specific for every species we have examined (man, hamster, rat, mouse, rabbit and cow). The use of stable genetic markers is routine practice in all genetically oriented stock keeping: now that such markers are available for cell culture work, their incorporation into the material to be cultured is the best guarantee against contamination.

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[1] Rothfels, K. H., Axelrad, A. A., Siminovitch, L., McCulloch, E. A., and Parker, R. C., in *Proc. Third Canadian Cancer Res. Conf. 1958* (edit. by Begg, R. W.), 189 (Academic Press, 1959).

[2] Defendi, V., Billingham, R. E., Silvers, W. K., and Moorhead, P., *J. Nat. Cancer Inst.*, **25**, 359 (1960).

[3] Brand, K. G., and Syverton, J. T., *J. Nat. Cancer Inst.*, **28**, 147 (1962).

[4] Gartler, Stanley M., *Nat. Cancer Inst. Mono.*, **26**, 167 (1967).

[5] Gartler, S. M., and Linder, D., *Symp. Quant. Biol.*, **29**, 253 (1964).

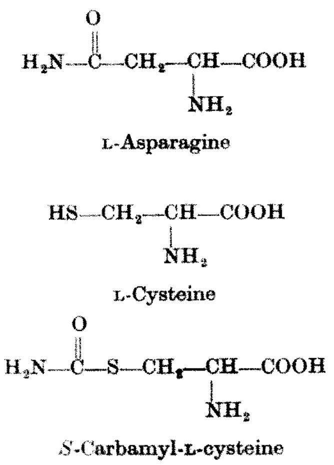
PATHOLOGY

Anti-tumour Activity of S-Carbamyl-cysteine

L-ASPARAGINASE has been shown to inhibit the growth of various tumours in the mouse, rat and dog and is currently being used in clinical trials[1-5]. Unlike normal cells, the cells of tumours that respond to this enzyme have a requirement for the nutritionally non-essential amino-acid, L-asparagine.

Treatment of tumours with L-asparaginase is tempered, however, by the possibilities of reaction to foreign protein and of endotoxin contamination of the enzyme isolated from *E. coli*.

Several compounds structurally related to L-asparagine were therefore investigated in the hope that these analogues might interfere with the uptake and/or utilization of L-asparagine. *S*-Carbamyl-L-cysteine (SCC, Fig. 1), structurally related to L-asparagine and L-cysteine, was tested for anti-tumour activity *in vitro* and *in vivo* against tumours which were sensitive and non-sensitive to L-asparaginase. SCC has previously shown anti-tumour activity against a transplanted mouse adenocarcinoma[6].



$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-CH_2-\underset{\underset{\displaystyle NH_2}{|}}{CH}-COOH$

L-Asparagine

$HS-CH_2-\underset{\underset{\displaystyle NH_2}{|}}{CH}-COOH$

L-Cysteine

$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-S-CH_2-\underset{\underset{\displaystyle NH_2}{|}}{CH}-COOH$

S-Carbamyl-L-cysteine

Fig. 1.

S-Carbamyl-L-cysteine was tested *in vitro* against *L*5178*Y* and *L*1210 cells in static culture as previously reported[7]. Cell counts were taken at 24 and 48 h using a Coulter counter. The effects of SCC at various concentrations are reported in Table 1. SCC seemed to be equally cytotoxic against the *L*5178*Y* L-asparaginase sensitive and the *L*1210 non-sensitive cells.

Table 1. CYTOTOXIC EFFECT OF *S*-CARBAMYL-L-CYSTEINE AGAINST CELLS IN TISSUE CULTURE

Cell line	SCC concentration (μg/ml.)	Percentage inhibition of growth of controls at 24 h	at 48 h
*L*5178*Y*	50	97	99
	25	94	98
	10	35	40
	5	0	0
*L*1210	50	68	97
	25	61	88
	10	26	40
	5	0	0

Table 2. ACTIVITY OF *S*-CARBAMYL-L-CYSTEINE AGAINST VARIOUS TRANSPLANTED TUMOURS

Tumour	Range of doses used (mg/kg)	Optimal dose (mg/kg)	Percentage increase in median survival over control	Percentage inhibition of average control tumour weight
*L*5178*Y*	50–400 d* 400 eod†	400 eod	30	—
*L*1210	250–300 d 400 eod	400 eod	40	—
*P*388	300 d 300–400 eod	400 eod	37	—
*P*1798	200–300 d	300 d‡	—	70

* d, Daily.
† eod, Every other day.
‡ Five injections (day 1–5), mice killed on day 10 and tumours excised and weighed.

S-Carbamyl-L-cysteine was also effective against several tumours *in vivo* (Table 2). SCC seemed more effective in inhibiting the growth of the *P*1798, L-asparaginase sensitive lymphosarcoma than in increasing the median survival of mice bearing leukaemias sensitive (*L*5178*Y*) or non-sensitive to L-asparaginase. The mechanism of the anti-tumour effect of SCC is unknown, but it, or one of its metabolic products, may interfere with the oxidative energy supply supporting protein synthesis[8]. The equivalent activity of SCC against *L*5178*Y* and *L*1210 *in vitro* does not lend support to the hypothesis that SCC is an antimetabolite of L-asparagine.

Further studies on the mechanism of action of SCC and studies of the anti-tumour activity of other analogues of L-asparagine are in progress.

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[1] Kidd, J. G., *J. Exp. Med.*, **98**, 565 (1953).
[2] Boyse, E. A., Old, L. J., and Stockert, E., *Nature*, **198**, 800 (1963).
[3] Broome, J. D., *Nature*, **191**, 1114 (1961).
[4] Kwak, K. S., Jameson, E., Ryan, R. M., and Kurtz, H. M., *Cancer Res.*, **21**, 44 (1961).
[5] Old, L. J., Boyse, E. A., Campbell, H. A., Brodey, R. S., Fidler, J., and Teller, J. D., *Cancer*, **20**, 1066 (1967).
[6] Skinner, C. G., McKenna, G. F., McCord, T. J., and Shrive, W., *Texas Rep. Biol. and Med.*, **16**, 493 (1955).
[7] Adamson, R. H., Hart, L. G., DeVita, V. T., and Oliverio, V. T., *Cancer Res.* (in the press).
[8] Rabinovitz, M., and Fisher, J. M., *J. Nat. Cancer Inst.*, **28**, 1165 (1962).

Reduced Binding of Carcinogenic Hydrocarbons to DNA of Mouse Skin during Inhibition of DNA Synthesis

CARCINOGENIC polycyclic hydrocarbons have been shown to bind to DNA in mouse skin[1-3], but no details are known of the conditions in which this binding occurs *in vivo*, for example the phase of the cell cycle, the functional status of DNA or whether the DNA is replicating or resting. Data are only available for *in vitro* binding to denatured and native DNA[3,4]. It could be assumed that replicating, histone-free DNA is a better target for hydrocarbons than resting DNA, in which case binding should be reduced if DNA synthesis is blocked. To test this idea we used hydroxyurea in different organisms[5,6]; this is a potent and rather specific inhibitor of DNA synthesis, and inhibits more than 90 per cent of DNA synthesis in mouse skin.

To measure DNA synthesis, 7 week old male NMRI mice were given ^{3}H-methyl-thymidine (1 μCi/g mouse intraperitoneally) and were killed 50 min later. The back skins (~7·7 cm^2) of these mice that had been previously shaved were scraped under liquid nitrogen, incubated in 3 per cent acetic acid overnight, homogenized and precipitated by 10 per cent trichloroacetic acid (TCA)[7]. After several washings with TCA, ethanol and ether, the residue was dried, weighed and burnt to give tritiated water which was counted in a liquid scintillation counter. The dose-response curve (thymidine incorporation against hydroxyurea) is shown in Fig. 1. All animals survived with a single dose of hydroxyurea of up to 0·66 mg/g body weight. Synthesis of DNA is effectively inhibited very quickly and remains so for 3–4 h (Fig. 2). When the effect of hydroxyurea wore off, DNA synthesis increased to more than the normal rate: if DNA synthesis has been blocked for 9 h the rate of DNA synthesis more than doubled after 24 h.

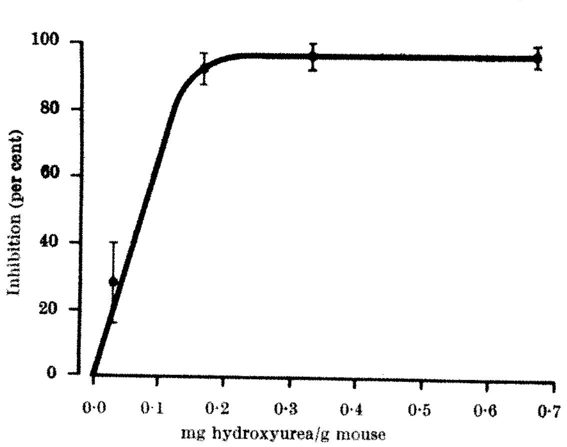

Fig. 1. Effect of different doses of hydroxyurea on DNA synthesis in mouse skin 70 min after intraperitoneal injection. Standard errors of the mean are indicated (four experiments).

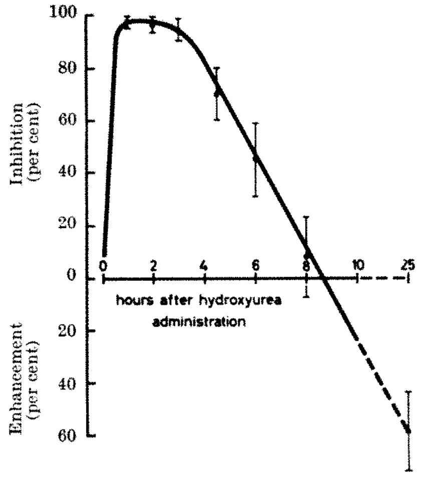

Fig. 2. Effect of hydroxyurea on DNA synthesis in mouse skin at different times after intraperitoneal injection (0·5 mg/g of mouse). Standard errors of the mean are indicated (four experiments).

A concomitant inhibition of RNA synthesis was not observed. This was followed by measuring the incorporation of ^{3}H-5-uridine (2 μCi/g intraperitoneally) according to essentially the same procedure as that described for thymidine. Thus hydroxyurea seemed to be a specific inhibitor of DNA synthesis in mouse skin as well as in other organisms[5,6]. To test whether DNA synthesis is essential for the binding reaction, five male mice were injected with hydroxyurea and incubated with tritiated 3,4-benzpyrene (BP) or 7,12-dimethylbenzanthracene (DMBA), dissolved in tricaprylin (0·3 ml./30 g of mouse). Unlabelled BP and DMBA were added to give a final concentration of hydrocarbon of 0·66 μM and 5 μCi/g of body weight, a dose known to produce tumours if later developed with croton oil. We preferred the oral route to local painting for two reasons: if hydrocarbons are locally applied, it is rather hard to eliminate residual hydrocarbons which might interact *in vitro* during the DNA isolation procedure. Furthermore, slight variations in the size of the painted area change the specific activities of labelled DNA considerably: the more untreated areas are included, the lower the specific activities will be. On the other hand, oral application ensures a rather more homogeneous distribution of hydrocarbons to mouse skin.

DNA was isolated after 4 or 12 h, a time which has been found to be long enough to allow maximum binding of BP or DMBA to mouse skin (unpublished results). The isolation procedure used was essentially that of Colburn and Boutwell[8]. RNA and proteins were removed by digestion with pancreatic RNase and pronase/trypsin. DNA preparations were characterized by the ratio of their wavelengths (260/280 usually > 1·83; 260/230 > 1·91), by their buoyant densities at equilibrium in solutions of caesium chloride (one peak only at 1·702), by their RNA contents (usually < 1·5 per cent) and by their protein contents (< 2 per cent). The specific activity of the DNA was measured by combusting the weighed TCA precipitate as before.

The experiments show that (1) carcinogenic hydrocarbons bind to mouse skin DNA even after oral administration, and (2) the binding can be reduced by pretreatment with hydroxyurea. At a first glance the reduction does not seem to be too overwhelming. Considering the fact that only 1–2 per cent of the total population of skin cells are engaged in active DNA synthesis[9,10], however it can be anticipated that only this small number of cells would respond to the inhibitory action of hydroxyurea. On this basis, the relative binding of carcinogenic hydrocarbons both to replicating and resting DNA can be determined. Assuming a tritium index of 1·5 per cent and 6 h for the *S*-phase, it can be calculated that during the 4 h of the BP experiment approximately 1 per cent (1·5 × 0·66) of the epidermal DNA had been replicated. Because 57 per cent of BP-binding is sensitive to hydroxyurea or dependent on DNA-synthesis, the ratio of hydroxyurea-sensitive to hydroxyurea-insensitive bindings per cell amounts to (57/1 : 43/99 =) 131. This ratio means that BP selectively binds to replicating DNA of mouse skin with an error of less than 1 per cent. Similarly, for DMBA we found a ratio of 45.

Table 1. EFFECT OF HYDROXYUREA (0·5 MG/G MOUSE) ON THE BINDING OF CARCINOGENIC POLYCYCLIC HYDROCARBONS TO MOUSE SKIN DNA

Hydrocarbon	Hydroxyurea	D.p.m./mg of DNA	Percentage inhibition
3,4-Benzpyrene-^{3}H	+	489 ± 11	57·4
	–	852 ± 38	
7,12-Dimethylbenz(a)anthracene-^{3}H	+	256 ± 8	31·0
	–	371 ± 56	

Standard errors of the mean are indicated (four experiments).

It is not certain, of course, whether replicating DNA is itself a preferential target for carcinogenic hydrocarbons, or whether these hydrocarbons first bind to DNA precursors which fail to be incorporated into DNA under the influence of hydroxyurea. Whatever the reason for the reduced binding of BP or DMBA to DNA, this manipulation of binding may provide a tool for deciding whether the complex of DNA and hydrocarbon, as Brookes[11] postulates, or protein binding, as Heidelberger[12] claims, is essential for tumour induction. If the tumour yield is unaffected by blocking DNA synthesis (simultaneously reducing binding to DNA), it might be safe to conclude that this binding reaction is not necessary for the initiation process. If, however, the contrary were true, this would support the idea that the initiation process is somehow correlated with DNA synthesis, as suggested by other results[13,14]. Similar ideas are now being tested for rat hepatoma induced by diethylnitrosamine (personal communication from M. F. Rajewsky,)

Mice and materials were supplied by Professor E. Hecker.

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[1] Brookes, P., and Lawley, P. D., *Nature*, **202**, 781 (1964).
[2] Goshman, L. M., and Heidelberger, C., *Cancer Res.*, **27**, 1678 (1967).
[3] Miller, E. C., Juhl, U., and Miller, J. A., *Science*, **153**, 1125 (1966).
[4] Poirier, L. A., Miller, J. A., Miller, E. C., and Sato, K., *Cancer Res.*, **27**, 1600 (1967).
[5] Pollak, R. D., and Rosenkranz, H. S., *Cancer Res.*, **27**, 1214 (1967).
[6] Kim, J. H., Gelbard, A. S., and Perez, A. G., *Cancer Res.*, **27**, 1301 (1967).
[7] Wiest, W. G., and Heidelberger, C., *Cancer Res.*, **13**, 246 (1953).
[8] Colburn, N. H., and Boutwell, R. K., *Cancer Res.*, **26**, 1701 (1966).
[9] Doermer, P., Tulinius, H., and Oehlert, W., *Z. f. Krebsforschg.*, **66**, 11 (1964).
[10] Elgjo, K., *Epidermal Cell Population Kinetics in Chemically Induced Hyperplasia*, Oslo, 36 (1966).
[11] Brookes, P., *Cancer Res.*, **26**, 1994 (1966).
[12] Heidelberger, C., *J. Cell Comp. Physiol.*, **64** (suppl. 1), 129 (1964).
[13] Frei, J. V., and Harsono, T., *Cancer Res.*, **27**, 1482 (1967).
[14] Sachs, L., in *Molekulare Biologie des Malignen Wachstums*, 17, *Coll Ges. Physiol. Chem.* (edit. by Holzer, H.), 242 (Berlin, 1966).

BIOCHEMISTRY

Association of Acid Mucopolysaccharides with Isolated Amyloid Fibrils

IN a review of amyloidosis, Cohen[1] concludes that "mucopolysaccharide does not constitute a significant portion of the material. Several reports in the literature indicate that an unusual mucopolysaccharide containing heparitin sulphate may be present in amyloid. To date these studies have been on whole amyloid tissues, not on isolated fibrils". Experiments now in progress on the nature of acid mucopolysaccharides associated with isolated amyloid fibrils are reported here.

Samples of liver and spleen obtained at autopsy from six cases of secondary amyloidosis (confirmed by thioflavine *T* staining at *p*H 1·0) (ref. 2) and twenty normal controls were stored at −20° C until required. Fibrils were isolated using the method of Cohen and Calkins[3] and the final top layer further purified by centrifugation at 18,000*g* for 1 h in 60 per cent sucrose, the deposit being retained and the process repeated three times. The final deposit was resuspended in 0·9 per cent saline, dialysed against distilled water for 48 h and lyophilized. All these procedures were carried out at 4° C. A further sample of fibrils isolated by the same method was kindly provided by R. B. Nichols of the University of Bristol (sample *A2*—Nichols and Clamp[4]). The fibrillar nature of the isolated material was confirmed by electron microscopy.

Mucopolysaccharides were isolated from whole tissues and fibrils after digestion with papain, separated by electrophoresis on cellulose acetate membrane and identified by methods described by Manley[5]. Samples of chondroitin sulphate and hyaluronic acid and mucopolysaccharides isolated from human aorta were used as markers.

The sialic acid content of papain digests of a known weight of isolated fibrils was determined by the method of Warren[6] after hydrolysis in 0·1 N sulphuric acid for 1 h at 80° C. Uronic acid was determined by the modified carbazole method of Bitter and Muir[7] before and after dialysis in heated viscose tubing. The optical density of the reaction mixture without addition of carbazole was used as a blank. There was no significant difference between results before and after dialysis. These estimations were also made on the supernatants of control samples of amyloid fibrils incubated in the digestion mixture without the addition of the enzyme.

Non-amyloid control tissues contained a large chondroitin sulphate fraction—less than 5 per cent of which was resistant to hyaluronidase—and a small amount of hyaluronic acid. Amyloid tissues showed three fractions: a small hyaluronic acid fraction; a chondroitin sulphate fraction, a variable portion of which was resistant to hyaluronidase; and a prominent heparan sulphate fraction representing 22–73 per cent of the total material. Tissues without histological evidence of amyloid but taken from patients with positive histology in other organs had the same pattern as normal controls. These results are shown in Table 1.

Results of analyses of isolated fibrils are shown in Table 2. All the fibrils examined contained a small amount of uronic and sialic acids. No uronic or sialic acid was found in the supernatants of non-papain-digested control fibrils and hyaluronic acid was not found in any of the samples tested. A chondroitin sulphate fraction was present in all the samples but showed marked variation in sensitivity to hyaluronidase. There was a prominent heparan sulphate band in five of the seven samples examined, and it was the predominant fraction in four of them. Although the pattern varied, it is important that it is essentially the same in fibrils isolated from different tissues in the same patient (case 4).

Heparan sulphate has been isolated from whole amyloid tissues in increased amounts[8-10] and recent histochemical studies[11,12] suggest that it is the principal acid mucopolysaccharide present in amyloid deposits, associated with chondroitin sulphate and some material containing sialic acid.

The results presented here confirm the increase in heparan sulphate in whole tissues and demonstrate its association with some isolated fibrils. Hyaluronidase digestion alone must be regarded as unsatisfactory for examining the chondroitin sulphate fraction, so the possibility that this fraction may include the second type of heparan sulphate found in whole amyloid tissues[10] must be considered. Further chemical characterization of the fractions isolated is in progress.

Table 1. RESULTS ON WHOLE TISSUES

Tissue	Case number	Histology for amyloid	Percentage of total mucopolysaccharide C.S.	H.S.	H.A.	Percentage of C.S. resistant to hyaluronidase
Liver	1	+	62	38	T	< 5
Spleen	1	Negative	100	0	T	< 5
Liver	2	+	39	53	8	< 5
Liver	3	+	24	73	3	67
Liver	4	+	48	46	6	38
Spleen	4	+	58	40	2	28
Liver	5	+	82	18	T	< 5
Spleen	5	+	52	35	13	< 5
Liver	6	Negative	100	0	T	12
Spleen	6	+	78	22	T	< 5
Liver	20 controls	Negative	85–100	0	0–15	< 5
Spleen	20 controls	Negative	89–100	0	0–11	< 5

Percentage given to nearest whole number.

T, Trace (less than 2 per cent); C.S., chondroitin sulphate; H.S., heparan sulphate; H.A., hyaluronic acid.

Table 2. RESULTS ON ISOLATED FIBRILS

Tissue	Case number	Percentage of dry weight Uronic acid	Sialic acid	Percentage of mucopolysaccharide C.S.	H.S.	H.A.	Percentage of C.S. resistant to hyaluronidase
Liver	2	0·302	0·625	35	65	—	< 5
Liver	3	0·318	0·487	66	34	—	100
Liver	4	0·256	0·597	23	77	—	17
Spleen	4	0·293	0·634	35	65	—	28
Spleen	5	0·154	0·476	100	—	—	< 5
Spleen	6	0·217	0·964	100	—	—	< 5
Liver	*A*2	0·388	0·505	19	81	—	< 5

Mean uronic acid, 0·275 per cent ±0·075; mean sialic acid = 0·613 per cent ±0·168.

Two glycoproteins with carbohydrate—predominantly neutral hexose in one and sialic acid in the other—have been isolated from fibrils (case 4 liver and *A*2) examined in this study[4]. Thus although the sialic acid content of isolated fibrils is low, it reflects the presence of an important moiety. The mean sialic and uronic acid contents of the fibrils examined here are similar to the figures of 0·56 and 0·34 per cent, respectively, given by Cohen[13] and it would not be surprising if the low uronic acid content also reflects the presence of an important moiety in the amyloid fibrils.

It is difficult to assess the extent to which isolated fibrils may be contaminated by loosely bound ground substance materials, but attempts to purify them further result in a higher, rather than lower, uronic acid content[13]. The present work shows that the uronic acid reflects the presence of material which must be very firmly bound to the isolated fibril to survive repeated washings in the extraction procedures used, a conclusion supported by the fact that none of this material could be isolated without enzymatic digestion. Acid mucopolysaccharides thus constitute a small but significant and integral part of the amyloid fibril.

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[1] Cohen, A. S., *New Engl. J. Med.*, **277**, 522 (1967).

[2] Burns, J., Pennock, C. A., and Stoward, P., *J. Pathol. Bacteriol.*, **94**. 337 (1967).

[3] Cohen, A. S., and Calkins, E., *J. Cell. Biol.*, **21**, 481 (1964).

[4] Nichols, R. B., and Clamp, J. R., *Clin. Chim. Acta*, **17**, 415 (1967).

[5] Manley, G., *Brit. J. Exp. Pathol.*, **46**, 125 (1965).

[6] Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).

[7] Bitter, T., and Muir, H., *Anal. Biochem.*, **4**, 330 (1962).

[8] Linker, A., Hoffman, P., Sampson, P., and Meyer, K., *Biochim. Biophys. Acta*, **29**, 443 (1958).

[9] Okuyama, T., and Turumi, K. I., *Clin. Chim. Acta*, **8**, 140 (1963).

[10] Bitter, T., and Muir, H., *J. Clin. Invest.*, **45**, 963 (1966).

[11] Mowry, R. W., and Scott, J. E., *Histochemie*, **10**, 8 (1967).

[12] Pennock, C. A., Burns, J., and Massarella, G., *J. Clin. Pathol.* (in the press).

[13] Cohen, A. S., *Lab. Invest.*, **15**, 66 (1966.

Biliprotein from the Ova of a Key-hole Limpet

THE mature ova of some limpets and key-hole limpets are green in colour[1]. In an attempt to identify the pigment responsible for the green colour of the ripe ova of the key-hole limpet *Diodora graeca* we have isolated from the ova a blue chromoprotein, which has a bile pigment as prosthetic group.

The key-hole limpets were collected in Malta from October to December, and ripe ovaries were dissected and weighed: 150–200 animals were found to give about 5 g of tissue. The material was homogenized with distilled water (250 ml.) and crushed ice (250 g) in a Waring blender. Octyl phenoxy polyethoxyethanol ('Triton *X*-100') (1 ml.) was added and the homogenate was stirred for 1 h and left at 4° C for 18 h. The homogenate was then centrifuged at 5,000*g* at 4° C for 1 h. The green precipitate was washed once in the centrifuge with distilled water (100 ml.). It was then dispersed in distilled water (100 ml.), tricalcium phosphate gel[2] (25 g) was added, and the mixture was stirred and centrifuged. The calcium phosphate was then washed with 0·1 M phosphate buffer, *p*H 7·0 (100 ml.), and subsequently with 0·1 M phosphate buffer, *p*H 7·0, containing 1 M sodium chloride (2 × 100 ml.), which gave a blue eluate. This eluate was half-saturated with ammonium sulphate, and the blue precipitate was collected by centrifuging at 5,000*g* at 4° C for 1 h. The precipitate was then dissolved in the desired buffer and dialysed against the same buffer at 4° C. Some protein which precipitated during dialysis was removed by centrifuging.

The blue protein solution showed a single component which migrated towards the cathode on cellulose acetate membrane electrophoresis with 0·1 M phosphate buffer, *p*H 7·0. Gel-filtration through a 50 × 2·5 cm column of 'Sephadex *G*-200' equilibrated with 0·05 M *tris* hydrochloric acid, *p*H 7·5, containing 0·1 M potassium chloride[3] indicated a molecular weight of 300,000 for the protein.

The prosthetic group was isolated as follows. A blue precipitate was obtained on dialysis of a solution of the protein in 0·1 M phosphate buffer, *p*H 7·0, against distilled water. The precipitate was collected by centrifuging and extracted with methanol at room temperature. The extract was evaporated to dryness in a current of air. The blue residue was soluble in chloroform as well as methanol. Thin-layer chromatography on silica gel *G* showed a single component with an average R_F of 0·6 in each of the following solvent systems: *iso*-propanol–concentrated ammonia–water (10 : 0·5 : 1, v/v); *n*-butanol–acetic acid–water (12 : 3 : 5, v/v); and chloroform–methanol–acetic acid (15 : 4 : 1, v/v).

The prosthetic group was identified as a bile pigment because it gave a positive Gmelin reaction; a solution of the pigment in aqueous methanol turned violet, then red, and finally colourless after the addition of concentrated nitric acid.

The absorption spectrum of the pigment in 5 per cent (w/v) methanolic hydrochloric acid showed maxima at 615, 396 and 325 mμ and inflexions at 420, 365 and 270 mμ. The zinc complex salt of the pigment showed maxima at 610 and 405 mμ in chloroform solution, and exhibited weak green fluorescence in ultraviolet light. On oxidation with iodine in the presence of ammonia, it showed maxima at 610, 515 and 365 mμ and an inflexion at 405 mμ.

The pigment seems to be a bilidiene in view of its blue colour, absorption spectrum, reaction with nitric acid, and formation of a zinc complex with fluorescence in ultraviolet light.

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[1] Ward, J., *Bull. Mar. Sci.*, **16**, 685 (1966).
[2] Swingle, S. M., and Tiselius, A., *Biochem. J.*, **48**, 171 (1951).
[3] Andrews, P., *Biochem. J.*, **96**, 595 (1965).

Effect of Orotic Acid on Activity of Nuclear DNA-dependent RNA Polymerase and Polyribosomal Profiles in Deficient Chick Liver lacking Vitamin B_{12}

Orotic acid, like vitamin B_{12}, increases the RNA[1] and free nucleotides[2] in the liver of chicks deficient in vitamin B_{12} and stimulates the incorporation of phosphate labelled with phosphorus-32 into these structures[3]. More recently, a significant increase in the incorporation of ^3H-formate into RNA of subcellular fractions of liver and into acid-soluble free nucleotides has been found in chicks treated with orotic acid[4].

This communication concerns a comparison of the effect of orotic acid and vitamin B_{12} on the activity of nuclear DNA-dependent RNA polymerase and on polyribosomal profiles in chick livers deficient in vitamin B_{12}.

We have investigated the part played by orotic acid in the metabolism of nucleic acid. Orotic acid is a growth factor for chicks and rats which are fed on a diet deficient in vitamin B_{12}[5,6].

One day old New Hampshire × White Leghorn chicks were divided into three groups. One group received a diet deficient in vitamin B_{12}[7], the other two groups received the same diet supplemented with either 10 μg of vitamin B_{12} or 10 mg of orotic acid in every 100 g of diet. After 5 weeks, six chicks from each group were injected with sodium ^3H-formate in a sterile solution (50 μCi/100 g body weight). The chicks were killed 2 h later and the livers were quickly removed. Ribosomes were obtained from the mitochondria-free supernatant of a whole liver homogenate treated with bentonite[8], and were treated with sodium deoxycholate (final concentration 1·3 per cent w/v). Ribosomes suspended in a medium according to Tata and Widnell[9] were layered over a 15–30 per cent w/v linear sucrose density gradient. After centrifuging for 3 h at 63,000*g*, 0·5 ml. fractions were collected; extinction was measured at 260 mμ and radioactivity was determined by adding scintillator fluid[10] in a Nuclear Chicago automatic spectrometer. Sedimentation coefficients were calculated[11,12].

Table 1. EFFECT OF OROTIC ACID AND VITAMIN B_{12} ON DNA-DEPENDENT RNA POLYMERASE ACTIVITY IN VITAMIN B_{12}-DEFICIENT CHICK LIVER

Group	Animals in experiment	RNA polymerase activity (percentage of control) Activated by Mg^2	Activated by $Mn^{2+}/(NH_4)_2SO_4^-$
1	Vitamin B_{12}-deficient (control)	100	100
2	Orotic acid-treated	131	261
3	Vitamin B_{12}-treated	107	271

Results are the averages of three determinations of pooled livers (three livers per pool). Duplicate experiments agreed within ±10 per cent. Magnesium-activated RNA polymerase activity in the control was 148 μμmoles of ^{14}C-AMP incorporated/15 min/mg of DNA. Manganese-activated RNA polymerase activity in the control was 98 μμmoles of ^{14}C-AMP incorporated/45 min/mg of DNA.

RNA polymerase activity, dependent on nuclear DNA, was assayed in liver of other animals from the same experimental groups. Both the reactions which are dependent on magnesium and those dependent of manganese and ammonium sulphate were assayed by measuring the incorporation of ^{14}C-AMP into the acid-insoluble RNA[9]. The radioactivity of samples labelled with carbon-14 dissolved in formic acid was measured by adding ethyleneglycol monomethylether and the liquid scintillator[10].

Profiles of the sedimentation of polyribosomes from chicks treated with orotic acid (Fig. 1, graph 2) as well as from those treated with vitamin B_{12} (Fig. 1, graph 3) show higher radioactivities in the peaks *d*, *e* and *f*, which correspond to 206 *S*, 259 *S*, and 280 *S* polyribosomal aggregates, respectively, when compared with the profile of chicks deficient in vitamin B_{12} (Fig. 1, graph 1).

The results for the activity of DNA-dependent RNA polymerase show that the reaction activated by manganese and ammonium sulphate is significantly increased in

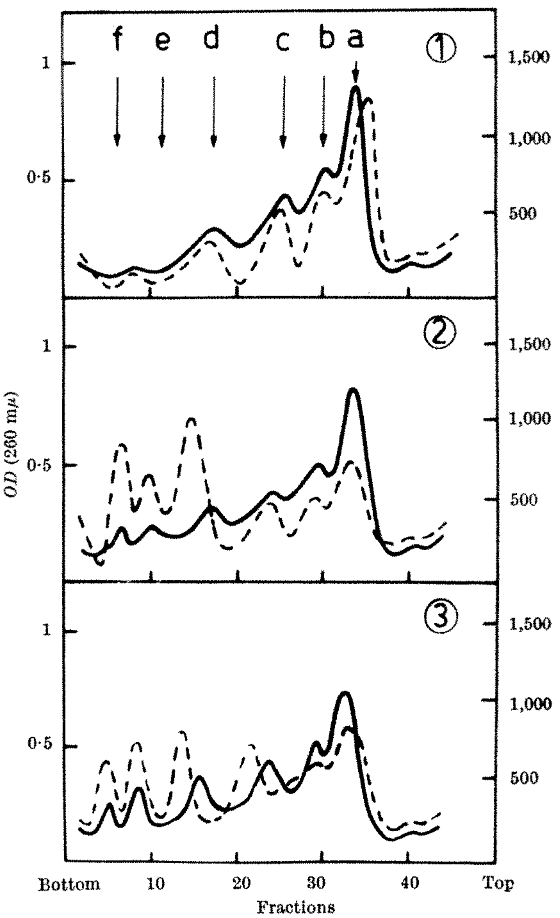

Fig. 1. Effect of orotic acid and vitamin B_{12} on profiles of polyribosomal sedimentation chick liver deficient in vitamin B_{12}; Graph 1, vitamin B_{12}-deficient; graph 2, treated with orotic acid; graph 3, treated with vitamin B_{12}; (peak *a* = 73 *S*; *b* = 113 *S*; *c* = 147 *S*; *d* = 206 *S*; *e* = 259 *S*; *f* = 280 *S*). ———, Optical density at 260 mμ; - - - -, radioactivity.

the liver of chicks treated with orotic acid or vitamin B_{12}, while no changes are found in the reaction activated by magnesium. The higher optical density and radioactivity of the regions corresponding to the polyribosomal aggregates of 5, 7 and 8 repeating units indicate a positive effect of orotic acid not only on the ability of ribosomes to be aggregated, but also on the levels of rapidly labelled cytoplasmic RNA. This is in agreement with the increased manganese/ammonium sulphate-activated RNA polymerase activity, observed in the orotic acid-treated chicks. According to Widnell and Tata[13], the product of the reaction activated by manganese and ammonium sulphate represents (in terms of base composition and nearest-neighbour frequency) a more DNA-like RNA, whereas that of the reaction activated by magnesium is a ribosomal type of RNA.

These results lead us to suggest that orotic acid has a positive effect on the biosynthesis of *m*RNA.

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[1] Marchetti, M., Caldarera, C. M., and Moruzzi, G., *G. Biochim.*, **9**, 378 (1960).
[2] Marchetti, M., Caldarera, C. M., and Moruzzi, G., *Biochim. Biophys. Acta*, **55**, 218 (1962).
[3] Marchetti, M., Caldarera, C. M., and Puddu, P., *G. Biochim.*, **10**, 519 (1961).
[4] Caldarera, C. M., Barbiroli, B., and Marchetti, M., *Experientia*, **23**, 521 (1967).
[5] Marchetti, M., Viviani, R., and Rabbi, A., *Nature*, **178**, 805 (1956).
[6] Moruzzi, G., Viviani, R., and Marchetti, M., *Biochem. Z.*, **333**, 318 (1960).
[7] Spivey Fox, M. R., Brigg, G. M., and Ortiz, L. O., *J. Nutrit.*, **70**, 283 (1957).
[8] Petermann, M. L., *The Physical and Chemical Properties of Ribosomes*, 44 (Elsevier Publ. Co., Amsterdam, 1964).
[9] Tata, J. R., and Widnell, C. C., *Biochem. J.*, **98**, 604 (1966).
[10] Bray, G. A., *Analytical Biochem.*, **1**, 279 (1960).
[11] Martin, R. G., and Ames, B. N., *J. Biol. Chem.*, **236**, 1372 (1961).
[12] Wettstein, F. O., Staehelin, T., and Noll, H., *Nature*, **197**, 430 (1963).
[13] Widnell, C. C., and Tata, J. R., *Biochim. Biophys. Acta*, **123**, 478 (1966).

Polyoma Virus-induced Release of Inhibition of Cellular DNA Synthesis caused by Iododeoxyuridine

THE halogenated pyrimidine nucleosides, 5-iodo-2′-deoxyuridine (IUdR) and 5-bromo-2′-deoxyuridine (BUdR), are incorporated instead of thymidine into the DNA of mammalian cells for one division cycle. The synthesis of DNA is then delayed and takes place only in a small fraction of the cells[1]. At high concentrations of these compounds (or at low concentrations when 5-fluoro-2′-deoxyuridine (FUdR) or amethopterin is present and inhibits endogenous synthesis of thymidine) the DNA doubles once; no further increase in the DNA content of the cultures is observed, even after the analogues have been removed[2,3].

The mechanism of inhibition of DNA synthesis by the halogenated pyrimidine nucleosides has been attributed to either (1) a malfunction of the IUdR-containing genes involved in the regulation of DNA synthesis, or (2) the presence of IUdR at special sites in the DNA which results in steric hindrance and prevents the replication of DNA[1-5]. The experiments described here were an attempt to differentiate between these possibilities.

Infection with polyoma virus of cells in stationary phase, or of X-irradiated cells that have lost their ability to synthesize DNA, results in the resumption of cellular DNA synthesis[6-8]. Cells that have been treated with IUdR before infection can support the growth of polyoma virus[9], and so it was interesting to determine whether such cells would also regain their ability to synthesize cellular DNA.

Primary mouse embryo (ME) cells grown in Eagle's medium[10] containing 3 per cent dialysed serum (EDS) were infected with the small plaque variant of polyoma virus[11] (multiplicity of infection, approximately 20 PFU/cell). The determination of the incorporation of ^{14}C- or ^{3}H-adenine, ^{3}H-deoxycytidine, and ^{14}C-thymidine or ^{3}H-thymidine into DNA, as well as the extraction and isopycnic centrifugation in caesium chloride of DNA, have been described before[12]. Cellular DNA was separated from viral DNA on methylated–albuminin–kieselguhr columns (MAK), also as described before[13].

We determined first the degree of incorporation of IUdR into the DNA of mouse embryo cultures and whether these cultures are able to resume DNA synthesis after removal of the analogue. One day after seeding, the cultures (containing approximately $1·5 \times 10^6$ cells/90 mm Petri dish) were incubated in EDS with FUdR (2 μg/ml.) and IUdR (20 μg/ml.) for 3 days with daily changes of medium. Cultures treated identically but which were supplied with thymidine (20 μg/ml.) instead of IUdR served as controls. The degree of replacement of IUdR for thymidine in the DNA of the IUdR-treated cultures was ascertained from the buoyant density of the DNA in caesium chloride in the Spinco Model *E* analytical centrifuge, as described before[14]. Two peaks of DNA appeared: one of these peaks, containing approximately one third of the DNA in the sample, consisted of normal thymidine-containing DNA ($\rho = 1·701$ g/cm^3); the second peak had a buoyant density of 1·757 g/cm^3 and consisted of hybrid DNA composed of one strand of normal, thymidine-DNA and one in which about 95 per cent of the thymidine has been replaced by IUdR. The presence of normal thymidine-containing DNA in the sample indicates that not all the DNA of the cells in the culture replicated during the period of incubation with IUdR.

Table 1. EFFECT OF TREATMENT WITH IUdR BEFORE INFECTION ON DNA SYNTHESIS IN POLYOMA VIRUS-INFECTED AND UNINFECTED CELLS

	IUdR	Thymidine
	(c.p.m./sample)	
Uninfected	1,520	7,100
Infected	10,900	14,291

Cultures treated with IUdR, as described before, were washed extensively and were infected with polyoma virus. Twenty-four hours later, ^{3}H-thymidine (0·5 μCi/ml.; specific activity, 416 mCi/mM) was added for 1 h. The cultures were collected and the amount of the label in the DNA was determined. Table 1 shows that incorporation of thymidine into DNA was reduced in non-infected cultures by about 80 per cent after treatment with IUdR. The infected IUdR-treated cultures synthesized, however, only 23 per cent less DNA than the infected cultures treated with thymidine. Similar results were obtained when the incorporation of ^{3}H-adenine into DNA was tested. The type of DNA (viral or cellular) synthesized by the infected IUdR-treated and thymidine-treated cultures was determined. In both cases, approximately 85 per cent of the label was found in cellular DNA as indicated by its elution pattern from MAK columns. Thus cultures which have incorporated IUdR into DNA regain the ability to synthesize cellular DNA after infection with polyoma virus. The cultures treated with IUdR, however, contained cells that had not replicated in the presence of IUdR, shown by the fact that a fraction of the DNA of these cultures consisted of normal DNA containing thymidine, and it was therefore necessary to determine whether DNA molecules containing IUdR had actually replicated after infection. One day after seeding, mouse embryo cultures were incubated for 8 h with ^{14}C-thymidine (0·06 μCi/ml., specific activity, 31 mCi/mM). The label was removed and the cultures were incubated for 3 days with IUdR and FUdR, as already described. (At the end of this incubation period, DNA labelled with carbon-14 and with two different buoyant densities was present in the cultures. Of the radioactivity, 34 per cent was associated with normal thymidine-DNA and the rest with hybrid IUdR-DNA.) The cultures were washed extensively to remove IUdR, infected with polyoma virus, and incubated with unlabelled thymidine and ^{3}H-adenine for 48 h. IUdR had been removed from the medium before the addition of ^{3}H-adenine, and so this label would become associated with IUdR-hybrid DNA only if the molecules of hybrid IUdR-DNA present in the cultures at the time of infection were to replicate.

Table 2. REPLICATION OF IUdR-HYBRID DNA IN POLYOMA VIRUS-INFECTED AND UNINFECTED CELLS

	Tritium label in DNA			
	Infected		Uninfected	
	c.p.m./ sample	Percentage of total	c.p.m./ sample	Percentage of total
Thymidine DNA	28,354		9,365	
IUdR-hybrid DNA	15,943	37·6	1,136	10·7
Incorporation resulting from IUdR-hybrid DNA replication	31,886	75·2	2,272	21·5

Infected and uninfected cultures treated with IUdR before infection were incubated for 48 h in EDS containing thymidine (10 μg/ml.) and ^{3}H-adenine (1 μCi/ml.; specific activity, 7 mCi/mM). The DNA extracted from the cultures was subjected to equilibrium sedimentation in caesium chloride and the amount of tritium associated with the peaks of DNA was determined. Of the total ^{3}H-adenine in the DNA of the infected cells 5 per cent was in viral DNA (as determined by its ability to renature after heat denaturation). Viral DNA will consist of thymidine DNA, and so 5 per cent of the total counts in the sample was subtracted from the thymidine DNA before tabulation.

Table 2 shows the distribution of tritium in the peaks of hybrid-IUdR and normal thymidine DNA obtained from infected and uninfected cultures. In the uninfected cells, approximately 11 per cent of the label was associated with IUdR-hybrid DNA, and because the replication of a hybrid DNA molecule in the conditions described will give rise to one ^{3}H-thymidine-IUdR molecule and one ^{3}H-thymidine–thymidine molecule, 22 per cent of the total radioactivity incorporated into DNA was due to the replication of hybrid IUdR-DNA. The relative frequency at which the IUdR-hybrid DNA molecules replicated was thus considerably reduced as compared with the molecules containing thymidine, for 66 per cent of the DNA in the cultures was IUdR-hybrid DNA and only 22 per cent of the incorporation of label into DNA in the cultures resulted from the replication of this DNA. These results differ somewhat from those of Morris and Cramer[15,16], who found that half of the IUdR-containing molecules can replicate after removal of IUdR from the cultures.

The proportion of IUdR-hybrid DNA molecules that replicated was increased after infection of the cultures with polyoma virus (Table 2). Approximately 75 per cent of the incorporation into DNA was calculated to be caused by the replication of hybrid IUdR-DNA; this DNA comprised 66 per cent of the DNA in the culture, and infection with polyoma virus thus seems to have resulted in the complete resumption of the ability of IUdR-hybrid molecules to replicate.

We next tested whether hybrid IUdR-containing molecules would also replicate after infection when IUdR was not removed from the cultures, and whether DNA with IUdR in both strands would thus be synthesized. An experiment essentially analogous to the one already described was performed. Mouse embryo cells were incubated with FUdR (2 μg/ml.), IUdR (20 μg/ml.) and ^{3}H-deoxycytidine (2 μCi/ml.; specific activity, 1·25 Ci/mM) for 3 days. At the end of this incubation period, only DNA with a density corresponding to hybrid IUdR-DNA had become labelled. The cultures were washed, infected with polyoma virus, and incubated with FUdR (2 μg/ml.), IUdR (20 μg/ml.), deoxycytidine (10 μg/ml.) and ^{14}C-adenine (0·04 μCi/ml.; specific activity, 55 mCi/mM) for 48 h. The cells were collected and DNA was extracted and subjected to isopycnic centrifugation in caesium chloride. Fig. 1 shows that all the ^{14}C-adenine incorporated into the DNA of uninfected cultures banded in a position characteristic of hybrid IUdR-DNA, and that no DNA molecules with IUdR in both strands were formed. In the infected cultures, however, about 30 per cent of the carbon-14 was present in DNA that banded in a position characteristic of double-stranded IUdR-DNA. This DNA consisted, at least partially, of cellular DNA, for tritiated DNA that had been present in the cells at the time of infection shifted to the position of double-stranded IUdR-DNA. (Cellular DNA is not degraded into an acid-soluble form after infection.) These experiments show clearly that whereas in uninfected cultures DNA synthesis is inhibited in most of the cells that have incorporated IUdR into one strand of this DNA, this inhibition is reversed after infection with polyoma virus.

Cellular DNA synthesis is renewed after infection of cells in which IUdR has been substituted for thymidine in one strand of DNA, and so the inhibitory effect of IUdR is probably not a result of its presence *per se* in the DNA and a consequent physical hindrance of replication. This renewal, however, may be caused by the synthesis in the infected cells of a DNA polymerase which, in contrast to the polymerase present in uninfected cells, is able to use IUdR-hybrid DNA as a template. The renewal of DNA synthesis may reflect also the fact that the incoming viral genome carries the information for the formation of proteins (enzymes or regulatory proteins) necessary for the replication of cellular DNA, these proteins not being functional when coded by DNA which contains IUdR. On the other hand, the regulatory mechanisms normally controlling cellular DNA synthesis may not be operative in cells infected with polyoma virus.

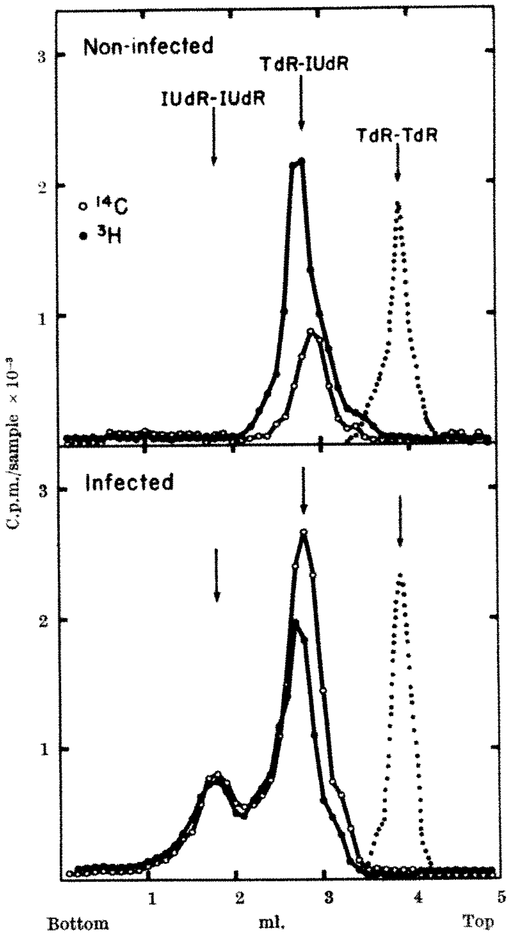

Fig. 1. Formation in cells infected with polyoma virus of cellular DNA containing IUdR in both strands. See text for experimental details. TdR, thymidine.

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[1] Simon, E. H., *Exp. Cell Res.*, suppl., **9**, 263 (1963).
[2] Hakala, M. T., *J. Biol. Chem.*, **234**, 3072 (1959).
[3] Littlefield, J. W., and Gould, E. A., *J. Biol. Chem.*, **235**, 1129 (1960).
[4] Toliver, A., and Simon, E. H., *Exp. Cell Res.*, **45**, 603 (1967).
[5] Kajiwara, K., and Mueller, G. C., *Biochim. Biophys. Acta*, **91**, 486 (1964).
[6] Dulbecco, R., Hartwell, L. H., and Vogt, H., *Proc. US Nat. Acad. Sci.*, **53**, 403 (1965).
[7] Weil, R., Michel, M. M., and Ruschmann, G. K., *Proc. US Nat. Acad. Sci.*, **53**, 1468 (1965).
[8] Gershon, D., Hauser, P., Sachs, L., and Winocour, E., *Proc. US Nat. Acad. Sci.*, **54**, 1584 (1965).
[9] Munyon, W., Hughes, R., Angerman, J., Bereczky, E., and Dmochowski, L., *Cancer Res.*, **24**, 1880 (1964).
[10] Eagle, H., *Science*, **130**, 432 (1959).
[11] Stanners, C. P., *Virology*, **21**, 464 (1963).
[12] Ben-Porat, T., and Kaplan, A. S., *Virology*, **25**, 22 (1965).
[13] Ben-Porat, T., Coto, C., and Kaplan, A. S., *Virology*, **30**, 74 (1966).
[14] Kaplan, A. S., and Ben-Porat, T., *J. Mol. Biol.*, **19**, 320 (1966).
[15] Morris, N. R., and Cramer, J. W., *Mol. Pharmacol.*, **2**, 1 (1966).
[16] Cramer, J. W., and Morris, N. R., *Mol. Pharmacol.*, **2**, 363 (1966).

L-Asparaginase Activity in Human and Animal Sera

AN outstanding characteristic of the amino-acid enzyme L-asparaginase is its ability to inhibit the growth of certain rodent leukaemias[1-3]. This was established by Broome[4], who followed up the observation by Kidd[5] that administration of guinea-pig serum inhibited the growth of the Gardner lymphosarcoma (tumour *6C3HED*) in *C3H* mice. L-Asparaginase can be extracted not only from guinea-pig serum but also from certain types of *Escherichia coli*[6,7].

Administration of preparations of *Escherichia coli* asparaginase to three dogs with lymphosarcoma produced remission in each[8,9]. A child with lymphoblastic leukaemia, resistant to conventional therapies, was given an extract of asparaginase from guinea-pig serum[10]; this caused amelioration of the leukaemic process, but it also caused severe side-effects. Three patients with acute leukaemia treated by Hill *et al.*[11] with an *Escherichia coli* asparaginase showed no immediate toxic effects, however, and there were definite signs of a favourable response. Further therapeutic evaluation of this material is limited by the difficulty in obtaining sufficient quantities of it.

Asparaginase activity can be determined by measuring the ammonia liberated in the transamination and hydrolysis of asparagine[12]. Precipitation by ammonium sulphate increases the specific activity of asparaginase from guinea-pig serum, but the more purified preparations have shown a decrease in biological activity[13]. L-Asparaginase from *E. coli* exists in two forms[14] with widely differing biological activities[15,16].

We have measured asparaginase activity by the method of Meister[17], the liberated ammonia being determined by treatment of the protein-free supernatant with Nessler's reagent. The units of asparaginase activity are defined as the number of μmoles of ammonia liberated at the maximum rate from 1 ml. of test solution in 1 h in the conditions of the reaction.

L-Asparaginase activity was not detected in any of twenty-five human sera from control subjects. Similarly, sera from a variety of animal species (horse, cow, pig, sheep, dog, monkey, rabbit and hen) were also assayed and in none of these was any activity found. Holmquist[18] found similar results with four human sera. We further tested the effect of the addition of various sera on the asparaginase activity of guinea-pig sera and coliform extracts (Worthington Biochemicals), having established that dilution of these with borate buffer did not influence the recovery of the enzyme. The test system consisted, of two aliquots of solution containing asparaginasee diluted with either borate buffer (x') or the serum to be tested (x). After thorough mixing, samples were taken into the buffered incubation medium for asparaginase assay.

Table 1. UNITS OF ASPARAGINASE ASSAYED IN GUINEA-PIG SERA IN BUFFER (x') AND AFTER THE ADDITION OF HUMAN SERUM (x)

Units assayed after addition of borate buffer (x')	Units assayed after addition of serum (x)	$\frac{x-x'}{x'}$ (per cent)
80·2	96·5	20·2
63·1	77·6	23·5
98·4	104·6	6·3
92·1	97·5	5·9
50·5	54·9	8·7
23·0	27·8	20·8
58·7	64·5	9·9
37·9	43·1	13·8
40·5	45·2	12·4

Table 2. UNITS OF ASPARAGINASE ASSAYED IN GUINEA-PIG SERA IN BUFFER (x') AND AFTER THE ADDITION OF HUMAN SERUM (x)

Species	Animal No.	Units assayed after addition of borate buffer (x')	Units assayed after addition of serum (x)	$\frac{x-x'}{x'}$ (per cent)
Horse	1*a*	151·1	155·0	2·6
	1*b*	65·8	61·1	—
Cow	1*a*	134·5	135·6	0·7
	1*b*	126·5	127·0	0·4
	3	128·0	132·0	3·2
	4	126·5	128·5	1·6
Dog	1	99·7	109·9	1·2
Rabbit	1	84·9	86·1	1·4
Monkey	1	171·3	178·3	3·5
Hen	1	126·8	126·0	—
Sheep	1*a*	124·1	141·8	14·3
	1*b*	90·2	105·5	17·0
Pig	1*a*	126·5	138·2	9·3
	1*b*	117·3	126·3	7·6
	1*c*	96·0	107·5	12·0
	2	128·0	125·0	—
	3	128·0	128·0	—
	4	126·0	127·6	1·6

Table 3. UNITS OF ASPARAGINASE ASSAYED IN COLIFORM EXTRACT IN BUFFER (x') AND AFTER THE ADDITION OF HUMAN SERUM (x)

Units assayed after addition of borate buffer (x')	Units assayed after addition of serum (x)	$\frac{x-x'}{x'}$ (per cent)
64·4	78·7	21·8
55·4	78·1	41·0
62·4	71·1	14·0
88·0	107·7	22·4
124·0	144·4	16·4
116·3	139·0	19·5
105·4	140·8	32·8
112·8	138·1	22·6
95·6	122·4	27·8
80·5	108·3	34·6

Table 4. UNITS OF ASPARAGINASE ASSAYED IN THE COLIFORM EXTRACT IN BUFFER (x') AND AFTER THE ADDITION OF ANIMAL SERUM (x)

Species	Animal No.	Units assayed after addition of borate buffer (x')	Units assayed after addition of serum (x)	$\frac{x-x'}{x'}$ (per cent)
Horse	1	126·4	155·5	23·0
	1	112·9	138·3	20·0
Cow	1	111·3	139·0	25·7
	2	116·2	130·7	21·0
Dog	1	113·4	143·6	26·5
Rabbit	2	125·0	136·3	9·0
Monkey	1	114·3	138·6	21·0
Hen	1	114·4	134·4	17·5
Sheep	1	113·3	143·0	26·0
Pig	1	113·7	132·1	17·0

All human sera which were tested enhanced the asparaginase activity of the guinea-pig sera by 6 per cent to 23 per cent and the degree of enhancement did not correspond to the initial asparaginase content (Table 1). Of the animal sera tested, all except the sheep and pig gave clearly negative results (Table 2). Sera from four pigs were assayed and only one, tested on three occasions, showed any enhancement; sheep serum consistently enhanced the asparaginase activity of guinea-pig serum.

With the coliform extract the pattern of results is different, in that the addition of all human and animal sera gave enhanced activity (Tables 3 and 4).

We concentrated the protein fraction from human serum by ultrafiltration through a collodion membrane over an ice bath and tested both the protein concentrate and the filtrate containing the soluble salts and amino-acids, for their effect on the assay of asparaginase from guinea-pig serum. The asparaginase enhancing effect was contained in the protein moiety, the protein-free fraction having no such effect.

Experiments are to be undertaken to define further the serum factors responsible for the enhancement of asparaginase activity and to attempt to determine whether this has any biological counterpart.

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[1] *Lancet*, i, 431 (1967).
[2] Boyse, E. A., Old, L. J., and Stockert, E., *Nature*, **198**, 800 (1963).
[3] Boyse, E. A., Old, L. J., Campbell, H. A., and Mashburn, L. T., *J. Exp. Med.*, **125** (1), 17 (1967).
[4] Broome, J. D., *J. Exp. Med.*, **118**, 99 (1963a).
[5] Kidd, J. G., *J. Exp. Med.*, **98**, 565 and 583 (1953).
[6] Mashburn, L. T., and Wrinston, J. C., *Biochem. Biophys. Res. Commun.*, **12**, 50 (1963).
[7] Mashburn, L. T., and Wrinston, J. C., *Arch. Biochem. Biophys.*, **105**, 450 (1964).
[8] Old, L. J., Boyse, E. A., Campbell, H. A., Brodley, H. S., Fidler, J., and Teller, J. D., *Cancer*, **20** (7), 1066 (1967).
[9] Old, L. J., Boyse, E. A., Campbell, H. A., Brodley, H. S., Fidler, J., and Teller, J. D., *Lancet*, i, 447 (1967).
[10] Dolowy, W. C., Henson, D., Cornett, J. A., and Sellin, H., *Cancer*, **19** (12), 1813 (1966).
[11] Hill, J. M., Roberts, J., Loeb, E., Khan, A., McLellan, A., and Hill, R. W., *J. Amer. Med. Assoc.*, **202**, 882 (1967).
[12] Meister, R. A., Sober, H. A., Tice, S. V., and Frazer, P. E., *J. Biol. Chem.*, **197**, 319 (1952).
[13] Broome, J. D., *J. Exp. Med.*, **118**, 121 (1963b).
[14] Roberts, J., Prager, M. D., and Bachynsky, M., *Cancer Res.*, **26** (10), 2213 (1966).
[15] Hiramoto, R., Tate, C., and Hamlin, M., *Proc. Soc. Exp. Biol. and Med.*, **121**, 597 (1966).
[16] Campbell, H. A., Mashburn, L. T., Boyse, E. A., and Old, L. J., *Biochemistry*, **6** (3), 71 (1967).
[17] Meister, R. A., *Methods of Enzymology*, **2**, 380 (Academic Press, London and New York, 1955).
[18] Holmquist, M. D., *Proc. Soc. Exp. Biol. and Med.*, **113**, 444 (1963).

GENETICS

Albumin Máku: a New Variant of Human Serum Albumin

AT least six different electrophoretic variants of human serum albumin have been found in man; three migrate more rapidly than normal and three less rapidly[1,2]. One of these, a rapidly migrating variant, occurs at a frequency of 5–30 per cent in several tribes of North American Indians[3]. As one aspect of a multidisciplinary study of the Yanomama Indians of southern Venezuela and adjacent parts of Brazil[4], sera from 1,111 individuals residing in twenty-five villages were examined for albumin type. Although no example of the rapidly migrating variant which is common in North American Indians was observed, we did note, when testing 75 of 108 persons residing at the village of Borabuk (2° 45′ N. and 62° 05′ W.), four individuals who possess a variant which also migrates more rapidly towards the anode in starch gel electrophoresis than does normal albumin.

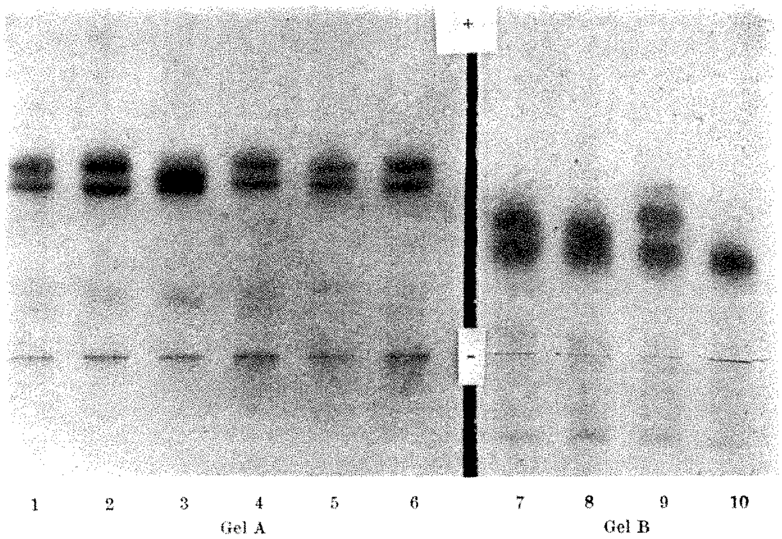

Fig. 1. Albumin Máku. Comparative vertical starch gel electrophoresis using the three types of rapidly migrating albumin variants and slight modifications of the electrophoretic conditions detailed in Weitkamp *et al.*[1]. Gel A: Electrophoresis for 5 h in the *tris*-EDTA-borate system, *p*H 6·9 (starch hydrolysed, Lot 245–1, Connaught Laboratories, Toronto) Gel B: Electrophoresis for 19 h in the acetate-EDTA system, *p*H 5·0 (Lot 174, Electrostarch Co., Madison, Wisconsin). Positions 1, 4, 6, 8: Albumin Máku; 2, 9: "very fast"; 3: "fast"; 5, 7: "faster".

The fact that this variant is different from the three previously described types of rapidly migrating albumin variants[1] can be seen in the photograph of two starch gel electropherograms in Fig. 1. The new variant clearly migrates more rapidly than the "fast" type in Gel A and more slowly than the "very fast" type in Gel B. The "faster" type of variant, which is polymorphic in North American Indians, migrates less rapidly compared with the new variant in Gel A and more rapidly compared with the new variant in Gel B.

The four individuals who are heterozygous for normal and variant albumin are related as indicated in the pedigree in Fig. 2. Three of five possible mates of I-1 and

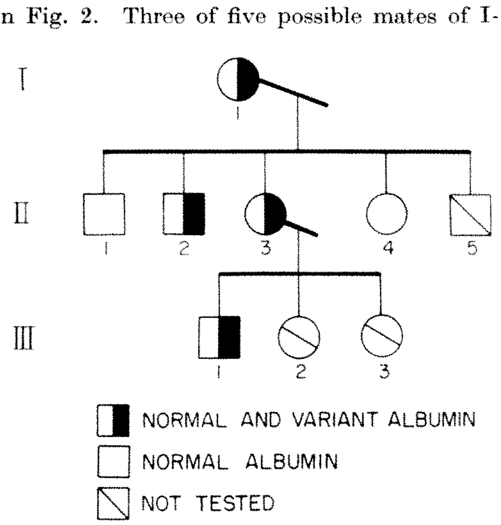

Fig. 2. Pedigree of the family from Borabuk showing albumin phenotypes. Three of five possible spouses for I-1 and one of two possible spouses for II-3 were tested and demonstrated normal albumin only. (Squares designate males, circles females.)

one of two possible mates of II-3 were tested and showed normal albumin only. Although the albumin types for the other possible mates are not known, this variant, because it is uncommon in the village, is probably inherited through the individuals identified in the pedigree. All of the individuals heterozygous at the albumin locus are homozygous at the *Gc* locus; consequently, the family does not provide an opportunity to test the possibility of close autosomal linkage between loci for serum albumin and *Gc* protein which has been described in families segregating for other albumin variants[2,5,6].

The new albumin variant was introduced into the Yanomama village of Borabuk by individual I-1 (Fig. 2), a woman captured from the Máku tribe[7] in a raid some time during the early 1930s. In 1925 the Máku were still a viable cultural entity, maintaining settlements of their own on the Rio Uraricoera[8]. During an eastward expansion by the Yanomama, however, the Máku were reduced to virtual extinction: by 1964 only three individuals were known to be able to speak the Máku language[9]. Although a number of Máku may have been incorporated into a second Yanomama group on the Rio Uraricaá[9], there seem to be few individuals who have survived to reproduce within Yanomama society. The woman who did survive, however, was not subjected to the traditional Yanomama customs relating to marriage and reproduction because she was an outsider[10]. She provided several men in the village with an opportunity to reproduce earlier in their lives—men who might otherwise not have obtained a spouse until later, or perhaps not at all. She was, in turn, provided with reproductive opportunity which was somewhat better than usual, as witnessed by the fact that she has five surviving children (compared with an average of 3·2 for post-menopausal Yanomama women)[11]. The probability of retention of the new gene which this woman introduced into the village may therefore have been partly determined by cultural factors affecting her reproductive behaviour.

The new albumin variant, apparently foreign to the Yanomama population, was introduced by a captive member from the nearly extinct Máku tribe. In consideration of this once numerous and culturally significant but now dying people, we propose to name the new variant Albumin Máku, at least until a proper name which identifies the structural alteration in the variant can be assigned.

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[1] Weitkamp, L. R., Shreffler, D. C., Robbins, J. L., Drachmann, O., Adner, P. L., Wieme, R. J., Simon, N. M., Cooke, K. B., Sandor, G., Wuhrmann, F., Braend, M., and Tárnoky, A. L., *Acta Genet. Stat. Med.*, **17**, 399 (1967).

[2] Weitkamp, L. R., Robson, E. B., and Shreffler, D. C., *Abst. Amer. Soc. Hum. Genet., Toronto*, No. 44, 42 (1967).

[3] Melartin, L., and Blumberg, B. S., *Science*, **153**, 1664 (1966).

[4] Arends, T., Brewer, G., Chagnon, N., Gallango, M. L., Gershowitz, H., Layrisse, M., Neel, J. V., Shreffler, D., Tashian, R., and Weitkamp, L., *Proc. US Nat. Acad. Sci.*, **57**, 1252 (1967).

[5] Weitkamp, L. R., Rucknagel, D. L., and Gershowitz, H., *Amer. J. Hum. Genet.*, **18**, 559 (1966).

[6] Kaarsalo, E., Melartin, L., and Blumberg, B. S., *Science*, **158**, 123 (1967).

[7] Koch-Grünberg, T., in *Festschrift Eduard Seler* (Strecker und Schröder, Stuttgart, 1922); *Vom Roroima Zum Orinoco*, **1** (Dietrich Reimer, Berlin, 1917) and **3** (Strecker und Schröder, Stuttgart, 1923).

[8] Rice, A. H., *Geograph. J.*, **71**, 113, 209, 345 (1928).

[9] Migliazza, E., *Boletim do Museu Paraense Emilio Goeldi*, **22**, 1 (1964); **25**, 1 (1965).

[10] Chagnon, N. A., dissert., Univ. Michigan (1966).

[11] Neel, J. V., and Chagnon, N. A., *Proc. US Nat. Acad. Sci.* (in the press).

BIOLOGY

Discrimination between Heavy Water and Water by the Mouse

THE experiment described here indicates that mice are able to discriminate between D_2O and H_2O. Two male litter mates of *Mus musculus domesticus* (strain *T–O*) were kept in separate Harwell type cages, each fitted with two water bottles. In the experimental cage, one bottle contained D_2O and the other glass-distilled H_2O, and in the control cage each bottle contained glass-distilled H_2O. The bottles were refilled every 24 h and the quantity taken from each during the previous day was determined. The bottles were replaced in the cages in a random manner, following Fisher and Yates's table of random numbers. The experiment was continued for 14 days and Table 1 shows the quantities of liquid taken during that period by the two mice.

Table 1

Experimental cage		Control cage	
H_2O (ml.)	D_2O (ml.)	H_2O (ml.)	H_2O (ml.)
12·8	2·8	7·2	10·2
13·2	3·2	15·2	13·4
15·2	2·1	10·0	12·0
14·2	2·1	9·5	12·0
12·2	3·6	14·4	5·0
14·0	3·6	12·0	15·0
15·2	4·2	14·6	17·4
10·8	2·0	4·4	11·0
9·6	2·4	5·0	12·2
11·4	1·0	8·0	4·0
12·0	1·2	7·2	3·2
12·6	3·4	8·0	10·2
10·2	1·8	10·0	5·2
9·5	1·4	5·2	7·4

It is clear from the results in Table 1 that *Mus musculus* can distinguish ($P \leqslant 0·001$) between H_2O and D_2O. There was no significant difference between the amounts taken from the two bottles in the control cage ($P > 0·7$).

This result is of interest in two different contexts. First, it has been proposed that the decreased fluid intake of rodents presented with D_2O or deuterated water is the result of a decrease in excretion[1]. My results suggest that distaste for D_2O may also contribute to the observed diminution in excretion. Second, Shallenberger[2] has suggested that the formation of hydrogen bonds between a molecule and a gustatory receptor site plays an important part in stimulating the receptor cell. It is known[3] that the bonds attaching deuterium to oxygen are considerably more stable than those attaching hydrogen to oxygen. The ability of D_2O and H_2O to form hydrogen bonds is thus significantly different. It is possible that the mouse uses this difference as the discriminandem between D_2O and H_2O.

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Received January 30, 1968.

[1] Thomson, J. F., *Biological Effects of Deuterium*, 96 (Pergamon Press, 1964).

[2] Shallenberger, R. S., and Acree, T. E., *Nature*, **216**, 840 (1967).

[3] Wiberg, K. B., *Chem. Rev.*, **55**, 713 (1955).

Phylogenetic Relationships among Venomous Snakes of the Genus *Agkistrodon* from Asia and the North American Continent

AMONG a great number of venomous snakes, *Agkistrodon* is the only genus which is found in both Asia and the North American continent. The Asiatic members of *Agkistrodon* consist of seven species and range from Japan, Formosa, China, south-east Asia (excluding Celebes and Philippines), India, Central Asia and southern Siberia, to the borders of the Caspian Sea and the Ural River. Three species of *Agkistrodon* are distributed on the North and central American continents, and are found in the south-eastern portion of the United States, all of Mexico and most of Central America. The *Agkistrodon* of Asia and America are morphologically similar.

It has been shown by a number of investigators that the chemical properties of venoms can be used as an index for taxonomy and phylogenetic relationships[1-3]. It is therefore of interest to investigate and compare the immunological properties of venoms of morphologically similar snakes which are geographically widely separated.

For this study, venoms of *A. contortrix laticinctus* (broadbanded copperhead), *A. contortrix contortrix* (southern copperhead) and *A. piscivorus piscivorus* (eastern cottonmouth) were selected from the United States. *A. bilineatus* (tropical moccasin) from Central America, *A. halys* (mamushi) from Japan and two venoms of *A. rhodostoma* (Malayan pit viper) from Asia were also selected. Venom was extracted from *A. rhodostoma* captured in the vicinity of Bangkok, Thailand. Another sample of venom was milked from snakes captured in the northern part of Malaya.

Antibodies to *A. rhodostoma* were obtained from horses immunized with snake venoms of Thailand origin. Antibodies to *A. contortrix laticinctus* were prepared from rabbits (New Zealand White) following immunization.

Immunodiffusion was carried out at 3° C for 24 h in 1 per cent 'Difco' agar made 0·85 per cent in sodium chloride and 3·75 per cent in glycine. Immunoelectrophoresis was carried out in 1 per cent agar, in 0·0275 M barbital at *p*H 7·00. Electrophoresis was run for 3 h using 260 V and 13 A at room temperature. Diffusion was carried out for 24 h at 28° C.

Venoms were used at a concentration of 10 mg/ml. for species other than *A. rhodostoma* tested against its own antibody, in which cases 20 mg/ml. was used.

It can be seen from Fig. 1 that precipitin lines obtained from Ouchterlony double diffusion for *A. rhodostoma* of Thailand and Malayan origins fused together. Immunoelectrophoretic patterns of these two snakes were also very similar. This evidence indicates that *A. rhodostoma* of Thailand and Malayan origins are identical. Considerably different precipitin lines were observed, however, with other venoms. Venom of *A. halys* from Japan gave two lines which were considerably darker than those for venoms of North and Central American origin. The immunoelectrophoretic pattern (Fig. 2) also indicated that *A. halys* is more similar to *A. rhodostoma* than North and Central American species. The pattern of *A. bilineatus* of Central America had the least similarity to that of *A. rhodostoma*.

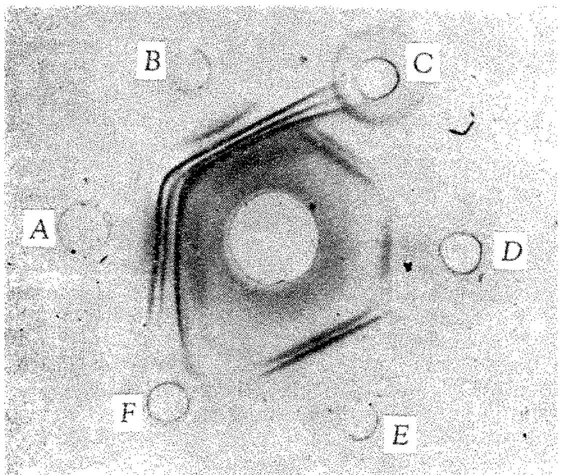

Fig. 1. Ouchterlony reaction of horse anti-*A. rhodostoma* venom of Thailand origin (centre well) against: (*A*) *A. rhodostoma* venom from Thailand; (*B*) *A. rhodostoma* venom from Malaya; (*C*) *A. contortrix contortrix* venom from North America; (*D*) *A. piscivorus piscivorus* venom from North America; (*E*) *A. halys* venom from Japan; (*F*) *A. contortrix laticinctus* venom from North America.

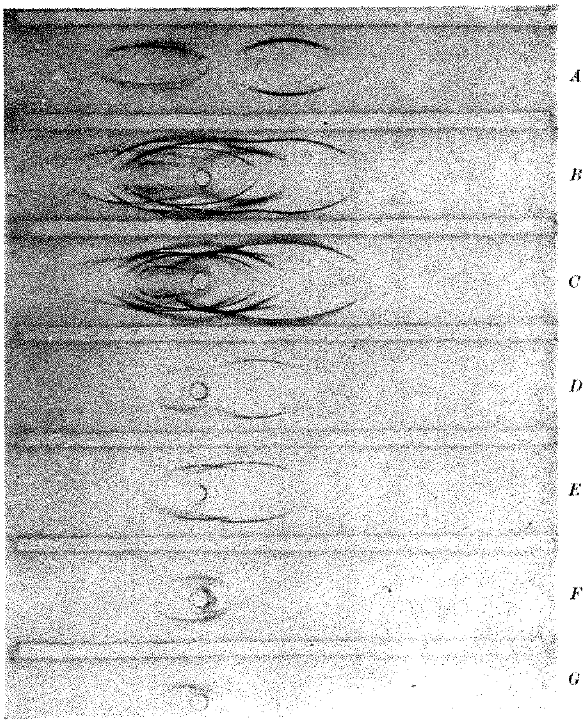

Fig. 2. Immunoelectrophoretic patterns of horse anti-*A. rhodostoma* of Thailand origin (in trough) against: (*A*) *A. halys* venom from Japan; (*B*) *A. rhodostoma* venom from Thailand; (*C*) *A. rhodostoma* venom from Malaya; (*D*) *A. contortrix laticinctus* venom from North America; (*E*) *A. contortrix contortrix* venom from North America; (*F*) *A. bilineatus* venom from Central America; (*G*) *A. piscivorus piscivorus* venom from North America.

It is of interest to note that a degree of similarity is related to geographical distance. Degree of kinship estimated from Fig. 1 and Fig. 2 is in the order *A. rhodostoma* (Thailand and Malaya), *A. halys* (Japan), three snakes from North America (*A. contortrix laticinctus*, *A. contortrix contortrix*, *A. piscivorus piscivorus*) and *A. bilineatus* (Central America).

When antibodies to *A. contortrix laticinctus* were used, immunoelectrophoretic patterns of the North American snake venoms became very similar (Fig. 3). Venom from *A. halys* of Japanese origin responded to antibodies to *A. contortrix laticinctus* more positively than venom of *A. rhodostoma* from either Thailand or Malaya. Venom from *A. bilineatus* of Central America gave sharp lines, but these were quite distinct from those of North American origin.

From these investigations, it can be concluded that in the order of the genetic kinship, *A. halys* from Japan lies between *A. rhodostoma* from south-east Asia and North American members of *Agkistrodon*. Phylogenetically, however, *A. halys* from Japan is somewhat closer to North American *Agkistrodon* than to south-east Asian *Agkistrodon*.

It is generally believed by zoologists that *Agkistrodon* of the North and Central American continent migrated from Asia when the American continent was connected to the Asiastic mainland, at the present Bering Strait area. This is also the case for many animals in North America. The Bering land bridge has been exposed or submerged to only shallow seas several times during prehistoric years; the most recent land bridge was about 12,000 yr ago[4]. Between 12,000 and 22,000 yr ago any migration of mammals was impossible because of the blockage by the western edge of the Cordilleran ice sheet[4]. Reptiles are more sensitive to cold climate than mammals. The migration of snakes to America must therefore have occurred earlier than 22,000 yr ago. Our immunological

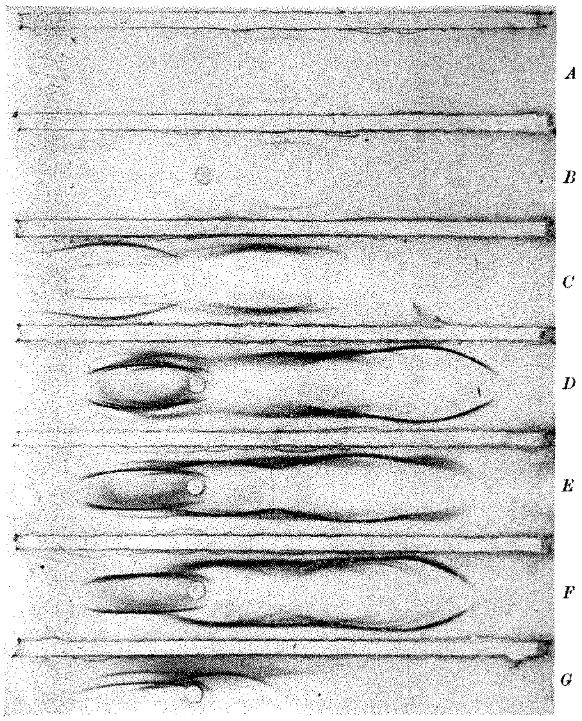

Fig. 3. Immunoelectrophoretic patterns of rabbit anti-*A. contortrix laticinctus* venom of North American origin (in trough) against: (*A*) *A. rhodostoma* venom from Thailand; (*B*) *A. rhodostoma* venom from Malaya; (*C*) *A. halys* venom from Japan; (*D*) *A. contortrix laticinctus* venom from North America; (*E*) *A. contortrix contortrix* venom from North America; (*F*) *A. piscivorus piscivorus* venom from North America; (*G*) *A. bilineatus* venom from Central America.

study indicated that differences between Asian and American snakes are considerable and it is very doubtful that such a large genetic difference could evolve in a time span of a mere 22,000 yr. It is known among geologists that Formosa was part of the Asiatic mainland some geological aeons ago; when we investigated the immunological difference of the Thailand cobra (*Naja naja siamensis*) and the Formosan cobra (*N. naja atra*), we found that the difference was very small[5].

We believe therefore that migration of snakes to America from Asia occurred much earlier than 22,000 yr ago.

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[1] Jimenez-Porras, J., *Toxicon*, **2**, 187 (1964).

[2] Tu, A. T., Toom, P. M., and Murdock, D. S., *Animal Toxins*, 351 (Pergamon Press, 1967).

[3] Tu, A. T., and Toom, P. M., *Austral. J. Exp. Biol. Med. Sci.*, **45**, 561 (1967).

[4] Hopkins, D. M., *The Bering Land Bridge* (Stanford University Press, California, USA, 1967).

[5] Tu, A. T., and Ganthavorn, S., *Toxicon*, **5**, 207 (1968).

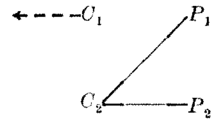
Adoptive Suckling in the Grey Seal

ALMOST all accounts of the natural history of the grey seal *Halichoerus grypus* give the impression that a cow invariably seeks and feeds only her own pup among the dozens or hundreds in a breeding colony which may be at an equivalent stage of development as her pup. Normal feeding fidelity may be represented thus

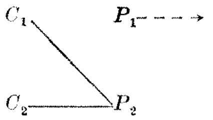
$$\begin{tikzcd} C_1 \arrow[r, dash] & P_1 \\ C_2 \arrow[r, dash] & P_2 \end{tikzcd}$$

etc.

Although there is no doubt that this is frequently the case, several patterns of adoptive feeding can and do occur both spontaneously and as a result of disturbance. Hewer and Backhouse[1] and others have reported a cow seen attending two pups, often of dissimilar size. Twins have not been recorded with certainty for this species, and so it can be assumed that one of these pups had been deserted by its mother. This can be called "foster feeding", for it replaces the normal pattern when one adult is absent temporarily or permanently, and can be represented as

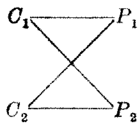


A marked feature of most grey seal breeding colonies around Great Britain is a high pup mortality which Coulson and Hickling[2] have correlated with population density according to the amount of accessible shoreline. When a proportion of the pups die within a few days of birth (lactation lasts about 18 days) some cows are left with milk, and observations have shown that the following pattern may occur



This can be called "supplementary feeding"; "fosterage" is not appropriate when the mother is still available to the second pup which feeds from both cows.

Observations have also shown that if the disappearance of a cow or a pup from the whelping ground is temporary, the following feeding pattern may occur



Both pairs of related animals remain available, neither being forced to replace a missing relative, and in this case also "fosterage" is not an appropriate term. I prefer "contingent feeding", which implies that it occurs when opportunities arise.

During extensive field studies of maternal relations in the grey seal in the Hebrides and Orkney in 1963–66 I have made the following observations. (1) The reactions of an individual cow to pups and to human presence vary greatly from day to day. (2) Supplementary and contingent feeding occurs in undisturbed as well as disturbed colonies. (3) Suckling usually follows soliciting by the pup. Acceptance by the cow may or not be preceded by olfactory investigation of the snout of the pup. (4) Between prolonged periods of sleep, strong pups will usually solicit cows and usually locate the teats readily. Weaker animals, which seldom sleep, have been seen sucking at seaweed, bulls, the boots of an observer and each other. (5) The precipitate departure of a suckling cow when disturbed may interrupt a pup's feeding only briefly because the pup may succeed in immediately resuming feeding from another cow. (6) A cow whose pup has been killed and removed may seem to search for it for a few days, but will accept and feed other pups meanwhile. (7) A cow suckling her own pup may be vigorously dispossessed by a strange cow which takes over the feeding temporarily.

The following provisional conclusions can be drawn. (1) A pup seen feeding from a cow is usually, but not necessarily, the offspring of that cow. (2) A pup abandoned by its cow will not necessarily starve to death. (3) A cow deprived of her pup will still feed other pups; she thus remains in the breeding colony and does not necessarily have a reduced chance of mating. (4) There is considerable power of recovery from disturbance which is not too frequent or prolonged.

These points are of considerable interest in relation to the way in which grey seal colonies, especially in the north-east Atlantic area, have elaborated into highly social groups. A puzzling feature of this development is that it increases pup mortality, a point which has been used by those who advocate the reduction of seal numbers on the grounds of alleviating the conditions of overcrowding. But a comparison with some herbivore populations does not apply to the grey seal; the animals choose this mode of life even when culled or when there is an abundance of space available into which they could spread.

Probably the grey seal was originally an ice-breeding species[3], but where it breeds on ice today it does so in ones and twos[4]. Only in temperate and therefore secondary habitats have the seals developed slum dwelling. More than three-quarters of the world's stock of the species now breeds in such conditions and in several places it has been reported to be increasing remarkably[5]. Thus despite an Arctic origin and a mortality that rises with density there is apparently some selective advantage in closely crowded colonies.

Coulson and Hickling, reviewing possible explanations for this[2], point out that pup mortality results in reduced recruitment to the breeding stock. Wynne-Edwards has suggested that social breeding is a means of preventing the undesirable effects of overpopulation by stabilizing numbers below a critical level[6]. Coulson and Hickling suggest that the self-induced pup mortality caused by grey seals choosing to breed in crowded conditions may be a means of preventing the numbers increasing beyond the limits of the food supply. But it is difficult to accept that the relatively few grey seals in the north-east Atlantic, whose feeding habits are catholic and opportunist, could be limited by the food available to the adults at sea. But this is a reasonable suggestion with regard to the food available before weaning.

The grey seal now has a remarkably shortened period of lactation and a dramatic rate of post-natal growth; it must obtain sufficient blubber for temperature control, streamlining and mechanical protection, and food reserve, as efficiently as possible. This situation is extreme among mammals and it is possible that pup mortality, which increases the opportunities for supplementary and contingent feeding, is a means of restricting recruitment to those fit enough to benefit in competition for milk. In other words, density dependent pup mortality could be a means whereby the milk supply in the colony or rookery as a whole is diverted to the genetically or physiologically fitter individuals. As Wynne-Edwards has observed[6], it is eugenically preferable to rear one or two healthy young than six or seven weak ones.

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[1] Hewer, H. R., and Backhouse, K. M., *Proc. Zool. Soc. Lond.*, **134**, 157 (1960).

[2] Coulson, J. C., and Hickling, G., *J. Anim. Ecol.*, **33** (3), 485 (1964).

[3] Davies, J. L., *J. Mammal.*, **38**, 297 (1957).

[4] Mansfield, A. W., *Fish. Res. Board Can. MS Rep. Ser. Biol.*, No. 846, 1-17 (1965).

[5] Smith, E. A., *J. Zool.*, **150**, 463 (1966).

[6] Wynne-Edwards, V. C., *Animal Dispersion in Relation to Social Behaviour* (Edinburgh, 1962).

Uptake of Organic Solutes by *Nereis virens*

WHILE investigating aspects of the trophic relationships of benthic animals, we have studied the uptake of amino-acids and other small organic molecules by the polychaete worm, *N. virens*, which lives in great numbers on the north side of the lower Thames Estuary[1]. Work on the isolation of possible organic solutes has already been reported[2], and while no specific substances have yet been identified from surface water collected on Thames Estuary flats at Southend-on-Sea, other workers have reported the presence of quantities of organic material in sea water from various sources (for example, Duursma[3]).

Stephens[4-6] has shown the uptake of nutrients by invertebrates. References to earlier work, which started with that of Pütter[7] and was continued by, among others, Krogh[8], will be found in Stephens's papers.

The work summarized here is a laboratory study of some aspects of the absorption of glutamic acid by specimens of *N. virens* weighing between 2 and 5 g. There are contradictory accounts of the food and feeding of *N. virens* in the literature, for example, Verrill[9] and Gross[10]. Our observations indicate that except during the summer months, the gut appears to be empty of obvious foodstuffs. A more thorough study of this is, however, being made.

After acclimatization in the marine aquaria of the laboratory, animals were weighed and placed individually in sterilized sea water containing glutamic acid labelled with carbon-14. At the end of the uptake period (usually 1 h) the specimens were each placed in 80 per cent ethanol (25 ml./g of worm wet weight) for 48 h, after which samples of the extracts were counted in a liquid scintillation counter together with samples of the original nutrient sea water. Uptake of glutamic acid was then calculated and expressed as μg/g wet weight/h. Similar results were obtained in experiments in which the animals were ligatured at both ends showing that absorption is independent of the gut.

Absorption from different concentrations of glutamic acid is shown graphically in Fig. 1, which shows that uptake is not directly proportional to concentration and thus is unlikely to be the result of simple diffusion. In these experiments it was also shown that glutamic acid was concentrated against a gradient, and further, that when the reciprocal of amino-acid uptake rate was plotted against the reciprocal of the external concentration, the resulting graph resembled that of a typical enzyme–substrate reaction (Lineweaver–Burk plot).

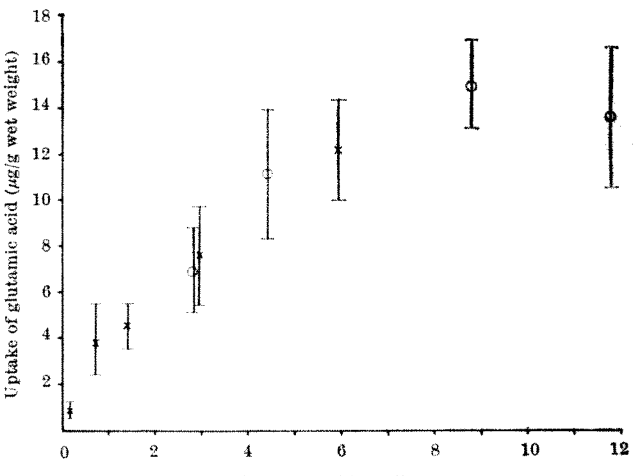

Fig. 1. Graph showing the effect of concentration on the direct uptake of glutamic acid by *Nereis virens*. ×, Experiment 12; ○, experiment 13. Lines represent standard deviations.

The active nature of the uptake process is also shown by experiments using amino-acids of similar configuration which acted as competitive inhibitors. Other unrelated amino-acids were shown to be absorbed independently.

Estimations of the amounts of glutamic acid which can be absorbed from a solution of 2×10^{-5} moles/l. could account for about 15 per cent of the animal's measured oxygen uptake if metabolized as an aerobic respiratory substrate. We have shown the animal to be able to take up a range of organic compounds including not only a variety of amino-acids, but also succinic, glycollic and citric acids and glucose. It is likely therefore that the overall process is significant in the worm's economy.

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[1] Brafield, A. E., and Chapman, G., *J. Mar. Biol. Assoc. UK*, **47**, 619 (1967).

[2] Chapman, G., and Rae, A. C., *Nature*, **214**, 627 (1967).

[3] Duursma, E. K., in *Chemical Oceanography* (edit. by Riley, J. P., and Skirrow, G.), **1**, 433 (1965).

[4] Stephens, G. C., *Biol. Bull. Wood's Hole*, **123**, 648 (1962).

[5] Stephens, G. C., *Comp. Biochem. Physiol.*, **10**, 191 (1963).

[6] Stephens, G. C., *Biol. Bull. Wood's Hole*, **126**, 150 (1964).

[7] Pütter, A., *Die Ernährung der Wassertiere und der Stoffaushalt der Gewässer* (Fischer, Jena, 1909).

[8] Krogh, A., *Z. Vergl. Physiol.*, **12**, 668 (1930).

[9] Verrill, A. E., *US Com. Fish and Fisheries*, Part 1, 295 (1873).

[10] Gross, A. O., *J. Exp. Zool.*, **32**, 427 (1921).

Gynodioecy in *Origanum vulgare*: Computer Simulation of a Model

THE population dynamics of the rare male sterility factors, or of gynodioecious species, which have a characteristically high proportion of female (male-sterile) and hermaphrodite individuals together in a population, is interesting to both evolutionists and plant breeders. Many workers have discussed the evolutionary significance of such a mating system, which promotes outbreeding with the proportion of females regulating the degree of outbreeding (for example, refs. 1–3). In *Origanum vulgare* (Labiateae), Lewis and Crowe[4] estimated that nearly 30 per cent, or even as many as 50 per cent, of females occur in various European populations. They showed that two loci govern the gynodioecy system; a dominant factor F causes anther suppression in combination with the recessive class hh to give $FFhh$ and $Ffhh$ as females, whereas HH, Hh suppress factor F to yield the other seven genotypes as hermaphrodites. Furthermore, certain segregation ratios suggested to them that the females are maintained through almost lethality of $ffhh$, lower viability of HH genotypes and poorer seed output of hermaphrodites than females. Thus if the fitness values are denoted by the matrix

$$\begin{array}{cc} & \begin{array}{ccc} FF & Ff & ff \end{array} \\ \begin{array}{c} HH \\ Hh \\ hh \end{array} & \begin{pmatrix} y & y & y \\ w & 1 & w \\ x & x & z \end{pmatrix} \end{array}$$

their data suggest that z is nearly 0, $y = 0{\cdot}25$ to $0{\cdot}50$ and $x > w$.

This basic model of Lewis and Crowe[4] can be readily generalized for theoretical studies by simulation on digital computer. Assume that loci F and H are linked with recombination value c, hermaphrodites practise mixed selfing (s) and random outbreeding ($t = 1 - s$), and that Hh have higher self-fertility than HH, hh classes so that the relative seedset from among the selfed ovules are in the ratio $s/a : s/b$, respectively (a, b are input constants). The value of x may be taken as constant between generations (model A) or varied by a frequency-dependent relation, say, $x_{n+1} = (k - \varphi_n)/\varphi_n$ (model B), where k is taken between 1 and 2, and φ_n = frequency of females in the preceding generation. A large number of deterministic sets were run with varying input values of selection and mating system parameters (a, b, s, c, x, y, w, z) of which only a few selected cases are reported here to illustrate briefly the interrelationships among these parameters in terms of the conditions allowing the maintenance of gynodioecy and, in particular, to discuss the selective forces postulated[4]. The simulation procedure involved the use of recursion equations between genotypic frequencies in the nth and $n+1$th generations solved iteratively for finding equilibria, the stability of which is tested either by using various initial frequency sets or by small perturbations in equilibrium frequencies. In most cases, initial allelic frequencies were assumed to be $p_F = 0{\cdot}01$ and $p_H = 0{\cdot}2$ or $0{\cdot}5$ so that females are initially rare depending also on the value of p_H. Note that (w,x) are fecundity parameters and (y,z) are relative viabilities.

Table 1. EQUILIBRIA UNDER MODEL A WITH $a = b = 1$ (INITIALLY $p_H = 0{\cdot}5$, $p_F = 0{\cdot}01$)

W	x	y	z	t, c	φ	p_F	p_H	$\overline{W}$
0·50	0·50	0·25	0	0·50, 0·01	0·307	0·608	0·390	0·704
				0·50, 0·50	0·238	0·607	0·437	0·640
				1·0, 0·01	0·296	0·604	0·391	0·666
				1·0, 0·50	0·234	0·579	0·430	0·625
0·90	0·90	0·25	0	0·50, 0·50	0·326	0·898	0·374	0·641
				1·0, 0·50	0·319	0·772	0·372	0·572
0·90	0·90	0·50	0·10	0·50, 0·50	0·216	0·850	0·508	0·701
				1·0, 0·50	0·206	0·699	0·490	0·668
0·75	0·75	0·50	0·10	0·50, 0·50	0·158	0·659	0·572	0·689
				1·0, 0·50	0·161	0·588	0·531	0·686

Table 2. EQUILIBRIA UNDER MODEL A WITH $w = x = 0{\cdot}75$, $z = 0$ (INITIALLY $p_H = 0{\cdot}50$, $p_F = 0{\cdot}01$)

		$a = 1$, $b = 1$			$a = 5$, $b = 1$			$a = 1$, $b = 5$		
t	c	φ	p_F	p_H	φ	p_F	p_H	φ	p_F	p_H
					$y = 0{\cdot}25$					
0·10	0·01	0·328	0·625	0·388	0·342	0·643	0·359	0·340	0·620	0·382
	0·50	0·301	0·839	0·397	0·321	0·839	0·370	0	0·028	1·0
0·50	0·01	0·324	0·624	0·376	0·335	0·635	0·364	0·336	0·626	0·369
	0·50	0·286	0·719	0·400	0·301	0·723	0·383	0·300	0·713	0·393
1·0	0·01	0·322	0·619	0·373	0·322	0·619	0·373	0·322	0·619	0·373
	0·50	0·282	0·654	0·396	0·282	0·654	0·396	0·282	0·654	0·396
					$y = 0{\cdot}35$					
0·10	0·01	0·295	0·583	0·424	0·327	0·624	0·379	0	0·011	1·0
	0·50	0·258	0·839	0·457	0·305	0·839	0·392	0	0·014	1·0
0·50	0·01	0·289	0·584	0·418	0·312	0·611	0·390	0·269	0·559	0·442
	0·50	0·243	0·708	0·453	0·275	0·716	0·413	0·208	0·682	0·496
1·0	0·01	0·283	0·582	0·412	0·283	0·582	0·412	0·283	0·582	0·412
	0·50	0·237	0·638	0·442	0·237	0·638	0·442	0·237	0·638	0·442

For both models A and B, the values of y and z were found to be critical in that $y > 0{\cdot}50$ and $z > 0{\cdot}25$ often gave equilibria with only hermaphrodites ($\varphi = 0$), $p_H = 1$, and locus F polymorphic unless heterozygote advantage was very strong ($0{\cdot}90 < x < 1{\cdot}0$, $w = 1$). For $y = 0{\cdot}25$ or $0{\cdot}50$ and $0 < z < 0{\cdot}10$ (compare ref. 4), on the other hand, the equilibrium proportion of females (φ_e) was usually in the range 0·25–0·40. Some examples of these equilibria are given in Table 1. Note that for y and z in low range, the other parameters have relatively little influence on the equilibrium values of φ, p_F and p_H. Both loci are maintained polymorphic in this case, although polymorphism at H is a sufficient condition for maintaining gynodioecy. Both inbreeding and linkage tend to give higher values of φ and $\overline{W}$, the mean population fitness, as measured by the usual frequency weighted selective values. The effect of self-fertility parameters, a and b, can be seen from Table 2; the set of values ($a = 5$, $b = 1$) simply yields an effectively higher selective advantage favouring Hh over HH, and vice versa in the case of set ($a = 1$, $b = 5$). As expected, the values of (t,c,y) become critical in certain combinations for the retention of gynodioecy in a population. Note that the critical value of t becomes greater as the value of y

Table 3. EQUILIBRIA UNDER MODEL B WITH $x=(1\cdot5-\varphi)/\varphi$, $a=1$, $b=5$, $w=1$, $z=0$

Initial	$y=0\cdot25$				$y=0\cdot30$				$y=0\cdot35$			
	$p_H=0\cdot5$		$p_H=0\cdot2$		$p_H=0\cdot5$		$p_H=0\cdot5$		$p_H=0\cdot5$		$p_H=0\cdot2$	
t, c	φ	p_F	φ	p_F	φ	p_F	φ	p_F	φ	p_F	φ	p_F
0·05, 0·01	0·344	1·0	0·344	1·0	0	0·010	0·250	0·808*	0	0·010	0	0·010
0·05, 0·50	0	0·052	0·344	1·0	0	0·014	0·252	1·0	0	0·012	0	0·012
0·10, 0·01	0·347	1·0	0·347	1·0	0·276	0·901*	0·276	0·951*	0	0·010	0	0·010
0·10, 0·50	0·347	1·0	0·347	1·0	0	0·017	0·277	1·0	0	0·014	0	0·014
0·15, 0·01	0·348	1·0	0·348	1·0	0·291	0·936*	0·291	1·0	0	0·012	0·185	1·0
0·15, 0·50	0·348	1·0	0·348	1·0	0	0·023	0·290	1·0	0	0·016	0·188	1·0
0·20, 0·01	0·344	1·0	0·344	1·0	0·300	0·953*	0·300	1·0	0·222	0·857*	0·222	1·0
0·20, 0·50	0·344	1·0	0·344	1·0	0	0·054	0·300	1·0	0	0·019	0·224	1·0

* Value in 500th generation; steadily approaching unity.

increases. In some cases linkage value can also be critical in terms of maintaining the females in population (for example, $y=0\cdot25$, $t=0\cdot10$, $b=5$). A similar pattern is noted for other sets of values of (w,x,t,c) with varying critical bounds in these situations.

Likewise, the interactions among the various parameters are rather complex for model B involving frequency dependent x (Table 3) with further effects of initial frequencies on the equilibria. Usually, for $p_H=0\cdot2$ where initially females are more frequent, wider conditions allow the maintenance of gynodioecy at equilibria than is the case with $p_H=0\cdot5$. The role of linkage and t depends, as already noted, on the value of y. Most nontrivial equilibria here too maintain females (φ_e) in the range 0·25–0·40 with $p_F=1$ and locus H polymorphic; in contrast, equilibria with $\varphi=0$ have $p_H=1$ and a low value of p_F at equilibrium. With model B and $a=1$, $b=1$ (that is, no self-sterility), in all cases stable equilibria were reached and t had little effect on the equilibrium values of φ and p_H as long as y and z are in rather low range. Thus most sets seemed to involve one or two peaks near *FFH*- or *FfHH*, especially when y and z play a critical part in comparison with the other parameters having little effect.

Thus the results from computer simulation support Lewis and Crowe's[4] model of selection which they arrived at through disturbed segregation ratios in certain progenies of wild individuals and not from any direct estimates of the selective forces in a natural population. The theory further points to the relative importance of various selection parameters which are critical for maintaining the gynodioecious system in *Origanum vulgare* and similar situations. Further data are needed, of course, to test certain ramifications of these models by direct estimates of selection and mating system parameters, for example, the amount of selfing, self-sterility and heterozygote advantage based on the experimental studies over generations. Male-sterile factors, in general, require rather strong selection for maintenance in natural conditions so that opportunities exist of measuring the selective differences in natural populations and are likely to be of great importance in the evolution of genetic systems, especially in predominantly selfing species[5] or homomorphic, self-fertile species[3]. Baker[3] recently discussed the evolution of breeding systems in *Armeria* and *Limonium* which suggest the origin of gynodioecy as a secondary system promoting outbreeding. An increase in the relative efficiency of the selfing component on hermaphrodites in a self-compatible form might impose counterpressure for greater outbreeding by maintaining a larger proportion of females and vice versa. Examples are now known of such a balance of various forces involved in the evolution of mating systems (for example, *Vicia faba*[6]). Ross (ref. 7 and personal communication) has recently described an interesting situation in *Plantago lanceolata* with a rather complex genetic system involving a combination of self-incompatibility and gynodioecy. Thus *Armeria*, *Origanum* and *Plantago* perhaps illustrate various combinations of the self-sterility and gynodioecy systems such as to represent three different stages in the evolution of self-compatibility from self-incompatibility. An understanding of the population dynamics of these mixed systems in terms of various selective forces and controlled breeding experiments would seem worthwhile.

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[1] Whitehouse, H. L. K., in *Darwin's Biological Work* (edit. by Bell, P. R., *et al.*) (Cambridge University Press, London, 1959).

[2] Crowe, L. K., *Heredity*, **19**, 435 (1964).

[3] Baker, H. G., *Evolution*, **20**, 349 (1966).

[4] Lewis, D., and Crowe, L. K., *Evolution*, **10**, 115 (1956).

[5] Jain, S. K., *Genetics*, **46**, 1237 (1961).

[6] Holden, J. H. W., and Bond, D. A., *Heredity*, **15**, 175 (1960).

[7] Ross, M. D., *Genetics*, **56**, 584 (1967).

Moss growing on Living Papuan Moss-forest Weevils

LARGE leaf-feeding weevils[1] of the genus *Gymnopholus* (subgenus *Symbiopholus*)[2], and certain other weevils, particularly of the genus *Pantorhytes*[3,4] (broad-nosed weevils), as well as certain cryptorhynchine weevils[3] and other beetles[3,5], all inhabiting fairly high moss forest ridges and summits, mostly in eastern New Guinea, have been reported to carry small gardens on their backs. The association seems to involve symbiosis, with the weevils providing a favourable environment for the plants, and the plants endowing the weevils with a protective resemblance to cryptogram-covered parts of plants, or with chemical protection.

Living in the plants are phytophagous oribatid mites[6] of a family known only from this association, and also sessile rotifers and minute nematodes, as well as other microscopic organisms.

The plants previously reported from the backs of the weevils (and sometimes on their femora) are fungi, algae, lichens and liverworts, as well as stray diatoms. Now the presence of moss can be reported. *Daltonia angustifolia* Dozy and Molk. (Figs. 1 and 2) of the Hookeriaceae has been determined from ten weevils of five species. These are *Gymnopholus* (*Symbiopholus*) *acarifer* Gressitt[2], Bulldog Rd., 2,400 m, G. A. Samuelson, 1966; *G.* (*S.*) *lichenifer* Gressitt[2], Bulldog Rd., 2,300 m, O. R. Wilkes, 1966; *G.* (*S.*) *fungifer* Gressitt[2], Saruwaged Range, 2,400 m, Samuelson, 1966; *G.* (*S.*) *reticulatus* Marshall[7], Daulo Pass, 2,500 m, Bismarck Range, L. Gressitt, 1955; and *G.* (*S.*) *carolynae* Gressitt and Sedlacek[8], Kubor Range, 2,950 m, Carolyn Gressitt, 1966.

The weevils on which the moss was found vary from about 22 to 36 mm long. Hepatics occur fairly abundantly on some of the weevils bearing moss, and in most cases fungi and algae are also present. Among the liverworts on these specimens are *Cololejeunea*, *Lejeunea* and *Metzgeria*. While one of us (J. L. G.) was in New Guinea in 1967, Dr R. Schuster visited the principal study area and identified *Odontolejeunea*, *Microlejeunea* and *Metzgeria* from cryptorhynchine weevils on Mount Kaindi. Dr Schuster also recognized a fern gametophyte on *Gymnopholus* (*S.*) *lichenifer*, but it was unfortunately lost. The moss-bearing weevils carried hardly any lichens.

The following plants have been recognized from the various weevils (partly identified by R. K. Benjamin, M. Hale, J. Proskauer and R. Schuster). Fungi–Fungi Imperfecti: ?Leptostromataceae, ?Nectrioidaceae; Ascomycetes: Helotiaceae, ?Micropeltaceae; Algae–Trentepohliaceae, Chroococcaceae, Nostocaceae, Oscillatoriaceae (or Scytonemataceae) and other algae; Lichens—Physciaceae (*Physcia* sp., *Anaptychia* sp.), Parmeliaceae (*Parmelia P.* (*Hypotrachyna*) *crenata* Kurok., *P. reticulata* Tayl., etc.); Hepaticae—Metzgeriaceae (*Metzgeria* sp.), Lejeuneaceae (*Cololejeunea* sp., *Lejeunea* sp., *Microlejeunea* sp., *Odontolejeunea* sp.); Musci—Hookeriaceae (*Daltonia angustifolia* Dozy and Molk.).

The weevils bearing the plants are mostly modified structurally in ways which provide niches and protection for the plants, particularly in their early stages. Among these modifications are pits, depressed areas surrounded by ridges and stiff setae, pubescent areas protected by tubercles, and others. It seems that evolution is progressing rapidly among these weevils. Populations on mountains separated by rather slight distances tend to be different, and several species-groups may be represented on one mountain. Already more than fifty species of *Gymnopholus* are known[2,8] and a similar number of *Pantorhytes*[4]. In spite of indications of rapid evolution, however, the plants and animals in this association must have been living together in a very humid environment for a considerable period of time. The weevils feed on a number of genera of dicotyledons[8,9].

Preliminary observations seem to verify the assumption that most of the plants growing on the weevils are those usually found on the surface of leaves and bark in the same environments. In the case of the lichens there is evidence of vegetative reproduction on the weevils. In the case of the moss, there are numerous protonemata on the weevils, which suggests that the origin may be from spores. It was earlier suggested that the fungivorous mites, of which up to seventy-five individuals have been found on one weevil, may transport propagules from one host to another.

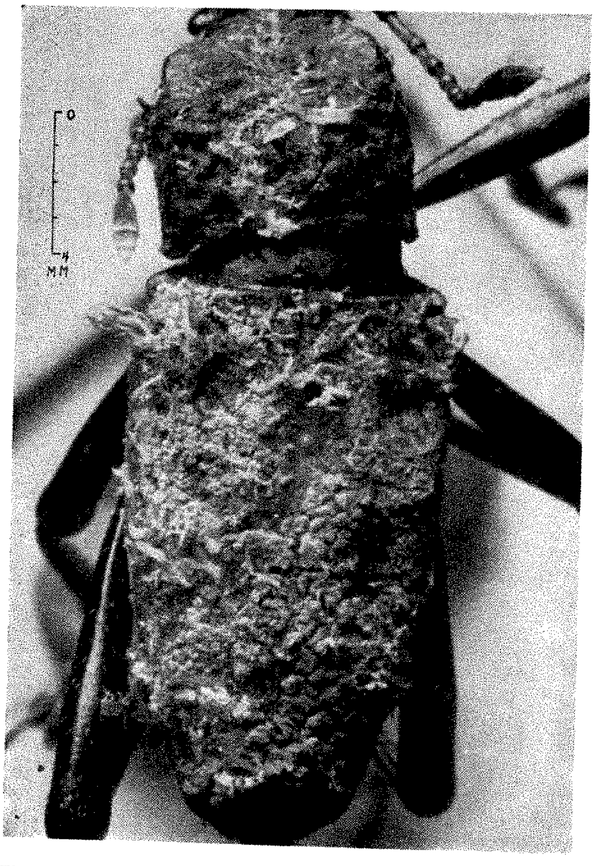

Fig. 1. *Gymnopholus* (*Symbiopholus*) *fungifer* Gressitt, Saruwaged Range, Huon Peninsula, north-east New Guinea, Samuelson, 1966, bearing the moss *Daltonia angustifolia* Dozy and Molk., as well as partial thick mat of fungi and algae and numerous hepatics.

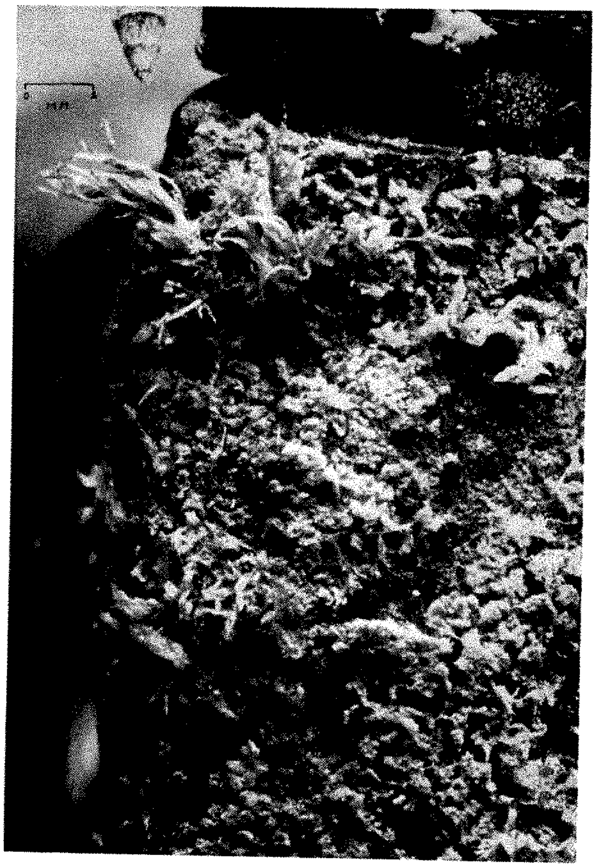

Fig. 2. *Gymnopholus* (*Symbiopholus*) *fungifer* Gressitt, Saruwaged Range, Samuelson, 1966, showing *Daltonia angustifolia* on base of left elytron, and the hepatics *Cololejeunea* sp. and *Metzgeria* sp. near centre of figure and *Lejeunea* sp. near bottom of figure.

Field observations so far suggest that predation of the weevils is relatively uncommon. The weevils may be distasteful to predators, or their large, heavily sclerotized bodies may be a deterrent, and (or) the plants growing on them may offer camouflage or be distasteful to predators. For more than a year individuals of *G.* (*S.*) *lichenifer* have been marked and released on the summit of Mount Kaindi, 2,350 m. Many of the weevils were collected on the upper slopes of the mountain, in forest, but released on host-bushes planted around the periphery of the flattened and cleared summit, around the communications repeater station. Although the weevils are thus in a very exposed situation, maintaining their habit of climbing to the top shoots of the plants at night and in foggy weather, almost no evidence of predation has been observed. Recovery rates of marked individuals have been as high as 43 per cent for individuals released within a few weeks preceding[8]. At least one weevil has been seen more than a year after marking. Several weevils have been observed to walk away into the forest after release, and some have been observed to die apparently of old age, so the reduction is obviously not primarily from predation. Only one killed individual was found, and this was on a log at night, so the enemy may have been a mammal. The weevil was not chewed up; only the abdomen had been removed.

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[1] Gressitt, J. L., Sedlacek, J., and Szent-Ivany, J. J. H., *Science*, **150**, 1833 (1965).

[2] Gressitt, J. L., *Pacific Insects*, **8**, 221 (1966).

[3] Gressitt, J. L., *Pacific Insects*, **8**, 294 (1966).

[4] Gressitt, J. L., *Pacific Insects*, **8**, 915 (1966).

[5] Samuelson, G. A., *Pacific Insects*, **8**, 290 (1966).

[6] Aoki, J., *Pacific Insects*, **8**, 281 (1966).

[7] Marshall, G. A. K., *B.P. Bishop Museum Occasional Papers*, **22**, 71 (1959).

[8] Gressitt, J. L., and Sedlacek, J., *Pacific Insects*, **9**, 481 (1967).

[9] Szent-Ivany, J. J. H., *Papua and New Guinea Sci. Soc. Trans.*, **6**, 30 (1965).

New Glycolipid specific to Nitrogen-fixing Blue-green Algae

The polar lipid fractions from blue-green algae are usually simpler than those from higher algae and leaves, and normally contain only those four acyl lipids that are commonly associated with chloroplasts, namely, mono- and di-galactosyl diglyceride, sulphoquinovosyl diglyceride and phosphatidyl glycerol[1,2]. Occasionally, other components are reported[1,3].

Thin layer chromatography on silica gel of chloroform–methanol extracts from three nitrogen-fixing blue-green algae (*Anabaena cylindrica*, *Anabaena flos-aquae* and *Chlorogloea fritschii*) has, however, indicated the presence in all three of a substance which has not yet been detected in any other class of photosynthetic tissue.

In the chromatographic conditions used, this substance migrated a little faster than standard phosphatidyl ethanolamine (Fig. 1). When the chromatograms were reacted with sulphuric acid[4], the unknown substance gave a purplish-red colour commonly observed with glycosides, and a positive reaction with a Schiff–periodate reagent[4] was also obtained. It gave negative reactions with reagents specific for phosphate groups[5].

To obtain further analytical data for this material, the lipids of *A. cylindrica* were fractionated by column chromatography on DEAE cellulose[6]. The unknown lipid was slowly eluted from the adsorbent with 5 per cent methanol in chloroform, the later fractions displaced by this solvent containing increasing quantities of a compound which during thin layer chromatography seemed to be slightly more polar than the principal fraction, but which gave similar colour reactions with specific reagents. No clear cut separation of the two glycoside fractions could be achieved by column chromatography and for the preliminary studies described here they were examined as a mixture.

The crude glycoside was freed from contaminating pigments by crystallization from 90 per cent aqueous methanol to give a colourless amorphous solid, melting point 95°–102° C.

The principal absorption peaks obtained by infrared spectroscopic analysis of the glycoside were caused by methylene and hydroxyl groups. No significant absorption at wavelengths associated with ester or amide groups was observed, indicating the absence of acyl residues.

The glycoside was stable to aqueous and alcoholic alkali, but was hydrolysed when refluxed with 2 N sulphuric acid in methanol–water (95 : 5 v/v) for 28 h. After dilution with water, the reaction mixture was extracted with chloroform. The aqueous phase from this extraction gave a positive Molisch test and paper chromatography of a portion of this phase yielded two chief fractions which gave positive reactions with aniline phthalate[7] and ammoniacal silver nitrate[8] reagents, and which had R_F values similar to those for galactose and glucose.

Analysis of the chloroform layer by thin layer chromatography on silica gel using chloroform–methanol (90 : 10 v/v) as mobile phase gave two spots: a principal one of R_F 0·6 and a minor one of R_F 0·5. Thus the heterogeneity of the crude glycoside of *A. cylindrica* may be the result of, in part at least, the presence of two aglucone components of differing polarities. Infrared analysis of the crude aglucone showed strong adsorption at frequencies corresponding to methylene and hydroxyl groups. The aglucone fraction of the glycoside of *A. cylindrica* probably consists therefore of a heterogeneous mixture of fatty alcohols, members of which may contain differing numbers of hydroxyl groups.

Chromatographic studies on the corresponding lipid fractions from the two other nitrogen-fixing blue-green algae suggest that these are also heterogeneous, and it would seem that these organisms possibly contain families of alcoholic glycosides in which differences in composition of both the aglucone and glycoside moieties occur.

Such glycosides seem to be specific to nitrogen-fixing blue-green algae and we have been unable to detect comparable compounds in blue-green algae which do not fix nitrogen (including *Anacystis nidulans*, *Anabaena variabilis*, *Spirulina platensis*), yellow algae (*Vaucheria sessilis*, *Monodus subterraneus*), brown algae (*Fucus serratus*, *Fucus vesiculosus*), a red alga (*Porphyridium*

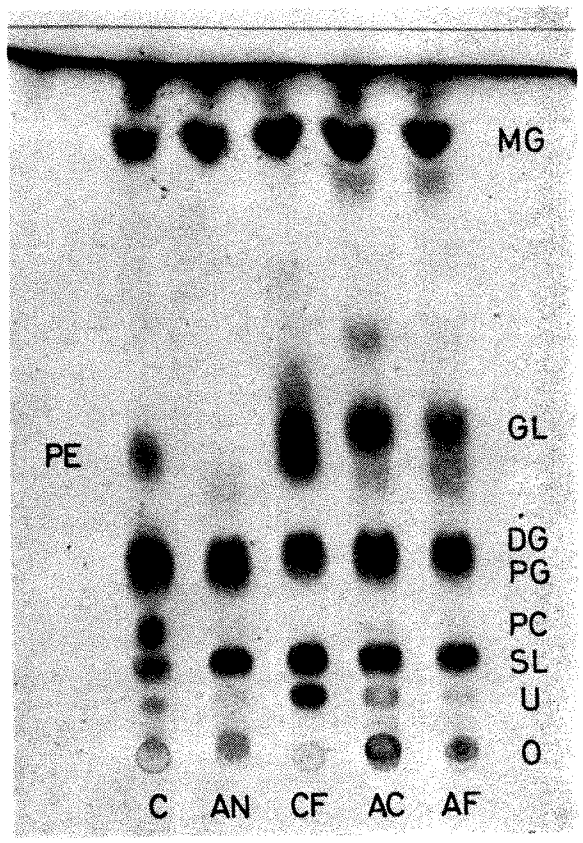

Fig. 1. Thin layer chromatography of lipid extracts from *Chlorella vulgaris* (C), *Anacystis nidulans* (AN), *Chlorogloea fritschii* (CF), *Anabaena cylindrica* (AC) and *Anabaena flos-aquae* (AF). Stationary phase: silica gel containing 10 per cent gypsum. Mobile phase: chloroform–methanol–acetic acid–water (85 : 15 : 10 : 3·7 v/v). Visualization: 25 per cent sulphuric acid at 220° C (5 min). Identification of components: monogalactosyl diglyceride (MG); the unknown glycolipid (GL); phosphatidyl ethanolamine (PE); digalactosyl diglyceride and phosphatidyl glycerol (not separated) (DG, PG); phosphatidyl choline (PC); sulpholipid (SL); an unidentified lipid (U); origin (O).

cruentum), green algae (including *Chlorella vulgaris*, *Chlamydomonas pulsatilla*), a marine diatom (*Phaeodactylum tricornutum*), a freshwater diatom (*Nitzschia palea*) *Euglena gracilis* and *Ochromonas danica*. They were also absent from lipid extracts from the leaves of several species of higher plants.

Present work is directed towards the preparation of homogeneous glycoside and aglucone fractions in order to determine their detailed chemical structure.

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[1] Nichols, B. W., Harris, R. V., and James, A. T., *Biochem. Biophys. Res. Commun.*, **20**, 256 (1965).
[2] Nichols, B. W., and Wood, B. J. B., *Lipids*, **3**, 46 (1968).
[3] Allen, C. F., Hirayama, O., and Good, P., in *Biochemistry of Chloroplasts* (edit. by Goodwin, T. W.), **1**, 195 (Academic Press, London, 1965).
[4] Nichols, B. W., *Biochim. Biophys. Acta*, **70**, 417 (1963).
[5] Dittmer, J. C., and Lester, R. L., *J. Lipid Res.*, **5**, 126 (1964).
[6] Nichols, B. W., and James, A. T., *Fette Seifen Anstrichmittel*, **66**, 1003 (1964).
[7] Partridge, S. M., *Nature*, **164**, 443 (1949).
[8] Partridge, S. M., *Nature*, **158**, 270 (1947).

Availability of Nitrogen to Plants in Acid Coal Mine Spoils

MINING of coal by either strip (open cast) or deep-mining methods leaves large quantities of raw rock overburden and waste on the surface and revegetation of such spoils presents great reclamation problems. Survival and growth of plants are hampered by a variety of physical and chemical features of the spoils; in particular, poor growth has been attributed to lack of plant nutrients, especially nitrogen. Nevertheless, investigations of anthracite coal mine spoils in the vicinity of Tamaqua, Pennsylvania, showed that the nitrogen available to plants may be very abundant in certain spoils composed of black pyritiferous shales.

Cornwell[1] classified the spoils of the Tamaqua area into five lithological groups: black pyritic shales; grey calcareous shales; buff sandstones; "oxidized-surface" spoils coloured yellow by oxidized iron compounds; and "surficials"—mixed deposits of subsoil and unconsolidated substratum. Small areas of "placeland" (remnants of native soil) were scattered among the spoil heaps.

The apparent total nitrogen content of the < 2 mm fraction of these materials was determined by a standard Kjeldahl procedure employing overnight predigestion. The results reported in Table 1 show high nitrogen contents in the black shales. Two successive digestions of the black shales revealed that 88 per cent of the total nitrogen was removed in the first digestion.

Table 1. APPARENT TOTAL NITROGEN OF LITHOLOGICAL SPOIL TYPES OF THE TAMAQUA AREA

		Total nitrogen (%)	
	n	Mean	Range
Black acid shales	24	0·54	0·30–0·69
Grey shales	3	0·17	0·12–0·21
Buff sandstones	8	0·14	0·10–0·23
Oxidized-surface	5	0·16	0·14–0·16
Surficials	4	0·10	0·07–0·10
Placeland*	3	0·26	0·18–0·34

Analyses were based on the unground < 2 mm fractions and single Kjeldahl digestions.

* A horizon.

Differences in availability of nitrogen among spoils were suggested by the appearance of gray birch, *Betula populifolia* Marsh., which was the most common tree on all spoils and essentially the only plant on the most acid ones. Birch saplings on the buff sandstone spoils had thin narrow crowns and small pale green leaves; those on the black acid shales had dense crowns and large, dark green leaves. Such differences were borne out by analyses of foliage samples from all the spoils and placeland, as shown in Table 2. The nitrogen status of birch on the strongly acid black shales (*p*H in water 2·8–3·9) at least equals that on placeland, and is markedly higher than that from the other rock spoils. These results can only be attributed to the release of fossil nitrogen from the shales.

Table 2. NITROGEN CONTENT OF MATURE *Betula populifolia* FOLIAGE ON VARIOUS SPOIL TYPES

Buff sandstones	Grey shales	Oxidized-surface	Surficials	Place-land	Black acid shales
1·51	1·68	1·88	2·34	2·41	2·47

n = 20: older and younger leaves from ten sites on each spoil type, each sample from ten trees. Values not connected by a horizontal line differ at the 5 per cent level.

It has been known for several decades that rocks, particularly sedimentaries, contain appreciable amounts of nitrogen as "fixed ammonium", kerogen and possibly other organic forms[2–5]. Recently, both Stevenson[6] and Southard[7] have shown that fixed ammonium constitutes a large proportion of the nitrogen in various carbonaceous shales. The release of nitrogen from the Tamaqua spoils is suggested by conventional analyses of the < 2 mm fraction for readily soluble nutrients[8]; soluble NO_3–N and NH_4–N did not exceed 10 p.p.2m. except on the strongly acid black shales where NH_4–N ranged from 11 to 25 p.p.2m.

Further evidence of release was provided by analysis of soluble salts accumulated on spoil materials during two greenhouse trials. Both used spoils from two locations on both the black and grey shales; the bulk samples were passed through 0·25 or 1 in. sieves, and 350 g or 650 g of the sieved spoil was placed in small containers to which distilled water was supplied from below through nylon wicks. After approximately 11 months, the salt-encrusted upper spoil was extracted with water and the soluble constituents were determined. Selected results, expressed on the basis of the entire 350 or 650 g, are shown in Table 3. Both spoil types contained pyrite (FeS_2) which oxidized to yield the large quantities of sulphate shown.

Table 3. CONTENT OF WATER SOLUBLE ELEMENTS IN PYRITIC SPOILS AFTER 11 MONTHS WEATHERING WITHOUT LEACHING, UNDER GREENHOUSE CONDITIONS

		Al	NO_3	NH_4	SO_4
			(mequiv./kg)		
Grey shales					
Sample 1	< 1″	0·8	0·17	0·39	94
	< 0·25″	0·4	0·22	0·21	69
Sample 2	< 1″	1·6	0·06	0·47	171
	< 0·25″	1·1	0·06	0·21	171
Black shales					
Sample 1	< 1″	104	0·32	8·5	226
	< 0·25″	22	0·05	3·7	62
Sample 2	< 1″	59	0·16	4·5	165
	< 0·25″	21	0·06	3·8	66

In the grey shale extracts, the sulphate anion was largely neutralized by calcium and magnesium, possibly derived from authigenic and cementing carbonates. In strong contrast, reactions from 2·7 to 3·2 in the black shale extracts and the abundance of soluble aluminium indicated an intensely acid weathering environment in which some destruction of silicate lattices must have occurred. Such destruction would necessarily liberate "fixed ammonium" as well as organic compounds containing nitrogen. If the latter are resistant to microbial attack, as has been supposed, the nitrogen values of Table 3 may largely represent fixed ammonium. In any case, the ammonium liberated from the black shales over the period of 11 months is of the order of 1–2 per cent of the total nitrogen (assuming 0·6 per cent) and certainly the quantities are large enough (100–240 p.p.2m.) materially to affect vegetation and to account for the high foliar contents shown in Table 2.

Certain inferences follow (*a*) The active weathering fraction in pyritic spoils includes fragments of any size which contain oxidizable sulphur, rather than being confined to the silt and clay-sized fractions, as in most soils Rapid weathering of the acid black shales in the field was indicated by coatings of secondary iron compounds, pronounced rounding of fragments and reprecipitated gypsum (*b*) Percolates from such spoils must contribute substantial amounts of nitrogen, as well as other elements, to outflowing ground-water and streams (*c*) Spoils containing little oxidizable sulphur, or having a high ratio of calcium and magnesium to sulphur, do not undergo much silicate destruction, and may therefore release little nitrogen, regardless of total content or form. Although Schramm[9] found that nitrogen deficiency limited plant growth on black shale wastes, the *p*H of his study materials ranged from 4 6 to 5 2, indicating minimal sulphide oxidation and silicate destruction

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[1] Cornwell, S M , thesis, Cornell Univ (1966)
[2] Hall, A D , and Miller, N H J , *J Agric Sci , Camb* , 2, 343 (1908)
[3] Rayleigh, Lord, *Proc Roy Soc* , A, 170, 451 (1939)
[4] Stevenson, F J , *Science*, 130, 221 (1959)
[5] Stevenson, F J , *Geochim Cosmochim Acta*, 26, 797 (1962)
[6] Stevenson, F J , *Bull Amer Assoc Petrol Geol* , 44, 1257 (1960)
[7] Southard, A R , thesis, Cornell Univ (1963)
[8] Greweling, T , and Peech, M , *Cornell Univ Agric Exp Stn Bull* , 960 (1965)
[9] Schramm, J R , *Trans Amer Phil Soc* , 56 (1966)

PSYCHOPHARMACOLOGY

Effects of Drugs on Conditioned "Anxiety"

THE behavioural phenomenon known as conditioned suppression was first demonstrated by Estes and Skinner[1] They maintained the bar-pressing activity of rats by a schedule of food reinforcement and found that this behaviour decreased in frequency during a stimulus which ended with an unavoidable shock Estes and Skinner suggested that the suppression of responding was an indirect measurement of "anxiety" Subsequent workers[2] have argued that the temporal relationships between the shock and the stimulus which precedes it satisfy the requirements for classical (respondent) conditioning to occur After a number of pairings, the conditioned stimulus (pre-shock stimulus) elicits the respondents which were formerly elicited only by the unconditioned stimulus (shock)

This interpretation has led to research programmes in which conditioned suppression has been used as a model of clinical anxiety. For example, the responding of rats is less suppressed during a pre-shock stimulus if they have recently received electro-convulsive shocks[3] It might therefore be suggested that conditioned "anxiety" is alleviated by this treatment.

In 1956, Brady investigated the effects of amphetamine and reserpine on conditioned suppression[4]. The results are summarized by his illustration (Fig 1). These cumulative records show (top) the effects of the Estes–Skinner procedure in control conditions (saline) When amphetamine was administered, the baseline rate of responding increased, but the number of responses emitted during the pre-shock stimulus decreased in comparison with control animals Reserpine produced opposite effects the baseline behaviour decreased in frequency but more responses were made during the pre-shock stimulus than had been made in the control conditions Brady claimed that "the method described does provide an approach to the selective assessment of specific drug-behaviour relationships in the affective sphere while providing a control for the general behavioural and motor disturbances that frequently develop as non-specific side effects of such

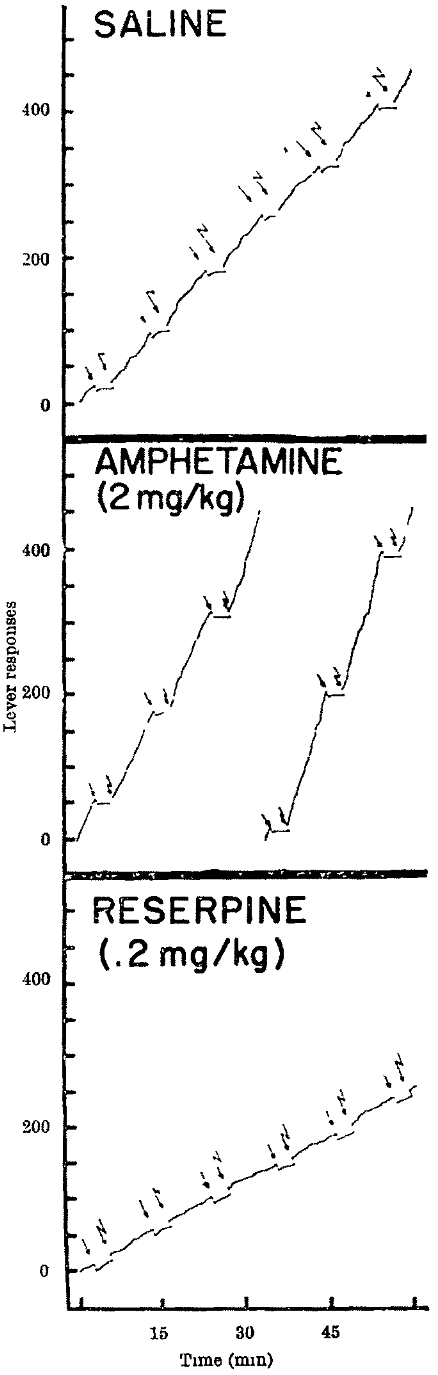

Fig 1 The effects of amphetamine and reserpine on conditioned suppression The pen of the cumulative recorder was deflected during the pre-shock stimulus (Reproduced from the article by J V Brady, *Science*, 123, 1033, 1956)

drug administration". Thus reserpine reduced "anxiety", because more responses were emitted during the pre-shock stimulus although the baseline responding became less frequent

A recent report[5] casts doubt on this claim In this experiment, attention was directed only to observable behavioural events; conditioned suppression was studied without regard to affective states such as "anxiety" The experiment was designed to discover whether frequency of responding can act as a determinant of conditioned suppression when all other variables known to be relevant are controlled (for example, frequency of reinforcement, shock intensity, deprivation, etc) This was done by means of paced variable interval schedules[6] Two rats were used so there was a control for possible sequence effects Both rats were first exposed to a condition (*A*) in which bar-presses were reinforced by food at variable intervals which averaged 1 min Then reinforcements became available just as before, but rat 1 obtained these only if it responded at a high rate, and rat 2 only if it responded at a low rate (*B*) Finally (*C*), these conditions were interchanged. Conditioned suppression was obtained with a standard procedure in each phase of the experiment

The results are summarized in Fig 2 They are strikingly similar to those reported by Brady, although no drug was used The behavioural baselines were affected by the pacing procedures in a way similar to that caused by Brady's two drugs Responding during the pre-shock stimulus also changed as it had done in Brady's experiment When the overall response rate was high, there was less responding during the pre-shock stimulus than there was in the control conditions. Similarly, when the overall response rate was low, responding occurred more frequently during the pre-shock stimulus than it had in control conditions These results suggest that Brady's findings do not necessarily show a specific pharmacological effect on "anxiety" His changes in effect were measured by conditioned suppression The changes in suppression may have been the direct result of changes in the behavioural baseline, rather than an effect exhibited despite contrary changes in this baseline. Brady's results alone are not firm evidence that amphetamine enhances "anxiety", nor that reserpine attenuates it.

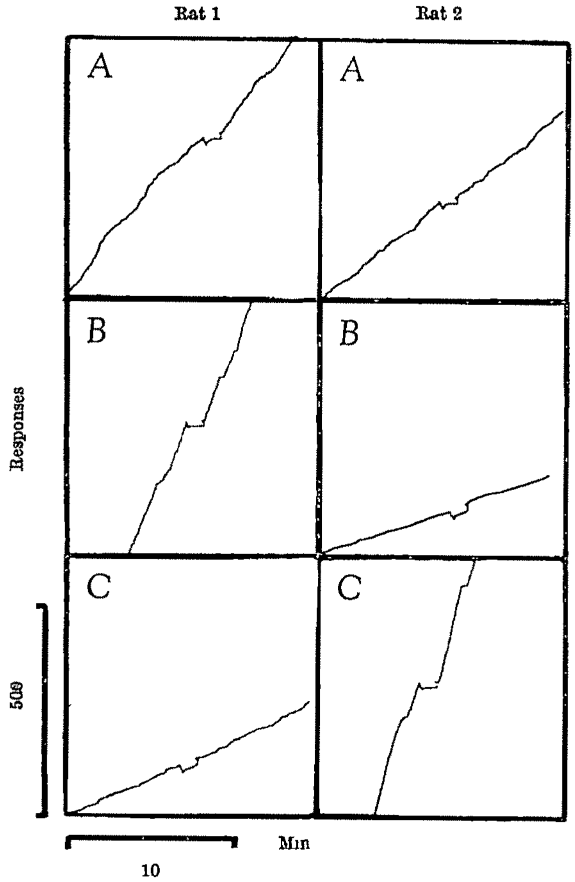

Fig 2 The effects of response pacing on conditioned suppression (Adapted from Blackman[5])

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Received September 29, 1967.

[1] Estes, W K , and Skinner, B. F , *J. Exp Psychol* , **29**, 390 (1941)

[2] Kamin, L. J , in *Classical Conditioning a Symposium* (edit by Prokasy, W F), 118–147 (Appleton-Century-Crofts, New York, 1965)

[3] Hunt, H F , and Brady, J V , *J Comp Physiol Psychol* , **44**, 88 (1951), Brady, J V , *ibid* , **44**, 507 (1951), Hunt, H F , Jernberg, P , and Brady, J V , *ibid* , **45**, 589 (1952), Geller, I , Sidman, M , and Brady, J V , *ibid* , **48**, 130 (1955)

[4] Brady, J V , *Science*, **123**, 1033 (1956)

[5] Blackman, D E , *Quart J. Exp Psychol* , **19**, 170 (1967).

[6] Ferster, C B , and Skinner, B F , *Schedules of Reinforcement* (Appleton-Century-Crofts, New York, 1957)

PHYSIOLOGY

Human Power Output

It has been known for a long time that power output in man increases rapidly with decreasing duration of exercise, but there have been few systematic studies of this problem Indeed, measurements of the power output of the body during a single movement lasting one-tenth of a second or less have not been made

We have investigated the peak power output in forty-seven men and eight young women, whose physical details are given in Table 1, during the performance of a standing vertical jump off both feet from a force platform Each

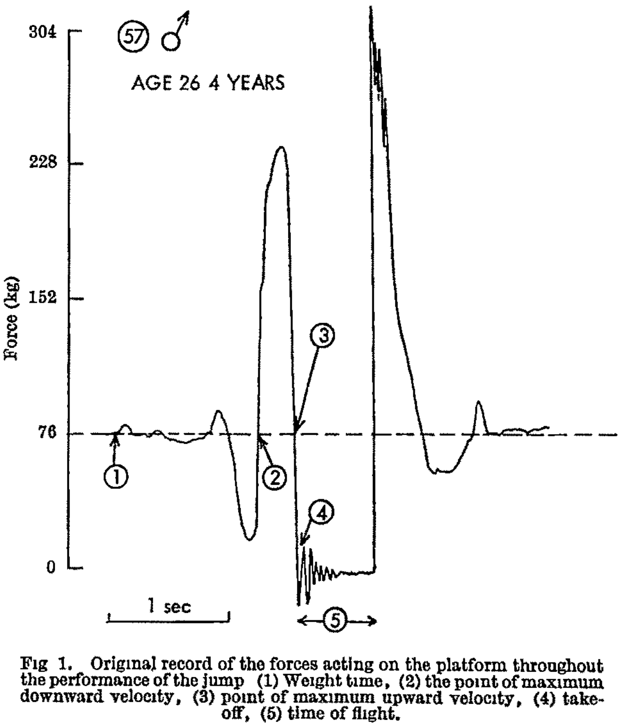

Fig 1. Original record of the forces acting on the platform throughout the performance of the jump (1) Weight time, (2) the point of maximum downward velocity, (3) point of maximum upward velocity, (4) take-off, (5) time of flight.

subject was allowed three practice jumps and the best of three experimental jumps was recorded and later analysed in the following way

Table 1 PHYSICAL CHARACTERISTICS OF THE FIFTY-FIVE SUBJECTS INVESTIGATED

Subjects	Age (yr)		Weight (kg)		Height (cm)	
	Mean	*S D*	Mean	*S D*	Mean	*S D*
Males ($n = 47$)	32 7	8 9	74 9	10 0	172 9	6 6
Females ($n = 8$)	22 1	3 9	60 0	7 5	163 6	6 0

The trace (Fig 1) was measured to an arbitrary scale at intervals of 0 02 s using an Oscar trace assessor (Benson–Lehner, Ltd) and the readings were compared with measurements of zero and known body weight to obtain a measure of force Acceleration (A) of the centre of gravity was found from $A = \dfrac{9\ 81 \times (F - W)}{W}$ M/s^{-2}, where F is force and W is body weight, and velocity was obtained by integration of the area under the A curve Values for instantaneous power were determined from the product of force and velocity Typical examples of such analysis are shown in Fig 2

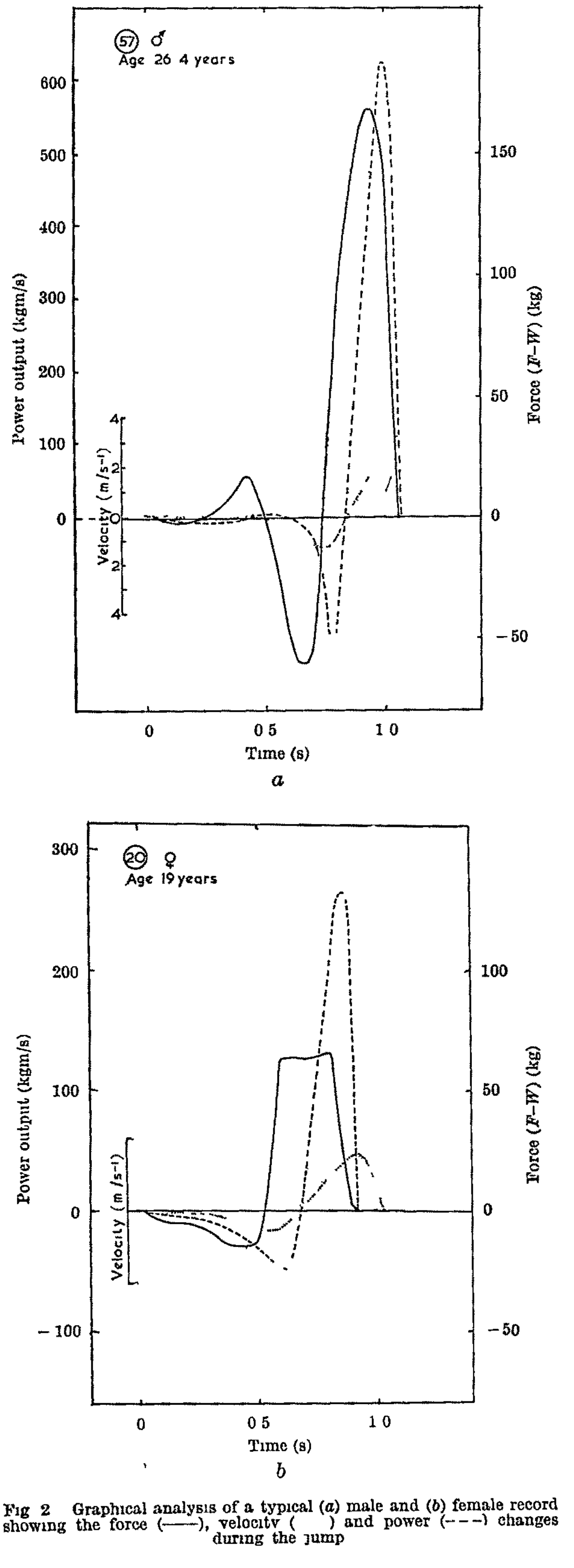

Fig 2 Graphical analysis of a typical (*a*) male and (*b*) female record showing the force (——), velocity ( ) and power (- - -) changes during the jump

Table 2 COMPARISON OF MAXIMUM POWER OUTPUT DURING SUSTAINED WORK ON A BICYCLE* ERGOMETER AND A STANDING VERTICAL JUMP FROM FORCE PLATFORM

Subjects	Power output (h p)			
	Bicycle ergometer		Force platform	
	Mean	*S D*	Mean	*S D*
Males ($n = 47$)	0 33	0 04	5 23	1 19
Females ($n = 8$)	0 25	0 03	3 15	0 48

* The power output was determined from direct measurements of oxygen consumption during the sixth to eighth minute of maximal work as a separate experiment

The results obtained on the fifty-five subjects expressed in terms of horsepower (h p) are shown in Table 2 The mean peak power output for the men was 5 22 ± 1 56 h p. and for the women 3 15 ± 0 48 h p These figures are among the highest ever recorded—they are some fifteen times greater than the corresponding values for maximum aerobic power achieved during sustained exercise on a bicycle ergometer (Table 2), and extremely close to the theoretical maximum postulated for man

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Promotion of 4-Hydroxy-3-methoxyphenylglycol Formation in Man by Reserpine

The ability of reserpine to bring about the depletion of biologically active amines in mammalian tissues[1] is well known This alkaloid also has several other biochemical effects which have recently been reviewed[2], and we report a new and potentially important action of reserpine on catecholamine metabolism which was revealed during the course of another experiment[3]

Six normal male volunteers, aged from 24 to 46, were given 1 25 mg of reserpine intravenously Urine was collected after 1, 3, 6, 9, 12 and 24 h. One month earlier, each subject had received an intravenous injection of placebo, and urine was collected at similar time intervals Samples were acidified with 6 N hydrochloric acid to *p*H 2, and stored in the cold until assayed for 4-hydroxy-3-methoxymandelic acid (VMA) (ref 4) and 4-hydroxy-3-methoxyphenylglycol (HMPG) (ref 5)

In agreement with the observations of other authors[6,7], there was a significant increase in the output of VMA during the test compared with control periods (Fig 1) This reached a peak in the 3–6 h collection and fell to normal by 12–24 h Predictably, but apparently for the first time, we also noted that reserpine gave rise to a significantly increased excretion of HMPG (Fig 2) which similarly reached a peak after 3–6 h The output of this compound was still significantly raised in the 12–24 h sample

Both compounds derive from the intermediate aldehyde formed from the oxidative deamination of noradrenaline and adrenaline (for review see ref 8) Perhaps the most interesting finding in this work is that there was a proportionately greater increase in the output of HMPG in response to reserpine injection (Fig 3), pointing to some diversion of the intermediate aldehyde from an oxidative to a reductive pathway of metabolism It may be relevant that Rutledge and Weiner[9] have produced *in vitro* data to suggest that noradrenaline, synthesized endogenously in the rabbit atrium from labelled dopamine, is predominantly inactivated to the glycol This tendency is exaggerated when the animal has previously been reserpinized (although the authors do not comment on this finding)

The mechanism of the reserpine-induced shift described here is not yet understood Others have observed a similar change after ethanol administration[10,11], both in the noradrenaline pathway and in the analogous 5-hydroxytryptamine route of metabolism[12] While a possible increase in the concentration of NADH$_2$ has been invoked to explain the shunting effect provoked by ethanol[11], it is more likely that some other mechanism is responsible for our finding, for Tyce *et al* [13] could not detect any alteration in ^{14}C-5-hydroxytryptamine metabolism in perfused reserpine-pretreated rat liver despite the predicted metabolic shift after ethanol perfusion

Disulfiram[10] and calcium carbimide[14] will induce similar *in vivo* changes, presumably by inhibition of aldehyde dehydrogenase, although it is just as likely that the reserpine effect is produced by activation of the complementary enzyme, aldehyde reductase, whether this latter enzyme is a distinct entity or whether one enzyme catalyses both reactions is still unknown[14].

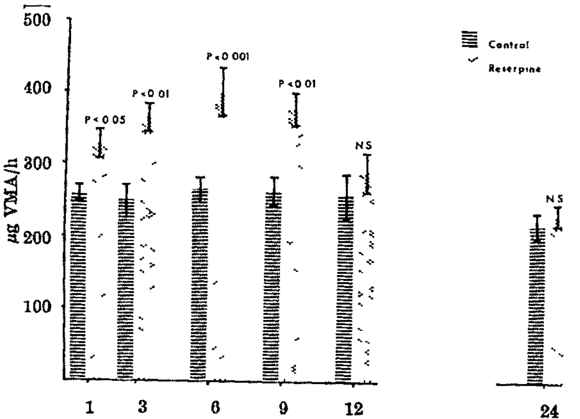

Fig 1 Means ± *S E* of timed urinary output of 4-hydroxy-3-methoxymandelic acid (VMA) (μg/h) in six subjects before and after a single intravenous injection of 1 25 mg of reserpine N S, Not significant

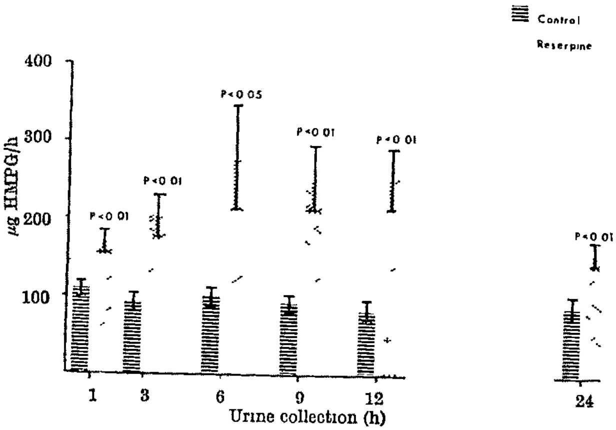

Fig 2 Means ± *S E* of timed urinary output of 4-hydroxy-3-methoxyphenylglycol (HMPG) (μg/h) in six subjects before and after a single intravenous injection of 1 25 mg of reserpine

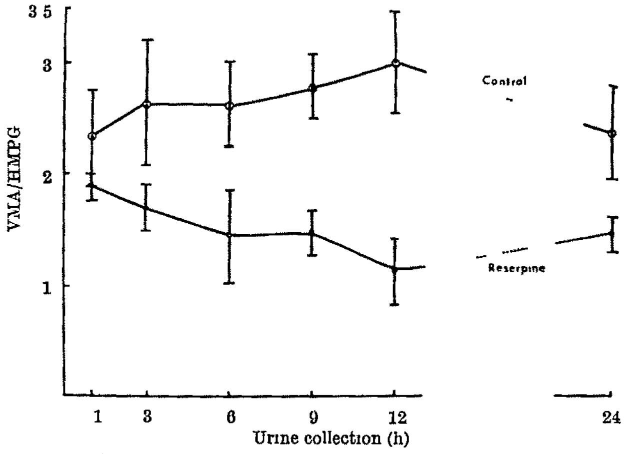

Fig 3 Ratios of means ± *S E* of urinary VMA to HMPG output in six subjects before and after a single intravenous injection of 1 25 mg of reserpine

There is one further possibility which deserves consideration It may be that the enzyme which brings about further oxidation of the aldehyde lies within the noradrenaline storage granule to which reserpine impairs access, thus facilitating aldehyde reduction.

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Received December 27, 1967

[1] Shore, P A, Silver, S L, and Brodie, B B, *Science*, **122**, 284 (1955)
[2] Carlsson, A, in *Heffter's Handbuch der Experimentellen Pharmakologie* (edit by Erspamer, V), 529 (Springer, Berlin, 1966)
[3] Sandler, M, and Youdim, M B H, *Biochim Appl*, *Parma* (in the press)
[4] Pisano, J J, Crout, J R, and Abraham, D, *Clin Chim Acta*, **7**, 285 (1962)
[5] Ruthven, C R J, and Sandler, M, *Clin Chim Acta*, **12**, 318 (1965)
[6] McDonald, R K, and Weise, V K, *J Pharmacol Exp Ther*, **136**, 26 (1962)
[7] Anton, A H, and Greer, M, *Clin Pharmacol Ther*, **7**, 727 (1966)
[8] Sandler, M, and Ruthven, C R J, in *Progress in Medicinal Chemistry* (edit by Ellis, G P, and West, G B), **6** (in the press, Butterworth, London, 1968)
[9] Rutledge, C O, and Weiner, N, *J Pharmacol Exp Ther*, **157**, 290 (1967)
[10] Smith, A, Gitlow, S, Gall, E, Wortis, S B, and Mendlowitz, M, *Clin Res*, **8**, 367 (1960)
[11] Davis, V E, Brown, H, Huff, J A, and Cashaw, J L, *J Lab Clin Med*, **69**, 787 (1967)
[12] Davis, V E, Brown, H, Huff, J A, and Cashaw, J L, *J Lab Clin Med*, **69**, 132 (1967)
[13] Tyce, G M, Flock, E V, and Owen, jun, C A, *Fed Proc*, **26**, 729 (1967)
[14] Goldstein, M, Friedhoff, A J, Pomerantz, S, and Contrera, J F, *J Biol Chem*, **236**, 1816 (1961)

Effect of Cocaine, Desipramine and Angiotensin on Uptake of Noradrenaline in Tissues of Pithed Rats

SYMPATHETIC nerves have been shown to be the site of uptake of circulating noradrenaline The extent of sympathetic innervation of an organ seems to determine the uptake of radioactive noradrenaline after intravenous injection if allowance is made for the volume of blood

Table 1. EFFECT OF COCAINE, DESIPRAMINE AND ANGIOTENSIN ON NORADRENALINE UPTAKE AND ON BLOOD PRESSURE RESPONSES TO NORADRENALINE IN PITHED RATS

Treatment	Predrug BP (mm Hg)	Maximum ΔBP (mm Hg)	Total radioactivity (mμCi/g) Heart	Skeletal muscle	Kidney	Adrenal gland	Aorta
Saline	60 ± 2.6	81 ± 3.0	92 ± 4.8	3.5 ± 0.3	125 ± 12.4	30 ± 3.4	16 ± 2.4
Cocaine	68 ± 2.8*	118 ± 1.9*	38 ± 1.9*	3.5 ± 0.2	144 ± 13.0	18 ± 1.8*	11 ± 3.0*
Desipramine	74 ± 3.0*	114 ± 3.3*	37 ± 1.8*	4.0 ± 0.4	150 ± 21.7	16 ± 0.4*	8 ± 0.9*
Angiotensin	64 ± 1.5	92 ± 2.4*	94 ± 5.3	3.9 ± 0.6	130 ± 9.3	33 ± 2.2	14 ± 1.0

Each blood pressure (BP) value or heart total radioactivity value is the mean ± S.E.M. of ten or eleven animals.
Each skeletal muscle, kidney, adrenal gland and aorta total radioactivity value is the mean ± S.E.M. of five or six animals.

* Denotes a significant difference ($P < 0.05$) from corresponding saline control values.

flow to the organ and its endogenous noradrenaline content[1].

Cocaine and desipramine have been shown to be potent inhibitors of noradrenaline uptake by the isolated perfused rat heart[2], and the potentiation of noradrenaline responses by cocaine and desipramine has been demonstrated in the isolated vas deferens of the rat[3]. Angiotensin (at a high concentration) is known to cause some inhibition of noradrenaline uptake by spleen, thoracic aorta and brain stem slices *in vitro*[4].

We have studied noradrenaline uptake *in vivo* utilizing the pithed rat preparation to determine whether the effects of cocaine, desipramine and angiotensin were similar in a variety of tissues. Exactly 30 min after pithing—while the blood pressure was being recorded—rats were injected with cocaine (10 mg/kg) or desipramine (1 mg/kg) or infused with angiotensin (0.03 µg/kg/min) or saline (0.004 ml./min). Fifteen minutes after injection or the start of an infusion, 2 µg (10 µCi)/kg of ^{3}H-7-DL-noradrenaline was injected intravenously. After 2 min the animal was killed and the total radioactivity of the heart, thoracic aorta, skeletal muscle, kidney and adrenal glands was determined by the procedure described by Van Zwieten, Widhalm and Hertting[5].

The blood pressure and radioactivity results obtained in this study are shown in Table 1. The variation of radioactivity recoverable was probably a result of the pressure of different numbers of sympathetic nerve endings in these tissues. The uptake of noradrenaline by heart, adrenal gland and thoracic aorta was significantly inhibited by cocaine and desipramine but not by angiotensin. The uptake of noradrenaline by skeletal muscle and kidney apparently was not affected by any of the agents. Studies of noradrenaline uptake in intact and immunosympathectomized rats[6] indicate that uptake by skeletal muscle and kidney is significantly reduced in the presence of a subnormal population of sympathetic nerve endings, and therefore it was expected that noradrenaline uptake in these tissues would be reduced by treatment with cocaine or desipramine. These experiments did not show whether the accumulation of noradrenaline by skeletal muscle and kidney from the circulation was significantly influenced by the relative blood flow to each tissue, by the ability of the tissue to extract the delivered noradrenaline[7] or by the more persistent noradrenaline blood concentrations resulting from the apparently defective uptake by other tissues.

The blood pressure responses to noradrenaline after cocaine, desipramine and angiotensin had been given were significantly facilitated. There seems to be a correlation between facilitation of noradrenaline blood pressure responses and inhibition of noradrenaline uptake after administration of cocaine and desipramine. None of the tissues utilized seemed to give any indication of a defective uptake of noradrenaline in the presence of a physiological concentration of angiotensin sufficient to cause a facilitation of the noradrenaline blood pressure responses.

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[1] Iversen, L., *The Uptake and Storage of Noradrenaline* (Cambridge, London, 1967).

[2] Iversen, L., *J. Pharm. Pharmacol.*, 17, 62 (1965).

[3] Benevenuti, F., Bonaccorsi, A., and Garrattini, S., *J. Pharm. Pharmac.*, 19, 477 (1967).

[4] Palaic, D., and Khairallah, P., *J. Pharm. Pharmacol.*, 19, 396 (1967).

[5] Van Zwieten, P., Widhalm, S., and Hertting, G., *J. Pharmacol.*, 149, 50 (1965).

[6] Zaimis, E., Berk, L., and Callingham, B., *Nature*, 206, 1220 (1965).

[7] Kopin, I., Gordon, E., and Horst, D., *Biochem. Pharmacol.*, 14, 753 (1965).

Fixation Tremor in relation to Eyeball–Muscle Mechanics

THE fixation tremor of the eyes can be recorded by using a small accelerometer which is fixed to a contact lens[1]. This technique has made it possible to determine the changes in the spectral composition of the tremor in various circumstances. In particular, we report here the results obtained when weights are fixed to the lens, so increasing the effective moment of inertia of the eyeball. These results yield information about the mechanics of the eyeball–muscle system.

The subject, wearing a close-fitting contact lens, lies supine and fixates on a black spot 3 mm in diameter at a distance of 2 m. The accelerometer is plugged into a 'Perspex' tube which is cemented to the lens. Small brass weights are stuck to this tube with 'Plasticine'. The output voltage from the accelerometer is amplified, passed through a band-pass filter (5–250 Hz), and then integrated electrically, thus obtaining an angular velocity-versus-time record of the tremor. The record is stored on the screen of a storage oscilloscope, and later photographed on 35 mm film. A specially designed digital potentiometer[2] is used to sample ordinates of the traces at intervals of 3 ms. The ordinate values are supplied to a digital computer, which is programmed to compute the autocorrelation function, and, from this, the velocity spectral density (VSD) function in the usual way[3].

Fig. 1 shows a typical series of VSD graphs. Each graph has been computed from 800 data points. When the eye carries only the contact lens and accelerometer (graph *a*) there is a main peak in the spectrum at 40 Hz. As the inertia is increased, this peak is seen to move downwards in frequency. There is also a reduction in the amplitudes of the high frequency components of the spectrum. These results can be interpreted in mechanical terms. The tremor waveform can be considered to be the "output" of a mechanical filter, the "input" to which is the force developed by the active component of the muscles in normal tone. The characteristics of the filter are fixed by the moment of inertia of the eyeball, the elastic stiffness of the muscles and the viscous damping of the system[4]. When considering VSD graphs, the filter is of the band-pass type. A peak of transmission is expected at the mechanical resonance frequency of the system. This frequency is lowered as the inertia of the system is increased. The main peak in the graphs of Fig. 1 is identified with the mechanical resonance frequency of the eyeball–muscle system.

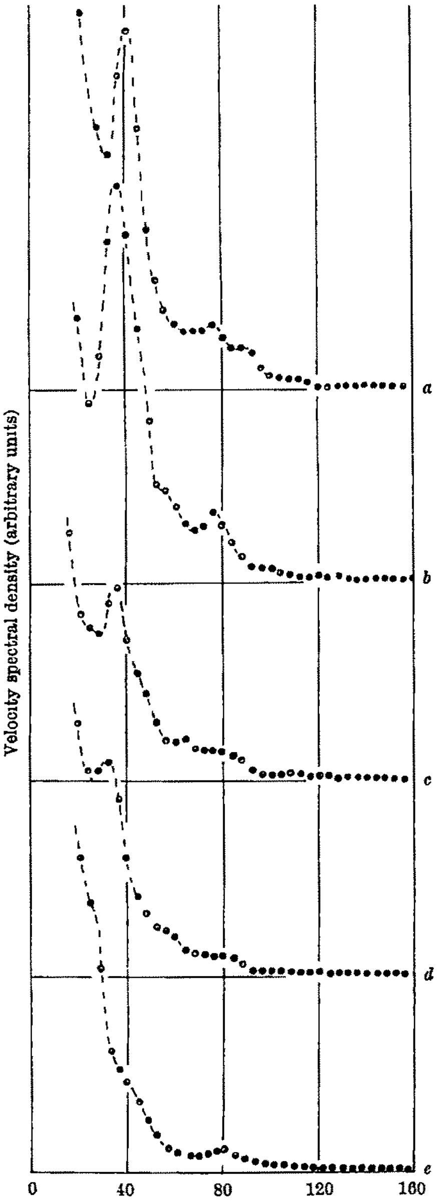

Fig 1 Velocity spectral density graphs which have been computed from records of elevation–depression tremor The graphs show the effect of loading the eye by fixing weights to the contact lens, thus increasing the moment of inertia of the eyeball The values of the added inertia for graphs *a*, *b*, *c*, *d*, *e* are 5, 6·1, 7·3, 10 and 12 g cm² respectively

From experiments in which the eye is vibrated by external forces, Thomas has formulated a model of the eyeball–muscle system[4]. The resonance frequencies deduced from the present work are 20–30 per cent lower than those which have been predicted from the model, but, in spite of this discrepancy, the present results must be considered to give strong support to the general validity of the model, in contrast to the model proposed by Robinson[5] (supported by Childress and Jones[6]) in which the system is stated to be " . heavily overdamped, has no resonant frequency and is little affected by the mass of the eyeball".

A small peak at 70–80 Hz can be seen in the graphs of Fig 1 When a correction is made for the loading effect of the lens and its attachments, or when tremor is recorded by a method which does not impose any loading of the eye[7], this peak is much more pronounced Its frequency is not changed by loading the eye, and so it is associated not with the mechanics of the system but with the "input" to the system—that is, with the fluctuations in muscle tension, and thus with the stream of tonic impulses to the muscles

The attenuation of the high frequency components of the tremor which is caused by inertial loading will have occurred in the experiments of previous workers who have used a mirror on a contact lens to record eye movements

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[1] Thomas, J G , *J Opt Soc Amer* , 55, 534 (1964)
[2] Bengi, H , and Thomas, J G , *J Sci Instrum* , 44, 951 (1967)
[3] Blackman, R B , and Tukey, J W , *The Measurement of Power Spectra* (Dover, New York, 1958)
[4] Thomas, J G , *Kybernetik*, 3, 254 (1967)
[5] Robinson, D A , *J Physiol* , 174, 245 (1964)
[6] Childress, D S , and Jones, R W , *J Physiol* , 188, 273 (1967)
[7] Bengi, H , and Thomas, J G , *Med and Biol Eng* (in the press)

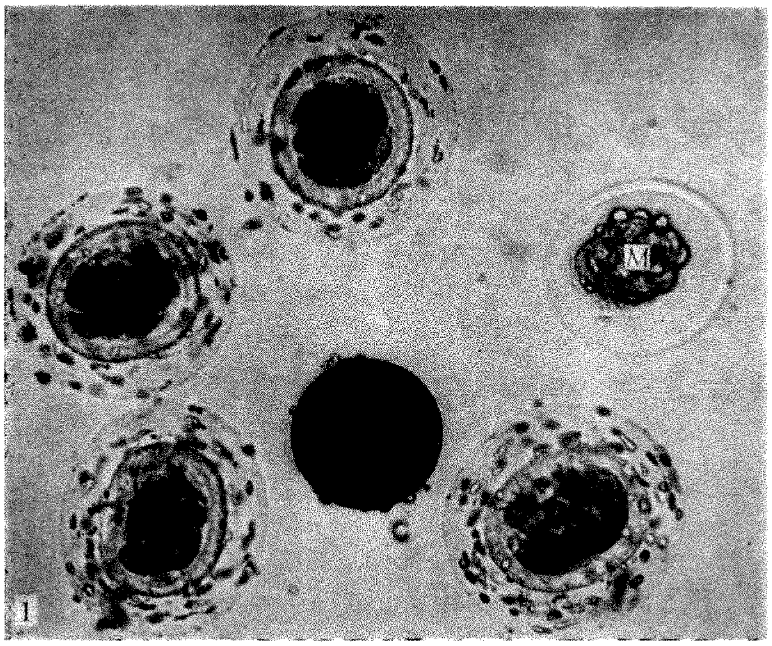
Effect of an Intra-uterine Device on Uterine Motility in the Rhesus Monkey

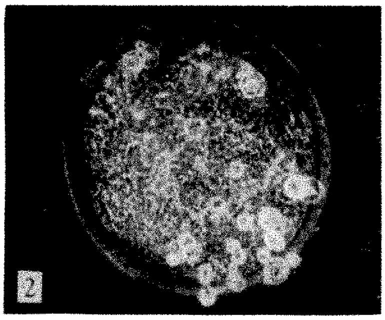
WE recently observed[1] that although an intra-uterine device (IUD) did not affect ovulation and fertilization, it was contraceptive in the rhesus monkey. The pattern of tubal transport was not markedly disturbed, but eggs could not be recovered from the uterus in IUD monkeys It was inferred that the principal contraceptive effect of the IUD is probably exerted within the uterus, to cause either premature expulsion and/or premature destruction of the fertilized egg The presence of an IUD may therefore disturb the pattern of uterine motility in the rhesus monkey

We have attempted to explore this possibility by recording the pattern of uterine motility in eight IUD and seven control rhesus monkeys at various times during consecutive menstrual cycles A chronically implanted, strain gauge transducer[2] was used and this was attached to the ventral serosal surface of the uterus, connected to a miniature plug in the vagina, and thence to a twin-channel recording system ('Oscillo-riter', Texas Instruments, Inc) An external transducer was attached to the monkey's chest to monitor both respiratory activity and gross bodily movements Recordings were made over a period of 1–3 h with the conscious monkey seated in a restraint chair The monkey was habituated to this restraint and tolerated it without undue stress

Some convincing records of uterine motility were obtained, but usually the record was distorted by respiratory or gross bodily movements Thus although the transducers were sensitive to variations in uterine motility and abdominal pressure, they did not provide a direct and quantitative means of comparing uterine motility

The possibility that eggs are prematurely expelled from the uterus in the presence of an IUD was then tested by determining the fate of artificial and natural substitutes for rhesus monkey eggs after they had been surgically transferred to the uterus Initially, groups of three silver (110*m*)-coated resin spheres (R A eggs) were used as artificial substitutes These R A eggs were similar in size and density to natural monkey eggs, and resembled those used by previous workers[3] In subsequent experiments, fertilized rabbit eggs were transferred as natural substitutes together with a single R A egg, which acted as a marker to facilitate transfer





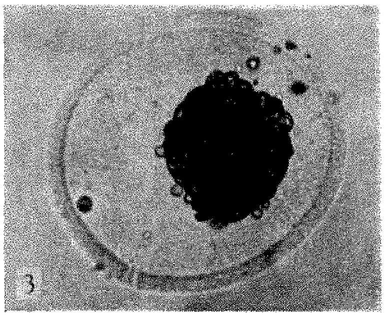


Fig. 1. Four rabbit eggs, one radioactive egg and a normal monkey morula (*M*) recovered from a control monkey on day 18.

Fig. 2. Degenerate, unfertilized monkey egg from the uterus of an IUD monkey on day 17.

Fig. 3. Degenerate monkey morula from the uterus of an IUD monkey on day 18.

R.A. and rabbit eggs were transferred to twelve control and twelve IUD monkeys on menstrual day 15, and to two IUD monkeys on day 18. The animals had regular menstrual cycles and had been caged with a fertile male for at least 3 days before transfer. At transfer, all except five monkeys had ovulated (Table 1): two of these animals had inactive ovaries and three had ovaries in a pre-ovulatory condition. The eggs were transferred in < 10 μl. of physiological saline by using a fine, nylon cannula attached to a micrometer syringe. At autopsy, the entire genital tract was removed and divided so that the uterus, cervix and vagina could be separately flushed with physiological saline. The uterus and cervix were then also monitored with a Geiger counter. With one exception, when eggs were flushed from the cervix, the transferred eggs were recovered only from the uterus.

The results of these experiments are summarized in Table 1. There was no difference in the recovery of transferred eggs between the control and IUD monkeys examined 48 h after transfer. Most of the R.A. and rabbit eggs could be recovered, and there was no evidence to suggest that premature expulsion from the uterus had occurred in the IUD monkeys.

At recovery, the appearance of the R.A. and rabbit eggs suggested that they were resistant to destruction within the monkey uterus (Fig. 1). The rabbit eggs had an intact and apparently normal zona pellucida, and the "albumen" layer, which had coated most of the eggs at the time of transfer, also seemed to be unchanged. The transferred eggs had virtually disappeared from the uteri of IUD monkeys examined at 72 h, and from the control monkeys at 96 h. Moreover, one control monkey examined 72 h after egg transfer yielded two rabbit eggs and an R.A. egg in the cervical flush, but nothing in the uterine flush. This evidence suggests that the transferred eggs were eventually expelled.

Table 1. RADIOACTIVE AND RABBIT EGGS RECOVERED FROM THE UTERUS AT AUTOPSY

Autopsy (h after transfer)	Group	Total monkeys (yielding transferred eggs/examined)	Total radio-active eggs (recovered/transferred)	Total rabbit eggs (recovered/transferred)
Following transfer on day 15				
48	Control	3/4*	7/12	—
	IUD	4/4	10/12	—
48	Control	2/2†	2/2	10/12
	IUD	4/4	4/4	20/27
72	Control	4/4	4/4	10/28
	IUD	1/4	0/4	1/28
96	Control	1/2‡	0/2	1/14
Following transfer on day 18				
48	IUD	2/2*	2/2	13/18

* One monkey had not ovulated.

† Two monkeys had not ovulated.

‡ One monkey ovulated between laparotomy and autopsy.

It is interesting that at 72 h after transfer (Table 1) only one egg could be recovered from the IUD monkeys, and yet all the R.A. eggs and 36 per cent of the rabbit eggs were found in the controls. The results might suggest that the transferred eggs had a somewhat shorter uterine life in the presence of an IUD. It is not possible, however, to draw any precise conclusions on uterine survival from groups of animals which had to be examined at time intervals 24 h apart.

Additional results were obtained, at autopsy, incidentally to these experiments. Rhesus monkey eggs were recovered from two out of four control and two out of eight IUD monkeys, which were suitable for the recovery of naturally ovulated eggs and had ovulated by menstrual day 15. On day 18 a normal morula of forty cells (Fig. 1) was flushed from the uterus of one control monkey while an abnormal, eleven celled morula was recovered from the cervix of the other. In contrast to our previous observations[1], the two IUD monkey eggs were recovered from the uterus. One egg, recovered on day 17, was degenerate and unfertilized, but the other was a degenerate morula of more than twenty cells on day 18 (Figs. 2 and 3). The corpora lutea associated with the four eggs were similar in structure: according to Corner's histological criteria[4], they were approximately 6 days old. These naturally ovulated eggs had probably survived within the uterus for 2 or 3 days.

Our results do not provide convincing evidence that the contraceptive action of an IUD in the rhesus monkey results principally from the premature expulsion of eggs from the uterus. By inference, the evidence favours the alternative explanation that in the presence of an IUD fertilized rhesus monkey eggs undergo premature degeneration once they have entered the uterus.

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[1] Kelly, W. A., and Marston, J. H., *Nature*, **214**, 735 (1967).
[2] Bass, P., and Callantine, M. R., *Nature*, **203**, 1367 (1964).
[3] Harper, M. J. K., Bennett, J. P., Boursnell, J. C., and Rowson, L.E.A., *J. Reprod. Fertil.*, **1**, 249 (1960).
[4] Corner, G. W., *Contrib. Embryol. Carnegie Inst.*, **31**, 117 (1945).

Activation of Malathion by the Locust Bodywall

WE reported earlier[1] that dilution of malathion in the mineral oil KEB (kerosene extract bottoms) not only increases its toxicity to mature adults of the desert locust, *Schistocerca gregaria*, but also the speed of its action, when applied topically on the hind wing base. We later discovered[2] that the killing power of a 10 µg/g dose of malathion 2·5 per cent solution in KEB varies considerably with the site of application on the locust body. Thus it yielded a 100 per cent kill when applied topically to the neck membrane, no deaths at all on the hind femur and intermediate values for other sites of application. Injections into the body cavity were always less toxic than topical application in the same general area of the body. There was, however, no significant difference in the killing power of malaoxon (2·5 per cent solution in KEB) topically applied to the neck membrane when compared with its injection through the same membrane[3]. This led us to believe that topically applied malathion is converted to its $P=O$ analogue, malaoxon (which is more toxic and more rapid in action than malathion) by the locust bodywall and that the KEB only helps its penetration and conversion by spreading the insecticide on to a wider area of the insect bodywall. The experiments reported here were designed to show this activation by the bodywall as well as penetration differences and to explain the differences in toxicity and speed of action reported earlier[1].

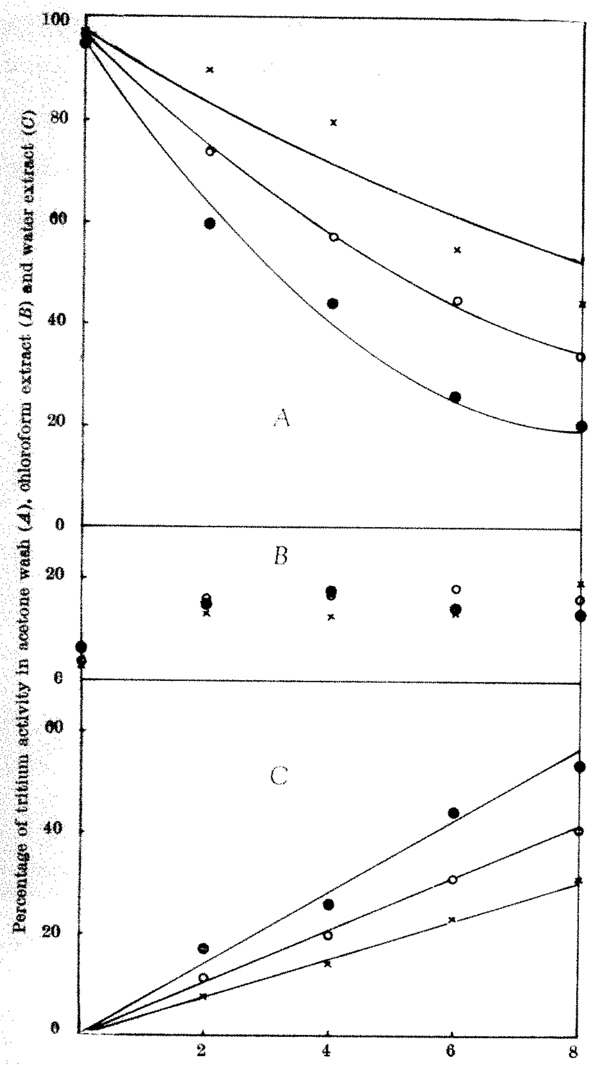
Malathion labelled with sulphur-35 (both atoms labelled) was mixed with tritiated malathion (the carboethoxy part of the molecule labelled), in the ratio of approximately 1 : 5. Three solutions (w/v) were then prepared from this mixture using KEB as the diluent and mature males of *Schistocerca gregaria* were dosed at a rate of 40 µg/insect, applied on the hind wing base as a single drop of 0·2, 0·8 and 1·6 µl./insect of 20, 5 and 2·5 per cent solutions, respectively. An 'AGLA' syringe driven by a micrometer was used for dosing in each case and the insects were kept at 31° C after dosing. At intervals of 0, 2, 4, 6 and 8 h after dosing, each insect was washed thoroughly with acetone, dried and homogenized together with any excreta and extracted with chloroform and water[4]. A portion of each extract and acetone wash was counted in a Packard 'Tri-Carb' liquid scintillation spectrometer to determine the recoveries of sulphur-35 and tritium in each phase. Corrections were made for quenching of each nuclide and self-decay of sulphur-35 where necessary. Tritium activity thus provided a measure of the unabsorbed portion of the dose (acetone wash), its accumulation in the insect body as malathion and/or malaoxon[4] (chloroform extract) and the portion hydrolysed (water extract) at different time intervals. The loss of activity of sulphur-35 over that of tritium, on the other hand, provided a measure of the conversion of malathion to malaoxon (a 50 per cent loss being equal to a 100 per cent conversion).

Table 1. RECOVERIES OF SULPHUR-35 AND TRITIUM FROM DIFFERENT PHASES AFTER DOSING EACH INSECT WITH 40 µg OF DOUBLE LABELLED MALATHION IN THREE DIFFERENT SOLUTIONS OF KEB

Percentage of malathion in KEB (w/v)	Hours after dosing	Nuclide	Radioactivity recovered (%)				Loss (−) or gain (+) of ^{35}S over ^{3}H (%)
			Unabsorbed	Chloroform extract	Water extract	Total	
20	0	^{35}S	96·5	3·4	Nil	100	Nil
		^{3}H	97·1	2·9	Nil	100	
	2	^{35}S	91·8	14·0	4·3	110·1	−0·3
		^{3}H	89·6	13·0	7·7	110·3	
	4	^{35}S	81·5	13·7	11·8	107·0	+0·8
		^{3}H	79·4	12·8	14·0	106·2	
	6	^{35}S	56·5	14·4	19·6	90·5	−1·2
		^{3}H	55·0	13·3	23·4	91·7	
	8	^{35}S	46·3	21·5	23·6	91·4	−3·7
		^{3}H	44·7	19·8	30·6	95·1	
5	0	^{35}S	95·9	4·1	Nil	100	Nil
		^{3}H	96·4	3·6	Nil	100	
	2	^{35}S	77·4	17·0	6·8	101·2	−0·6
		^{3}H	74·3	15·8	11·7	101·8	
	4	^{35}S	60·8	18·3	15·5	94·6	+0·3
		^{3}H	57·9	16·7	19·7	94·3	
	6	^{35}S	46·5	19·0	24·5	90·0	−3·7
		^{3}H	44·8	17·9	31·0	93·7	
	8	^{35}S	35·9	17·4	34·7	88·0	−3·3
		^{3}H	34·3	16·4	40·6	91·3	
2·5	0	^{35}S	93·3	6·7	Nil	100	Nil
		^{3}H	93·6	6·4	Nil	100	
	2	^{35}S	62·8	16·0	13·6	92·4	+0·6
		^{3}H	59·9	14·9	17·0	91·8	
	4	^{35}S	46·0	19·1	20·7	85·8	−1·6
		^{3}H	43·9	17·5	26·0	87·4	
	6	^{35}S	27·5	14·4	37·6	79·5	−5·0
		^{3}H	26·6	13·7	44·2	84·5	
	8	^{35}S	21·7	14·1	43·7	79·5	−7·6
		^{3}H	20·7	12·9	53·5	87·1	

The results, an average of three replications in each case, are presented in Table 1. The results are complicated by two factors. One is that the sulphur-35 product of malathion metabolism is soluble in organic solvents and results in a change of the ratio of sulphur-35/tritium even in the acetone wash. This sulphur containing by-product is eventually lost, possibly in the form of a gas, resulting in an overall increase with time in the recoveries of tritium as compared with sulphur-35, thus showing clearly the formation of malaoxon. This increase was higher in the case of the 2·5 per cent solution than in the 5 or 20 per cent solutions, where there are differences, although not well synchronized, showing that more malaoxon was formed in the case of the dilute than the concentrated solutions of malathion in KEB. Quantitative estimations of malaoxon formation in the three solutions were not possible because of the recovery of excess sulphur-35 activity in the organic solvents. The second factor which made quantitative estimations difficult was the decrease in total recoveries of tritium activity with time. This is because of the difficulty of homogenizing the locust wings and legs which in time become radioactive[3], presumably as a result of the movement of hydrolysis by-products of malathion into these regions. The fact that water extracts always had a higher activity of tritium than sulphur-35 also indicates that hydrolysis takes place chiefly, if not entirely, from malaoxon rather than directly from malathion.

In Fig. 1, the tritium activities recovered in acetone wash, chloroform and water extracts are plotted against time. It will be seen that increased rates of penetration in cases of dilute solutions were counterbalanced by a corresponding increase in the rates of hydrolysis. If hydrolysis represents detoxification[5], and the chloroform extract has only malathion in it, then the differences exhibited here are so trivial that the significant differences reported earlier[1] in the toxicity of these three solutions can scarcely be explained satisfactorily. In fact, at the end of the experimental period the portion of the applied dose which could be extracted with chloroform was lowest in the case of more toxic 2·5 per cent solution and highest in the least toxic 20 per cent solution. These results can only be explained by assuming that in the case of dilute solutions more malathion is desulphurized into malaoxon, which is a more labile compound than malathion. This conversion results in corresponding increases in the rates of hydrolysis, and because the rates of penetration decrease with time more rapidly in the dilute solutions than the concentrated ones, whereas the rate of hydro-



Hours after application of 40 μg of labelled malathion/insect as 20 per cent (×), 5 per cent (○) and 2·5 per cent (●) solutions in KEB.

Fig. 1.

lysis in all cases is linear, at the end of the experimental period the chloroform extractable portion of the dose applied as dilute solution goes down.

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Ahmed, H., and Gardiner, B. G., *Nature*, **214**, 1338 (1967).
Ahmed, H., and Gardiner, B. G., in *Symposium on the Activity of Pesticides, Soc. Chem. Indust., London, Monograph*, 29 (in the press).
Ahmed, H., and Gardiner, B. G., *Bull. Entomol. Res.* (in the press).
Krueger, H. R., and O'Brien, R. D., *J. Econ. Entomol.*, **52**, 1063 (1959).
Metcalf, R. L., *Ann. Rev. Entomol.*, **12**, 229 (1967).

Part played by Ciliate Protozoa in Rumen Function

IN general, the population of ciliate protozoa in the umen is large with more than 10^6 organisms/g of rumen ontents and a mass approximately equal to that of the umen bacteria[1]. These protozoa are known to possess ctive systems for hydrolysing carbohydrates, proteins nd lipids, but their effects on the efficiency of utilization of nutrients are not clear. In some experiments, removal of the fauna of the rumen has not influenced performance[2–4] while in others the patterns of rumen fermentation, growth rate and efficiency of food utilization were altered[5–7]. Protozoa have been implicated as causal agents in bloat, and Clarke[8] has shown that the introduction of an anti-protozoal agent to the rumen of cattle reduces the rate of rumen fermentation and the susceptibility to bloat.

Considerable day-to-day variation in the pattern of rumen fermentation (as measured by the molar proportions of steam volatile fatty acids present in the rumen liquor) has been observed in this laboratory, especially on diets low in fibre content. It has been postulated that this variation can be caused by a fluctuating population of ciliate protozoa in the rumen.

A short-term *in vitro* fermentation has been used in an attempt to study the effect on rumen fermentation of the ciliate protozoa of the rumen in mixed culture with rumen bacteria. In the three experiments reported, rumen liquor was donated by a rumen-cannulated sheep fed 800 g of concentrate and 92 g of hay daily. The ciliate protozoa were almost completely removed mechanically by centrifugation.

Centrifuged ("defaunated") and un-centrifuged rumen liquor were incubated for 1 h at 39° C either alone, or after adding a substrate consisting of 1 g of the concentrate fed to the donor sheep. Net production rates of total and individual steam volatile fatty acids were calculated by subtracting total production in the absence of substrate from that in the presence of substrate. The results are presented in Table 1.

The fall in the rate of production of *n*-butyric acid when ciliate protozoa were excluded from rumen liquor was striking, particularly in the first and third experiments. This fall was largely compensated by an increase in the rate of production of propionic acid. In all three experiments, the exclusion of ciliate protozoa raised the rate of production of *n*-valeric acid and depressed that of iso-valeric acid. Effects on the production of acetic acid were variable, there being a marked decline in rate of production in the first experiment but only slight reductions in the second and third experiments.

In the first two experiments, removal of the ciliate protozoa reduced the total rate of production of steam volatile fatty acids, a finding consistent with the removal of part of the biomass. In the final experiment, however, no such difference was observed. These variable effects on the rate of fermentation may be related to the initial size of the population of ciliates but, as yet, no attempt has been made to describe the population quantitatively.

Table 1. NET *in vitro* RATES OF PRODUCTION OF STEAM VOLATILE FATTY ACIDS IN RUMEN LIQUOR WITH OR WITHOUT THE PRESENCE OF CILIATE PROTOZOA

	With protozoa		Without protozoa	
	mequiv./100 ml.	M percentage	mequiv./100 ml.	M percentage
	Experiment I			
Acetic	0·798	41·8	0·395	30·4
Propionic	0·737	38·6	0·820	63·1
n-Butyric	0·300	15·7	0·022	1·7
Iso-butyric	0·015	0·8	0·011	0·8
n-Valeric	0·026	1·4	0·046	3·5
Iso-valeric	0·031	1·6	0·006	0·5
Total acids	1·907		1·300	
Final *p*H	5·70		6·16	
	Experiment II			
Acetic	1·559	53·0	0·862	50·2
Propionic	0·843	28·7	0·670	39·0
n-Butyric	0·382	13·0	0·102	5·9
Iso-butyr c	0·040	1·4	0·031	1·8
n-Valeric	0·040	1·4	0·049	2·9
Iso-valeri c	0·076	2·6	0·003	0·2
Total acids	2·940		1·717	
Final *p*H	6·29		6·77	
	Experiment III			
Acetic	1·084	47·7	1·047	45·8
Propionic	0·834	36·7	1·119	48·9
n-Butyric	0·251	11·1	0·023	1·0
Iso-butyric	0·040	1·8	0·035	1·5
n-Valeric	0·020	0·9	0·030	1·3
Iso-valeric	0·042	1·8	0·033	1·4
Total acids	2·271		2·287	
Final *p*H	5·57		5·90	

The final *p*H of the rumen liquor was consistently higher after fermentation in the absence of ciliate protozoa.

Similar but less dramatic effects on the fermentation process were observed when rumen liquor was fermented in the presence of an antiprotozoal agent (dimetridazole, May and Baker, England).

Changes in rumen fermentation of the magnitude observed in these experiments occurring *in vivo* could have important effects on the efficiency of utilization of energy for growth and milk production. Clearly further studies both *in vitro* and *in vivo* are necessary to determine more precisely the role of ciliate protozoa in the production of steam volatile fatty acids and to investigate factors, such as *p*H, which may determine the level of protozoal activity in the rumen.

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[1] Hungate, R. E., *Ann. Rev. Microbiol.*, **4**, 53 (1950).
[2] Becker, E. R., and Everett, R. C., *Amer. J. Hyg.*, **11**, 362 (1930).
[3] Bryant, M. P., and Small, N., *J. Dairy Sci.*, **43**, 654 (1960).
[4] Eadie, J. M., *J. Gen. Microbiol.*, **29**, 563 (1962).
[5] Abbou Akkada, A. R., and El-Shazly, K., *Appl. Microbiol.*, **12**, 384 (1964).
[6] Abbou Akkada, A. R., and El-Shazly, K., *J. Agric. Sci.*, **64**, 251 (1965).
[7] Christiansen, W. C., Kawashima Ryoji, and Burroughs, W., *J. Animal Sci.*, **24**, 730 (1965).
[8] Clarke, R. T. J., *N.Z. J. Agric. Res.*, **9**, 437 (1966).

Extracellular Recording from Single Neurones in the Olfactory Centre of the Cockroach

Very few reports have been published concerning the olfactory responses from single units in the central nervous system of invertebrates. This communication is concerned with the successful extracellular recording of single unit responses evoked by olfactory stimulation. It is emphasized that the response to odours can be classified into three categories: namely, "on" response; "on–off" response; and "off" response.

We used the adult American cockroaches *Periplaneta americana*, bred in the laboratory. An unanaesthetized cockroach was secured with pins and tape on a cork plate; its respiration was unhindered. A square section of the dorsal exoskeleton of the frons was removed to expose the olfactory centre. Electrolytically tapered tungsten needles with the insulation removed at the tip were used initially as recording microelectrodes, but these were later replaced by glass pipettes filled with 3 M potassium chloride with diameters of 0·5–2·0μ at the tip. The latter type of electrode was able to isolate single units more easily. The amplification and recording system was the same as that described earlier[1]. The microelectrode was inserted into the dorsal sensory neurone area of the deutocerebrum, and was advanced as far down as the outer margin of the ventral motor neurone area.

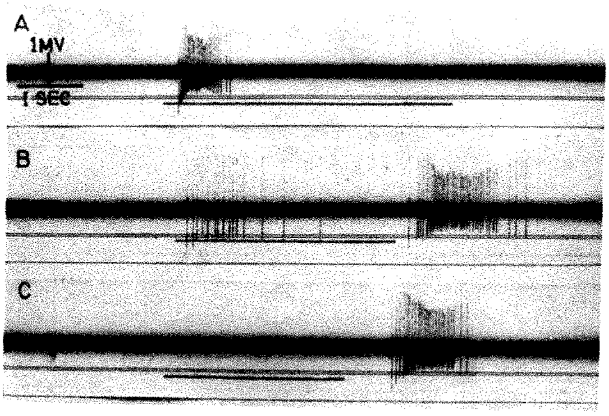

Fig. 1. Responses of a single olfactory neurone to odour stimuli. *A*, Propionic acid; *B*, acetic acid; *C*, acetic acid (10 s after the preceding stimulation shown in *B*). In each record, the horizontal black bar below the record indicates the period of stimulation.

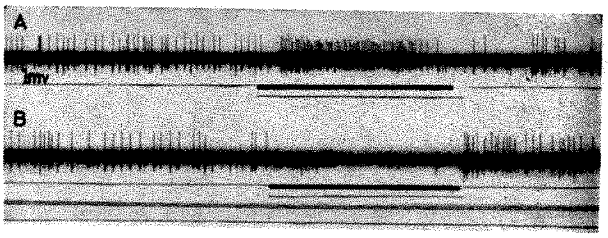

Fig. 2. Responses of olfactory neurones to propionic acid (*A*) and methyl-ethyl ketone (*B*). Time: 60 Hz.

Odorous air was applied to the surface of the antenna by squeezing a polyethylene bottle containing an odorous substance. During the course of penetration of the electrode, odour stimuli were repeatedly applied at regular intervals in order to ensure that the electrode did not miss neurones which were not spontaneously active. The odorous substances applied were propionic acid, acetic acid, acetone, methyl alcohol, hydrochloric acid and methyl-ethyl ketone. The experiments were performed at room temperature, about 20° C.

Unitary discharges were always observed when the microelectrode was advanced into the antennal lobe (the olfactory centre). Most units fired spontaneously, but some showed little or no spontaneous discharge. Fig. 1 shows the responses to odour stimuli of an olfactory neurone which shows no spontaneous discharge. Three kinds of response patterns were evoked by two types of odour stimuli. In Fig. 1*A*, the neurone stimulated by propionic acid produced an initial burst of discharges which soon ceased despite continuous stimulation ("on" response). Fig. 1*B* shows a typical "on–off" response caused by acetic acid. Fig. 1*C* shows an "off" response to an acetic acid stimulation which was applied 10 s after the initial stimulation (Fig. 1*B*). These "off" responses gradually became more pronounced as the time interval of acetic acid stimuli was shortened. The neurone that responded to propionic acid and acetic acid also responded to hydrochloric acid, but it failed to respond to other types of odour stimuli which included methyl-ethyl ketone, acetone and methyl alcohol, regardless of the concentration.

These results suggest that the neurones in the olfactory centre have a certain specific sensitivity to odour stimuli. An example of another type of sensitivity is shown in Fig. 2: the recording was made from a deeper region than that in Fig. 1, and spontaneous discharges were observed. The spontaneous activity permitted observation of the inhibitory as well as the excitatory effects of odour stimulation. Judging from the irregular spike heights, the responses may have been derived from more than one neurone.

Two types of odour, propionic acid and methyl-ethyl ketone, had different effects: propionic acid excited the neurones to produce a burst of discharges during the period of stimulation (Fig. 2*A*). The background impulses were temporarily inhibited immediately after the beginning of the stimulation. This is a kind of the "on" response shown in Fig. 1*A*. On the other hand, methyl-ethyl ketone inhibited the spontaneous discharges (Fig. 2*B*). The neurones that were inhibited by odour stimulation usually showed an increased activity immediately after the cessation of the stimulation. This resembles the "off" response shown in Fig. 1*C*.

In primary olfactory receptors of insect antennae, excitation ("on" response) and inhibition ("off" response) are known to be components of the odour response pattern of a receptor. The "on–off" response pattern has not, however, been recorded from the periphery[1–4].

It is interesting to note that the three patterns of responses observed in the olfactory centre of the cockroach are quite similar to those in the optic lobe of insects[5] and of the ganglion cells of the mammalian retina[6].

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[1] Yamada, M, *Appl Ent Zool*, 2, 22 (1967)

[2] Boeckh, J, *Z vgl Physiol*, 46, 212 (1962)

[3] Schneider, D, Lacher, V, and Kaissling, K E, *Z vgl Physiol*, 48, 632 (1964)

[4] Boeckh, J, *Proc Second Intern Symp on Olfaction and Taste* (edit by Hayashi, T), 721 (Pergamon Press, Oxford, 1967).

[5] Burtt, T E, and Catton, T W, *J Physiol*, 154, 479 (1960)

[6] Kuffler, S W., *J Neurophysiol*, 16, 37 (1953)

Conductance or Resistance?

IN circulatory physiology, there may be difficulty in expressing quantitatively the response of a vascular bed in which blood flow has altered If change of flow is used to express the response, then a large change in arterial pressure may be overlooked For this reason it has been customary to express responses as changes of resistance, vascular resistance being the pressure difference across the vascular bed divided by the flow through it

One disadvantage of this method can be appreciated when two simple examples are considered First, if flow changes from 50 ml /min to 2 ml /min, while the pressure difference remains constant at 100 mm of mercury, then the resistance changes from 2 to 50 units Second, if flow changes from 50 ml /min to 1 ml /min, while the pressure again remains constant at 100 mm of mercury, then resistance has changed from 2 to 100 units. In these examples the flow changes—48 ml /min and 49 ml /min—are similar and many flow meters would fail to register the difference between the two responses, but the resistance changes are 48 and 98 units, the difference between the two being large

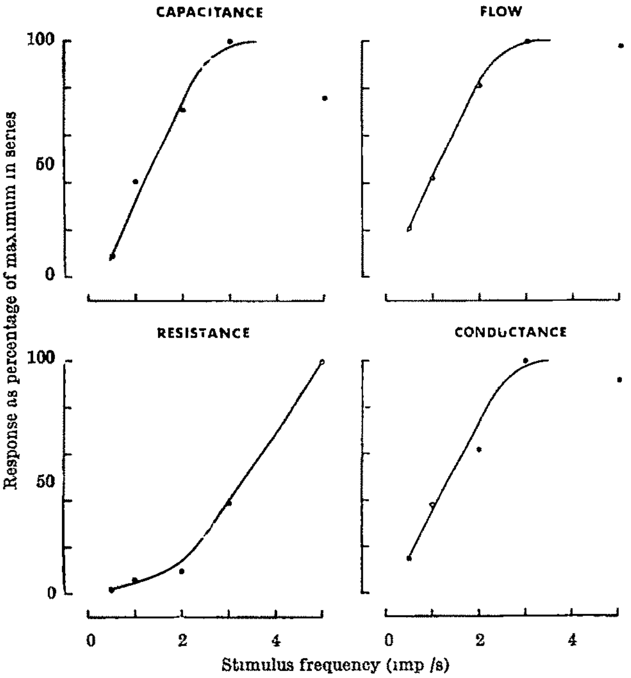

Fig 1 Capacitance, flow, resistance and conductance responses during splenic nerve stimulation These responses are measured as the differences between the pre-stimulation values of splenic weight, splenic artery flow, resistance and conductance and the respective values at the end of the second minute of nerve stimulation The differences are expressed as percentages of the maximum changes in each parameter (ordinates) and are plotted against the frequency of nerve stimulation (abscissae) Splenic weight change is a measure of capacitance change For details of the methods see ref 1

Vascular resistance is a derived term and is inversely related to blood flow. When vascular smooth muscle contracts, flow decreases and capacitance decreases but resistance increases Is it not then misleading to compare capacitance with resistance in conditions of nerve stimulation or drug infusion ?

Conductance, which is obtained by dividing flow by pressure difference, incorporates pressure but has a direct relationship to smooth muscle length In electrical terms it is more significant to compare conductance (A/V) with capacitance (A × s/V), rather than resistance (V/A) with capacitance In circulatory research, it is usual to disregard the pressure denominator when evaluating capacitance

Fig 1 shows the responses obtained in one cat when the splenic nerves were stimulated at frequencies between 0 5 and 5 impulses/s The changes in splenic weight (capacitance), splenic artery flow, resistance (pressure/flow) and conductance (flow/pressure) are expressed as percentages of the maximum responses in the series and plotted against the frequency of stimulation Smooth curves have been drawn Capacitance, flow and conductance each have a similar relation to frequency, but the resistance curve shows a marked separation from that of capacitance.

In conclusion, I feel that conductance is the most satisfactory method of expressing responses of a vascular bed in which blood flow or perfusing pressure changes

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[1] Greenway, C V, Lawson, A E, and Stark, R D, *J Physiol* (in the press)

Heparin and the Stabilization of Fibrin

IN experiments to compare the activity of factor XIII (fibrin stabilizing factor) in serum and plasma, it was necessary to activate the factor XIII in plasma by addition of calcium, but to prevent the plasma from clotting Heparin was therefore used as an anti-coagulant Addition of heparin to the test system resulted in the factor XIII deficient substrate clot being rendered insoluble in 5 M urea or 1 per cent monochloracetic acid (MCA) without addition of a source of factor XIII An investigation of the effects of heparin on the solubility of fibrin in these reagents was therefore undertaken

During the final stages of blood coagulation, fibrinogen is converted, by the enzymatic action of thrombin, to fibrin monomer which will then spontaneously polymerize to form a fibrin clot This fibrin is soluble in reagents which will split hydrogen bonds such as 5 M urea or 1 per cent MCA and is referred to as soluble or unstable fibrin[1].

A further enzyme, factor XIII, which is normally present in plasma reacts with this unstable fibrin in the presence of calcium ions and converts it into a form which is insoluble in 5 M urea or 1 per cent MCA but which is soluble in 0·1 M 2-mercaptoethanol The enzyme is believed to be a transamidase[2,3] which is activated from a precursor form by thrombin[4] The molecular weight of factor XIII has been estimated as 350,000 A tendency to dissociate into inactive sub-units of molecular weight 110,000 has been observed[5] and this dissociation was inhibited by cysteine

Purified human fibrinogen (Kabi Grade *L*) was clotted with thrombin (Maws bovine 5 U/ml) in the presence of 0 5 mM calcium chloride and 5 mM cysteine After incubation for 60 min at 37° C, the clots, when mixed with an equal volume of 2 per cent MCA and left at room temperature, showed no sign of dissolution within 12 h When similar clots were formed without calcium or without cysteine they were soluble in 1 per cent MCA within 30 min If before the addition of MCA the clots were mixed with a 1/6 volume of heparin (Boots or Evans 160–170 U/mg, 1 mg/ml) and incubated for a further 60 min, they no longer dissolved when an equal volume of 2 per cent MCA was added A similar alteration of solubility in MCA could be produced by incubation with other heparinoids such as chlorazol fast pink or dextran sulphate (Pharmacia, molecular weight 50×10^3)

The insolubility of the heparin treated clots in MCA could be reversed by incubation with protamine sulphate, polybrene or toluidine blue Unlike the clots stabilized through the action of factor XIII, the clots treated with heparin were not soluble in 0 1 M 2-mercaptoethanol

When a human fibrinogen preparation, purified by DEAE chromatography (my unpublished work), or a fibrin monomer preparation purified by repeated clotting and dissolution of a fibrin clot[4] was clotted in the presence of calcium and cysteine, the clots were soluble in 5 M urea or 1 per cent MCA When treated with heparin, these clots were also rendered insoluble in MCA Plasma obtained from a case of congenital factor XIII deficiency, however, remained soluble in MCA despite treatment with heparin

Clots made from citrated plasma by addition of thrombin were found to be insoluble in 1 per cent MCA, whether formed without added calcium or with addition of calcium and cysteine If 0 2 mg/ml (final concentration) of polybrene, 0 4 mg/ml of protamine sulphate or 20 mg/ml of toluidine blue was added to the plasma before clotting the clots which formed without added calcium were then soluble in MCA while those with calcium added remained insoluble When clots were formed from plasma anticoagulated with 5 mg/ml of ethylenediamine tetraacetic acid–disodium salt (EDTA) they rapidly dissolved in MCA, but if these clots were incubated in heparin they, too, became insoluble in MCA These observations are summarized in Tables 1 and 2

The effect of other thrombin inhibitors was studied, but neither hirudin, soya bean trypsin inhibitor (SBTI) nor tosyl-l-arginine methyl ester (TAMe) could affect the solubility of the clot

The significance of these observations is difficult to assess The fact that heparin in the absence of ionic calcium renders fibrin clots insoluble both in MCA and in 2-mercaptoethanol suggests that the mechanism involved is distinct from that by which clots are stabilized through the action of factor XIII Similar considerations prevail if the stabilization of the purified fibrinogen and of the fibrin monomer was the result of trace contamination with factor XIII The possibility that the effect of heparin on MCA solubility was caused by the quantity of calcium which this reagent contained and which would allow activation of traces of factor XIII within the clot was excluded by estimation of the calcium present This revealed only trace amounts and neutralization of this calcium with EDTA did not prevent the alteration of MCA solubility by heparin

Table 1

Citrate plasma	+	+	+	+	+
Calcium + cysteine	+	−	−	−	−
EDTA	−	−	−	+	+
Protamine	−	−	+	−	−
Thrombin	+	+	+	+	+
			30 min at 37° C		
Heparin	−	−	−	−	+
			30 min at 37° C		
0 6 ml of 2% MCA	+	+	+	+	+
			30 min at room temp		
Reaction with MCA	1	1	S	S	1

Table 2

Purified fibrinogen 0 25 mg/ml	+	+	+	+	−	−	−
Factor XIII deficient plasma	−	−	−	−	+	+	+
Calcium + cysteine	+	−	+	−	+	+	−
Thrombin	+	+	+	+	+	+	+
				30 min at 37° C			
Heparin	−	−	+	+	−	+	+
				30 min at 37° C			
0 6 ml of 2% MCA	+	+	+	+	+	+	+
				30 min at room temp			
Reaction with MCA	S	S	1	1	S	S	S

To 0 1 ml of the fibrin source were added 0 1 ml amounts of 2 5 mM calcium chloride, of 25 mM *l*-cysteine, of 5 mg/ml of EDTA or of 2 mg/ml protamine sulphate The volume was made up to 0 4 ml with 0 05 M glyoxaline saline buffer and 0 1 ml of bovine thrombin (Maw's topical thrombin, 5 U/ml) was added The clots were incubated at 37° C for 30 min, freed from the tube side by flicking and 0 1 ml of heparin 1 mg/ml or 0 1 ml of buffer was added The tubes were replaced at 37° C for 30 min and 0 6 ml of 2 per cent MCA then added The solubility of the clots was observed after a further 30 min at room temperature +, −, Presence or absence of reagent

S, soluble } 30 min after addition of MCA
I, insoluble }

Heparin is known to affect the zeta potential of fibrinogen[6] and to precipitate fibrinogen and other plasma proteins in certain conditions. If heparin were to stabilize clots by precipitating a "skin" of fibrin around the clot this precipitation might later be reversed by heparin antagonists EDTA has been observed to have a potentiating effect on the precipitation of fibrinogen by heparin by causing a reduction of *p*H, while serum inhibits the precipitation, possibly because heparin co-factor in the serum competes with fibrinogen for the available heparin[7] It is, however, not possible on these grounds to explain the failure of heparin to alter the solubility in MCA of clots prepared from the plasma congenitally deficient in factor XIII, nor the inhibition of stabilization of normal citrate plasma clots by protamine sulphate

The interpretation of these observations within the confines of the current theories of fibrin stabilization is difficult and their significance with regard to the physicochemical changes of fibrin stabilization remains to be explained

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[1] Laki, K , and Lorand, L , *Science*, 108, 280 (1948)

[2] Loewy, A G , *Trans Conf Intern Comm Blood Clotting Factors*, Gleneagles, Scotland, 1963, *Thromb Diath Haemorrh* , Suppl 13, 109 (1964)

[3] Loewy, A G , Dahleberg, J E , Dorwart, jun , W V , Weber, M J , and Eisele, J , *Biochem Biophys Res Commun* , 15, 177 (1964)

[4] Buluk, K., Januszko, T , and Olbromski, J , *Nature*, 191, 1093 (1961)

[5] Loewy, A G , Dahlberg, A , Dunathan, K , Krid, R , and Wolfinger, jun , H L , *J Biol Chem* , 236, 2634 (1961)

[6] Sheppard, E , and Wright, I S , *Arch Biochem. Biophys.*, 52, 426 (1954)

[7] Temperley, I J , *Irish J. Med Sci* , 448, 159 (1963)

APPLIED SCIENCE

Ammonia–Oxygen Fuel Cell

EARLIER publications have shown that the 'Teflon'-bonded platinum black electrodes developed by Niedrach and Alford[1] for use in fuel cells with aqueous electrolytes are mechanically stable over a temperature range from 25° to 200° C and perform well with both oxygen and air and with a variety of fuels and electrolytes[1-6] This communication presents the initial results that we have obtained using these electrodes in a fuel cell operating with gaseous ammonia and oxygen and using 54 weight per cent aqueous potassium hydroxide as electrolyte Similar results have been obtained with aqueous caesium hydroxide as electrolyte[7]. The cell and auxiliary electrical equipment were essentially the same as those used for the methanol–oxygen fuel cell[2]

The performance curves (Fig 1) show the variation with current density of the resistance-free voltages between the ammonia anode and the oxygen cathode and between each electrode and a hydrogen reference electrode in the same electrolyte The platinum catalyst loading of each electrode was 51 mg/cm^2, and the total cell resistance was about 0 03 ohm As expected from previous experience with hydrogen–oxygen cells[1], the cathode curve showed no limiting current density up to at least

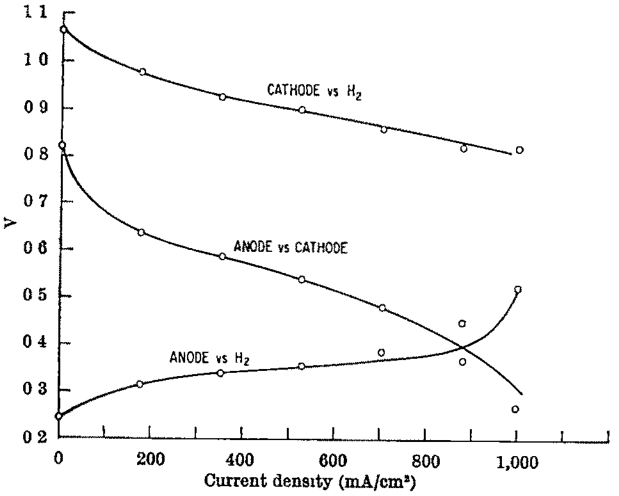

Fig 1 Performance of ammonia–oxygen fuel cell (No 73B) with 54 per cent KOH at 140° C IR-losses not included

1,000 mA/cm^2 The overall cell performance was limited by the anode, the voltage of which rose sharply at current densities above 700 mA/cm^2 Another anode, with a thicker layer of porous 'Teflon' on the gas side and with a lower limiting current density as a result, was operated over the range of temperatures and electrolyte concentrations shown in Fig 2 For current densities between 20 and 200 mA/cm^2 the anode performance improved by almost 75 mV as the temperature was increased from 109° to 191° C

The performance levels achieved in these ammonia–oxygen cells exceed those achieved with any fuels other than hydrogen or hydrazine Results have been reported[8] on an ammonia–oxygen fuel cell operating at 143° C with a mixed alkali hydroxide electrolyte containing only 12 per cent water and with porous nickel electrodes impregnated with platinum catalyst for the anode and silver catalyst for the cathode The Lockheed[8] cell voltage dropped to 0 5 V at a current density of only 35 mA/cm^2, even though its open circuit potential was about 130 mV higher than that shown in Fig 1 All other data published for ammonia–oxygen fuel cells[9,10] were obtained with either aqueous or fused alkali hydroxides immobilized in a porous matrix and with electrodes made of porous nickel or carbon and impregnated with platinum as the anode catalyst The maximum current densities reported for operation below 150° C were about 40 mA/cm^2 at a cell voltage of about 0 4 V, current densities of 120–130 mA/cm^2 were achieved only above 200° C

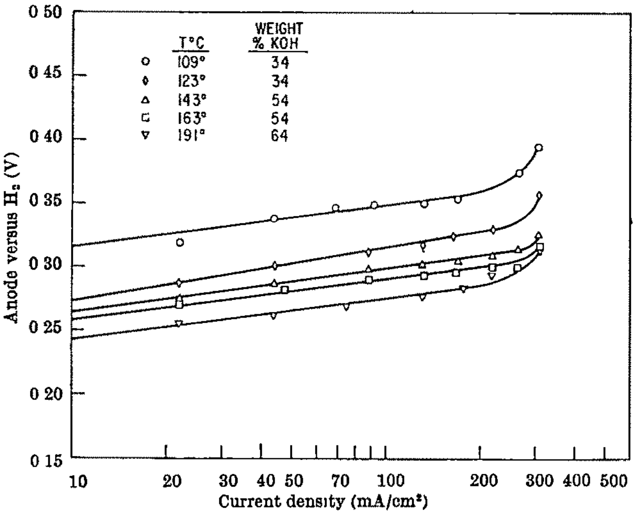

Fig 2 Effect of temperature upon anode performance (Cell 74) —○—, 109° C, 34 weight per cent potassium hydroxide, —◊—, 123° C, 34 weight per cent potassium hydroxide, —△—, 143° C, 54 weight per cent potassium hydroxide, —□—, 163° C 54 weight per cent potassium hydroxide, —▽— 191° C, 64 weight per cent potassium hydroxide

The authors appreciate the technical assistance of D C Bartosik and the encouragement given them by Dr H A Liebhafsky

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[1] Niedrach, L W, and Alford, H R, *J Electrochem Soc*, 112, 117 (1965)

[2] Cairns, E J, and Bartosik, D C, *J Electrochem Soc*, 111, 1205 (1964)

[3] Grubb, W T, and Michalske, C J, *Proc Eighteenth Ann Power Sources Conf*, May 1964

[4] Cairns, E J, and Holm, G J, paper presented at Fall Meeting, Electrochem Soc, Washington, DC, Oct 1964, Abstr 30 See also *Extended Abst*, *Battery Div*, 9, 75 (1964)

[5] Cairns, E J, *J Electrochem Soc*, 113, 1200 (1967)

[6] Cairns, E J, in *Hydrocarbon Fuel Cell Technology* (edit by Baker, B S), 465 (Academic Press, New York, 1965)

[7] General Electric Co, British Patent Specification 1,082,575, Improvements in Fuel Cells, Sept 6, 1967

[8] Lockheed Missiles and Space Co, *Basic Studies on Fuel Cell Systems*, Final Report, May 19, 1961–July 19, 1963

[9] Wynveen, R W, *Fuel Cells* (edit by Young, G J), 2, 153 (Reinhold Publishing Corp, New York, 1963)

[10] Electrochimica Corp, *Direct Ammonia-Air Fuel Cell*, report No 1 (July 1–October 1, 1963) and report No 2 (October 1, 1963–January 1, 1964)

GENERAL

Holographic Model of Temporal Recall

THE communication by Longuet-Higgins[1] has prompted me to suggest a different system of diffuse memory storage, which is based on holography like that described by Gabor[2] but differs from it in not requiring coherent repetitive vibrations Many systems of diffuse memory storage based on the interference principle are possible, holography being one The following requirements are essential

(1) The stimulus at each input point should produce a pattern of negative and positive values of some parameter over all the points in the storage area, which need not be a plane. (2) This pattern should be unique for the particular input point (3) Patterns from particular points are summated at the storage area according to the positive and negative values, producing a complex pattern (4) This complex pattern will represent uniquely a particular pattern of input stimuli at different input points, at various amplitudes (5) A suitable decoding system must be provided to convert a pattern in the storage area back to a corresponding pattern at the input area

A system of this kind can be made to have many of the properties found in holography The following are important (1) Many patterns in the storage area can be superimposed and decoded independently. (2) Ablation of large portions of the storage area will not drastically impair function (3) Part of the input pattern will serve as the code for extracting the remainder of the input pattern, for example, in holography the input pattern may be an object *A* plus a reference beam *a* *A* can be recovered by presenting *a*. Similarly, we can superimpose *B* with a different reference beam *b* and recover *B* independently We can also record object *C* together with a quite irregular reference beam *c* formed by the reflexion of light from an irregular object It does not have to be a plane mirror

We have here the elements of an associative memory If *D* plus *d* is recorded, sensory pattern *D* is recovered by putting in *d*, if *D* alone is recorded without *d*, then half of *D* will be the code for recovery of the other half

In the brain, the parameters which are summated at the storage area are excitation and inhibition The pattern is formed in the following way In the central nervous system, pulses are of constant amplitude. The intensity of a stimulus is represented according to the frequency of the pulses. The functional unit is a small group of synapses on the surface of a nerve cell, some of which carry excitatory and some inhibitory signals. These produce a transmitter substance and an inhibitor substance which enter the synaptic space and are summated there. When a sufficient excess of transmitter substance is built up, the cell fires and the substances disappear into the cell. Signals are thus summated at the functional unit in a way which is linear within certain limits.

Each neurone branches many times, ending in up to five thousand terminals which end as synapses on the surface of many cells. A simplified scheme showing three rows of cells is shown in Fig. 1*a*. Each dot represents a cell. The branches and synapses of two cells are shown and the others are assumed to be the same. One set of branches is shown in Fig. 1*b*. The figures represent the strength of the stimulus at each terminal. Some are inhibitory with a value of −1 and some excitatory with a value of +2. (In real life the inhibitory fibres have an internuncial neurone.) Assuming the simple additive relationship at the synapse, if a single cell in the first row of Fig. 1*a* is stimulated it is possible to deduce by simple arithmetic that the pattern of stimulation at the third row is as shown in Fig. 1*c*. The same information is shown in graphic form in Fig. 1*d*. The ganglion cells in the retina are already known to respond in this way—that is, with an excitatory receptive field surrounded by an inhibitory one.

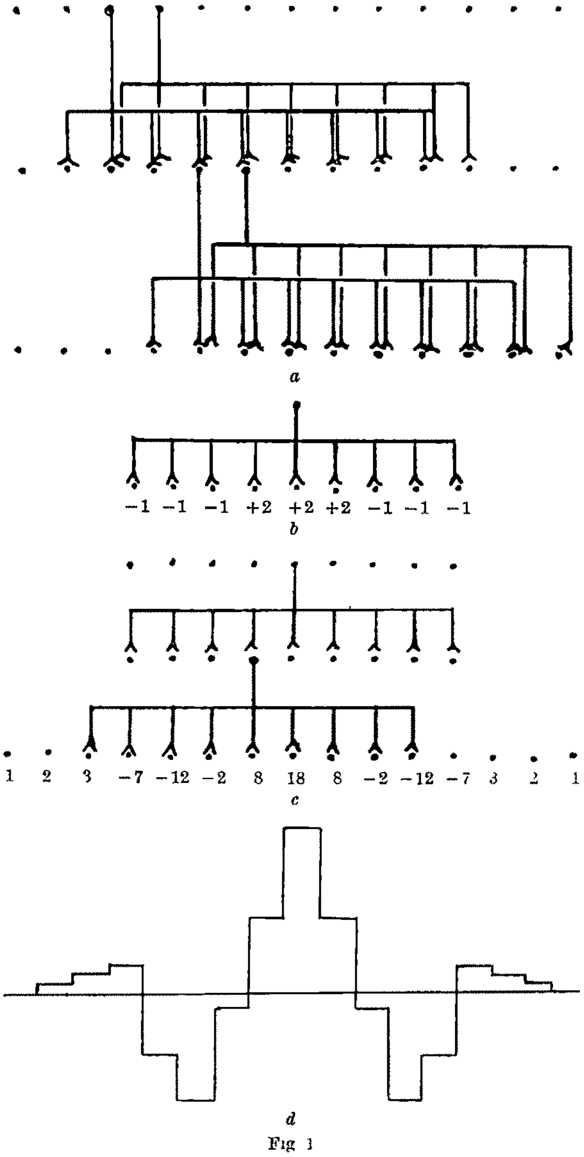

Fig. 1

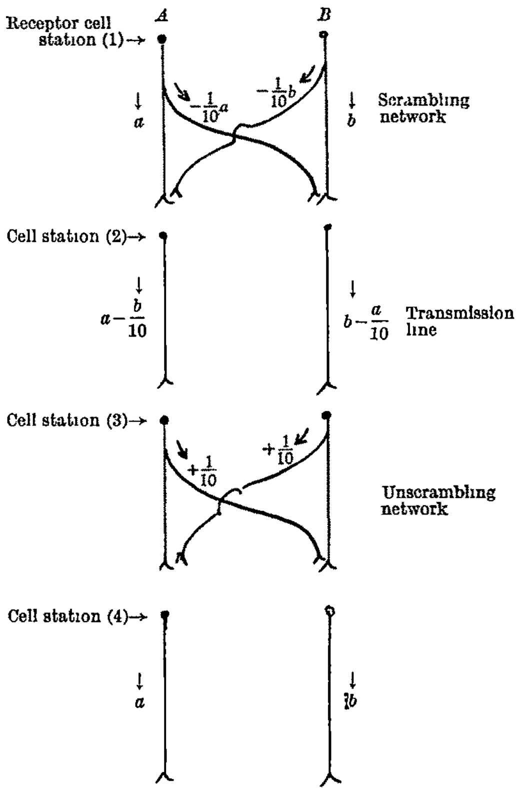

Fig. 2

In the brain the picture is more complex, with seven or more rows of nerve cells between the receptor and the cerebral cortex and branches from one cell are not confined to the next layer. The stimulation of a single receptor produces a complex pattern over the cortex and the pattern varies with each receptor.

For decoding, first consider Fig. 2, which shows two receptors A and B handling signals a and b. These pass to the second row of cells by one direct path carrying straightforward signals of value a and b and there is lateral inhibition of value, for example, of $-a/10$ and $-b/10$. Simple algebra shows that the decoding network is by a pair of excitatory side branches, as shown in Fig. 2.

Even for the most complex network, with many cells using some excitatory and some inhibitory branches, a decoding network could be designed by a suitable complementary arrangement. When making a computer with diffuse storage this could be designed, but in the brain it must be a random process. Cells which form the terminals corresponding to the decoded signal are self selected by a learning process. These cells which again hold the information in discrete form are widely scattered and are not contiguous. Most probably they are duplicated many times and there is considerable redundancy.

Information is stored in diffuse form in the synapses, which are changed by a signal passing through so that following signals are either decreased or increased. Either would serve as a basis for storage. It may well be that short term memory depends only on partial depletion of transmitter substance in the *boutons terminaux*. Long term memory involves a synthetic process and this may depend on a small increase in synaptic effectiveness over a large number of synapses in particular positions.

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[1] Longuet-Higgins, H. C., *Nature*, **217**, 104 (1968).
[2] Gabor, D., *Nature*, **161**, 777 (1948).

BOOK REVIEWS

BRITISH HYDROGRAPHY

The Admiralty Hydrographic Service 1795–1919

By Vice-Admiral Sir Archibald Day. (Ministry of Defence.) Pp. 378+13 plates. (London: H.M. Stationery Office, 1967.) 105s.

In 1885 Commander L. S. Dawson published *Memoirs of Hydrography*, a description of the growth of marine surveying in Britain from 1750 until the time when he was writing. Concentrating mainly on work done in the field, he gave a detailed account of surveys undertaken under naval auspices at home and abroad from the pioneering days of the Mackenzies and Captain Cook until the mid-nineteenth century when British ships had charted about three-quarters of the world's coastlines.

For some years after its foundation in 1795, the Hydrographic Office had little influence over surveys and it was not until Hurd's term of office (1808–23) that hydrographers began to have some say in choosing new projects. Gradually the department developed from its modest beginning as a clearing house for hydrographic material sent to the Admiralty by officers who took an interest in such work to the organization principally responsible for all surveys carried out by HM ships and for the production of charts which were available not only to the navy but to the general public.

When this happened the history of the Hydrographic Department and the progress of marine surveying became inseparable, and Admiral Day in *The Admiralty Hydrographic Service* has used the original departmental papers to produce a book specifically designed to supplement and continue Dawson's account, by describing the internal development of the Hydrographic Office, to which Dawson paid little attention, until 1884, and then carrying up to 1919 both the department's history and that of the surveys carried out under it. In this latter part of the book Admiral Day follows Dawson's original pattern, devoting a chapter apiece to the four hydrographers who held office between 1884 and 1919.

This chronological scheme precludes a sustained discussion of any single subject, but many themes run right through the book, notably the development of the concept of the department's role by successive hydrographers and their struggle to extend both its scope and its means. Notwithstanding repeated opposition from the Treasury in the form of cuts, as in 1851, and from the Navy Board, which was prone to object that the service "locks up" ships and men in its activities, it grew from the small office set up under Dalrymple in 1795 at an estimated cost of £470 per annum to an organization employing 350 people (on land) and spending nearly £150,000 per annum by 1919.

Admiral Day shows how, at the same time, the work of the surveyors changed, the broad sweeps of the early nineteenth century giving way to more painstaking coverage with techniques of increasing accuracy. The atmosphere of the service was so altered that in 1891 Wharton recommended increased pay for the crews of survey ships because they no longer had the "glamour of exploration" to compensate for their hard work. Increasingly their exploration was of a more scientific nature with contributions to many branches of science, especially oceanography and meteorology.

Admiral Day's book is much more than the story of the Hydrographic Department. It is a work of reference giving information both on its organization and on the many-sided activities which came under its control. It follows the progress made in producing Admiralty charts at all stages from the drawing board to their issue to the fleet and is illustrated with examples of the work of different periods, some of it of extreme beauty. It tells, with facts and figures, of what lay behind the exploits of the great surveyors of the nineteenth century which Admiral Ritchie described in *The Admiralty Chart* (1967), to which Admiral Day's book is, as he points out, complementary. *The Admiralty Hydrographic Service* is a most valuable addition to the growing number of books about the discovery of the oceans and its value would be enhanced if a reprint of Dawson's original *Memoirs* could be produced to accompany it.

MARGARET DEACON

ASTRONOMERS OF OLD

Their Majesties' Astronomers

A Survey of Astronomy in Britain between the Two Elizabeths. By Colin A. Ronan. Pp. 240+20 photographs. (London: The Bodley Head, 1967.) 30s. net.

The title of this book might suggest that it chronicles the activities of Astronomers Royal or governmental: the sub-title gives a better idea of its contents, though, of course, the survey could not possibly have been restricted to the work of British astronomers. Actually, it will serve to provide the general reader with a lucid account of the onward sweep of the science of the heavens during the past four hundred years.

A brief introductory chapter covers developments up to the mid-sixteenth century when the new Sun-centred cosmology began to inspire the English Copernicans, Recorde, Dee, Thomas Digges and the rest. Next, the urgent problems of navigation, hastening the foundation of the Royal Observatory, introduce the reader to Flamsteed and his immediate successors, to Harrison and his chronometer, and, through the beginnings of lunar theory, to Newton's achievements in celestial mechanics and in optics. Advances in the technique of telescope making lead on to the pioneer work of the Herschels and Lord Rosse in stellar and nebular astronomy. Following a chapter devoted to "The Neptune Scandal", Mr Ronan traces parallel developments in precise astronomy (revealing the phenomena of aberration and parallax) and in spectroscopy. He has been permitted to draw on the personal recollections and to consult the private papers of Mr William Christie, grandson of Sir W. H. M. Christie, the eighth Astronomer Royal. The concluding two chapters respectively survey the modern revival of interest in cosmology (in the fullest sense of the word) and endeavour to chart "The Way Ahead".

The reader of this book will encounter certain imprecisions. The stars do not all exhibit their maximum aberrational displacements northward or southward at the same time of year (page 139). The point about Gill's determination of the solar parallax at Ascension Island was not that he had "a base-line of some fourteen hundred miles to Greenwich", but that a calculable base-line was marked out by the Earth's rotation and orbital motion between evening and morning observations (page 153). The stellar distance cited on page 151 requires three more ciphers. There are not 86,000 but 86,400 sec in one day (page 198). Samuel Molyneux, Bradley's colleague, is confused with his father William (page 93), "the elder Cassini" is confused with his son Jacques (page 137). Lockyer's first name was Joseph, not John (page xi and facing page 176). There are, indeed, besides obvious misprints, many mistakes in the spelling and initialling of proper names in both text and index, and several wrong dates. The book requires, and deserves, a thorough revision before proceeding to a second edition.

A. ARMITAGE

HALLUCINOGENS

The Hallucinogens

With a Contribution by T Weckowitz By A Hoffer and H. Osmond Pp ix+626 (New York Academic Press, Inc , London Academic Press, Inc (London), Ltd , 1967) 200*s*

THE hallucinogens, almost totally unknown twenty years ago, currently enjoy a sinister notoriety engendered by their social misuse This has tended to obscure the most valuable contribution they have made to neurological science A study of their mode of action has led to advances in biochemistry and pharmacology as well as throwing what little light there is on the biochemical basis of schizophrenia They have also been used widely in psychotherapy and form an interesting facet of pharmacoanthropology This book surveys all these aspects of the known hallucinogens, covering the literature to 1965 The following fields are covered origins, chemistry, biochemistry, pharmacology, neurophysiology, psychology, clinical implications and use and anthropology. Naturally some of these are dealt with more fully than others, particularly detailed accounts are given of the psychological and clinical effects of these drugs and interesting details of the use of the plants from which they are derived by various cultures

Some 150 pages are devoted to adrenochrome and its relatives The controversies which have raged over this compound are described by the authors, who have long battled in defence of the "adrenochrome theory" of schizophrenia Some of the bitterness of this dispute is reflected in their comment "adrenochrome, at one time, became a dirty word".

This involves three problems: (1) whether adrenochrome is a hallucinogen, (2) whether it occurs *in vivo*, and, if so, (3) whether it plays any part in the biochemical lesion of schizophrenia Reports on the psychological effects of adrenochrome have been conflicting, but a recent double-blind study carried out in Czechoslovakia by Grof *et al* [1] supports the claim that it produces a psychotomimetic reaction.

In considering the question as to whether adrenochrome actually occurs *in vivo*, Hoffer quotes twenty authors who found "fluorescent derivatives of adrenaline in body fluids or tissues". However, if one refers to these sources quoted, one finds that there has been some confusion for example, (i) Utevskii *et al* [4] report that whereas brain contains "substances which exhibit the properties of intermediate products of quinoid oxidation of catechol amines" they go on to state "adrenaline, adrenochrome or adrenolutin was not detected" Fischer and Landtsheer[5] injected the adrenochrome themselves to trace its metabolism, and Bacq *et al* [6] and Goldenberg *et al* [7] did not detect any adrenochrome at all, but merely used the Von Euler method of estimating adrenaline by converting it themselves to adrenochrome Fischer and Lecomte[8] curiously quote Bacq *et al* [6] as stating that 10–20 per cent of the injected adrenaline was excreted as an indolic compound—a fact not mentioned in this paper Green *et al.*[9] did not actually detect anything but merely suggested a conversion of adrenaline to adrenochrome as a hypothesis to explain the inhibitory action of ferritin on the action of adrenaline on capillaries Gershenovich *et al* [10] found adrenochrome only in brains of rabbits dying of oxygen poisoning (exposed to 3 5–6 atmospheres pure oxygen) Three other references were untraceable I did not consult the rest Moreover, Hoffer does not quote any of the authors who failed to find adrenochrome in the blood, notably Axelrod[2] Thus the matter remains quite unresolved It is still possible that adrenochrome or one of its relatives may be formed in the brain in schizophrenia, although there is no direct evidence to support this The indirect evidence presented is not very convincing In our present state of ignorance concerning the biochemistry of schizophrenia it would, however, be unwise to be dogmatic in any direction But I would urge in general that workers in this field should recall Popper's dictum that a theory should be tested with the direct aim of trying to refute it Only by surviving such tests can a theory become valid

The authors include an attack on the "double-blind" methodology in trials of psychotropic drugs. Although there is much to be said for their criticism of much of the methodology in this field, I remain convinced that the "double-blind" precaution remains quite essential and the failure to observe it has led many investigators wildly astray J R SMYTHIES

[1] Grof, S , Vojtechovsky, M , Vitek, V , and Prankova, S , *J Neuropsychiat* , 5, 33 (1963)

[2] Szara, S , Axelrod, J , and Perlin, S , *Amer J Psychiat* , **115**, 162 (1958)

[3] Feldstein, A , *Amer J Psychiat* , **116**, 454 (1959)

[4] Utevskii, A M , and Osinskaya, V O , *Chem Abst* , **60**, 14796 (1964)

[5] Fischer, P , and Landtsheer, L , *Experientia*, **6**, 305 (1950)

[6] Bacq, Z M , Fischer, P , and Lecomte, J , *C R Soc Biol* , **143**, 583 (1949)

[7] Goldenberg, M , Avanow, H , Smith, A A , and Faber, M , *Arch Intern Med* , **86**, 823 (1950)

[8] Fischer, P , and Lecomte, J , *Bull Soc Chim Biol* , **33**, 569 (1951)

[9] Green, S , Mazur, A , and Shore, E , *J Biol Chem* , **220**, 237 (1956)

[10] Gershenovich, Z S , Krichevskaya, A A , and Alekseenko, L P , *Chem Abst* , **49**, 10470 (1955)

BIOLOGY OF THE UTERUS

Cellular Biology of the Uterus

Edited by Ralph M Wynn. Pp xi+524 (Amsterdam North-Holland Publishing Company, New York Appleton-Century-Crofts, 1967) 180*s*.

RESEARCH into the anatomy, physiology and biochemistry of uterine and placental function has increased very considerably during recent years So far, however, few textbooks have attempted even a limited survey of the progress that has been made Ralph Wynn has brought together fourteen other authors in a very comprehensive review of what he has chosen to call uterine cellular biology This has enabled him to concentrate attention on the many different aspects of normal uterine function

Dr S R M Reynolds in a thoughtful opening chapter reviews many of the important advances in the field of reproductive physiology and morphology during the past 40 years He sets the standard for the excellent chapters that are to follow He rightly claims "that so large has this field of study become that today a person must of necessity become a student in, rather than of, the subject"

Jack Davies reviews the embryology of the uterus both in the human and other animals Elizabeth Ramsey describes the vascular anatomy of the uterus, while John Morris discusses the vascular physiology

The genetic, biochemical and hormonal mechanisms in the regulation of uterine metabolism are considered in reasonable detail by Kenneth McKerns This chapter includes a consideration of hormone biosynthesis in the ovary and human placenta as well as the steroid relationships between the human foetal adrenal and placenta These, in fact, are the only aspects of placental metabolism that are discussed, but in a book devoted essentially to the uterus this is acceptable.

The only British contributors to this symposium are Dorothy Needham and Catherine Shoenberg from Cambridge They provide a very well documented account of the biochemistry of the myometrium listing more than 200 references Indeed, most chapters include a very up to date and a very complete number of references Other chapters are devoted to oestrogens, nucleic acids and protein synthesis in uterine metabolism, decidualization, the ultrastructure of the myometrium, the ionic basis of electrical activity in uterine smooth muscle, and hormonal regulation of myometrial activity and its clinical applications

This book should prove invaluable to all those who are interested in reproductive physiology and biochemistry

Concentrated within its 499 pages is a wealth of biological scientific detail. Perhaps other writers could be encouraged to publish a similar synthesis of important biological information. In particular, there is a considerable need for a comparable book devoted to the placenta. Meanwhile, Ralph Wynn has set a very high standard for any who may care to follow his method of presentation.

N. F. MORRIS

TOOTH BIOLOGY

Structural and Chemical Organization of Teeth

Vol. 1. Edited by A. E. W. Miles. Pp. xv+525. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 220s.

THERE can be little doubt that the first volume of this two volume treatise contains much of interest and significance to biologists in general as well as to dental research workers. The reason for this probably lies in the fact that most of this volume deals with dental development which exhibits many fundamental processes of biology such as induction, morphogenesis and mineralization. The relationship between the neural crest cells and dental development, not yet fully understood but well reviewed in this volume, provides a good example of how things dental have application to the wider realms of biology.

It is a little more problematical to assess the value of this book to the dental research worker. Its encyclopaedic character means that those chapters dealing with subjects where advances have been rapid are to some extent dated. The initial overtures to the contributors were made some five years ago and, although the editor has tried, by the addition of addenda, to keep abreast of recent developments, advances in some fields have been so rapid as to make this an impossible task. This criticism must not be allowed, however, to detract from the value of the volume as a whole. It provides an excellent source of reference and this alone justifies the effort that has gone into its production. I am, however, a little perturbed by the cost of the volume. Together with its companion this exceeds twenty pounds, which virtually limits its sale to libraries. Whether large, expensive, multi-author treatises are the best means of collecting and sifting information can be questioned. The eleven chapters in this volume could equally well have been published in the form of separate monographs at a cost allowing individual purchase over a period of time. Even so, the contents of this book needed to be published and the editor, together with the publishers, has succeeded magnificently in what must have been no mean feat of production.

A. R. TEN CATE

GENES IN ACTION

The Message of the Genes

By Navin Sullivan. Pp. 198. (London: Routledge and Kegan Paul, Ltd., 1968.) 25s. net.

THE past two decades have witnessed enormous advances in our understanding of what genes are and how they function. It is not unimportant that the layman should have an opportunity of learning about these developments. After all, it is he who eventually finances research, the discoveries made will, no doubt, have an ever increasing impact on the society in which he lives and, if he is able to share in some of the excitement which research generates, so much the better. Mr Sullivan's book is not for the specialist.

The Message of the Genes opens with a description and interpretation of Mendel's experiments on the inheritance of certain traits in peas. This is followed, in the second chapter, by a brief account of chromosomes and the construction of genetic maps. The next two chapters describe the chemical components of which cells are made, the function of proteins as enzymes and hence why nucleic acids, as the information carriers, are so important. This lays the foundation for the next six chapters, which are concerned with modern molecular biology: how genetic information is duplicated and how it is expressed. Mechanisms for gene expression begin the final chapter, which concludes with a short section on memory.

In all this Mr Sullivan presents the relevant experiments with a fresh enthusiasm; far from being a textbook, this account is written in an engaging style which will draw the reader on to follow the line of thought or the connexion between seemingly unrelated observations. This is accompanied by the occasional aside in which the author relates how the physical difficulties of solving the problem were overcome by the scientist, or the excitement of the meeting at which the results were first presented.

The specialist may find several unimportant errors in the book: the structures of ammonium cyanate and urea on page 42 are incorrect, the legend to Fig. 24 gives the wrong impression of the position on an *s*RNA molecule to which the amino-acid is attached, and a couple of people are credited with work which was done by others. A reader with a small knowledge of chemistry might have expected the book to be more chemical in outlook, particularly those sections which describe the biosynthesis of proteins and nucleic acids. These criticisms should not deter the lay reader. The book, the message of which comes across as a clear and lucid presentation, is to be recommended at its modest price.

M. S. BRETSCHER

EXPERIMENTS IN BEHAVIOUR

Experiments in Animal Behaviour

By Marguerite D. Hainsworth. Pp. x+206+8 plates. (London: Macmillan and Co., Ltd.; New York: St Martin's Press, 1967.) 35s.

TEACHERS at all levels have learned by bitter experience the problems of class experiments in behaviour. They usually require a long time in which to carry them out, the observations only producing a pattern which demands explanation after numerous notes on factors which may or may not be crucial have been made. Animals are wayward creatures liable to make just those reactions which theory finds it hardest to explain. Miss Hainsworth attempts to come to the teachers' help by drawing together a number of possible experiments on the behaviour of animals from all parts of the animal kingdom. She does many a service in this, for not only does she continually pose questions the answers of which lie in the observations made but she also harps on the need for quantitative results. Indeed, criticism of work on behaviour by other scientists is frequently that it is uncritical in its methodology, but this book endeavours to instil into the student a sense of the importance of questioning not only what he is studying but also how he is studying it.

Part of the point of the book is that it shows that any part of an animal's activities is worth studying in the context of behavioural investigations. It is not necessary to look at courtship, striking though this essential behaviour is and despite the amount of space given to it in the literature, how an animal moves may be closely observed and analysed, or its food preferences, or its reaction to light or a slope. These details of behaviour, however, may too easily be considered minutiae by a student, not worthy of great attention and quite unexciting. Successful experiments, in terms of class morale, are ones that stand a good chance of working in the sense that they produce discussable results. But great faith is needed to carry out very many of these suggestions and I cannot help wondering whether some of them are as useful as they appear But it must be admitted that what may be successful with one class is not necessarily so with another, the user makes his own selection

The experimental details are often good, for example, it is pointed out that the gas supply in some districts may no longer contain the respiratory poisons so useful in anaesthetizing protozoa, yet the important warning for these times that glassware used for protozoa must not be washed in detergent is not there The importance of identifying an animal and the use of comparative studies are both stressed, yet inexplicably all sea anemones are lumped together as if their behaviours would be the same. And in her desire to hang the experiments on a framework of theory, the author makes some remarkable statements, surely, for example, the molluscs can hardly be called an "aberrant" group ? It is perhaps misleading to suggest that the reaction of *Hydra* to glutathione has something in common with the reactions of minnows to alarm substances Although a chemical stimulus is involved in both, the behaviour evoked is quite different and almost certainly the receptors involved bear little likeness to each other

Nevertheless, this book contains many useful ideas which should stimulate teachers to try again with experimental work in behaviour They will not lack direction for their theoretical reading, for Miss Hainsworth supplies a good list of selected references The book is attractively produced and, apart from the transposition of two legends, the illustrations are clear and informative

J D Carthy

WATCHING BIG GAME

A Field Guide to the National Parks of East Africa

By John G Williams Pp 352+32 plates (London William Collins, Sons and Co , Ltd , 1967) 45*s* net.

This book has three sections which describe the wild life areas, their mammals and their rarer birds

Sixty faunal areas covering about 50,000 square miles are discussed Places of ornithological interest such as the Uganda forests are detailed, while some areas like the 11,000 square mile Selous are treated briefly and yet others, Ankole, for example, although faunistically interesting, are scarcely mentioned However, due emphasis is placed on such new and exciting parks as the Ruaha, Kidepo and Meru Accompanying these descriptions are comprehensive check lists of birds and mammals with maps and details of communications, which will be useful to tourists when planning their itineraries

The mammal section occupies a sixth of the book and is disappointingly brief about such conspicuous creatures The larger mammals and bats, of which the author is particularly knowledgeable, are emphasized while Murids, with their attendant taxonomic problems, are dismissed as "seldom seen unless specially searched for "

Concise descriptions of the mammals include details of their distribution and habitat, but I would also welcome descriptions of their seasonal movements and, for night identification, their calls Some of the information in the literature on demography, nutrition and ethology would also be relevant Such information when given is occasionally misleading, for example, we are told, without further qualification, that the "hippo" is 14 ft long

Why is far more space given to the rare Uganda cormorant than to elephants, despite the latter's importance in many park ecosystems today ? There is, for instance, no mention of their huge mobile ears as useful characteristics for distant identification or of the Congo race found in the Semliki Valley The answer lies partly in John Williams's years of experience in the Coryndon Museum, Nairobi, and his intimate contact with the living material while on safari, from which he has acquired unparallele knowledge of their taxonomy

His taxonomic approach is essential in the section o rarer birds It is here that the Peterson method indicatin diagnostic characters on the plates by pointers is mos valuable The plates drawn by Rena Fennessy, famou for current wildlife issues of East African postage stamp although superb would benefit from a scale This sectio supplements the author's previous field guide to th commoner birds, to which there are abundant cross references and without which visiting ornithologists wi be lost

This book will require regular revision to keep pac with scientific work, new areas and sightings such a greater kudu and oryx now confirmed from the Kidep or to delete species like the Queen Elizabeth Park huntin dog

Our changing knowledge is emphasized by an exampl from the area with which I am most familiar Ishash lions do not climb trees so much to see over the grass which is in any case short, but to avoid flies and heat

This then is a guide for the taxonomically orientate tourist and the ornithological enthusiast I hope that i will stimulate others, including East African school children and potential ecologists, to a greater awarenes of these little known areas Christopher R Field

OBITUARIES

Professor J H. Taylor

James Haward Taylor, AM, PhD, FGS, FRS, who hel the chair of geology at King's College, University o London, was one of Great Britain's most distinguishe geologists His death by drowning while engaged on underwater research in the Seychelles is a sad loss to th science

Educated at Clifton College and King's College, London, he obtained a Henry Fellowship to Harvard in 1933, returning to take up an appointment to HM Geological Survey From this he was appointed professor of geology at his old college in 1949

His initial interest was in igneous rocks, which he studied in Leicestershire and the Isle of Man His work at Harvard, on the Little Belt Mountains, Montana, led him to a study of mineralization, while his early work with the Geological Survey in the west Midlands stimulated his interest in the sedimentary formations These interests coalesced when, during the Second World War, he was engaged on the study of the Northamptonshire iron-ore field He made the petrology of these ores his special study, and his memoir on them has become a standard work on the subject At the same time he maintained an active interest in their field occurrence, and with S E Hollingworth and G A Kellaway established the importance of periglacial superficial movements in their disposition This in turn led to a study of the Pleistocene sequence and the geomorphology of the area

A student mapping project in the central Weald revived his interest in these sediments, and his studies of this sequence are among his latest publications He retained his interest in economic ores, however, extending his work to Broken Hill, Rhodesia He was greatly interested, through earlier studies of Jurassic limestones in the English Midlands, in the genesis of these rocks, and in 1963 initiated a project to investigate recent limestone formation on the Seychelles Banks Some of his students' work on this had been published, and the project contained the promise of exciting contributions in this new and expanding field He travelled widely, in Europe, Africa and North America, and had a wide and varied knowledge of geology outside his special interests

All Professor Taylor's work was marked by a meticulous attention to accuracy and to scientific discipline He set himself the very highest standards of investigation and exposition He applied the same conscientiousness to his numerous other duties, he served on the councils of the Geological Society, the Geologists' Association, the Mineralogical Society and the Institute of Mining and Metallurgy, being president of the last two, he was elected to a fellowship of the Royal Society in 1960, served on its council and became chairman of the British National Committee for Geology He was a member of the Natural Environment Research Council and of the Iron and Steel Federation and the British Ceramic Research Association During the past year he presided over the meetings of the International Sedimentological Congress at Reading and Edinburgh All these, and his many other duties, he carried out with a zeal and an efficiency which were a constant source of amazement to all who knew him

His public manner, apparently controlled and urbane, concealed a deeply shy and reserved nature Despite this, however, all who were in contact with him immediately sensed a warmth of understanding Unfailingly courteous, his time and energies were always at the service of his colleagues He enjoyed the respect of all, and leaves a multitude of friends J E PRENTICE

Dr P. J. du Toit

DR P J DU TOIT, who died in Pretoria on November 13, 1967, at the age of 79, was one of South Africa's greatest men of science—first in the role of a brilliant young veterinary research worker, then as Dean of the Faculty of Veterinary Science at the Transvaal University College of South Africa, where he succeeded Sir Arnold Theiler in 1927, and later as Director of Veterinary Services in South Africa (1927–48), a responsibility which included guiding the destinies of the world-renowned Veterinary Research Institute at Onderstepoort

While the careers of many lesser men may have ended there, at retirement, du Toit went on with characteristic enthusiasm and stamina to become the second President of the South African Council for Scientific and Industrial Research (CSIR) in 1951, succeeding Sir Basil Schonland Later (1951–60) he was appointed President of the Scientific Council for Africa South of the Sahara (CSA)—a non-governmental body that has since become a victim of political change in Africa During these years, du Toit guided the activities of CSA at numerous meetings of the Commission for Technical Co-operation in Africa South of the Sahara (CCTA) held in East, Central, West and southern Africa

Du Toit's academic qualifications included a BA (Hons); PhD (with honours) from Zurich University, and a doctorate in veterinary science, with honours, from Berlin He held six honorary doctorates at the time of his death, four from South African universities which recognized his outstanding contribution to science in Africa, and one each from the universities of Utrecht, Holland and Glasgow

Du Toit was a Fellow of the Royal Society and belonged to twelve scientific societies all over the world He served on many commissions, enquiring into education, veterinary science, agricultural science, medicine, museums and so on, and represented South Africa at many international conferences He wrote more than 140 publications

His early interest during the First World War, when he was allowed to continue his research in Germany despite the fact that he held a British passport, was leukaemia in cattle He received a doctorate in veterinary science for this work from the University of Berlin and later collaborated with Professor P Knuth on tropical veterinary problems He was co-author with Knuth of a standard textbook (in German) on tropical diseases in domestic animals

Du Toit returned to South Africa in 1919 to carry on with the fundamental research into lamsiekte (bovine botulism) begun by Sir Arnold Theiler This was to have far-reaching results in the development of the study of mineral deficiencies By 1928 he had focused his attention on protozoology and virus diseases as professor at the Transvaal University College of South Africa A year before, he had visited Nigeria and reported on veterinary matters there at the request of the Colonial Office in the United Kingdom

Despite growing administrative burdens, du Toit's research continued in earnest until after he retired as Director of Veterinary Services of the South African Department of Agriculture, and Dean of the Faculty of Veterinary Science, University of Pretoria, in 1948 A cross-section of his publications reveals interests ranging from rinderpest to iodine in the nutrition of sheep, from problems of immunity in animal diseases to studies of the mineral content and feeding value of natural pastures in South Africa

It is difficult to write an appreciation of a scientist such as P J du Toit, for he would have been the first to contend that the praise was undeserved and his achievements were mainly due to the efforts of his colleagues He will be remembered as an outstanding administrator with a mind comprehensive in its grasp of things and simple and precise in its setting out of a problem In addition to his scientific and academic responsibilities he gave much time to public work of various kinds, including active membership of the South African Historical Monuments Commission

As a personality, he will be remembered for his keen sense of humour and charming companionship He was in great demand as a raconteur and after-dinner speaker, accomplishments which earned him the affectionate sobriquet "P J " He never flaunted his own achievements nor underrated the work of others S M NAUDÉ

Professor G. Z. Dimitroff

GEORGE Z DIMITROFF, emeritus professor of astronomy at Dartmouth College, Hanover, New Hampshire, and former superintendent of the Harvard Observatory, died on January 1 at his home in Hartland, Vermont, at the age of 66 He had been a member of Dartmouth's astronomy department for twenty years before his retirement in June 1966

A native of Svistove, Bulgaria, Dimitroff graduated from the University of Boston in 1927 and took his MA and PhD degrees at Harvard He taught physics at Colorado State College and astronomy at Harvard and Radcliffe

For five years before he went to Dartmouth, Dimitroff was superintendent of the Harvard Observatory where he helped to build some of the largest Schmidt cameras in existence before that at Mount Palomar was built He advised the Mexican government about the building of their astrophysical observatory at Puebla, and was co-author of a book on telescopes and accessories

For hundreds of Dartmouth undergraduates Astronomy 1 and Professor Dimitroff were synonymous In addition to this introductory course in stellar astronomy and the astronomical universe, popularly known as "stars", he also taught more advanced courses in astrophysics and observational astronomy. His open house "Evening with the Stars" at the Shattuck Observatory gave pleasure to many Hanoverians, young and old

As a commander in the US Navy in the Second World War, Dimitroff undertook a variety of scientific missions in Europe and Japan, including studies of optical developments there On leave from Dartmouth in 1956, he surveyed astronomical developments in Europe and the Middle East, and at that time was scientific liaison officer with the US embassy in London He went to Russia in 1958 as a member of the International Astronomical Union and returned there in 1963 as part of a world tour of observatories Impressed with Russian scientific advances, he predicted that in twenty years Russia would be the leading scientific nation

University News :

Bristol

Dr M A Epstein, reader in experimental pathology in the Middlesex Hospital Medical School, has been appointed professor of pathology in succession to Professor T F Hewer

Manchester

Dr K R Parthasarathy, at present reader in probability theory in the University of Sheffield, has been appointed to the chair of mathematical statistics

New South Wales

Professor P T Fink, formerly P N Russell professor of mechanical engineering in the University of Sydney, has been appointed professor of mechanical engineering and head of the School of Mechanical and Industrial Engineering

Reading

Dr E H Roberts, lecturer in horticulture in the Department of Botany, University of Manchester, has been appointed professor of crop production

Salford

Dr R R Birss, at present senior lecturer in physics, University of Sussex, has been appointed to a second chair of physics in the Department of Pure and Applied Physics

Appointments

Dr R L Mitchell, at present deputy director of the Macaulay Institute for Soil Research, has been appointed director of the institute in succession to Dr A B. Stewart

Announcements

Ege University at Bornova, Izmir, Turkey, is setting up a Natural Science Museum which will be attached to the Faculty of Science It is hoped that the proposed six halls, each 250 square metres, will be finished by the end of 1970. At the preliminary stage of organization of the museum the Dean, Professor Dr Yusuf Vardar, and his staff are seeking advice from various museums in the world on organization, administration and activities They would now appreciate help from any other institution in the form of publications covering methods of collection, preservation, maintenance and registration of exhibits Any material (zoological, botanical or geological) which can be donated would also be greatly appreciated

The following have been elected officers of the American Crystallographic Association for 1968. *President*, Dr S C Abrahams, Bell Telephone Laboratories, Murray Hill, New Jersey; *Vice-President*, Dr W C Hamilton, senior chemist at Brookhaven National Laboratory, Upton, New York

Dr Sol Spiegelman, professor of microbiology at the University of Illinois, has been awarded the 1968 Bertner Foundation Award for his work involving replication of genetic material in controlled laboratory conditions.

Meetings

Modern Techniques in Food Analysis, March 19, Grimsby College of Technology (Head of Science Department, College of Technology, Nun's Corner, Grimsby, Lincs)

Training of Food Scientists and Technologists, April 4–5, Grimsby College of Technology (Head of the Science Department, College of Technology, Nun's Corner, Grimsby, Lincs).

Turbulence of Fluids and Plasmas, April 16–18, New York City (MRI Symposium Committee, Polytechnic Institute of Brooklyn, 333 Jay Street, Brooklyn, NY 11201)

Computer-based Information Retrieval Systems, April 18–19, Liverpool (Bernard Houghton, School of Librarianship and Information Work, College of Commerce, Department of Professional Studies, Tithebarn Street, Liverpool 2)

Mechanical Design, May 6–10, University of Bradford (The Registrar, University of Bradford, Bradford 7)

Cryogenics Engineering, May 7–10, Brighton (Conference Secretary, ICEC 2, "Cryogenics", 32 High Street, Guildford, Surrey)

Surging Glaciers, June 6–8, Banff (C M Lockwood, Assistant Director, Department of Extension, The University of Alberta, Edmonton, Alberta)

Canadian Botanical Association, June 11–14, Ontario (D R Lindsay, c/o Canadian Botanical Association, Lakehead University, Port Arthur, Ontario)

Corrosion and Protection of Pipes and Pipelines, June 12–14, Earls Court (Technical Exhibitions Ltd, 3–4 Clements Inn, London WC2)

Third Congress of the European Society of Ophthalmology, June 18–21, Amsterdam (The Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands)

The Solar Terrestrial Environment, July 10–12, Sheffield (Miss G M White, Department of Applied Mathematics and Computing Science, The University, Sheffield).

Organic Geochemistry, September 16–18, Amsterdam (Dr P A Schenck, Secretary of the Organizing Committee, 4th International Meeting on Organic Geochemistry, Volmerlaan 6, Rijswijk (Z H.), The Netherlands).

Mass Spectroscopy Group meeting, September 16–18, University College, London (Secretary, Mr R. M Elliott, GEC-AEI (Electronics), Ltd, Scientific Apparatus Division, Barton Dock Road, Urmston, Manchester).

Electronics Design, September 23–27, University of Cambridge (The Conference Department, Institute of Electrical Engineers, Savoy Place, London WC2).

Stresses in Composite Materials, September 25–27, College of Aeronautics, Cranfield (The Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

Cellular and Humoral Mechanisms in Anaphylaxis and Allergy, October 3–5, Toronto (Professor Henry Z Movat, Department of Pathology, University of Toronto, 100 College Street, Toronto 2, Canada)

Astronautical Congress, October 13–19, New York (Miss Mireille Gérard, American Institute of Aeronautics and Astronautics, 1290 Avenue of the Americas, New York, NY 10019)

Meeting of the Clay Minerals Society, October 14–17, Indiana University (Dr John B Droste, Department of Geology, Indiana University, Bloomington, Indiana 47401)

Crystallography, August 7–27, 1969, Roswell Park Memorial Institute, Buffalo (August 7–12), State University of New York at Stony Brook (August 14–21), Stony Brook campus and Brookhaven National Laboratory, Long Island (August 23–27) (Eighth International Congress of Crystallography, Congress Office, State University of New York, Stony Brook, Long Island, New York).

Erratum In the communication "Stimulative Vaporization of Phenylmercuric Acetate by Mercury-resistant Bacteria" by R Tonomura *et al* (*Nature*, 217, 646, 1968) the fresh culture medium (line 3, paragraph 4) contained 2,000 μg of inositol and 400 μg of nicotinic acid as well as the components listed

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, February 26

University of London (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Professor A W Merrison "The Science and Engineering of Elementary Particle Physics" (further lectures on March 4 and 11) *

Tuesday, February 27

Royal Statistical Society (Medical Section) (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1)—Dr I Sutherland "When was the Great Plague ?"

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 2 30 p m —Colloquium on "Developments Towards a Computer-Based Information Service in Physics, Electrotechnology and Control"

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W1), at 4 p m —Symposium on "Cement Automation"

Imperial College of Science and Technology (in Lecture Theatre A, Department of Mechanical Engineering, Exhibition Road, London, SW7), at 5 30 p m —Professor W S Elliott "The Computer—Master or Servant ?" (Inaugural Lecture)

Royal Institution (at 21 Albemarle Street, London, W1), at 5 30 p m —Professor Ronald King "Metals" (Lecture for Sixth Form Boys and Girls from Schools in London and the Home Counties To be repeated on February 28, March 5 and 6)

University of Aston in Birmingham (at Gosta Green, Birmingham, 4), at 5 30 p m —Professor E J. Davies "The Challenge of Electrical Machines" (Inaugural Lecture)

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Professor S Cohen "Structure and Biological Properties of Immunoglobulins" (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)*

University of London (in the Engineering Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Professor E H Brown "Man, the Geomorphological Process" (Inaugural Lecture) *

University of London (at the Institute of Archaeology, 31–34 Gordon Square, London, WC1), at 5 45 p m —Dr C F A Schaeffer (Paris) "Ras Shamra—Ugarit" (further lecture on February 29) *

Wednesday, February 28

Royal Aeronautical Society, Agricultural Aviation Group (at 4 Hamilton Place, London, W1), at 10 a m —All-day Meeting on "Operational and Safety Aspects of Agricultural Aviation"

Royal Institution, History of Science Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Professor Robert Siegfried (University of Wisconsin) "Humphry Davy's Attitude to the Uses of Science" *

Society of Chemical Industry, Food Group and the Microbiology Group (at the School of Pharmacy, Brunswick Square, London, WC1), at 2 30 p m —Meeting on "Microbial Problems of Food Handling Equipment"

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Dr E. T Hall "Measurement as an Aid to Archaeological Research"

Society of Apothecaries of London, Faculty of the History of Medicine and Pharmacy (at Apothecaries' Hall, Blackfriars Lane, London, EC4), at 5 30 p m —Sir George E Godber "General Practice, Past and Present" (Monckton Copeman Lecture)

Institution of Electronic and Radio Engineers, Communications Group (at 9 Bedford Square, London, WC1), at 6 p m —Mr R Gibson and Mr S M Edwardson "Ultrasonic Delay Lines and their Uses in Television"

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Dr J G Wistreich "Impact of Automation of Steel Manufacture on Plant Design" (John Player Lecture)

Society for Analytical Chemistry, Thin-Layer Chromatography Group (at "The Feathers", Tudor Street London, EC4), at 6 p m —Discussion Meeting on "Radiometric Microanalysis Employing Thin-Layer Chromatography".

University of London (at the Institute of Archaeology, 31–34 Gordon Square, London, WC1), at 6 p m —Miss E Vollans "Sources of Maritime Archaeology—Trade and Ports in Britain" *

Thursday, February 29

Royal Society (at 6 Carlton House Terrace, London, SW1), at 10 30 a m —Discussion Meeting on "Science and Technology of Aerosol Pollution in Modern Society" organized by Professor A R Ubbelohde, FRS, and Professor T M Sugden, FRS

University of London (in the Anatomy Theatre, University College London Gower Street London, WC1), at 1 20 p m —Mr D H Girmes "Is Logic Absolute and Complete ?"*

University of London (at Imperial College, London, SW7), at 1 30 p m —Mr Pat Carpenter "Art and Science VIII—Art and Science Now—the Possibilities of Convergence" *

Fertiliser Society (at the Geological Society, Burlington House, Piccadilly, London, W1), at 2 30 p m —Mr W C Weber and Mr I S Mangat "The Production of High Nitrogen NPK Granular Fertilisers"

Institution of Civil Engineers Hydrological Group (at Great George Street, Westminster, London, SW1), at 5 30 p m —Report on Leningrad Symposium on Floods and on Soviet hydrological research introduced by Mr M Mansell-Moullin and Mr J C Rodda (Informal Discussion)

University of London (at the Institute of Child Health, Guilford Street, London WC1), at 5 30 p m —Dr N A Mitchison "Recognition of Antigen" (Last of fifteen lectures on "The Scientific Basis of Medicine", organized by the British Postgraduate Medical Federation)*

Institution of Mechanical Engineers, Process Engineering Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Development in Desuperheated Techniques"

Friday, March 1

West Cheshire Central College of Further Education (at Carlett Park, Eastham, Wirral, Cheshire)—Symposium on "Advances in Polarography"

Linnean Society of London (at Burlington House, Piccadilly, London, W1), at 10 55 a m —Symposium on "The Experimental Taxonomy of Flowering Plants"

Royal Institution, Photochemistry Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Mr M B Ledger "Photoreactions in Water" *

Institute of Rural Life at Home and Overseas (at Friends International Centre, Courtauld House, Byng Place, London, WC1), at 1 15 p m —The Rev Merfyn Temple "Problems of Development for Rural Communities in Zambia" *

University of London (at the Royal Postgraduate Medical School, Du Cane Road, London, W12), at 4 p m —Dr C N Armstrong "The Diagnosis of Sex" *

Institution of Electrical Engineers (at Savoy Place, London, WC1) at 5 30 p m —Mr H B Williams and Mr S C Chu "Computer Solution of e h v Underground Cable Cooling Problems"

Society for Analytical Chemistry (at the County Hall, Durham City), at 5 30 p m —Annual General Meeting, followed by Mr L J Clark "North Sea Gas"

University of London (at Queen Elizabeth College, Campden Hill Road, London, W8), at 5 30 p m —Professor L Hough "The Chemical Architecture of Carbohydrate Molecules" (Inaugural Lecture)

Royal Institution (at 21 Albemarle Street, London, W1), at 9 p m —Dr Anne McLaren "Life Before Birth—the First Few Days"

Saturday, March 2

Inner London Education Authority (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3 30 p m —Zoe, Lady Hart Dyke "Silk Farming in England" *

Monday, March 4

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 10 a m —Colloquium on "Pulsed Code Modulation"

University of London (at the Royal Postgraduate Medical School, Du Cane Road, London, W12), at 4 p m —Professor R D Girdwood "Folic Acid Deficiency" *

Institution of Civil Engineers (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "The Organization of the Port Planning Team and the Role of the Engineer" introduced by Mr J E G Palmer

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Need or Otherwise for Suppression of Harmonics Generated by Convertor Equipment" opened by Mr H J Sheppard

University of London (at the Royal Free Hospital School of Medicine, 8 Hunter Street, London, WC1), at 5 30 p m —Docent Annica Dahlström (Stockholm and Goteborg) "Monoamine-containing Neurones in Mammals I Monoamine-containing Neurones in Mammals as Studied by Fluorescence Microscopy" *

Institution of Mechanical Engineers, Applied Mechanics Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Designing Components Subjected to High Surface Pressure"

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W1), at 6 p m —Mr F W. Hyde "Progress in Radio Astronomy"

Society of Chemical Industry, London Section (joint meeting with the Chemical Engineering and Heavy Organic Chemicals Groups, at 14 Belgrave Square, London, SW1), at 6 30 p m —Sir Kenneth Hutchison, CBE "New Sources of Natural Gas for Britain" (Jubilee Memorial Lecture)

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer in the Laboratory of General Physiology in the School of Biology—The Registrar, The University, Leicester (February 28)

Lecturer or Assistant Lecturer (with good academic qualifications in sociology, teaching experience and an interest in the sociology of education) in Education—The Registrar, The University, Keele, Staffs (March 1)

Research Assistant (with a Ph D degree in enzymology or a good honours degree with research experience in enzymology) to Dr B T Cromwell, in the Department of Botany, to carry out research on enzyme systems involved in the biogenesis of alkaloids—The Registrar, The University, Hull (March 1)

Assistant Lecturer in Philosophy—The Secretary, Birkbeck College (University of London), Malet Street, London WC1 (March 4)

Assistant Lecturer (with special interests in either anatomy and/or microstructure or in aspects of the biology of algae) in the School of Plant Biology—The Registrar, University College of North Wales, Bangor, North Wales (March 4)

Assistant Lecturer in Civil Engineering—The Secretary of the University Court, The University, Glasgow (March 8)

Superintendent (graduate in physics, preferably with experience of administration in industry in the government service or in universities) of Laboratories in the Department of Physics—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (March 8)

Reader/Senior Lecturer in Electronic Control or Electronics—The Registrar, The University, Canterbury, Kent, quoting Ref A7/68 (March 9)

CHAIR OF GENETICS—The Secretary of the University Court, The University, Glasgow (March 15)

LECTURERS or ASSISTANT LECTURERS (3) in the DEPARTMENT OF MATHEMATICS, The Royal University of Malta—The Inter-University Council, 33 Bedford Place, London, WC1 (March 15)

CHAIR OF MATHEMATICAL STATISTICS—The Registrar, The University, Hull (March 16)

UNIVERSITY RESEARCH FELLOW IN THEORETICAL PHYSICS (Atomic Scattering and Classical Mechanics)—The Secretary (NAT), University of Stirling, Stirling, Scotland (March 16)

ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Keele, Staffs (March 18)

CHAIR OF APPLIED MATHEMATICS and HEADSHIP OF THE DEPARTMENT—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (March 18)

LECTURERS (2) IN CHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, March 29)

RESEARCH FELLOW in the DEPARTMENT OF GEOGRAPHY, Research School of Pacific Studies, Australian National University, to work in cultural, economic or social geography in South-east Asia or the Pacific Islands—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, April 1)

ASSISTANT PROFESSOR (with a Ph D with emphasis on environmental physiology and energy metabolism, and teaching and research experience) of ANIMAL PHYSIOLOGY in the DEPARTMENT OF ANIMAL SCIENCE—Head of Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (April 15)

UNIVERSITY LECTURER (preferably with endocrinological interests) in HUMAN ANATOMY—Professor G W Harris, CBE, FRS, Department of Human Anatomy, University of Oxford, South Parks Road, Oxford (April 27)

LECTURER IN MICROBIOLOGICAL CHEMISTRY in the DEPARTMENT OF ORGANIC CHEMISTRY—The Registrar, The University, Newcastle upon Tyne

LECTURER in PHYSICS to be attached to the SOLID STATE GROUP (Electron Diffraction Section)—Professor M Blackman, FRS, Physics Department, Imperial College, London, SW7

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London Series A Mathematical and Physical Sciences No 1129 Vol 262 (14 December, 1967) Asymptotic Solution of Initial Boundary-Value Problems for Hyperbolic Systems By B Granoff and R M Lewis Pp 387–411 (London The Royal Society, 1967) 9*s* , $1 35 [1512

Titles of Dissertations approved for the Ph D , M Sc , and M Litt Degrees in the University of Cambridge during the Academical Year 1966–67 Pp 38 (Cambridge The University, 1967) [1812

Greater London Council—Scientific Branch Annual Report of the Scientific Adviser 1966 Pp 83 (London Greater London Council, 1967) 5*s* [1812

Proceedings of the Society for Psychical Research, Vol 55, Part 200 Thomas Bayes and the Bundle of Sticks By Colin Rollo Pp 23–64 (London Society for Psychical Research 1967) 10*s* , $1 50 [1812

Ambassade de France, Service de Presse et d'Information The French Press Pp 27 (London Ambassade de France, Service de Presse et d'Information, 1967) [1812

Other Countries

Smithsonian Institution—U S National Museum Bulletin 255 Revision of the Moths of the Subfamily Prodoxinae (Lepidoptera Incur variidae) By Donald R Davis Pp vii+170 (Washington, D C Govern ment Printing Office, 1967) $1 [111

Advances in Molecular Relaxation Processes, Vol 1, No 1 (November 1967) Pp 1–108 Subscription price per volume £10 9*s* 6*d* or Dfl 90 00 plu postage (Amsterdam Elsevier Publishing Company, 1967) [111

World Meteorological Organization World Weather Watch Plannin Report No 21 Design of Optimum Networks for Aerological Observin Stations Pp 58 Planning Report No 22 The World Weather Watc and Meteorological Service to Agriculture By L P Smith Pp 14 (Geneva World Meteorological Organization, 1967) [111

Institut Royal Météorologique de Belgique Annuaire—Résultats de Observations des Marées Terrestres en 1964–1965, Centre de Physique du Globe, Dourbes Pp 188 Bulletin Mensuel—Observations Ionosphériques Octobre, 1967 Pp 26 (Uccle-Bruxelles Institut Royal Météorologiqu de Belgique, 1967) [121

Deutschen Wetterdienstes Annalen der Meteorologie (Neue Folge), Nr 3 Die Meteorologen-Tagung in Munchen vom 27 bis 30 April 1966 Pp 224 (Offenbach a M Selbstverlag des Deutschen Wetterdienstes, 1967) [131

Australia Commonwealth Scientific and Industrial Research Organiza tion Annual Report of the Wool Research Laboratories, 1966–67 Pp 65 Annual Report of the Division of Forest Products, 1966–67 Pp 84 (Melbourne Commonwealth Scientific and Industrial Research Organization 1967) [131

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Irrigation Research Laboratory, 1966–67 Pp ii+55 (Griffith, N S W Commonwealth Scientific and Industria Research Organization, 1967) [1512

United States Department of Commerce National Bureau of Standards Miscellaneous Publication 293 1967 Technical Highlights of the Nationa Bureau of Standards Annual Report, Fiscal Year 1967 Pp iv+162 (Washington, D C Government Printing Office, 1967) $0 55 [1512

Commonwealth of Australia Department of Supply Defence Standard Laboratories—Jubilee Annual Report, 1966–67 Pp 87 (Maribyrnong, Vic Defence Standards Laboratories, 1967) [1512

A Neuropsychiatric and Genetical Investigation of Acute Intermittent Porphyria By Lennart Wetterberg Pp 87 (Stockholm Svenska Bokförlaget/Norstedts, 1967)

Science Council of Canada Report No 2 The Proposal for an Intense Neutron Generator Initial Assessment and Recommendations Pp v+12 (Ottawa Queen's Printer, 1967) $0 25 [1812

Australia Annual Report of the Museum of Applied Arts and Sciences, Sydney Pp 20 (Sydney Museum of Applied Arts and Sciences, 1967) [1812

Canada, National Research Council Annual Report on Support of University Research, 1966–67 Pp viii+279 (Ottawa National Research Council 1967) $0 50 [1812

Indian Forest Records (New Series) Entomology Vol 10, No 12 Effect of Leaching Out Water Soluble Substances in the Bamboo, *Bambusa nutans* Wall , as Protection Against Insect Borers By M L Roonwal, P N Chatterjee and R S Thapa Pp 249–256+plate 1 (Delhi Manager of Publications, 1966) [1912

Republic of Botswana Ministry of Commerce, Industry and Water Affairs—Geological Survey Department Annotated Bibliography and Index of the Geology of Botswana to 1966 Pp 172 (Lobatsi, Botswana Geological Survey Department, 1967) R 1 00 c [2012

The Centennial History of the Auckland Institute and Museum Prepared by A W B Powell S G Brooker Christina O Troup and E G Turbott. Pp 88 (Auckland, New Zealand Auckland Institute and Museum, 1967) [2112

Directorate for Scientific Affairs, Organization for Economic Co-operation and Development Reviews of National Science Policies—United States Pp xii+701 (Paris OECD, 1967) [2112

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Mr Crosland's Little Joke

Mr Anthony Crosland, president of the Board of Trade for half a year, is not merely an eminently reasonable man but resourceful as well, which is why there are few who can be entirely surprised that the project to build a third airport for London in the rolling green fields of Essex has finally come to grief. The story that he deliberately engineered this upset of his predecessor's scheme by sanctioning re-alignment of the runways at Stansted, which was bound to provoke the wrath of the Council of Tribunals, may be a gross misstatement, but it is one of those stories which is too good to be entirely untrue. In any case, to retreat from a well-prepared but irrational position as Mr Crosland has done is not only courageous but a welcome break with what seems to be the style of modern government. The residents of Stansted, and air travellers in the seventies, will understandably be delighted with the way things have turned out. The affair should encourage all those who believe that there is, or should be, some merit in pure reason.

The brief history of the Stansted project is worth repeating even though some of it has now become a part of British folk-lore. The hunt for a site for a third London airport began almost before the second airport, at Gatwick, came into service a decade ago. By 1963, a committee consisting largely of government officials came to the conclusion that Stansted was the best of all possible sites for the third airport, and towards the end of 1965 a public inquiry was begun to assess the weight of the objections to the proposal which had, by then, become apparent. The report of the inspector holding the inquiry was unambiguous—in his view, the siting of the third airport at Stansted was a "calamity" for the locality, and there should be an independent "review of the whole problem". In the event, the Government carried out an internal review which, for practical purposes, denied the validity of the inspector's objections. From there it was a simple step for the then president of the Board of Trade, Mr Douglas Jay, to insist that the project must go forward with despatch, and for the Government to dragoon first its supporters in the House of Commons and then the House of Commons as a whole into acquiescence. Throughout its struggle with good sense, the Government has been able to count on the shrill support of Mr Peter Masefield, the chairman of the British Airports Authority, who has turned out in the past year to be one of the most prolific writers of letters to the daily newspapers.

It is perhaps understandable that the way in which this decision has been stood on its head has made many of the Government's supporters in the House of Commons consider that they have been cheated or led up the garden path. Elsewhere, people will have been provided with a chilling illustration of how little can be done, with present arrangements, to divert a government from a chosen course, however foolish. But these are long-range matters, which is not to suggest that nothing more should be heard of them. It is, however, an immediate problem to know just how a sensible site should be chosen for the third London airport. How, one way or another, will it be possible to prevent the result of the inquiry now to be mounted seeming as if some official has stuck a pin at random into the map of south-east England? How is the machinery which has functioned so badly in the past few years now to be nudged towards a sensible choice?

One set of considerations ought by now to be so compelling that nobody can fail to grasp it. Briefly, in densely populated countries such as Britain, where the coastline is mercifully quite substantial, the obvious place for major airports is on the sea. Any other arrangement is bound to mean that increasingly noisy aircraft will increasingly distract ordinary people. It is true, of course, that it is sometimes necessary to destroy amenities—such as absence of unnatural noise—for the sake of some other social objective, and it may even occasionally be necessary to attempt some kind of balance between amenity and its cost. Yet there is no question that the noise which abounds in built-up areas near international airports is usually a kind of civic indecency. If there is any alternative to building near places which will eventually be occupied by people, and if the difference of cost in doing so is not more than, say, a third of the cost of building anyway, then the opportunity should be seized of avoiding unnecessary assaults on people's peace and quiet. But this case for building the next London airport near the sea is reinforced, for an airport due to come into service when supersonic aircraft may be flying, by the uncertainties which surround the social acceptability of supersonic bangs. Will supersonic transports ever be allowed to fly supersonically over land? And if they are, will not inland airports be a great nuisance to the airlines as well as to people living on the ground? With these uncertainties, who in his senses would now put money into inland airports when there are reasonable alternatives near the sea? It is curious, even now, that these uncomplicated considerations were given so little attention during the official arguments for and against Stansted as a site for the third airport, although the Government's most recent summary of the position a year ago did grudgingly acknowledge that

Stansted would have been a greater nuisance on the the grounds of noise than an airport at one of the several alternative sites on the Thames Estuary

There are, however, more serious charges to be made against the way in which the site at Stansted was selected. Looking back, it is clear that the case for Stansted was unreasonably influenced by the pre-existence of a single concrete runway at the site—a circumstance which could not have saved more than ten per cent of building on the site even on the basis of the Government's calculations Also included in these calculations was the comic guess that it would cost no more than £6 million of capital to improve existing transport links with the proposed airport to the point at which they could be considered good enough for the year 2000 (when the third London airport will presumably be still in service) But to argue like this is absurd In setting out to compare the costs of alternative sites, the Government should seek to do for airports what it insists the nationalized industries should do when making decisions about the new investments on capital equipment—some attempt should be made to lump together running costs and capital costs And the truth is, of course, that in circumstances in which the annual flow of passengers through the new airport is likely to be several tens of millions in the eighties, cheeseparing now about the initial cost is likely to be an exceedingly false economy The real need is for a much more realistic appraisal of the full costs of alternative sites from the point of view of the whole function of the airport and the whole complex of potential users It is particularly important to graft on to the study which is now to be undertaken—and Mr Crosland has promised that it will be a thoroughly comprehensive inquiry even though there are misgivings about the time that that will take—a proper study of the surface transport, freight as well as passengers The object should be to regard the new and non-existent airport as a part of a huge and integrated system and not as an isolated facility by itself It is, in other words, a splendid opportunity It will be interesting to see how well the Government rises to the occasion after making such a mess of its first attempt to choose a site

What Takers for Dr Dainton's Cure?

THREE years almost to the day after the appointment of the committee under Dr F S Dainton which has been looking into the causes for the drift away from science in British schools, the final report has been published On the whole, it is a perceptive and a liberal document Although the committee was charged with responsibility simply for the problem of the diminishing flow of young people into science and technology at the interface between British schools and the various establishments of higher education, it has creditably taken a wider view of its task In particular, it has quite accurately concluded that much of what it wishes to accomplish can only be done by reconstruction of the British educational system (To be accurate, the Dainton Committee has also acknowledged what seems to be the superiority of the Scottish system in comparison with what happens south of the border, and urges that the Scots should set their faces against supposed reforms of their system the effect of which would be to make the interaction between schools and universities in Scotland resemble more closely the relationship which now obtains in the rest of Britain) Fortunately, the need for change is now more widely appreciated than it has ever been, and it is not unreasonable to hope that a measure of change may actually come about In the circumstances, the Dainton Report is likely to find a more sympathetic reception than would have seemed likely even a year ago By the same test, unfortunately, its historical importance is likely to be less than if it had appeared sooner, when the going was still rough.

The swing away from science in British schools is, of course, a comparative affair The groups of young people following science studies in the sixth forms of British schools are still a growing proportion of the age group as a whole, but the groups following other kinds of studies are growing much more rapidly The result is that the balance between science studies and other studies in the senior classes of British schools is changing rapidly and to the detriment of the sciences One consequence is the difficulty there has been in recent years in recruiting people to fill the science departments at universities to their best advantage The Dainton Committee is right to take the view that this trend cannot be a healthy one at a time when there is a large unsatisfied demand for technical people in commerce and industry, and when science and technology as a whole are capable not merely of changing the character of life but of exciting the imagination as well Sensibly enough, however, the committee has set its face not merely against expedients that would artificially persuade young people to suppress their natural inclinations but also against attempts to develop artificial rules for saying how many people with which kinds of training British higher education should provide

So what, then, should be done ? Schools and universities have separate parts to play, but it is entirely proper that the Dainton Committee should put high on its list of remedies the view that British schools should provide a much broader curriculum for their pupils This, of course, is a crying need which even Scotland should not ignore The question which now needs to be answered is what kind of reform should now be undertaken It has been an open secret for several months that the Dainton Committee would urge that only in the most exceptional circumstances should pupils in British schools be allowed to abandon mathematics, which is entirely sensible But what form should the rest of the curriculum take ? The Dainton Committee has obviously been attracted by the talk in recent months of schemes in which students preparing for higher education would study three or four subjects as well as mathematics, and its own preference is for a mixture of science, social science and arts for everybody So far, so good The real problem is to know what should be done to bring about these changes

The committee would have done a useful service if it had been more outspoken on this issue Events in the last six months suggest that the time has come when several distinguished heads should be knocked forcibly together, and the Dainton Committee could have made a useful start For one thing, it is important that the Schools Council, which has responsibility for the British school curriculum, should be dissuaded from its introspective preoccupation, the pressure of external examinations (and the need somehow to diminish it) Its own proposals are narrowing, not broadening, and have served so far chiefly to undermine confidence in the council's capacity to function sensibly But it is also important that work should promptly be put in hand to design the kinds of curricula which the logic of the committee's proposals would imply, and on recent form there is very little to suggest that the Schools Council, which ought to be in charge, will be fit and able to do what is expected of it But there are also problems concerning teachers' organizations—who, for example, is going to persuade the science teachers whose enthusiasm has been chiefly responsible for experiments in science teaching in recent years that the time has now come to have less, not more, formal teaching in the schools ? One of the valuable discoveries which the Dainton Committee has to report is that students following science courses tend to be well looked after by well qualified teachers The question now is who should tell these talented people that they must spend less time behaving as if they were teaching in universities To be sure, it will be valuable to have Dr Dainton's support for the cause of more in-service training for teachers, more laboratory technicians in British schools and better means of persuading young people to take up science teaching The trouble, unfortunately, is that these needs have also been obvious for some time The most urgent need has become that of making them materialize It is, of course, a great absurdity that the British educational system has remained archaic for so long, but the prize will go to the one who can show how change can happen quickly

What the committee has to say about the universities follows naturally enough—universities must be more flexible about their requirements for entry By good fortune, it seems now very much as if the Standing Conference on University Entrance will be given advice in this direction in the months ahead The article by Professor L Rosenhead, a member of the Dainton Committee, on page 806, is one man's vision of how universities might quickly become better suited to the real needs of the schools But here, too, it is all too likely that the formal processes of consultation and the traditional unwillingness of those concerned with British education to consider seriously any but the most moderate of changes will blunt the present tendency towards reform

This, perhaps, is where the Government could help As it has turned out, and as it should be, the Dainton Report is not a piece of special pleading on behalf of science teachers but yet another comment on the inadequacies of British education It also stands out among public documents as a sensible statement of the part which should be played in education by science studies and of the reasons why some acquaintance with science should be considered important not merely for professional scientists but for everybody The trouble, of course, is that in the present balance between British schools and British universities, there is no room for this kind of appraisal On the whole, the schools are too preoccupied with specialist studies and the universities are forced as a result to be too preoccupied with the need for a broader education It is hard to see how this pattern can be changed except by the simultaneous agreement of the host of interested parties, but it is also plain that the need for change is urgent In the circumstances the Government could do worse than organize the kind of conference at which many interested parties could be persuaded to hammer out answers to the question which excessive modesty has persuaded Dr Dainton to leave alone

The Magic of Numbers

THE dead-pan send-up is a literary genre that seems now to be flourishing in the United States A few weeks ago there was the *Report from the Iron Mountain* Now there has appeared Professor D J de S Price's *Research on Research* (Journeys in Science, University of New Mexico Press, 1967) It is true that Professor Price appears to be entirely serious in what he has to say, but many of his readers will be bound to suspect that he has devised a witty demonstration of the inadequacies of what is called the science of science

That hindsight makes it seem as if scientific discovery points to a logical method of procedure has led some people to suppose that there must be an analogous logic in the way in which science is carried on —a logic the investigation of which merits a new and distinct discipline of social enquiry One approach of this "discipline" is to examine some numerical parameter assumed to be indicative of the forward march of science, such as the number of all papers published, and if possible to describe its variation in terms of some mathematical function From this kind of exercise, scientists of science expect, some useful insight may eventually emerge Among the principal difficulties of this argument is the assumption that the part of the individual in scientific discovery is either so predictable or so negligible that it can be altogether ignored. The crude macroscopic indices considered by the scientist of science can be used to demonstrate that, so inexorably does the juggernaut of science roll forward, not even a genius can make a significant contribution to its momentum. But it is not easy to know whether Professor Price is mocking when he says that "Science is, so to speak, a much more regular thing in its behaviour than are people". This assumption, a necessary point of departure for the science of science as usually construed, is an absurdity which lies at the other extreme from the Cleopatra's Nose school of history. Can it seriously be supposed that if Archimedes, Newton and Einstein had never existed, the present state of science would be no different?

Granted that the individual is insignificant, Professor Price shows how several intriguing "laws" may be set up. The "First Law of Research on Research" is that the "size" of science increases exponentially, whether judged by the number of scientists, scientific journals or scientific papers. It follows that "87½ per cent of all the scientists who have ever been are alive now". This is an impressive figure indeed and doubtless deserves its place in the *Guinness Book of Records*, along, perhaps, with a note that a similar figure would probably have been valid in 450 B.C. But what does it show? Professor Price offers this, that "science runs so much faster than people, so much more rapidly than civilization". This would seem to cast the man at the bench in the position of the Red Queen, which may be Professor Price's way of saying that the size of science bears no relation to such wholly immeasurable aspects as its quality or rate of progress at any given time. Alas that the latter aspects, which alone are of significant interest, fall outside the crude scope of the science of science.

No first law is complete without a second, and Professor Price announces the Second Law of Research on Research which states that the distribution of quality among scientific institutions, men or journals follows the approximately inverse square law typical of the Pareto law of distribution of income. "For men as for institutions", Professor Price explains, "the chance of doubling the size of achievement is uniformly about one in four, no matter what the size already achieved." From this follows the premise implicit in the foundation of the argument, that in science (though not, apparently, in art) the individual counts for nothing. "If Beethoven had not existed, other men would have written quite different symphonies, Beethoven's private property is unmistakable. If Planck, however, had not made his particular discovery, somebody else would have to have made it and rather quickly."

As the culmination of his essay, Professor Price produces statistics to show that the size of each country's research effort is proportional to its gross national product. Size is measured by the percentage of papers of each national origin indexed in *Physics Abstracts* and *Chemical Abstracts*. (He calls these "National Brownie Points", which is another reason for believing that he may not be entirely serious.) From this analysis it emerges that the Soviet Union, whose share of the world's GNP is 15·6 per cent, contributed 15·6 per cent of physics papers, while the equivalent figures for the United States are 32·8 and 31·6, respectively. This is a remarkable concurrence, but is it significant? Concurrences in historical and social phenomena have an unfortunate habit of being less meaningful than their counterparts in scientific experiments. George I, II, III and IV all died on a Saturday, but this does not by itself serve to establish the existence of a baneful influence between the Sabbath day and the House of Hanover. The science of science has far to go before it attains respectability. Only Professor Price can know whether his ingenious essay was written to help it on its way or as another obstacle in its path.

Mr. R. Brightman

THE death of Mr R. Brightman at his home in Cheshire last week-end is a particularly sad blow for *Nature*, for he played an important part in its production for the whole of forty years. He was a chemist by trade, and served for many years in the Dyestuffs Division of ICI Ltd. He had a passion for books and for the scientific literature, which is why no doubt he spent much of his professional energy on the management of the literature. But he also had a great affection for his work for *Nature*, which began with book reviewing and quickly graduated to other tasks. In the years since the Second World War, when many men of his age would have settled back in their retirement, he found himself writing a great proportion of the leading articles which appeared in *Nature*. More recently, he took on the job of working through *Hansard* each week in search of parliamentary news, and he was at the same time hard at work on a volume intended to celebrate the centenary of *Nature* next year. He would have wished no better monument.

Gloomy Post Office

THE prospects of the General Post Office for its final year before being established as a public corporation are described in a Government White Paper, just published (HMSO, 1*s*. 9*d*.), and presented to Parliament last week by the Postmaster-General. A Bill for the establishment of the corporation will appear later in the year. For the past five years a financial target of 8 per cent was set for the Post Office as a whole, but this has not been achieved. The overall return is expected to be nearer 7·6 per cent, the return from telecommunications, which reached the target, compensating for comparative failures of the postal services. Under the current reorganization these two sections will be managed separately and, because of the different nature of their activities, will aim for different targets. A net return target of 8·5 per cent for telecommunications (after historic depreciation but before interest and supplementary depreciation) has been set, but for the postal service, which is less highly capitalized, a margin of 2 per cent on total expenditure is being attempted. At present prices, it is unlikely that these targets will be reached. In fact, the postal service expects to make a loss, and, faced with capital expenditure for expansion and increased productivity over the next five years of £2,000 million, the Post Office says that charges must be brought into line with costs The position is being reviewed by the Prices and Incomes Board

The telecommunications section is aiming to increase the size of the system by 50 per cent without increasing overall manpower, by using more machinery Improvement of service for the existing 7 7 million subscribers will be the most important task in the coming year, and a net increase of half a million subscribers is expected The various local, trunk and overseas lines are all being increased, as are exchanges and the number of telex lines By March 1969 it is expected that 98 per cent of customers will be on automatic exchanges Automation is progressing in such fields as international telex and telegrams, data transmission services are being expanded, and additional submarine cable links with various European countries are being laid Aluminium conductors have been successfully used instead of copper in telephone cables, and further use of aluminium is expected to benefit the economy because of the high price of imported copper

The postal section will see two important developments in the coming year—the two-tier post system in the autumn with lowest rates of postage being open only to mail in "post office preferred" envelopes, and the inauguration of the National Giro Service at its Lancashire headquarters Modern techniques of management and marketing are being examined for staff and stamps, respectively, and post codes will be allocated to parts of London and twenty-five other towns and cities During the year, twenty mechanized sorting offices will be brought into service

New Units for Industry

THE process of changing all Britain's units from the old imperial system to the metric system is a messy business Colonel J S Vickers, chairman of the Planning Group of the British Standards Institution, which is planning the change, calls it "an agony" But at least everyone agrees that the medicine, once taken, will do the patient good The arguments are about who should take the medicine first In most countries which have made the change, the retail trade, which includes packaging, has adopted the new units first, but in Britain the opposite will happen The plans for the retail trade will depend on the recommendations of the Joint Committee on Metrication, a Ministry of Technology body whose chairman is Mr Arthur Wynne The committee is widely expected to accept a recommendation made to it by the CBI (see *Nature*, **216**, 1157, 1967) and propose the formation of a Metrication Board

Last week the BSI was able to announce its plans for the engineering industry After consultation with industry which seems to have been somewhat one-sided (of 1,600 firms to which the BSI sent its original questionnaire, only 250 replied), the BSI has prepared a draft programme which it is circulating to industry through trade associations The period of transition has been set at 1970–75 The terminal date was fixed by a Board of Trade decree, so all that really needed deciding was the starting date 1970 seems to have been the overwhelming favourite The first industry to go metric, chosen because its problems were likely to be the greatest, will be the engineering equipment manufacturers, who use the basic engineering materials to produce equipment which is then used by the process industries This time-scale will, the BSI says, fit in with the plans of the construction industry, which is on the way to metrication already Some 20,000 copies of the draft programme have been sent to engineering companies through their trade associations, and they have been invited to send comments to the BSI by March 29 Companies not members of any trade association can get copies from the BSI.

Another New Reactor

DESPITE its great cost, which might be expected to dictate caution, nuclear reactor design remains astonishingly diverse Almost every conceivable combination of coolant, moderator and fuel has been studied, on paper if not in actual hardware, although most of these systems are unlikely to evolve into commercial power reactors. Although Britain has so far tended to favour gas cooling for thermal reactors, the United Kingdom Atomic Energy Authority last week invited the Duke of Edinburgh down to Winfrith to open its latest reactor, which is water cooled In the event, the considerable skill of the authority at public relations had to be directed at those who thought the royal visit had cost a great deal too much, but the virtues of the reactor were not entirely forgotten It had, after all, cost £16 million itself

The reactor is called, somewhat inelegantly, the steam generating heavy water reactor, abbreviated to SGHWR It is a system in which moderation by heavy water is combined with cooling by ordinary water, which boils and drives the turbine generators in a direct cycle The design uses a number of pressure tubes, rather than a single pressure vessel, an arrangement with several important advantages The fuel, slightly enriched uranium dioxide, is contained in thin tubes made of zirconium alloy, 36 such tubes go to make up each fuel element Cooling water passes up the tubes, which are surrounded by an aluminium calandria (vessel) containing heavy water moderator

The electrical output of the station is 100 MW, which is fed into the National Grid The reactor can be refuelled either on-load or off-load, although the advantages of on-load refuelling seem much overrated. If the reactor is refuelled off-load, the whole job can be done in a weekend so that most customers are likely to choose this option The pressure tube design allows much of the fabrication work to be done in the workshop instead of on-site, which is a valuable advantage in places where welding technology is less advanced than it is in the United States The authority is tentative about quoting costs, but says that capital costs should work out at about £60 per kilowatt, with generating costs of 0 50 pence a kWh or less The reactor is a good plutonium producer, very much better than the advanced gas cooled reactor, and about the same as a boiling water reactor The design can also be adapted for natural, rather than enriched uranium The authority says that there would be no difficulty in building an SGHWR as big as 1,000 MW.

So far, however, the whole contracting position is a mystery The authority firmly says that "the team which built this reactor will take on contracts anywhere" Under these circumstances, the AEA would be the prime contractor and no move seems to have been made to license the consortia of industrial companies to build the design. The AEA has already submitted tenders in Finland, and has signed a contract for the supply of information to Japan; it seems determined to keep the SGHWR to itself. Meanwhile, the Minister of Technology is still wondering what to do with the nuclear power industry in Britain. He should think quickly, before the authority answers his questions for him.

Experimental Factories

from Angela Croome

A SECOND stage in the Akademgorodok (science city) experiment in Siberia is now actively under way. This involves the development of areas outside the main centre, containing experimental factories, design offices and special training facilities to speed the transition from good idea to industrial production. The scheme was put forward by the Siberian Academy of Sciences led by Academician Mikhail Lavrentiev in 1966 and adopted by the Soviet Government last year. The site chosen is about 2.5 miles from Akademgorodok on the banks of the Ob Sea and is to be called Pravye Chomy. The population will be limited to 15,000—much less than the total of 40,000 which Akademgorodok attained last year, its tenth anniversary.

The object is to improve the ways in which basic research is turned into industrial practice. The ideas, which will come from the research institutes of Akademgorodok, will pass to design offices at Pravye Chomy linked with the appropriate institute. Designs produced here will then be developed at centres on the spot which are described as "something between laboratories and factories". In these centres development will go at least as far as the production of prototypes, still under the guidance of the original inventor. Academician Lavrentiev commented, "We have recently come to the conclusion that the traditional 'conveyor belt'—academic institute/design office/industry—by which scientific discoveries are brought into practical use in industry, often works too slowly. The Siberian Design Office experiment, whereby valuable scientific ideas are brought to the point of practical industrial use under the direct control of the inventors themselves, has proved highly successful." The Institute of Hydrodynamics at Akademgorodok has pioneered the procedure. The hydraulic impact forging techniques introduced by the institute are estimated to hav esaved the Soviet oxygen equipment industry alone 100 million roubles a year. An interesting feature of the Pravye Chomy scheme is that most of the capital cost is being found by industry.

The scheme will be mostly operated by young people. This is seen as a unique opportunity to provide, after the 3–4 year development phase, not only effective designs but the people to put them to work on the shop floor. Linked to this is the introduction of a new type of degree to be given by Novosibirsk Technical College, shortly to be opened. This qualification will be intermediate between that of the full engineer and skilled factory hand. Novosibirsk University is adding an engineering faculty soon in support of the new technological developments. A large workshop block with a floor area of 1,500 square metres is nearly ready at Pravye Chomy.

French Scientists on Strike

from our Paris Correspondent

IF French politicians of all political parties rarely show an active and informed interest in research—election speeches contain polite but woolly references to the "progress of science" but are singularly lacking in precise policies, and the discussion over finance each year is carried on before a virtually empty House—scientists themselves seem to have long ago made up their minds to direct the attention of politicians and the Government to their future.

This is why an order to strike was given by two trade unions two weeks ago at the Centre for Nuclear Research at Saclay. The immediate aim was to protest against a plan for professional classification which would harm certain categories of personnel. In the longer term the question was that of fixing a date for a possible reorganization of the Atomic Energy Commission (the CEA). Is the CEA, which provides work for approximately 30,000 people and which, because of the size of its budget, is in French science a considerable *force de frappe*, threatened with disruption? In political as in research circles, many people would very much like to reduce this tremendous power by giving to various other organizations functions which originally were mistakenly attributed to the CEA for reasons which are no longer valid. The CEA, even under this system, would remain, if only for military reasons, an institution of considerable importance. Inevitably, however, a certain amount of reorganization would take place. At all events, it is doubtful whether strike action would weigh very heavily on Government decisions which will probably be taken at the highest level and in accordance with well known political and military strategy.

Despite this, another strike took place on February 20 and 21, organized this time on a national scale by the three research workers' unions. It seems that the strike was 70–80 per cent effective. It is the mode of operation of the National Centre for Scientific Research or CNRS (6,000 research workers, 8,500 technicians) which is the issue at stake, or, rather, the method of professional promotion agreed on by the responsible ministry, the Education Ministry. Having decided to push the research workers into the industrial sector, by force if necessary, the minister, M. Alain Peyrefitte, has created a bottle-neck between the first grade of the CNRS hierarchy, that of research assistant, and the second grade, that of research organizer. This year, seventy-nine assistants, all holders of doctorates and having the recommendation of the commissions concerned, have not been able to enjoy the promotion they were expecting, which is virtually forcing them to leave the CNRS once and for all.

Those who defend this measure argue that the almost automatic passing from one grade to another leads to "functionalization" of research and threatens to make the CNRS, which ought to be a dynamic body, stagnate. Their opponents, while agreeing that this argument does have some justification, deplore the improvised nature of this new policy and see in it a threat to basic research. The desire which the minister professes for "irrigating" industry is for them based on a dangerous illusion. French business men who are extremely timid in their attitude towards research are certainly disinclined to pour capital investment into this field and thereby offer scientists attractive posts. Furthermore, most firms are wary of research workers with a university background and prefer, when recruiting scientists, to look for them in the major colleges such as the Polytechnique or the Centrale. And university lecturing, also suggested as an alternative solution for those excluded from the CNRS, has been completely transformed in recent years, with the result that a teaching background is insisted on for lecturers. It is not at all certain that the universities will find satisfactory recruits among the members of the CNRS who had chosen precisely this field in order to pursue their work without any teaching commitments. The only course for those affected by this measure—which from now on must be repeated year after year—seems therefore to be to yield sooner or later to the temptations of the brain drain. The American "head-hunters" indeed have been making conquests in France for some time, and young people in France are beginning to have a better knowledge of English than their elders.

Money and Health

THAT the "healthiest" countries (judged in terms of mortality) spend most on health services is one of the conclusions reached in the 1967 World Health Organization report, *An International Study of Health Expenditure* (price 12*s*.) covering 29 countries. The objectives of this report were two-fold: to compile comparable information on expenditure on health services and on methods of financing such services, and to develop a standardized framework for national health accounting that could be used as a routine aid to health planning in all countries.

Most of the figures in the report were drawn from questionnaires, and the countries covered were not, it is emphasized, necessarily representative of all countries in the world because of the difficulty of obtaining information from countries with lower standards of living. Estimates of health expenditure for each country are compared with gross national product and national income.

It seems that over the period of study Israel, Canada, and the United States spent the greatest proportion of national income on health services, while the proportion of Gross National Product devoted to health services varied from 2.5 per cent to 6.3 per cent in countries providing full information. Countries with relatively high standards of living tended to spend more on health expenditure than countries with a lower standard of living. As the latter countries have on the whole poorer health than richer countries, less was spent both absolutely and in relation to total resources where the need was greatest.

The report states that generally there is no evidence of correlation between health expenditure and the need for health services; rather, health expenditure is presumably influenced to a large extent by the "felt needs" of governments. No country was found in which less than 25 per cent of the money spent on health services came from public funds and among the high-income countries those receiving heaviest government support were not those allocating most money to health expenditure.

Breaking down the capital expenditure on health services, with the exception of Pakistan and France, 18 countries allocated 88 per cent or more to personal health services. Furthermore, hospitals took more than 74 per cent of capital expenditure in all countries except Chile, Czechoslovakia and Yugoslavia. The United Kingdom was well down the list with only 76.4 per cent provided through hospitals compared with 99.7 per cent in Canada, although a spokesman at the Ministry of Health said that 14 hospitals had in fact been built in England and Wales since 1948.

APPROXIMATE TOTAL EXPENDITURE ON HEALTH SERVICES (CAPITAL AND CURRENT) AS PERCENTAGES OF GROSS NATIONAL PRODUCT

	Year	Total health expenditure (£ millions)	As % of GNP
Israel	1961–62	40.71	6.3
United States	1961–62	1,303	5.8
Australia	1960–61	262	5.2
Poland	1960	311	3.7
United Kingdom	1961–62	1,016	4.2

Looking to the future, until cost-benefit analysis and other techniques become applicable to the selection of targets for health programmes, the report suggests that it is desirable that the costs of health services should be consistent with a chosen long-term plan, and that the plan should be firmly based on economic realities.

Developing Oxford

from our Oxford Correspondent

As *Nature* went to press, Congregation at Oxford was voting on a proposal for acquiring new sites in north and central Oxford, principally for the university's scientific and administrative institutions. The administration of the university has been conducted from various buildings in different parts of Oxford, the Registry being, for example, in the Clarendon Building in the centre, while the financial department is three-quarters of a mile away in Wellington Square. In 1965, it was proposed that the Registry should expand into the premises of the nearby Indian Institute, which would then be displaced if not dismembered. Much criticism was directed at this proposal and Bodley's librarian resigned on account of it. Now, because the university has bought a number of houses around Wellington Square and also because the Franks Commission recommended that the administrative buildings should be in one place, the Hebdomadal Council has decided to apply for planning permission to redevelop Wellington Square, and move all the administrative buildings into that area. But a new Indian Institute library is already being built on top of the new Bodleian. If, then, the new proposal is accepted, all but the ground floor of the Clarendon Building and the old Indian Institute will be empty.

The council has not yet put forward suggestions for using this extremely valuable area in the centre of Oxford, considering it "premature" until planning permission has been granted for the Wellington Square development. It is possible that Hertford College will take over some of the Indian Institute building, but part at least will continue to be used as a library. Similarly, it is suggested that the Taylorian Institute and Modern Languages Faculty will take up residence in one corner of Wellington Square, leaving another large central area empty. Here again no decision has yet been made on how to use the space.

In north central Oxford, where most of the present science faculty buildings stand, the university has done well to buy (or to be in the process of buying) one million square feet of buildings, after the recommendation of the Holford Committee which reported in 1963 New departments of psychology and zoology are at present being built and the Radcliffe Science Library, the School of Geography and the Physics Laboratories are being extended Since the Holford Committee sat, sixteen houses have been bought from colleges for the use of the Social Science Departments, and some of these have already moved into their new accommodation Congregation will now have voted on the proposal to buy another nineteen houses, with 100,000 square feet, for science facilities Despite the Government's statement of January 16, 1968, further projects may be included in the building programmes for 1970 A new computer library will be built, and the adjacent Departments of Engineering, Survey and Geodesy and Metallurgy will be extended The professors of these departments have already agreed to share as many facilities as possible, following the example of the zoology/psychology building, which will, the Hebdomadal Council states, guide many future developments

Reinforced Plastics

THE production of carbon fibre reinforced plastics (CFRP) is now going ahead even though licensing and financing arrangements between the National Research Development Corporation and the three firms involved—Courtaulds, Morganite and Rolls-Royce—have not yet been finally decided Rolls-Royce is producing the plastics for its own use, but Courtaulds and Morganite will sell CFRP in the general market Morganite is quoting prices of £35 per lb for batch fibre and £100 per lb for continuous fibre, but it is acknowledged that prices will fall considerably when mass production is achieved.

The development and applications of these very strong plastics were described by Mr L N Phillips, head of the Plastics Technology Section, Materials Department, of the Royal Aircraft Establishment, Farnborough, at a joint session of the plastics and polymer group of the Society of Chemical Industry and the London Section of the Plastics Institute, on February 22 Mr Phillips began by pointing to the considerable experience of RAE in the development of cellulose, asbestos and glass reinforced plastics for structural purposes. The general engineering opinion was that glass fibre has too low a Young's modulus, compared with metals, to be attractive for structural purposes Conversely, it was reasoned that a new fibre with a Young's modulus three to six times greater than glass would be of considerable interest

Attention was focused on carbon fibres some four years ago, because of the wide difference between the properties of commercial fibres then obtainable by rayon pyrolysis (Young's modulus 6–9 million lb per square inch, with ultimate tensile values of 50,000 lb per square inch), and the properties of graphite whiskers (Young's modulus between 100 to 147 million lb per square inch) Details of the RAE processes for making high-strength high-modulus carbon fibres could not be given for commercial reasons, but the principle is to preserve the orientation already existing in a highly aligned textile fibre as the polymer is destroyed by heat (The inventors are W Watt, W Johnson and L N Phillips) Experiments with the composites at RAE have shown that water absorption is not a difficulty and that the fatigue life under various conditions is excellent, the heat build-up during fatigue testing being much less than with glass and other non-conducting fibres Friction and wear tests carried out at Farnborough have shown that when carbon fibres are incorporated into thermoplastics and thermosetting resins, the coefficient of friction is lowered and the rate of wear enormously reduced It seems as if a new class of bearing materials has been created But many other uses for the materials have been uncovered—the Rolls-Royce Company has, for example, used them for very large fan blades in aircraft engines, which have a clear advantage in efficiency over titanium Aircraft structures, chemical plant, marine uses and space applications were also mentioned

Considerable increases in the stiffness of thermoplastics are obtained by incorporating small amounts of chopped carbon fibres—for example, 10 per cent weight addition can increase the modulus by 100 per cent This is important in the manufacture of large mouldings

Sensible suggestions for further uses of CFRP are welcomed by the Ministry of Technology

Crack Detector

THE development of a new instrument for detecting invisible cracks in iron and steel surfaces was announced on Monday by the National Research Development Corporation The new instrument works on the principle that a coil carrying an alternating current will induce eddy currents in nearby conducting materials When the coil is moved over a surface crack, the crack causes variations in the eddy currents leading to a change in the voltage across the coil This can be detected by a meter or made to sound a warning buzz in an earphone worn by the operator

Eddy current techniques in non-destructive testing are not new, but up to now they have not been widely applied to ferrous materials This has been because variations in the distance of the coil from the material under examination, such as might be caused by paint or rust layers, lead to interfering effects The advantage of the new instrument is that it can be compensated for variations in the gap between the coil and the metal This means that once the electronic circuit has been balanced for a particular specimen the instrument can be used over rough surfaces which may be covered by paint or rust, and over welds

The initial impetus for the design of a crack detector was provided by the discovery of cracks in the hull of the nuclear submarine HMS Dreadnought The instrument was developed by the Admiralty Materials Laboratory at Poole, and the patent rights were assigned to the National Research Development Corporation for exploitation Two licensees have been appointed to manufacture and sell the instrument

Because the new detector is simple, portable and rugged, it is likely to find many applications for testing structures in the field Time savings of more than 80 per cent are expected when compared with conventional magnetic methods of crack detection, which involve cleaning the surface under examination About

70 prototype crack detectors made by the Admiralty Materials Laboratory are at present in use in dockyards and research establishments The new instrument is proving of interest to British Rail, which has recently been seriously troubled by cracks in railway lines A special version is in the process of development for fitting to railway wagons so that the track can be inspected at high speed

Scientific Memorabilia

A PLEA for the preservation of unpublished scientific records, manuscripts and correspondence has been made by the President of the Royal Society and the Master of the Rolls The Royal Society and the Historical Manuscripts Commission have now formed a standing joint committee under the chairmanship of Sir Harold Hartley with the aim of locating, preserving and making accessible this material The committee is to prepare and publish a guide to the location and content of the personal papers of leading British scientists and technologists from the sixteenth century until, and including, the time of Lord Rutherford (but excluding scientists still living) The preparation of this guide has been made possible by a grant from the Goldsmiths' Company The librarian of the Royal Astronomical Society, Dr R E W Maddison, is to be editor Many of the papers are still in private possession, and Professor Blackett and Lord Denning are appealing to all owners of such papers to write to Dr Maddison (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W1) and to inform him of what they possess The papers will remain the owner's property—the aim is to locate the papers, to ensure their preservation, and to make them available in original or copy to historians

New Ways of Making War

A TWO-DAY conference on chemical and biological warfare last week in London served at least as an opportunity for breaking down what Lord Ritchie-Calder, chairman of the conference, described as the conspiracy of silence on the subject The conference was held under the auspices of the newly formed J D Bernal Peace Library, and was attended by scientists and science journalists from several countries Lord Ritchie-Calder set the tone for the proceedings with a reminder—culled from the late Professor Joliot-Curie—of how weapons like these might be used without the intended victims knowing

A long list of known chemical weapons was provided by Mr J Perry Robinson At one end of the spectrum are the so-called riot-control agents—lacrimators and the rest—which seem to be lethal in sufficiently high concentrations Nettle gases, dichlorformoxime, for example, sting hard Blood gases such as hydrogen cyanide block oxygen circulation Vesicants, of which mustard gas is typical, injure all kinds of tissues and can therefore be lethal Involatility enhances their effectiveness Then there are nerve gases which inhibit cholinesterase and which are therefore the most rapidly lethal of these agents Mr Robinson was also able to describe several means by which these agents can be disseminated—one of the essential tricks of the trade Defence, he said, would probably be too expensive for most countries

Academician I Malek from Prague had very little new to say, although he emphasized the self-spreading capacity of biological agents and their cheapness He argued that the only defence against biological weapons was an international agreement to forbid their use

The uses of defoliants were also discussed at the meeting, largely because of their use in Vietnam by United States forces According to Professor Arthur Galston, professor of plant physiology at Yale University, 1,000 square miles of Vietnam were dealt with in this way in the first 9 months of 1966 Dr F Kahn of the University of Paris held that agents like these can be toxic to human beings—notwithstanding official statements to the contrary Dr Kahn is a member of a scientific commission set up for the International War Crimes Tribunal which met last year in Stockholm His evidence for the lethality of defoliants is based on evidence collected orally by a government committee in North Vietnam and experiments carried out with ducks

Dr J H Humphrey, deputy director of the National Institute for Medical Research of the United Kingdom Medical Research Council, had a practical suggestion to make He argued that there would be great benefit in an arrangement by means of which the research establishments engaged on chemical and biological warfare were to become non-secret places, at least for a trial period Then, at least, taxpayers would know what was being done with their money

Listing Overseas Visitors

DIFFICULT as it is, tracing the names and numbers of overseas academic visitors at present undertaking research in Britain is not impossible Two almost up-to-date lists have been compiled for United States and Commonwealth visitors showing where they are and just what they are doing

The US directory of American university teachers and senior scholars for 1967–68 was prepared by the US Information Service of the American Embassy in London and contains over six hundred names The present location, field of study, the awards covering the research, and the university or institute which the visitors attended in the US are all quoted Data are based on information provided by registrars and other officers in British universities and, at this stage, copies are available only to university registrars (The US Embassy says that it is unable to supply further copies on demand)

The second list, prepared by the Association of Commonwealth Universities, is based on information furnished by the registrars of member universities and university colleges in overseas parts of the Commonwealth, and of former member universities in the Republic of South Africa Some 630 visitors are currently in Britain and there is a list of about 360 visitors shortly to arrive, dates of departure are given where known By circulating copies of the list to universities, learned societies and other interested bodies in Britain, the association hopes that this will assist contact between the members of these bodies and current or prospective visitors

Although the lists are incomplete, they provide a useful service which could usefully be taken up by countries outside the US and the Commonwealth which also have senior scholars in Britain

Preventing London Flood

THE possibility of catastrophic flooding in London has often been discussed, particularly since the disastrous floods in Florence. The idea of a barrier across the Thames to stop such a flood has been suggested on several occasions, but all schemes appear to have met with opposition, sometimes short-sighted, it seems. Professor Hermann Bondi, chairman of ESRO, who is very concerned about the possibility of flood, has just published a report called the *London Flood Barrier*. It seems that Professor Bondi was approached by the Ministry of Housing and Local Government in October 1966 to consider the whole question of a barrier, not only its desirability and practicality, but also its possible design. Professor Bondi admits that he is "not a civil engineer"—some may criticize the report on this ground alone—but a flood barrier may well be the type of problem which needs an outsider's view, cutting as it does across several interests. In any case, the report deserves serious consideration.

Professor Bondi could scarcely make the frightening consequences of a serious flood in central London more clear. As he says, "the case for preventing the flooding of a major part of London during a surge tide seems to me enormously strong. We are at risk all the time and further delay only invites the disaster". Statements like this, even if slightly exaggerated, should not be taken lightly. Rumblings like this were heard before the Aberfan disaster.

The advantages and disadvantages of a permanent rather than a movable barrier are also discussed by Professor Bondi. A permanent barrage or dam, with locks in the Woolwich region, although expensive, would be the best solution if seen in the context of a major plan for the development of the south-east. A barrier of this type would be an effective flood defence as well as adding to the amenities of the Thames. Regretfully, Professor Bondi concludes that this scheme would not answer the immediate problem. He therefore moves on to discuss a removable barrier, one which would only be put in place when there was imminent danger of a surge tide flooding central London. Such a barrier would have no use other than flood prevention.

He rejects the idea of a retractable barrier close to the mouth of the river. It should be possible to find a site further upstream where a narrower opening in the barrier would not seriously impede shipping. Three possible locations are suggested: just below the Ford Motor Works, just above Dagenham Dock, and in Woolwich Reach. In the case of the first two locations, a clear opening of 800 ft., or perhaps 750 ft., might be acceptable to shipping interests. In the case of Woolwich something in the range of 350–500 ft. might be considered if the river banks were cleared of mooring berths. These sites are compared with the barrier at Long Reach considered in previous reports of the consulting engineers which required minimum clear openings of 1,400 ft.

The Government appears not to be apathetic to proposals for a flood barrier. In a written answer to the House of Commons on February 20, Mr Anthony Greenwood, Minister of Housing and Local Government, mentioned that the Greater London Council has undertaken to investigate, in consultation with the Port of London Authority and navigation interests, the relative merits of the two types of barrier, fixed and movable, and the most suitable site for one. Presumably the Bondi report will be taken into account in these discussions. The minister is also going to consult the South East Economic Planning Council on the wider implications of the projects.

Parliament in Britain

Technological Community

THE Prime Minister, Mr Harold Wilson, was pressed last week to give more details of his proposals for a European Technological Community. Mr Norman St John Stevas suggested that it had been one of the Prime Minister's happier initiatives—the only one, another member interjected—and that it should be pursued with much more vigour. Dr David Owen said that it was foolishness to refuse to share the technology of Capenhurst nuclear fuel enrichment plant with other European countries. By doing this, the British Government was driving them to establish their own plants. Mr Heath thought that the Technological Institute was a better idea than the technological community, but that enrichment would be covered by the terms of the non-proliferation treaty. The Prime Minister was not very enlightening. He said that discussions about the community had been held with representatives of the Dutch Government; the West German Government had welcomed the idea. As for fuel enrichment, the British Government had said that it was prepared to join with Europe in the setting up of a system for the production of enriched uranium-235. Britain had more know-how than others, he said, but they had cheap electricity. (Oral answer, February 20.)

Research and Development

DR JEREMY BRAY, Joint Parliamentary Secretary at the Ministry of Technology, told Mr Gwynfor Evans, the only Welsh Nationalist member in the House, that research and development expenditure in England supported by his department in the year ending March 31, 1967, had cost £219.5 million. Alas for Mr Evans, Dr Bray revealed that only an additional £2 million had been spent in Wales in the same period. (Written answer, February 20.)

Channel Tunnel

MR JOHN MORRIS, answering questions for the Ministry of Transport, showed no inclination to cancel the Channel Tunnel project. Mr Clark Hutchinson said that the whole project was out of date, and asked when the Government would stop wasting public money and time on these futile projects. Mr Eric Lubbock asked for a pause in the plans to see how well the new hovercraft service using the SRN 4 went. Mr Morris said that the expected growth of traffic over the next thirty years showed that the tunnel was economically preferable to the alternatives. (Oral answer, February 21.)

Computers

MR WEDGWOOD BENN, Minister of Technology, said the number of computer installations in operation or on order in the UK in 1970 would be not less than 5,000. Not less than 1,500 installations would by then be at the planning stage. These revised estimates would mean that more computer personnel would be needed. By 1970, there would need to be 300 advanced programmers, 20,000 people falling into the category of systems designers or systems analysts, and 25,000 operators. (Written answer, February 22.)

NEWS AND VIEWS

Boom in Radio Astronomy

It seems already as if 1968 will be a vintage year for radio astronomy Last week, from Cambridge, there was described in *Nature* the discovery of radio signals from a pulsating radio source which seem as if they may turn out to be signals from one of the long-sought neutron stars Now, this week (see page 818), there comes evidence of a survey of the sky for radio sources at a frequency of 2,700 MHz which seems as if it may reopen some cosmological discussions which had seemed quite recently to have been finished

The signals which may come from neutron stars were recorded by the Mullard Radio Astronomy Observatory at Cambridge using a new radio telescope which can detect sources considerably fainter than previous surveys They are unusual because they consist of an emission of descending frequency which repeats itself every 1 337 seconds This repetition period is maintained with breathtaking accuracy—the true period is constant to better than one part in ten million Up to now, four of these peculiar sources have been discovered, although the position of only one of them was given in the article in *Nature* last week by Dr A Hewish and his colleagues (*Nature*, **217**, 709, 1968)

Neutron stars are believed to represent the final stages in the life of certain kinds of stars When the nuclear fuel sustaining these stars is exhausted, they contract to become what is an extremely dense star known as a white dwarf, the size of which is comparable with the Earth White dwarfs are difficult to detect because they are rather faint objects, but about 250 of them are known to exist The next stage in the evolution of these stars may be the gravitational compression of white dwarfs to neutron stars

Much of the observational interest of the new discovery stems from the way in which it is possible to use the character of the signals which are received at the Earth as a means of fixing an upper limit for the distance of these objects Briefly, ionized hydrogen in interstellar space should modify by dispersion the character of the radio pulses reaching the Earth, and this line of argument suggests an upper limit of 65 parsecs for the object which has so far been studied in detail, which in turn suggests that these pulsating radio sources may be comparatively common in the galaxy Obviously there is a staggering wealth of observation now to be undertaken

The new survey of the southern hemisphere is a different story For several years, radio astronomers have tried to distinguish between different models of the universe by making counts of radio sources One simple procedure has been to plot log N against log S, where N is the number of radio sources having a radio flux greater than S The result is sometimes a straight line, and the slope of this line has some importance for cosmologists On the simplest view of a Euclidean universe containing a uniform distribution of similar sources, the slope of the "log N–log S" curve should be -1 5 Apparently this is also the value to be expected if the steady state view of the universe holds

Up to now, counts of radio sources outside the galaxy have led to values of -1 8 for the slope of the log N–log S curve On page 818 of this issue of *Nature*, however, is a paper by an Australian group who have used the 210 feet Parkes radio telescope to obtain a slope of $-1{\cdot}4$ The chief difference between this and previous surveys is the choice of observing frequency, the new result refers to radio fluxes at 2,700 MHz whereas previous source counts were compiled at much lower frequencies

This suggested to the Australian group that the discrepancy between their result and the previous work may be explained by a detailed examination of the spectra of the sources used in the different surveys Bearing out this notion, there seems to be a large range in the form of radio source spectra As well as the problems posed by possible differences in the spectral content of the various surveys, the type of radio source observed seems to depend to some extent on the frequency at which the counts are compiled For instance, the new survey contains many quasi-stellar objects which were not in an earlier count made at Cambridge The outcome of all this uncertainty is that the value of the log N–log S curve as a means of distinguishing between theories of the universe is made even more questionable

Plants for South Kensington

The department of botany of the British Museum (Natural History) has received as a bequest the herbarium of the late Georges Le Testu of Caen The collection consists of about 15,000 specimens of flowering plants and ferns from Africa, and will be very useful to botanists working on the classification and identification of plants from this part of the world

Le Testu was trained as an agronomist and worked for an agricultural firm in Dahomey during 1900–1902, and for another in Mozambique from 1904 to 1906 After that he entered the French colonial service and served in Gabon and Oubangi-Chari from 1907 to 1934 Throughout his time in Africa, Le Testu collected plants as often as his duties allowed Of his collection, he gave one set of plants to the Muséum National d'Histoire Naturelle in Paris Many of these were type specimens, from which the original descriptions of species were made This Paris collection was used by F Pellegrin for a series of papers entitled "Plantae Letestuana Novae" published in 1923–1955 Le Testu's Paris collection was also the chief source for the *Flore du Mayombe*, and for various other works about the plants of this part of Africa

The plants received by the British Museum constituted the other part of Le Testu's collection, and are important because they include many isotypes, that is duplicates of the type specimens held in Paris The bequest is very valuable because many of these plants have not been available at the British Museum before The Le Testu bequest fills a gap that has been a cause of difficulty to botanists working in London on floras of, for example, tropical East Africa—a project which is being carried out jointly at the Museum and at the Royal Botanic Gardens, Kew

The British Museum's herbarium, consisting of preserved flowering plants, bryophytes, lichens, fungi and algae, is available to anyone engaged in work for which they require to study this plant material The value of the collection is mostly to those who are preparing floras—identification manuals—of the plants of a particular region, and monographs about particular groups of plants This sometimes requires considerable rethinking of classification, and it is preferable to have preserved specimens to refer to than to have recourse only to written accounts The collection at the British Museum is about two-thirds of the size of that at Kew, and one and a half times as large as the third national collection which is in Edinburgh

Marine Sediment Studies

THE meeting last Thursday of the Marine Studies Group of the Geological Society of London was devoted entirely to recent work on Holocene marine sediments near the British Isles Six papers at the meeting, organized by Mr A H Stride, dealt with sediment deposition in environments ranging from an estuary to the sea floor of the English Channel It was made clear how factors such as current strength, exposure and source of sediment material have affected sediment deposition in the past and still do Unfortunately, only half an hour was allowed for each paper, which was a pity, particularly for a paper on estuarine sediments read by Dr J E Prentice Dr Prentice is in charge of a research team from King's College and Sir John Cass College which has been working on sediments of the Thames Estuary for the past 6 to 7 years

Various aspects of the work of the team have been published before, but this meeting was the first time that the work as a whole has been summarized The importance of the research on the Thames cannot be underestimated, for it is inextricably bound up with long-term economic planning of the future of the Thames Only last week, a new scheme was proposed for a barrier across the river by Professor Hermann Bondi (see p 800) A further scheme in a wider context which would involve not only the river and the docks, but roads, motorways and airports as well, was also outlined by Mr John F F Clarke in last week's *New Scientist* (Vol 37, February 22, page 402)

The sediments of the Thames Estuary extend over an area of more than 500 square miles, are up to 100 feet thick and are made up of a variety of materials from finely laminated clays to coarse conglomerates What the sedimentological team found surprising was that all the sediments are mobile, and they would only stop moving if there were a substantial fall in the sea level Although there is no evidence of an overall increase in silting up of the estuary during the period of research, there had been many changes in the distribution of sediments in the outer regions A system of channels, the Edinburgh Channels, for example, which was navigable ten years ago is rapidly silting up—other systems of deep channels, however, seem to form to take its place

For the purpose of the research, the Thames was divided into three parts the inner estuary (as far down as Southend), the middle estuary and the seaward outer estuary In the inner estuary, mud deposition is dominant Fine clay in suspension enters from upstream and, on meeting the sea-water, flocculates and begins to fall through the water column This takes up silt and fine sand, and forms a dense suspension of black "fluid mud" which clings to the bed of the river It is normally kept in motion by water movement, but the fluid mud layer can also flow by gravity into channels and hollows Erosion of mud banks is apparently reduced to some extent because the Thames embankment is kept in a reasonable state of repair Shipping, however, has an effect because the washes help in the erosion of mud flats and in keeping the fluid mud in suspension In the middle estuary, the characteristic sediment is fine sand, although there are also fine clay and coarse pebbles The possible sources of these sediments were described by Dr Prentice

Mr N H Kenyon spoke on the phenomenon of sand ribbons—long ribbons of sediment, known to be up to 4 miles long and up to 200 yards wide, found on the sea floor around the British Isles in most of the areas where the tidal currents have maximum speeds near the surface of the sea of about two knots or more They can be used to infer present-day transport paths of the bed load, but, because they are not usually preserved, they are of little use as indicators of palaeocurrents The ribbon pattern may, however, be preserved in negative as linear shell beds, because living organisms have been seen to prefer the more permanent coarse material between the ribbons It was suggested that the material of the ribbons is a shelly sand that is in a continuous but restricted supply It is derived mainly from the reworking of relict sediment and from the shelly material of the local benthos

Looking Backwards for Resonances

from a High Energy Physics Correspondent

ONE of the most exciting aspects of high energy physics in recent years has been the discovery of very large numbers of largely unsuspected resonances and the determination of their properties A report of a recent experiment performed at the zero-gradient synchrotron at Argonne National Laboratory (A D Krisch *et al*, *Physical Review*, **164**, 1661, 1967) demonstrates clearly for the first time the value of a powerful yet simple new technique for studying such resonances in the pion–nucleon system

Because these resonances have such short lifetimes that they cannot be "seen" in any direct sense, the traditional methods of determining their masses, widths (lifetimes) and internal quantum numbers have been to look for anomalous variations of total cross-sections for scattering processes, and the enhanced groupings of two (or more) particles in inelastic experiments. Then the spins and parities of these states have been deduced from angular distributions of the outgoing particles in the elastic scattering events. The new idea is to study pions (in this case negatively charged ones) scattered at 180° from proton targets. When this backwards elastic differential cross-section is plotted against incident beam energy it exhibits a most striking structure, which can then be interpreted almost trivially in terms of resonance effects.

In the present experiment, where the incident energy range is from 1·6 to 5·3 GeV/c, there is evidence for some nine peaks and dips (many of which stand out quite vividly) superimposed on a smooth and steadily decreasing background. The interpretation of the background effect is for the present purposes unimportant, although models for it in terms of elementary or alternatively Regge baryon exchange have accounted for it in a reasonable manner, all that one requires is that it be smooth and should not change sign. Then each pronounced peak or dip is interpreted as the effect of the pion and proton forming a resonant state during the scattering so as to produce constructive or destructive interference with the background term. As the incident energy at which a peak or dip occurs is trivially related to the mass of the resonances, these masses are effectively measured directly. Perhaps the most elegant feature, however, is that resonances of positive parity interfere constructively to produce peaks, and negative parity resonances produce dips, so that the parity of a resonance can be read off from the curve at a glance. The determination of such other parameters as elasticity or partial width is on much less firm foundation, but at least some indication of their values—to within factors of two, say—may be obtained by detailed analysis.

The present experiment has not only shown the value of the technique but has also provided the theoreticians with at least two big problems. First, there is a dramatic destructive interference (which the reader is urged to view for himself) at around 2·2 GeV resonance mass, in which the cross-section drops by two orders of magnitude. Second, there is a large narrow peak at a resonance mass of about 3·25 GeV, suggesting a resonance there which has a width of the order of 1 per cent of its mass. That such a stable particle (half-life about 10^{-22} seconds) should exist at so high a mass will not be easy to explain.

Italian Crystallographers

from a Correspondent

THE newly founded Italian Crystallographic Association held its first general assembly in Perugia from January 11–13, 1968, in the Department of Chemistry of the University of Perugia, under the presidency of Professor F. Mazzi. The general assembly was combined with a symposium on the subject of four circle diffractometers. The two introductory lectures were given by B. T. M. Willis, who gave a general account of the whole subject.

Papers which followed covered the selection and operation of diffractometers. W. A. Wooster (Cambridge) maintained that the accuracy of 0·01° usually quoted by makers of four circle diffractometers is not necessary, except on ω- and 2θ-circles, when they are used for accurate measurement of lattice dimensions. He also discussed the relative merits of absolute-reading and difference-reading digitizers. J. Hornstra (Holland) described the PAILRED instrument, emphasizing the simplicity of a linear diffractometer which requires no computer or calculated angles. R. L. Beddoes (London) described the Hilger and Watts diffractometer and gave an account of its practical application. D. W. Beard (Cleveland, Ohio) dealt with crystal monochromators mounted in horizontal and vertical orientation and pointed out the relative merits of crystal reflected and filtered radiation. E. Curcio (Rome) described the GE diffractometer. A. J. H. Duisenberg (Holland) gave an account of the Nonius diffractometer and of some of the structural work carried out with it. E. R. Wolfel gave an account of two Stoe diffractometers. The first combines a Weissenberg photographic goniometer with a detector so that it becomes an equi-inclination diffractometer and the second is a four circle diffractometer with a special mounting for the X-ray tube. The last paper was given by H. Mayer (Germany), who described the Siemens four circle diffractometer, laying special stress on the accuracy of its mechanical construction.

More Allosterism

from our Molecular Biology Correspondent

IT has sometimes been difficult of late to avoid the disagreeable impression that the greater portion of biochemistry, if not indeed of chemistry, is gradually being engulfed by the allosteric hypothesis as particular cases of the universal truth. Among the all too large number of supposed allosteric phenomena in the current literature there is, however, an interesting group of articles on the enzyme aspartate transcarbamylase, which was studied by Gerhart and others, with the interesting upshot that the catalytic and regulatory sites occur on different sub-units.

This work has now been extended by Changeux, Gerhart and Schachman (*Biochemistry*, 7, 431 and 538, 1968), who have studied the binding of ligands to the protein and its sub-units. There are two catalytic and four regulatory sub-units, but it appears that four sites in all are present which will bind the substrate analogue, succinate, and four sites for the inhibitor, cytidine triphosphate (CTP). This situation can be accommodated by the capacious allosteric theory of regarding the enzyme as an isologous aggregate of four protomers (effective sub-units), each consisting of one regulatory sub-unit and half a catalytic sub-unit. In the intact protein, only the binding of succinate follows a sigmoidal saturation curve, so that the substrate sites evidently function in a co-operative manner. The reciprocal effect of substrate on inhibitor binding has also been demonstrated, uptake of CTP being partly reversed by succinate.

In the same issue of the journal, Gerhart and Schachman seek evidence for the conformational changes which are expected to accompany the attachment of ligands. It was in fact reported almost two years ago by Dratz and Calvin that the addition of succinate engenders in aspartate transcarbamylase a considerable change in optical rotatory dispersion which can reasonably be interpreted in terms of a difference in the content of α-helix. Gerhart and Schachman have, however, chosen a chemical approach—reactivity of sulphydryl groups. Reaction with mercurial leads to dissociation of the enzyme into its sub-units, but this turns out to be a co-operative process at intermediate stages of the reaction only intact unreacted and fully reacted dissociated species were present. The rate-determining factor is then the reactivity of the native enzyme (most probably in fact its dissociation into the reactive sub-units) and this can be measured Addition of the two substrate species, succinate and carbamyl phosphate, led to a large increase in the rate of reaction, which was partly annulled by the inhibitor CTP The substrates at the same time brought about a small diminution in sedimentation coefficient, which was again opposed by CTP From the absence of any dependence on enzyme concentration, it was surmised that the substrates induce a true change in frictional coefficient rather than a gross shift in dissociation equilibrium It does therefore appear that an appreciable structural change occurs when the substrates are attached Changeux and Rubin (*ibid*, 561) show that the experimental data can be satisfactorily fitted on the basis of the two-state allosteric model, such that the substrate binds only to one of the conformational isomers, the inhibitor preferentially to the other, and that in the absence of ligands the two states co-exist, with a four-fold preponderance of the catalytically active form

It is worth noting, as part of the discussion of allosteric mechanisms, an article by Sweeny and Foster (*ibid*, 561) in which a clear formulation is given of the generation of sigmoid binding curves by isolated catalytic sites The requirement is only that there shall be alternative reaction pathways to the protein–ligand complex Several such models are described Another interesting analysis of co-operative binding is given by Klapper and Klotz (*ibid*, 223) for situations involving an aggregation equilibrium in which the affinities of the associated and dissociated states for a given ligand differ Measurement of ligand affinities can be used to yield the equilibrium constant for the protein dissociation, and a striking application to the dissociation of haemerythrin is described

Symmetry of RNA Transcription

from our Cell Biology Correspondent

In 1966, Szybalski and his collaborators proposed that clusters of pyrimidine residues in DNA are somehow related to RNA transcription, possibly acting as initiation and termination signals for RNA polymerase At the time the evidence for this hypothesis was thin, and the theory itself may well be an oversimplification, but all the evidence obtained since is compatible with Szybalski's suggestion In the latest paper from Szybalski's laboratory, Summers and Szybalski (*Virology*, **34**, 9, 1968) report that more than 99·8 per cent of the T_7 and more than 99 per cent of the T_3 coliphage messenger RNA molecules that are made throughout the phage growth cycle are transcribed from only one of the two complementary strands of T_7 and T_3 DNA And the strand which is transcribed contains clusters of cytosine residues, while the strand that is not transcribed has neither cytosine nor thymine clusters

Summers and Szybalski used the DNA/RNA hybridization technique to study T_7 and T_3 transcription and the success of their experiments depended on their ability to separate the two complementary strands of T_7 and T_3 DNA They did this with the techniques that have been developed by Szybalski's group over the last three years for separating the DNA strands of phage and bacteria If one of the two strands of a DNA molecule contains more cytosine than the other, they can be fractionated by first heating the DNA to separate the strands, then binding a polyribonucleotide rich in guanine to the *C* rich strands, so increasing its density, and then separating it from the *C* poor strand in a caesium chloride gradient

Having separated T_7 and T_3 DNA strands in this way, Summers and Szybalski were able to show that all the messenger RNA made at both early and late times hybridizes specifically with the *C* cluster strand Control experiments proved that the two DNA strands are not self-complementary and that *C* poor strand is available for hybridization so that its failure to hybridize *m*RNA is not an artifact

Clearly only one strand of T_7 and T_3 DNAs, the one rich in cytosine clusters, acts as template for messenger RNA Similarly, Mamur and Greenspan (1963) reported that only the pyrimidine rich strand of phage *SP*8 hybridizes with *SP*8 *m*RNA Szybalski's group (Taylor, Hradneca and Szybalski, *Proc US Nat. Acad. Sci*, **57**, 1619, 1967) reported that both strands of λ DNA act as templates and this correlates well with the occurrence of pyrimidine rich clusters on both strands Also Habich *et al* (1966) found that although both strands of *Bacillus megatherium* DNA act as template for *m*RNA, the pyrimidine rich strand contains most templates And *m*RNA must also be transcribed off both strands of T_4 DNA because Streisinger *et al* (*J Mol Biol*, **31**, 607, 1968) have concluded from genetic experiments that the lysozyme and *r* II genes are translated in the opposite direction to T_4 head protein

Given the correlation between template function of DNA strands and their high content of pyrimidine clusters, it is tempting to speculate that *dC* or *dT* clusters act as initiation and possibly termination signals for RNA polymerase Two *in vitro* experiments with RNA polymerase support this speculation First, most *m*RNA made *in vitro* with *E coli* as templates has *A* or *G* at the 5′ terminus (Maitra and Hurwitz, 1965) Second, Chamberlain (1967) reported that when poly *dCdG* or poly *dTdA* are used as templates *in vitro*, RNA initiation and termination occur very frequently

What sort of signals can clusters of pyrimidines provide? Langridge (1967) found that synthetic double helical DNA with pyrimidines on one strand and purines on the other had slightly different structure from native DNA Summers and Szybalski suggest that this modified structure in the regions of native DNA with pyrimidine clusters could serve as a recognition region for RNA polymerase

Isoenzymes and Cancer

from our Medical Biochemistry Correspondent

It is now well known that lactate dehydrogenase (LDH) is found in five electrophoretically separable forms known as isoenzymes or isozymes The enzyme molecule is made up of four sub-units, each of which can be one of two types known as *H* and *M* sub-units The possible combinations of *H* and *M* sub-units give the five forms of LDH which can be separated by electrophoresis in suitable conditions

In a study of the LDH isozymes in normal human uterus, in myoma and in carcinoma of the uterine cervix, Okabe and co-workers (*Biochemistry*, 7, 79; 1968) have found, like many other workers working with many other kinds of tumours, that there is a preponderance of isozymes containing *M* sub-units in the malignant tissue from tumours. They found, for example, that the benign uterine tumour had an isozyme pattern very similar to the pattern in normal human uterus. They isolated the isozymes from the tissues and found that those from the malignant tissue showed a higher rate of reaction with nicotinamide adenine dinucleotide analogues than the other types.

An enhanced production of lactic acid from pyruvic acid (brought about by LDH) is the outstanding biochemical difference between malignant cells and normal cells. Indeed, when Warburg first discovered the high rate of glycolysis in tumours, he suggested that this was the cause of malignant growth. Opinion has recently tended to swing away from this theory, but there is now some evidence that the change in LDH isozymes may be one of the earliest alterations when a tissue becomes malignant and that this change in LDH may influence the rate of glycolysis.

In 1966, Latner, Turner and Way (*Lancet*, ii, 814, 1966) found that in specimens of pre-invasive carcinoma of the cervix obtained at operation, the amount of change in the LDH pattern seems to be correlated with the amount of tumour tissue. In some cases, however, the LDH pattern was changed even though there was no evidence of carcinoma when sections were examined. All the specimens must have contained some tumour cells, for cancer had been diagnosed from cervical smears. The LDH isozymes were changed before any change in the activity of lactate, glucose 6-phosphate or 6-phosphogluconate dehydrogenases was measurable.

Langvad (*Inter. J. Cancer*, **3**, 17, 1968) has now measured the ratio of LDH isozymes with M_3H_1 and M_1H_3 constitutions in samples from the tumour and from apparently normal colon of operation specimens from thirty-six patients with carcinoma of the colon. Each tissue sample was halved longitudinally, one half being used for the preparation of LDH isozymes and the other prepared for histological examination. The mean ratio for samples containing tumour tissue was 1·5–1·6, while the mean ratio for samples with no tumour tissue was 0·7–0·8. Usually the ratio decreased with distance from the tumour, but some specimens had local areas where the ratio rose considerably above 0·8 and approached the ratio found in the tumours. These patches where the ratio increased were often at considerable distances from the edge of the tumour. It is not unusual to find more than one primary tumour in patients with carcinoma of the colon, so these areas where the LDH ratio changes towards the tumour ratio may well be areas which will eventually become malignant. The change in LDH isozymes seems to occur before the tissue looks malignant, and may be one of the earliest detectable changes in a tissue which is developing a tumour.

Phage RNA Replication

from our Cell Biology Correspondent

FOR several years, there has been great interest in the mechanism by which the single-stranded RNA molecules of coliphage are synthesized. Weissmann's group (Feix, Pollet and Weissmann, *Proc. US Nat. Acad. Sci.*, **59**, 145, 1968) has now demonstrated that the minus strand RNA molecules of $Q\beta$ coliphage, which are inherently non-infectious, can serve as templates for the *in vitro* synthesis of $Q\beta$ plus strands. Last year Weissmann proposed that the minus strand—not a duplex molecule with plus and minus strands hydrogen bonded together—is the template for synthesis of progeny plus strands during the replication of the single-stranded RNA genome of $Q\beta$ (see *Nature*, **214**, 1287, 1967). Two observations supported this idea. First, some of the minus strands made *in vivo* in $Q\beta$ infected *E. coli* occur in a complex with molecules of replicase and with plus strands but are not hydrogen bonded to the plus strands. Secondly, neither of the two species of double-stranded RNA molecules—replicative form and replicative intermediate—which can be extracted with detergent and phenol from $Q\beta$ infected *E. coli* serves as a template for $Q\beta$ replicase *in vitro* unless it has been denatured by heat. The only way to test this hypothesis, however, was to isolate minus strands and investigate their template properties.

In November last year (see *Nature*, **216**, 639, 1967) Weissmann's group reported the isolation of pure $Q\beta$ minus strands. Now they have found that the minus strands do indeed direct a rapid synthesis of $Q\beta$ plus strands. In the presence of the four nucleotide triphosphates, $Q\beta$ minus strands promote rapid synthesis of RNA plus strands by $Q\beta$ replicase. In fifteen minutes the amount of RNA synthesized is three times the input of RNA, but with $Q\beta$ plus strands as template, less RNA and fewer infectious units are made. Furthermore, the enzyme is saturated by a much lower concentration of plus strands than minus strands and, at high template concentrations, incorporation into RNA is about seven times greater with minus strand than with plus strand templates. Feix *et al.* calculate that with minus strands as templates, a plus strand is made in about 100 seconds, which means that about 30 phosphodiester bonds are formed per second. These results are compatible with the assumption that the replicase preparation contains a small number of binding sites specific for plus strands and a larger number of binding sites specific for minus strands. Whether these two sites are on different sub-units of one protein or on two different proteins remains to be seen.

Spiegelman's group, unconvinced by the evidence obtained by others for the existence of a minus strand and its role in replication, has been doing experiments to convince itself. First, the group has satisfied itself that a minus strand exists and, in its latest contribution to the *Proc. US Nat. Acad. Sci.* (**59**, 141, 1968), Pace, Bishop and Spiegelman conclude that replicative form and intermediate, which they had chosen to call Hofschneider and Franklin structures, are in fact involved in $Q\beta$ replication and deserve their original names.

Also in the current *Proc. US Nat. Acad. Sci.* (**59**, 185, 1968), Nayak and Baluda describe the isolation, by phenol extraction, of replicative intermediate RNA molecules from chick embryo cells infected with equine influenza virus. Because replicative intermediates have also been found in cells infected with polio virus, it appears that all single-stranded viral RNA molecules are replicated by the same basic mechanism.

Action after the Dainton Reports

by
L. ROSENHEAD
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What are the reasons for the swing away from science at sixth form level?

THE Interim Dainton Report of 1966 and the recent Dainton Report of early 1968 bring a number of national and educational issues into sharp focus. These issues have become dangerously acute for Great Britain within just a few years. The danger lies in the fact that while our national welfare has become increasingly dependent on engineering, technology and science, the percentage of "dedicated young scientists" entering the sixth forms has dropped from 41.5 per cent in October 1961 to 31.4 per cent in October 1966. This means that the science-based activities of society are rapidly losing favour among the brightest of Britain's young people, just at a time when our national economy has been experiencing crisis after crisis. This deliberate turning away from engineering, technology, science, medicine and related work will have far-reaching effects on recruitment to those sectors of our national activities which are dependent on science, and will aggravate the effects on society of the brain drain at high levels of expertise.

This situation has developed at a time when the relevant age groups are smaller than those of the bulge years that immediately preceded them, with the result that both trends have already combined to produce in recent years a substantial numerical decrease in the number of dedicated scientists in our sixth forms. While this has been going on, the sixth forms themselves have been growing in size, but the increase has appeared in groups interested in "the traditional arts", "socio-economic studies", "craft, domestic and commercial subjects" and, quite appreciably, in a mixed "science-with-arts" group. The increases are therefore appearing in sectors which, under present conditions of university entrance, make the members of those groups ineligible for admission to courses of instruction currently given in faculties of engineering, technology, science and medicine—even if they desired to enter those faculties. In addition, I have recently been told that appreciable numbers of the apparently dedicated scientists of our sixth forms are moving over to the study of economics when they enter universities.

From these facts there emerge a number of important issues. Should we welcome these developing trends, or should we passively accept them? Are they in the national interest? Will they lead to appreciable unemployment in the near future among a substantial proportion of our university-trained people? These are matters meriting very careful consideration and early decision. If we are concerned with the public welfare, we would do well to remember a telling phrase from the recent government "Brain Drain Report" (Cmnd 3417) which reads "... the source of national wealth is in the creative, productive industries of this country", and another from Professor J. K. Galbraith's recent book *The New Industrial State* (Hamish Hamilton) which goes "It is the vanity of educators that they shape the educational system to their preferred image. They may not be without influence, but the decisive factor is the economic system".

There is an unresolved conflict between the ideas of those who favour "a general education" and of those who stress the importance of adjusting educational curricula and procedures to meet the changing needs of society. In this conflict we are confronted not with a problem of right versus wrong, but with a problem of right versus right, and in such a situation there is only one way in which to move ahead, and that is by way of compromise—a middle way *must* be found. I welcome the increased vitality shown by secondary schools in increasing the range of studies in their sixth forms—by so doing they have induced large numbers of young people to remain at school and try their hand at advanced work. But this growth of our sixth forms is occurring largely among those who do not seem to want to turn to the science-based activities of society. What are the reasons for this? Are they connected with the pattern of education in schools? Or the specialized entrance requirements of universities? Or the ideas of young people about society generally?

Young people sometimes complain that they were not warned of the existence of university entrance requirements before they started on their sixth form work. One knowledgeable headmaster told me that he makes a point of meeting individual members of his sixth form before they start work, and of discussing their subject groupings. Those who have attached themselves firmly to either science or arts present little difficulty, but the mixed science-with-arts people are warned that they are cutting themselves off from science, though not necessarily from arts, social science or economics. They are also warned that they will probably have to make many enquiries before they find a university that will accept them. The fact that sixth formers are not automatically accepted by universities on the basis of any grouping of sixth form studies, and the quite demanding requirements of the science-based faculties of universities, are, in the opinion of some headmasters, important factors in the "swing away from science".

According to some people the swing away from school science has been reinforced by the siren-like lure of the word "management" and its implications, but I feel that the spurious part of this attractiveness will become evident in a very short time. I also believe that it will soon come to be recognized that a training in engineering or science, reinforced by instruction in economics, and the ideas and techniques of management services, is the best preparation for management activities and decision making.

The reasons for the nature of current university entrance requirements in engineering, technology and science are that science-based university courses are, in Great Britain, all highly intensive in one way or another. They are geared to the production of high-level specialists. The tempo of university work is fast, and the time is short. Science-based university studies bear the mark of recent additions to knowledge and understanding, and the studies are hard and demanding. In order to achieve the current academic level of an honours degree in 3 years our science-based faculties demand some previous knowledge of certain basic disciplines, and these demands are embodied in entrance requirements which vary from university to university in accordance with the specialist outlooks of different academic communities. As the boundaries between the traditional sciences are collapsing the effect is that university science-based faculties are broadening the range of their entrance requirements; a knowledge of physics and mathematics, for example, is being increasingly demanded. These requirements are not just whims—they are based on valid specialist attitudes to engineering, technology, pure science and medicine, and on one further condition. This condition is that, in England and Wales alone of all the countries of the world, the standard of a high-level university "first degree" has to be achieved in only

3 years—as a result of pressure imposed by the appropriate finance-awarding authorities. In order to meet this situation schools, of their own free will, and in order to prepare their students for university entrance, have in the past introduced specialization into their sixth form studies to a level that many people feel is in conflict with the requirements of "a desirable general education". It is in this way that continuing externally imposed financial pressure has transmitted specialization through universities into schools. But schools now say, in effect, that as a result of their shortage of science-specialist teachers they can no longer keep up the academic pace. At the same time the schools say that they wish to broaden the range of sixth form studies in the interests of general education. They also say that some of their sixth forms contain a growing proportion (up to three-quarters) of young people who have no intention of going on to university. But this is precisely the reason why extra attention should be paid to the needs of that diminishing group who wish to serve society as engineers, technologists, scientists, doctors, dentists, lawyers, specialist teachers, and so on.

There is, I feel, a simple way of meeting this situation. If more places were made available in our universities, and if 3-year and 4-year honours courses were run, in parallel, in all universities, as a matter of principle, then the intensive nature of present entrance requirements would automatically be relaxed, schools could drop some of their specialist intensive sixth form work, and could devote themselves to a more flexible pattern of instruction whose principal aims would be the development of character, curiosity and self-reliance—as well as an appreciation of the arts and the sciences. All that universities would then need to demand would be evidence of education, to agreed levels, over a range of subjects wider than at present—and this range would, it is hoped, span the "two cultures". In this way the need to make decisions at the age of about thirteen or fourteen, which drastically biases subsequent career choices, would disappear entirely.

But career choices, broad or narrow, cannot be postponed indefinitely. Young people do have to make decisions about themselves and the basic question is "When ?" I do not know the answer to this—except that it varies from individual to individual—but I am sure that young people should not be compelled to make such decisions at about the age of thirteen or fourteen when they know little of the challenge, motivations and objectives of the science-based activities of society. At the same time, if a young person shows that he really knows what he wants, then I would not attempt to impose on him the elements of a completely non-oriented education in place of the ingredients of an oriented one. Enforced dispersion of studies may be quite as inflexible and as harmful as enforced concentration of studies. Education for living should not be planned on too many preconceptions, though some may, of course, be necessary. And, above all, flexibility of approach, within reasonable limits, should be the hallmark of our educational system. I feel it reasonable to expect that, if up to the age of about sixteen a young person has been exposed to stimulating, thought-provoking instruction, it should reinforce his out-of-school experience to such an extent as to produce in him some kind of career motivation, broad or narrow, and that by the age of about eighteen the career-decision should be, if not firm, then at least more sharply defined than at sixteen. This means that instruction between sixteen and eighteen should be more broadly based and less intensive than at present.

Even under my own private vision of a desirable pattern of further education, however, I can offer no guarantee that there will result a reversal of the current swing away from science, technology, engineering and medicine in our secondary schools. That is quite a different matter. A decision has to be taken "somewhere" that such an objective, the reversal of the swing, is in the national interest—and this is clearly a matter for highest governmental levels—and appropriate action has then to be set in train. To me it seems that the continuation of the expansion of our universities without some reference to national needs is bad policy and potentially very dangerous.

But before any action is taken the reasons for the swing away from science have to be diagnosed and brought into the light of day by enquiry, discussion and speculation—and the Dainton reports are important in this context in that they open the debate. Mr J. R. Butler's survey of literature on "Occupational Choice", which is mentioned in the report, is both fascinating and highly relevant. Changes in the current programme of education at primary, secondary and tertiary level are fundamental in order to enable our young people to make a "more informed career choice" than at present, but which way the informed choice will direct itself will depend on other matters. If our young people continue to regard industrial employment as they now appear to do, and if the teaching of science, arts and economics continues as at present, then the effect of postponing career-decisions may well be to accelerate the flow away from engineering, technology and science.

It is, I feel, very important that we find out what our young people are thinking about the world, Great Britain and themselves, of course we cannot hope to obtain clear and precise answers—probably nobody ever makes decisions about the future on the basis of clear and precise views—but even answers of a general nature might help us to understand. Of one thing I am sure, and it is that the "image of science" is no longer as exciting as it used to be. Very few of our young people see themselves as making world-shattering scientific discoveries in a small laboratory, isolated from the rest of the world. Science is no longer thought of as the harbinger of the new society, or the vital instrument in the creation of a future in which all men will be brothers. Science, they feel, leads to "the bomb", to bigger and faster aeroplanes, to gigantic electricity-generating stations, to computers of frightening flexibility and complexity, to submarines, to intercontinental ballistic missiles—and to the insignificance of individuals and themselves. This picture is distasteful to many, and positively repellent to some of our young people, but it is based on a false image of science and all that engineering, technology and science can do for people. Further, this attitude towards science, and to developments from it, has arisen in all the other highly technological societies of the world, both in schools where science teaching is said to be "good" and in those where it is said to be "bad". In the underdeveloped countries of the world, however, science still wears its bright and shining appearance. I believe that it is by making employment in science-based industries attractive, and by appealing to the idealism that is endemic among our young people, that we can repair and refurbish the image of science, and point to the excitements of scientific discovery, and to the service that they, the young people, can render to society and to the underdeveloped countries of the world by becoming engineers, technologists and scientists. In this context, both teachers and industry have a great deal of imaginative work to do to make science-based activities more attractive to our young people, and to make the social purposes of industry more understood. In spite of all that we have done, we still have only *started* to explore space, to understand the nature of matter, to probe the mysteries of the stars, the air, the land, the sea, to conquer the deserts and control the weather, to make two blades of grass grow where only one grew, to extend life and to increase vigour into old age—and to banish hunger, want, homelessness and fear from the world Engineering, technology and science still remain potent forces for good in planned social change

No one line of action will reverse the swing away from science Government, schools, universities and industry will all have their parts to play—and they will have to act in harmony At about the beginning of this century people in authority in Great Britain came to the conclusion that we were suffering from a pronounced lack of "educated manpower", particularly in science and engineering, and the universities of Great Britain were enlarged as a result of this realization Now, at the beginning of the final third of the twentieth century, we are confronted by challenges which are clearly outlined and clarified by the Dainton reports Perhaps the most fundamental of the challenging questions to be answered are these What have we to do to make the science-dependent activities of society more attractive to the brightest of our young people? How, through our educational system, can we influence our young people to turn to engineering, technology, science, medicine and so on? And how can we induce, through our educational procedures, an appreciation not only of human values in isolation, but also of engineering, technology and science in relation to human aspirations?

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The State of Public Science

by

Sir SOLLY ZUCKERMAN

The following is an extract from the speech given by Sir Solly Zuckerman, Chief Scientific Adviser to the British Government, and chairman of the Central Advisory Council for Science and Technology, to the Parliamentary and Scientific Committee

It is no exaggeration to say that today our public institutions for dealing with science and technology are passing through a phase of rapid evolution as they adapt—not without struggle—to the facts of the scientific and technological age in which we are immersed

The basic pattern which is now being transformed was set during the period of the First World War, to meet contemporary needs as seen at the time not only by politicians, administrators and industrialists, but also by scientists The problem as then defined was to place the responsibility for our national research institutions in the hands of a minister who was free from the pressure of administrative duties and who was therefore—so it was assumed—"immune from any suspicion of being biased by administrative considerations against the applications of the results of research". What happened—not surprisingly—was that the research council system which emerged out of this presumed principle of scientific independence—like the university world with which it became mainly associated—exercised little influence on the policies of those government departments which were concerned with related technological matters

If we are to continue as a powerful nation in the new world of industrial competition, it is my view—contrary to what Lord Haldane's committee supposed—that the applications of the results of scientific enquiry must necessarily be biased by political and administrative considerations It is in the light of this proposition that I feel that we should judge the major changes that have taken place in our public scientific institutions over the past three to four years, and of further changes which I see inevitably following in the years ahead

The basic fact that we have to bear in mind is that up to the Second World War, little more than twenty-five years ago, we in the United Kingdom not only covered but almost defined the nature of the water front of world science and technology Against the massive deployments of the USA and USSR, the technological resources we can mobilize now cover only a small part—although there is still a powerful tendency to spread ourselves too widely and too thin Our problem—like that of Europe as a whole—is to select, and to concentrate our scientific effort The challenge before us is to achieve a sophisticated view of the priorities for the deployment—during the next ten or twenty years—of our scientific and technological resources

The reorganization in the machinery of government of the past few years has brought into being the Ministry of Technology, a series of advisory bodies, and Parliament's new Select Committee on Science and Technology

The Ministry of Technology has quickly, and not surprisingly, grown into a major enterprise and has now become the principal department concerned, in the interests of the economy as a whole, with the problem of helping industry make as much use as it can of the fruits of modern technology These are early days, and those who are sometimes impatient with the progress of the new ministry forget the immensity of the task of shifting the focus of the Government's direct and indirect technological efforts from defence projects on to economically productive fields The Ministry has had to carry on with at least half the functions of the old DSIR, it has had to learn how to stepfather the AEA and NRDC, as well as emerge as the sponsor of the engineering, electronic and shipbuilding industries And, finally, and most arduous of all, it has had to become the manager of all the remaining functions of the old Ministry of Aviation

Experience will determine which way the ministry organizes the activities of the research and development establishments it inherited for the benefit of the industries whose efficiency it has the responsibility of fostering I have little doubt that industry itself will help determine what the right pattern of co-operation in research and development should be

We should treat the so-called Haldane principle of 1918 as a warning to be on our guard lest current arrangements and practices become codified into some new set of presumed principles The scale, the structure, the methods, and the directions and economic impact of industry change as technology changes—and no one benefits if the Whitehall machine fails to react in reciprocal fashion.

The same needs to be said of the network of advisory committees of independent scientists which characterize the Whitehall scene—although here I am bound to say, from personal experience, that there is little need to worry During the past twenty-five years or so, I have served—at one time or another—on most of these bodies They have changed so fast that many no longer exist What is perhaps surprising is that the fact that they did exist is often not known to their successors, whose footsteps then cover ground that has been well trodden before. Depending on the way you look at it, parliamentarians have an advantage here. Hansard is bound for future reference, but old advisory committee papers are normally burnt.

Two of the more significant changes of the past four years in this field are, first, the transformation of the old Advisory Council for Scientific Policy into today's Council for Scientific Policy, to which the Secretary of State for Education and Science turns for advice about the scale of resources required by the four research councils answerable to him, and about the division of the resources which the Treasury makes available and, second, the establishment of a new Central Advisory Council for Science and Technology, of which I have the honour to be chairman.

This body, which is answerable through the Prime Minister to the Government as a whole, was set up little more than a year ago, not to oversee the deliberations of departmental advisory bodies such as the Council for Scientific Policy or the Advisory Council on Technology, which was set up by the Minister of Technology, but to consider how best all the scientific and technological resources of the country can be deployed in the long-term national interest. We have not yet reported publicly, but our enquiries are proceeding apace, sometimes into questions of organization—on which, of course, we can only advise—but more frequently into matters which represent the core of our remit. For example, we want to uncover the factors which modulate the process of technological innovation, whether in industry or agriculture, we want to see whether criteria can be defined in order to help guide government investment in development projects, and we want to provide an intelligent appreciation of the likely priorities for our scientific and technological resources over the next ten years.

This leads finally to what I see as an extremely significant development in our national institutions for assuring the best deployment of our resources—the establishment of the Select Committee on Science and Technology, a development which the Parliamentary and Scientific Committee, so I understand, did much to foster.

I have read a fair amount of the literature—including the record of parliamentary statements—which deal with the formation of this new Select Committee, and I have also referred to the exhaustive report on nuclear energy which it has published. Considering the short time it has been operating, the impact of this new body has been formidable, and I have no doubt at all that it will increase even more, and that the committee will become a powerful force in the process of determining national policies.

A distinguished scientific writer, commenting on the Select Committee in an article which also dealt with the new Central Advisory Council for Science and Technology, remarked that "with any luck the two bodies will do their jobs so well that they will come into open conflict"—as the servants of the executive (that's where my council comes in) and of the legislature properly should. So far this has not happened, and I myself doubt—somewhat regretfully—that it ever will. What is certain is that the country at large, and departmental scientific advisory bodies in particular, cannot fail to benefit from the enquiries of the new Select Committee—whether or not they agree with its conclusions.

My main reason for saying this is that, however carefully the Select Committee has to tread in defining and agreeing its fields of enquiry with government departments, its proceedings are open and public. It has the power to invite—which is a polite way of saying summon—before it what witnesses it wishes to interrogate—ministers, government officials or private individuals, and "strangers" are not debarred—unless the committee so decides—from attending the sessions. This is a highly interesting departure. The committee will, I understand, not be concerned with matters which are within the arena of party controversy. But any issue of policy is controversial within the field with which it deals—otherwise it would not be debated—and among the "strangers" who listen may be potential witnesses who would be heard on some later date. As we all know, there is a lot of inertia—indeed, vested interest—in scientific and technological affairs, and interest is all the more likely to become vested if it is fostered behind locked doors.

This could help significantly in creating an informed climate of opinion about a great deal which now seems obscure. This applies as much to scientists and engineers as to industrialists, members of the public and parliamentarians. There are many issues relating to the exploitation of advanced technology which could only benefit from a greater public understanding. Not all advances are necessarily in the public interest. Industrial and commercial progress, as we are often reminded today, sometimes entails a not-immediately discernible social cost (and I am not just talking about matters like pollution here). Scientists and engineers themselves do not always appreciate this fact when it comes to advocating one of their specific projects. Furthermore, the point I made at the outset, that we can no longer attempt to cover the whole scientific and technological waterfront—however long ago this fact was clearly enunciated—is never, in my experience, appreciated when it comes to catering for particular interests. Here, too, the Select Committee could help, through its enquiries, to jerk us into the competitive realities of today from the delusions of a past grandeur, and to emphasize the need to select and concentrate our technological efforts—and those of Western Europe too. I can think of other ways it can and will help as it evolves through practice.

Excited Electronic States of Benzene and Naphthalene

by

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The excitation of the singlet and triplet π-electronic states of benzene and naphthalene by photons and by electrons is discussed in the light of recent experimental results, and suggests new techniques for the observation of these excited states.

PARISER[1] has characterized the ground and the singly excited π-electronic states of aromatic hydrocarbon molecules by three properties: multiplicity (singlet or triplet), symmetry (benzene A_{1g}, E_{2g}, B_{1u}, B_{2u} or E_{2u}, naphthalene A_g, B_{1g}, B_{2u} or B_{3u}), and sign (plus or minus in the linear combination of the molecular orbital configurations). The corresponding selection rules for electric dipole transitions are (*a*) multiplicity $1\leftrightarrow 1$, $3\leftrightarrow 3$; $1\leftarrow||\rightarrow$, (*b*) symmetry $g\leftrightarrow u$, $g\leftarrow||\rightarrow g$, $u\leftarrow||\rightarrow u$, (*c*) sign: $-\leftrightarrow +$; $-\leftarrow||\rightarrow -$, $+\leftarrow||\rightarrow +$

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The observed optical absorption transitions in benzene and naphthalene and their probable assignments are listed in Tables 1 and 2. The assignments of some of the states in the Platt free electron orbital model[2] are shown in parentheses. The ground state is ${}^1A^-_{1g}$ for benzene and ${}^1A^-_g$ for naphthalene (see, for example, ref. 3, this was incorrectly assigned ${}^1A^-_{1g}$ in ref. 1), and so the selection rules give allowed transitions to ${}^1X^+_u$ states (where X represents any specific symmetry type), but some forbidden transitions to ${}^1X^-_u$ and ${}^3X^+_u$ states are also observed. The lowest excited triplet state, which is ${}^3B^+_{1u}$ and ${}^3B^+_{2u}$ in benzene and naphthalene, respectively, may be populated by flash photolysis and allowed absorption transitions occur from there to ${}^3X^-_g$ states. The observed emission transitions (fluorescence and phosphorescence) are also included in the tables.

Electronic transitions in these molecules with electron excitation have been observed[4,5]. Tables 1 and 2 list optical data and the maxima observed in the threshold electron excitation spectrum of benzene and naphthalene[4] and their probable assignments. Included in Table 1 are the maxima from the electron energy loss spectrum of benzene for 300 V incident electrons[5].

The importance of low-energy electron impact spectrometry for the study of electron excitation, temporary negative ion formation and electron capture processes in molecules has been pointed out previously[22]. With low energy electron excitation the multiplicity selection rule (*a*) is relaxed because of electron exchange, as long as the incident electron energy E is not too high. Thus several singlet–triplet transitions have been observed in He($E\leqslant 50$ eV)[23], N_2($E\leqslant 15.7$ eV)[24], H_2($E\leqslant 15.7$ eV)[25], CO($E=30$–60 eV), CO_2, N_2 and He($E=50$ eV)[25]. For a number of larger molecules, however, no singlet–triplet transitions have been observed with electron excitation[26] at $E\geqslant 35$ eV. This indicates that for polyatomic molecules, such as the aromatic hydrocarbons, the incident electron energy E needs to be close to the threshold for the efficient excitation of a singlet–triplet transition. The three singlet–triplet transitions in benzene, which are observed with threshold electron excitation[4], are not detected with 300 V electron excitation[5], although the allowed singlet–singlet transitions are observed in both cases. This confirms that the electron impact excitation function for singlet–triplet transitions is a strongly peaked function of E near the threshold.

Three relatively strong minus–minus transitions are observed with threshold electron excitation, showing that the sign selection rule (*c*), which is also violated with photon excitation, is not stringent, probably because of vibrational interaction.

No *g*–*g* transitions are observed, indicating that the symmetry or parity selection rule (*b*) is valid with both high-energy electron and photon excitation. For low-energy electrons, however, *g*–*g* or *u*–*u* transitions may be weakly observed because of the impacting electron's form factor. Electric quadrupole *g*–*g* transitions have been observed both optically[27] and by electron impact[28], but such transitions are extremely weak, with a transition probability of only 10^{-8} that of an allowed electric dipole transition[27].

Threshold electron excitation of the ${}^1A^-_{1g}$ ground state (${}^1A^-_g$ for naphthalene) gives transitions to ${}^1X_u$ states, as with photon excitation, and also to ${}^3X_u$ states. Population of the lowest triplet ${}^3B^+_{1u}$ by flash photolysis gives allowed optical transitions to ${}^3X_g$ states, so that the two techniques of triplet excitation are complementary[29].

The only unidentified states in benzene below the ionization potential are ${}^3E^+_{2g}$, ${}^1E^+_{1g}$ and ${}^1E^-_{2g}$ with theoretical energies of 5.96, 8.20 and 8.36 eV, respectively[1]. Dipole transitions to these states from ${}^1A^-_{1g}$ are forbidden by selection rule (*b*). The ${}^3B^+_{1u}\rightarrow{}^3E^+_{2g}$ transition has not yet been observed by flash photolysis.

The states in naphthalene with theoretical energies[1] less than ~ 6 eV that have not yet been positively identified are the three *u*-states—${}^3B^+_{1u}({}^3B_b)$, 3.64 eV; ${}^3B^-_{3u}({}^3L_b)$, 4.02 eV, and ${}^3B^+_{2u}({}^3B_a)$, 4.22 eV—and the five *g*-states—${}^3B^+_{1g}$, 3.42 eV, ${}^3A^+_g$, 4.44 eV, ${}^1B^+_{1g}$, 5.51 eV, ${}^1A^-_g$, 5.73 eV, and ${}^1B^-_{1g}$, 5.98 eV.

Excluding the forbidden transitions to the *g*-states, the three *u*-states are candidates for the unidentified state X corresponding to the peak at 5.4 eV in the threshold electron excitation spectrum of naphthalene[4].

The ${}^3L_b$ state in benzene is observed at ~ 5.4 eV in the threshold electron excitation spectrum, and the corresponding state is therefore expected to have a rather lower energy in naphthalene. This seems to exclude the identification of X with ${}^3B^-_{3u}({}^3L_b)$. The ${}^1A^-_g\rightarrow{}^3B^-_{3u}$ transition in the threshold electron excitation spectrum of naphthalene is probably obscured by the strong ${}^1A^-_g\rightarrow{}^1B^+_{2u}$ transition.

The theoretical energy[1] (2.18 eV) of the lowest ${}^3B^+_{2u}$ state in naphthalene is 1.02 eV below the observed peak at 3.2 eV in the threshold electron excitation spectrum which is definitely assigned to the transition to this state. Addition of 1.02 eV to the theoretical energy of the second ${}^3B^+_{2u}$ state gives a predicted energy of 5.24 eV for the corresponding peak in the threshold electron excitation spectrum. DeGroot and Hoytink[20] have made improved calculations of the separations of the triplet states of naphthalene. Their value of 2.13 eV for the theoretical separation of the two lowest ${}^3B^+_{2u}$ states is a little higher than that of 2.04 eV obtained by Pariser[1]. Assignment of the 3.2 eV and 5.4 eV peaks in the threshold electron excitation spectrum to transitions to the two lowest ${}^3B^+_{2u}$ states, respectively, gives an observed separation of 2.2 eV, which agrees closely with the theoretical value of 2.13 eV. It appears reasonable, therefore, to assign X to the second ${}^3B^+_{2u}$ state. The state ${}^3B^+_u$ was not considered as a candidate for the X state because it is theoretically predicted[1] to lie too low in energy (3.64 eV). Furthermore, the ${}^3B^+_{3u}$ state is most likely associated with the peak around 3.6–4 eV observed in the threshold electron spectrum[4].

Additional support for the identification of the X state in naphthalene as the second ${}^3B^+_{2u}$ state comes from a comparison of the spectral resemblance between benzene and naphthalene[11]. If in going from benzene to naphthalene the degenerate triplet states ${}^3B_{a,b}$ in benzene split in the same way as the degenerate singlet ${}^1B_{a,b}$ states, then from the observed position of the ${}^3B_{a,b}$ states in benzene one concludes that the 5.4 eV peak in the naphthalene threshold electron spectrum corresponds to the energy of the second ${}^3B^+_{2u}({}^3B_a)$ state.

An experimental method has been proposed previously[20] for discriminating between singlet and triplet states excited by threshold electrons, and this is discussed more fully below.

An aromatic molecule excited into a higher singlet state internally converts, provided that it can lose its excess energy by collisions, to the lowest excited singlet state. It then either emits its characteristic fluorescence, undergoes intersystem crossing to the triplet manifold, or makes a radiationless transition to the singlet ground state. An aromatic molecule excited into a triplet state is similarly internally converted into the lowest excited triplet state. It then emits its characteristic phosphorescence, or undergoes radiationless intersystem crossing to the singlet ground state.

If the quantum efficiency of internal conversion is unity, as in most molecules in which competing dissociation processes do not occur, the fluorescence excitation spectrum $F(\bar{\nu})$ observed with threshold electron excitation will correspond to the singlet state excitation spectrum $S(\bar{\nu})$. Similarly, the phosphorescence excitation spectrum $P(\bar{\nu})$ will correspond to the triplet state excitation spec-

Table 1. ELECTRONIC TRANSITIONS IN BENZENE

Assignment of observed transition	Mode of excitation						Comments	Ref.
	Photons		Threshold electrons		300-eV electrons			
	0–0 Energy (eV)	Relative intensity*	Peak energy (eV)	Relative intensity*	Peak energy (eV)	Relative intensity*		
Temporary negative ion resonance	—	—	1·55 (0·9±0·3 onset)	—	—	—		6
$^1A^-_{1g}(^1A)\rightarrow{}^3B^+_{1u}(^3L_a)$	3·65	—					O_2 perturbation (solution)	7
	3·68	—					O_2 perturbation (crystal)	8
			3·9 (3·6 onset)	0·05				4
					Not observed			5
$^1A^-_{1g}\leftarrow{}^3B^+_{1u}(^3L_a)$	3·66	—					Phosphorescence	9, 10
$^1A^-_{1g}\rightarrow E^+_{1u}(^3B)$	4·58	—					Crystal absorption and O_2 perturbation	8
			~4·7 (4·4 onset)					4
					Not observed			5
$^1A^-_{1g}\rightarrow{}^1B^-_{2u}(^1L_b)$	4·64 (4·84 peak)	0·0024						11, 12
			4·80	0·26				4
					4·93	~0·0007		5
$^1A^-_{1g}\leftarrow{}^1B^-_{2u}(^1L_b)$	4·59	—	—	—	—	—	Fluorescence	13
$^1A^-_{1g}\rightarrow{}^3B^-_{2u}(^3L_b)$	Not observed	—	~5·4		Not observed		See ref. 4 for discussion	4
$^1A^-_{1g}\rightarrow{}^1B^+_{1u}(^1L_a)$	5·99 (6·11 peak)	0·14						11, 12
			6·16	0·71				4
					6·21	0·014		5
$^1A^-_{1g}\rightarrow{}^1E^+_{1u}(^1B)$	6·77 (6·89 peak)	1·00						11, 12
			6·96	1·00				4
					6·96	1·00		5
$^3B^+_{1u}\rightarrow{}^3E^-_{2g}$	>5·0						$^3B^+_{1u}$ populated by flash photolysis	14
							Theoretical value 3·59→8·36=4·77 eV	1

* Intensities expressed relative to corresponding $^1A^-_{1g}\rightarrow{}^1E^+_{1u}$ transition.

Table 2. ELECTRONIC TRANSITIONS IN NAPHTHALENE

Assignment of observed transition	Mode of excitation		Comments	Ref.
	Photons 0–0 Energy (eV)	Electrons Peak energy (eV)		
Temporary negative ion resonance	—	0·8		4
$^1A^-_g(^1A)\rightarrow{}^3B^+_{2u}(^3L^+_a)$	2·63		O_2 perturbation (solution)	7
	2·63		From delayed fluorescence excitation spectrum (crystal)	15
		3·2 (2·6 onset)		4
$^1A^-_g\leftarrow{}^3B^+_{2u}(^3L^-_a)$	2·63		Phosphorescence	16
$^1A^-_g\rightarrow{}^3B^+_{3u}(^3B_b)$	—	~3·6–4·0 (?)		4
$^1A^-_g\rightarrow{}^1B^-_{3u}(^1L_b)$	3·97			11, 12
		~4·5	See ref. 4 for discussion	4
$^1A^-_g\leftarrow{}^1B^-_{1u}(^1L_b)$	3·97		Fluorescence	17
$^1A^-_g\rightarrow{}^1B^+_{2u}(^1L_a)$	4·29			11, 12
		4·8		4
$^1A^-_g\rightarrow X$	Not observed	5·4	Sharp intense peak. See Section 5 for discussion of assignment	4
$^1A^-_g\rightarrow{}^1B^+_{3u}(^1B_b)$	5·63			11, 12
		6·0		4
$^1A^-_g\rightarrow{}^1B^+_{2u}(^1B_a)$ (?)	6·52		Platt[2] assigns to ^1C_b	11, 12
$^1A^-_g\rightarrow{}^1B^+_{3u}(^1C_b)$ (?)	~7·4		Platt[2] assigns to ^1B_a	11, 12
$^3B^+_{2u}\rightarrow{}^3B^-_{1g}$ (?)	3·13 (2·63→5·76)	—	$^3B^+_{2u}$ populated bv flash photolysis. Pariser[1] assigns to $^3A^-_g$. See refs. 19, 20 for discussion	18, 19
$^3B^+_{2u}\leftarrow{}^3B^-_{1g}$ (?)	3·13 (2·63←5·76)	—	Triplet–triplet fluorescence in glow discharge	21
$^3B^+_{2u}\rightarrow{}^3A^-_g$ (?)	3·64 (2·63→6·27)	—	$^3B^+_{2u}$ populated by flash photolysis. Pariser[1] assigns to $^3B_{1g}$. See refs. 19, 20 for discussion	18, 19
$^3B^+_{2u}\rightarrow{}^3A^-_g$	4·79 (2·63→7·42)	—	$^3B^+_{2u}$ populated by flash photolysis	18, 19

trum $T(\bar{\nu})$, plus a component $aS(\bar{\nu})$ of the singlet state excitation spectrum, where a is the quantum efficiency of intersystem crossing.

Experimentally, $F(\bar{\nu})$ should be the more readily observable of the two luminescence excitation spectra. Comparison of $F(\bar{\nu})$ with the total threshold electron excitation spectrum, the photofluorescence excitation spectrum and the optical absorption spectrum of the vapour would discriminate between singlet–singlet transitions and singlet–triplet transitions or negative ion resonances, and it would provide data about relative excitation cross-sections and about any deviations from Vavilov's law (fluorescence quantum yield independent of excitation energy), such as have been observed in benzene[30,31], alkyl benzenes[30,31], and alkyl naphthalenes[31]. To ensure efficient internal conversion, the vapour pressure needs to be high enough for sufficient collisions to occur within the molecular fluorescence lifetime (~20 ns for benzene, ~80 ns for naphthalene) to dissipate the excess excitation energy before emission.

Photon and threshold electron excitation of the $^1A^-_{1g}$ ground state gives transitions to $^{1,3}X_u$ states. The population of the lowest $^3B^+_u$ triplet state by flash photolysis enables allowed transitions to ^3X_g states to be observed. The one group of excited states that cannot be observed by existing techniques, because of the symmetry selection rule (b), are the ^1X_g states. An experimental method for the observation of such states is proposed, namely, the population of the lowest excited singlet state by flash photolysis and the observation of the transient singlet–singlet absorption spectra during the lifetime of the excited singlet state. The lowest excited singlet states in benzene, naphthalene, anthracene and the higher polyacenes are u-states, and allowed optical transitions from these states to higher ^1X_g states can occur.

Recent advances in pulsed laser technology have made feasible the singlet-state flash photolysis of the polyacenes. Experiments are in progress at the University of Manchester with a pulsed nitrogen laser which yields recurrent intense flashes of near ultraviolet radiation of 20 ns duration, and it is proposed to use this source for the singlet-state flash excitation of aromatic hydrocarbons and the observation of transient singlet–singlet transitions The technique is also applicable to the study of higher excited singlet states of excimers

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[1] Pariser, R , *J Chem Phys* ,**24**, 250 (1956)
[2] Platt, J R , *J Chem Phys* ,**17**, 484 (1949)
[3] Orloff, M K , *J Chem Phys* , **47**, 235 (1967)
[4] Compton, R N , Huebner, R H , Reinhardt, P W , and Christophorou, L G , *J Chem Phys* (in the press)
[5] Skerbele, A M , and Lassettre, E N , *J Chem Phys* , **42**, 395 (1965)
[6] Compton, R N , Christophorou, L G , and Huebner, R H , *Phys Lett* ,**23**, 656 (1966)
[7] Evans, D F., *J Chem Soc* , 1351 (1957)
[8] Colson, S D , and Bernstein, E R , *J Chem Phys* , **43**, 2661 (1965)
[9] Shull, H , *J Chem Phys* ,**17**, 295 (1949)
[10] Nieman, G C , thesis, California Inst Technol (1965)
[11] Klevens, H B , and Platt, J R , *J Chem Phys* ,**17**, 470 (1949)
[12] Jaffe, H H , and Orchin, M , *Theory and Applications of Ultraviolet Spectroscopy* (John Wiley and Sons, New York, 1962)
[13] Birks, J B , Braga, C L , and Lumb, M D , *Proc Roy Soc* , A, **283**, 83 (1965)
[14] Godfrey, T S , and Porter, G , *Trans Faraday Soc* , **62**, 7 (1966)
[15] Avakian, P , and Abramson, E , *J Chem Phys* , **43**, 821 (1965)
[16] Ferguson, J , Iredale, T , and Taylor, J A , *J Chem Soc* , 3160 (1954)
[17] Aladekomo, J B , and Birks, J B , *Proc Roy Soc* , A, **284**, 551 (1965)
[18] Porter, G , and Wright, F J , *Trans Faraday Soc* , **51**, 1205 (1955)
[19] Porter, G , and Windsor, M W , *Proc Roy Soc* , A, **245**, 238 (1958)
[20] deGroot, R L , and Hoytink, G J , *J Chem Phys* , **46**, 4523 (1967)
[21] Schuler, H , and Arnold, G , *Z Naturforsch* , **16a**, 1091 (1961)
[22] Christophorou, L G , and Compton, R N , *Health Phys* ,**13**, 1277 (1967)
[23] Chamberlain, G E , Heideman, H G M , Simpson, A , and Kuyatt, C E , in *Abstracts Fourth Intern Conf Physics of Electronic and Atomic Collisions*, 378 (Science Bookcrafters, Hastings-on-Hudson, New York, 1965)
[24] Heideman, H G M , Kuyatt, C E , and Chamberlain, G E , *J Chem Phys* , **44**, 355 (1966)
[25] Heideman, H G M , Kuyatt, C E , and Chamberlain, G E , *J Chem Phys* , **44**, 440 (1966)
[26] Skerbele, A , Dillon, M A , and Lassettre, E N., *J Chem Phys* , **46**, 4162 (1967)
[27] Herzberg, G , *Spectra of Diatomic Molecules*, second ed (D van Nostrand Co , Princeton, New Jersey, 1950)
[28] Skerbele, A , Dillon, M A , and Lassettre, E N , *J Chem Phys* , **46**, 4161 (1967)
[29] Birks, J B , in *The Triplet State*, 403 (Cambridge University Press, England, 1967)
[30] Braun, C L , Kato, S , and Lipsky, S , *J Chem Phys* , **39**, 1645 (1963)
[31] Conte, J C , thesis, Univ Manchester (1966)

Proton Magnetic Resonance and the Helix–Coil Transition

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The molecular weight of polypeptides has a marked effect on the nuclear magnetic resonance manifestation of the helix–coil transition The technique can be used for absolute determinations of helix contents and compared with optical rotatory dispersion data.

WE have suggested that the changes in the nuclear magnetic resonance spectra of polypeptides through a helix–coil transition would depend on the nature of the side-chain and also on the molecular weight of the polymer[1] A comparison of the available data on different polypeptides[2–8] demonstrates that the nature of the side-chain has a marked effect on the nuclear magnetic resonance manifestation of the helix–coil transition This article reports the effect of molecular weight variation on the nuclear magnetic resonance spectrum of poly-γ-benzyl L-glutamate and demonstrates that in favourable circumstances an absolute estimate of helix content can be made

Of all the polypeptides so far synthesized, the polyglutamate esters have been subjected to the most intensive physico-chemical studies and as a result are the best characterized systems Doty and his collaborators[9] have shown for poly-γ-benzyl L-glutamate in dioxan and formamide that polymers with a degree of polymerization (dp) of 5 were completely random and with a dp of 13 were 44 per cent helical, concluding that a dp of between 7 and 14 was necessary for the onset of the helix form The five samples which we have used were synthesized by W E Hanby of Courtaulds, Ltd , and their properties are summarized in Table 1 The molecular weights of the three smaller samples [439*A* (1), (2), (3)] were determined from vapour pressure measurements using a Perkin–Elmer model 115 molecular weight apparatus, the molecular weight of the *R* 10 sample was determined from osmotic pressure measurements of *m*-cresol solutions using a Hewlett–Packard series 500 membrane osmometer (we thank Dr J C Haylock of the University of Exeter for this measurement) The molecular weight of sample *S P.* 18.4 was determined from its reduced viscosity measured in dichloracetic acid using the curve of viscosity against molecular weight obtained by Doty, Bradbury and Holtzer[10]. It has been shown that low molecular weight poly-γ-benzyl L-glutamate can form β-structures[11], and so the infrared spectra of chloroform solutions were recorded on a Grubb Parsons grating instrument, the 'Spectromaster' These showed that the lowest molecular weight polymer 439*A* (1) contained about 20 per cent of the β-form, the 439*A* (2) sample contained a negligible amount of β-material (< 2 per cent) and the other samples none It was assumed from this that β-structures would be absent in trifluoroacetic acid/deuterochloroform solutions of all samples except 439*A* (1)

Nuclear magnetic resonance spectra were recorded at 60 MHz on a Perkin–Elmer *R* 10 spectrometer using a computer of average transients to collect and average 128 spectra, at 100 MHz on the Jeol Co '*JNM*–4*H*–100' spectrometer and finally a few spectra were recorded on the Perkin–Elmer *R* 14 100 MHz instrument. The time

Table 1 POLY-γ-BENZYL L-GLUTAMATE SAMPLES

Sample No	Molecular weight	Average dp	β-Content in chloroform
439*A* (1)	1,480	7	~20 per cent
439*A* (2)	2,790	13	Negligible
439*A* (3)	4,490	21	None
R 10	20,100	92	None
S P 18 4	140,000	640	None

averaged spectra at 60 MHz were obtained from 1.5 per cent w/v solutions at 33.5° C, but spectra of 5 per cent w/v solutions were found to be identical. Thus for single scan spectra recorded at 100 MHz, 5 per cent w/v solutions were used throughout. The optical rotatory dispersion curves of the same solutions as used for the nuclear magnetic resonance studies were recorded at the same temperatures on the Bendix spectropolarimeter, 'Polarmatic 62', and were analysed using the Moffitt and Yang equation[12] to give the b_0 parameter. With our system a b_0 of $+70$ was obtained for the random coil form in trifluoroacetic acid and -630 for the fully helical form, and so the scale $(-b_0+70)/700$ was used to estimate the helix content by this method.

The nuclear magnetic resonance spectra of the poly-γ-benzyl L-glutamate of dp 92 in 8 per cent trifluoroacetic acid/92 per cent deuterochloroform at different temperatures are given in Fig. 1. The assignments of the resonance peaks are as follows: 1.83 τ NH in the helical form, 2.05 τ NH in the random coil form, 2.72 τ C_6H_5, 4.95 τ benzyl CH_2; 5.55 τ αCH of the random coil form, 6.00 τ αCH in the helical form, 7.55 τ, γCH_2 and at 7.75 τ the βCH_2 resonance peak. The polypeptide undergoes an inverted helix–coil transition as can be seen from the b_0 values in the caption, being largely helical at 50° C and almost random at 0° C. The solvent system is the same throughout, and so the changes observed in the spectra are a consequence of the conformational change occurring in the polypeptide. In agreement with Ferretti[4], two peaks are observed for both the amide NH and αCH resonances, there is also evidence of a similar behaviour for the βCH_2 peak at 7.8 τ, for there is an apparent upfield shift of 28 c.p.s. on going from the helix to the random coil form. It can be seen from the spectra that the two components of the αCH peak are well separated (50 c.p.s.) and of comparable width, while the components of the amide NH peak are separated by only 22 c.p.s., and, furthermore, the helical peak is wider than the component arising from the random form. For these reasons we prefer to use the αCH peak for conformational analysis. The changes in relative areas of the αCH proton peaks can be seen to follow the b_0 values of the solutions. The effect of polymer size on the behaviour of these peaks is shown in Fig. 2. The solvent compositions were chosen so as to give approximately 50 per cent helix (from optical rotatory dispersion measurements) for all samples. The nuclear magnetic resonance spectra were recorded at 60 MHz in identical conditions and it can be seen that with increasing molecular weight the two components of the αCH resonance peak broaden markedly on going from dp 13 to dp 92. For the largest polymer of dp 640, however, the two peaks are no longer observable and there is a broad single peak at an intermediate position. Furthermore, it can be seen that for the smaller polymers the areas of the two αCH components indicate approximately 50 per cent helix content and are thus in agreement with the b_0 values.

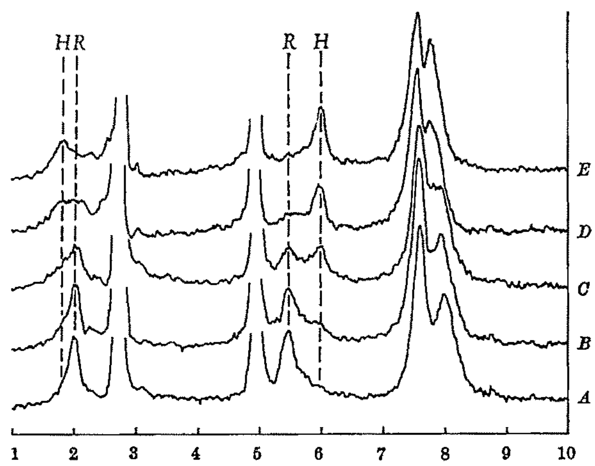


Fig. 1. Nuclear magnetic resonance spectra at 100 MHz showing the temperature induced helix–coil transition of poly-γ-benzyl L-glutamate, dp 92, in 8 per cent trifluoroacetic acid/92 per cent deuterochloroform. H, Helix, R, random. A, 0° C, $b_0=0°$; B, 6° C, $b_0=-100°$; C, 12° C, $b_0=-220°$; D, 25° C, $b_0=-340°$; E, 50° C, $b_0=-470°$.

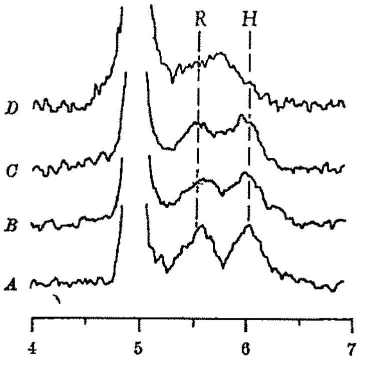


Fig. 2. Nuclear magnetic resonance spectra of poly-γ-benzyl L-glutamate at 60 MHz showing the effect of molecular weight on the components of the αCH peak. H, Helix, R, random. A, dp 13, $b_0=-305°$; B, dp 21, $b_0=-305°$; C, dp 92, $b_0=-285°$; D, dp 640, $b_0=-310°$.

At this point a more detailed study of the helix→coil transition in three samples of poly-γ-benzyl L-glutamate was undertaken at 100 MHz. The samples used were of dp 13, 92 and 640 to give a wide spread of molecular weights. The helix→coil transitions in the samples of dp 13 and 640 were controlled by increasing the trifluoroacetic acid content of the trifluoroacetic acid/deuterochloroform solvent system, while for the sample of dp 92 the temperature of the 8 per cent trifluoroacetic acid/92 per cent deuterochloroform solution was varied to give the inverted helix–coil transition. The results obtained for these polymers are given in Fig. 3a, b and c. There is little doubt from these spectra that, whereas two distinct and separate peaks are observed for the lowest molecular weight polymers, the polymer of dp 640 shows a single peak which is narrow for the random coil form, broadened at an intermediate position for the 50 per cent helical sample and very much reduced in area for the almost fully helical sample. It is evident, therefore, that the dynamics of the molecular motions are different for the highest molecular weight sample than for the smaller polymers.

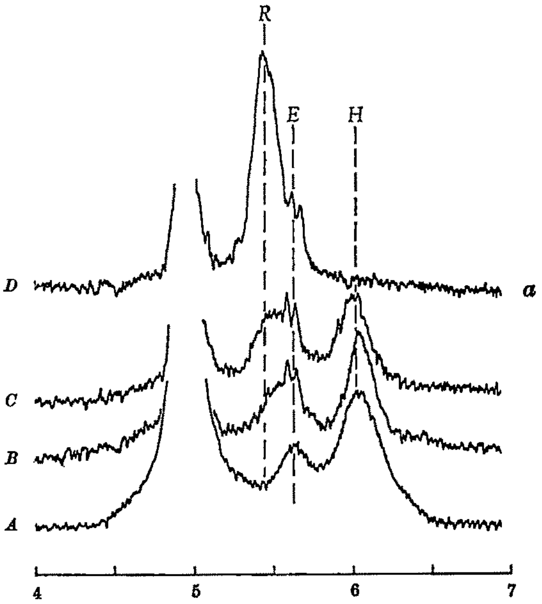
For the low molecular weight polymers the broadening of the two components of the αCH peak with increasing molecular weight is attributed to an increase in the overall correlation time of the molecule. As pointed out by Ferretti[4], the observation of two distinct peaks for the helix and random coil forms allows a lower limit for the lifetime t of the protons in the different conformations to be obtained from the inequality $t \geqslant [1/(2\pi|\nu_H-\nu_R|)]$ where ν_H and ν_R are the positions in c.p.s. of the peaks for the helix and random coil forms, respectively. For the αCH resonance the separation of the peaks is 58 c.p.s. giving $t \geqslant 2.8\times10^{-3}$ s. For the largest polymer, the 640 mer, the observation for the partially helical state of a single broadened peak intermediate in position between the positions of the peaks for the random coil and helix form indicates[13,14] that the polypeptide backbone protons are interconverting between the random coil form, which gives a sharp peak, and the helix form, which gives a much broadened resonance, at a rate which is faster than $2\pi|\nu_H-\nu_R|s^{-1}$. This gives a lifetime t for the proton in the helix and random coil forms as $t \leqslant 2.8\times10^{-3}$ s.

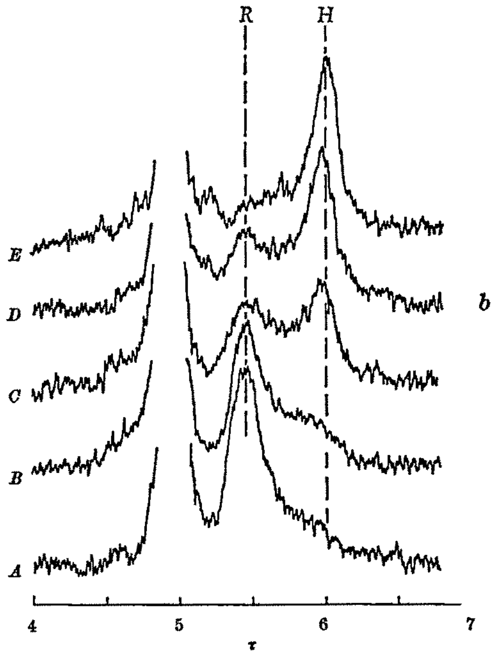
The observation of separate αCH peaks for the helix and random coil forms for the lower molecular weight poly-γ-benzyl L-glutamate allows an absolute determination of the proportion of the polypeptide in these forms

This is probably the only technique which permits an unambiguous determination of the helix content and can be used as an independent check on the use of b_0 for this purpose Ferretti[4] has pointed out that for poly-L-leucine and poly-β-methyl L-aspartate there is a correlation between the b_0 values and the area of the helical peak It is possible from the spectra given in Fig 3a and b to estimate the relative areas of the peaks for the two forms The overlapping curves were analysed by using the shape of the non-overlapped edges of the benzyl CH_2 resonance and the α*CH* resonance peak of the helical form to obtain the complete shape of the respective peaks By this method the scatter of results obtained was less than 5 per cent in the determination of the ratio

$$\frac{\text{area of the } \alpha CH \text{ helix peak}}{\text{total area of } \alpha CH \text{ peak}}$$

for the different solutions Fig 4 shows the inverted helix–coil effect for the polymer of dp 92 obtained from both optical rotatory dispersion determinations of b_0 and the contribution of the helical form to the α*CH* peak from the nuclear magnetic resonance spectra As can be seen, there is a very close correspondence between the two methods, a result which is in contradiction to that obtained by Stewart *et al* [3], who suggested from their nuclear magnetic resonance studies of poly-L-alanine that b_0 values gave a very poor estimate of helix contents A question which is of prime importance in the use of optical rotatory dispersion to estimate the helix content of proteins is whether the Moffitt–Yang equation holds for short helical lengths This question arises because Moffitt's original derivation of the equation[15] was based on considerations of helical chains of infinite length and it has been suggested that, for short helical segments, the value of b_0 would underestimate the number of residues in these segments[16], although certain theoretical considerations[17] suggest that most of the rotation characteristics of the helix are given by one turn of the helix The low molecular weight polypeptides used in this study allow a test of whether or not b_0 measurements hold for short helices In the sample 439*A* (2) of average dp 13, it could be expected that for chloroform solutions the average number of residues in the helices would be between 7 and 9 and for the sample 439*A* (3) of dp 21 in chloroform the average length of helical segments would include 15–17 residues, in trifluoroacetic acid/deuterochloroform solutions even shorter helical lengths would exist We have made an estimate from the nuclear magnetic resonance spectra of the proportion of residues in the helical form for these two samples and also for the polymer of dp 92 undergoing a temperature induced inverted helix–coil transition The ratios obtained were plotted against the helix contents calculated from b_0 values of the same solutions and this is shown in Fig 5 The broken line is that for a 1 1 correspondence and from a least square test this line is also the best fit to the experimental points





It is possible from the areas of the α*CH* peaks of the 13 mer and the 21 mer in chloroform to estimate the average number of residues not involved in the helix form and situated at the ends of chains. For both polymers this comes out to be two residues at each end A further point in connexion with these non-helical residues

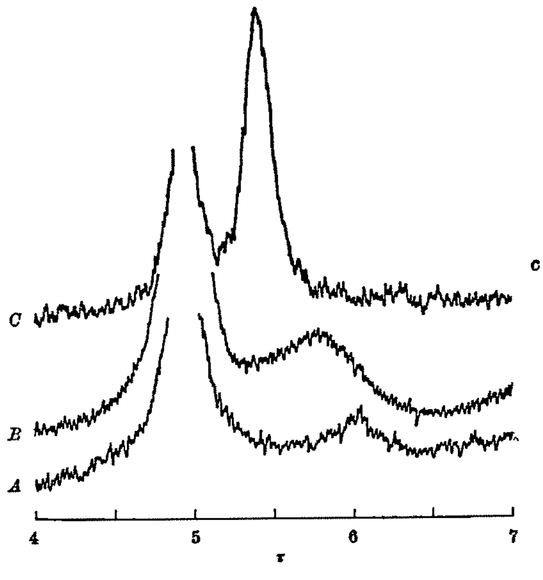


Fig 3 Nuclear magnetic resonance spectra of different polymers at 100 MHz showing the α*CH* peaks through the helix-coil transition *H*, helix, *R*, random, *E*, end groups

a, dp 13 *A*, 0 per cent TFA, $b_0 = -365°$, *B*, 5 per cent TFA, $b_0 = -330°$, *C*, 10 per cent TFA, $b_0 = -225°$, *D*, 20 per cent TFA, $b_0 = +30°$
b, dp 92 *A*, 0° C, $b_0 = 0°$, *B*, 6° C, $b_0 = -100°$, *C*, 12° C, $b_0 = -220°$, *D* 25° C, $b_0 = -340°$, *E*, 50° C, $b_0 = -470°$ *c*, dp 640 *A*, 6 per cent TFA, $b_0 = -600°$, *B*, 12 5 per cent TFA, $b_0 = - 410°$, *C*, 20 per cent TFA, $b_0 = +70°$

in chloroform solutions is, as can be seen from Fig. 3*a*, that the chemical shift of their α*CH* peak (5·60τ) is different from that of the random coil peak observed in 20 per cent trifluoroacetic acid/80 per cent deuterochloroform solution at 5·45τ. In the spectra of the solutions containing trifluoroacetic acid, however, there are also peaks at 5·60τ on the high field side of the main random peak which diminish with increasing molecular weight. It is thought that these higher field peaks result from residues at the end of the polypeptide chains and in the case of the chloroform solution these are the only residues which contribute to the random coil peak. Sternlicht and Wilson[18], however, have suggested that the α*CH* protons near the amino end of a helix may be shifted downfield by as much as 0·4 p.p.m. from α*CH* peak in an infinite helix. In the case given here the observation of the peak at 5·60τ for the fully random coil forms shows that it cannot have this origin. Furthermore, because the helical α*CH* peak in the spectra of the partially helical states of the dp 13 and 7 polymers in mixtures of trifluoroacetic acid and deuterochloroform has the same shift as the helical peak in the 92 and 640 mers, it is clear that residues on both ends of a helix have the same shift as those within long helical segments.

Concerning the difference in chemical shift between the random and helical components of the α*CH*, it has been suggested[1,6] that this may result from a specific interaction of trifluoroacetic acid with the helical regions of the polypeptide chain giving rise to a magnetic anisotropy not present in the helix itself and resulting in an upfield shift. This suggestion was made because it has been shown[7,19] that in water soluble polypeptides, poly-L-lysine, poly-L-glutamic acid[5] and copoly $(\text{glu}^{42}\,\text{lys}^{28}\,\text{ala}^{30})$, there is either no shift or only a very small shift in the α*CH* peak on going from the helical to the random coil forms. As shown here, however, the position of the peak for the helical form in deuterochloroform is the same as in the mixed solvents containing trifluoroacetic acid which indicates that the suggestion concerning the role of trifluoroacetic acid in this shift no longer holds.

The observation of a 0·6 p.p.m. chemical shift difference for the α*CH* peak of the random coil and helix forms differs from the result quoted by Sternlicht and Wilson[18] that there is no shift in this peak for poly-γ-benzyl L-glutamate. In this paper it is shown that there is a marked effect of the length of the polypeptide chain on the behaviour of the nuclear magnetic resonance spectrum in the helix–coil transition region. The behaviour of the low molecular weight samples confirms our earlier suggestion (ref. 1, case 2) that short polypeptide chains would have short overall correlation times and that this would lead to two components for the α*CH* resonance peaks having comparable widths for the helix and random coil forms. For the highest molecular weight sample the observation of a broadened time-averaged peak of intermediate position for the partially helical systems shows that the helical and random coil regions are interconverting at a rate faster than $360\ \text{s}^{-1}$. It is evident from this difference in behaviour between the various polymers that the rate of interconversion of the helix and random coil forms is dependent on molecular weight as well as on the nature of the side-chain. The much reduced apparent area of the α*CH* peak for the dp 640 polypeptide in 6 per cent trifluoroacetic acid/94 per cent deuterochloroform (which has an estimated helix content of 96 per cent from b_0 measurement) shows that for long helical segments the correlation time is insufficiently short to average out the magnetic anisotropy of the helix and considerable peak broadening occurs. This result is in contradiction to others[3,5], from which it was suggested that fully helical polypeptides give rise to well developed nuclear magnetic resonance spectra. As before[7] the width of the side-chain proton resonance peaks diminishes with increasing distance from the helical main chain.

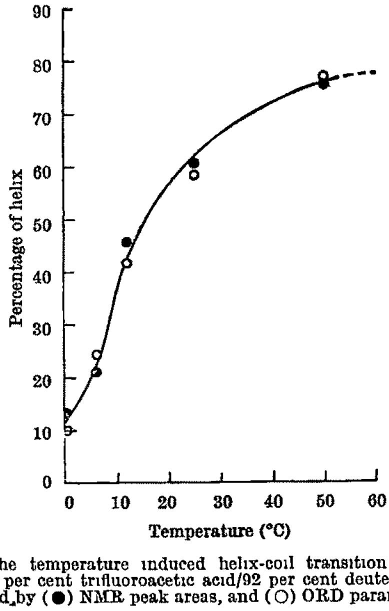


Fig. 4. The temperature induced helix-coil transition of polymer dp 92 in 8 per cent trifluoroacetic acid/92 per cent deuterochloroform followed by (●) NMR peak areas, and (○) ORD parameter b_0

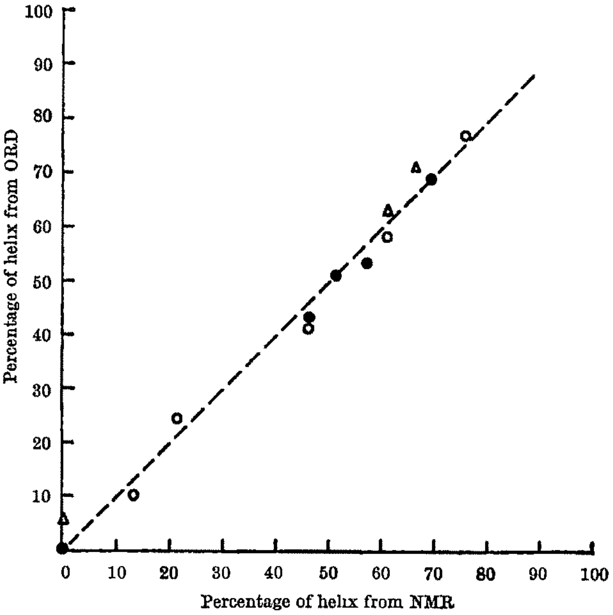


Fig. 5. The correlation of NMR and ORD estimates of helix content for three samples. (○) dp 92, (△) dp 21, (●) dp 13

Nuclear magnetic resonance spectra can give an absolute determination of helix content for two cases of polypeptide behaviour. The first is when the helix and coil regions are interconverting rapidly to give a single time-averaged peak, the position of which is proportional to the relative proportions of the two forms. For this case the estimation of helix content requires the resonance in the fully helical form to be prominent enough for its shift to be measured. Markley *et al.*[5] have used this method for the estimation of helix content. The second is the case elaborated here, where the appearance of two well resolved components for the α*CH* peak also allows an absolute determination of helical content and, furthermore, allows a test of the use of b_0 measurement for estimating the helix content in short helical lengths. The results from the polymer of dp 13 in deuterochloroform show that b_0 measurements of helix content are valid down to helical lengths involving eight residues and it is not unreasonable to suggest that because the correspondence also holds for the same polymer at lower helical contents in solutions of trifluoroacetic acid and deuterochloroform that b_0 measurements hold for helical segments involving fewer residues. From the point of view of biological applications of b_0 measurements to the determination of helix contents in proteins it is now reasonable to suggest that, provided there are no important contributions from side-chain chromophores or large proportions of conformations other than helical or random, a reasonable estimation of the helix content might be expected even if the helical segments are very short

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[1] Bradbury, E M, Crane-Robinson, C, and Rattle, H W E, *Nature*, **216**, 862 (1967)
[2] Goodman, M, and Masuda, Y, *Biopolymers*, **2**, 107 (1964)
[3] Stewart W E, Mandelkern, L, and Glick, R E, *Biochemistry*, **6**, 143 (1967)
[4] Ferretti, J A, *Chem Commun*, 1030 (1967)
[5] Markley, J L, Meadows, D H, and Jardetzky, O, *J Mol Biol*, **27**, 25 (1967)
[6] Bradbury, E M, and Rattle, H W E, *Polymer* (in the press)
[7] Bradbury, E M, Crane-Robinson C, Goldman, H, and Rattle, H W E, *Biopolymers* (in the press)
[8] Marlborough, D I, Orrell, K G, and Rydon, H N, *Chem Commun*, 518 (1965)
[9] Mitchell, J C, Woodward, A E, and Doty, P, *J Amer Chem Soc*, **79**, 3955 (1957)
[10] Doty, P, Bradbury, J H, and Holtzer, A M, *J Amer Chem Soc*, **78**, 947 (1956)
[11] Doty, P, Holtzer, A M, Bradbury, J H, and Blout, E R, *J Amer Chem Soc*, **76**, 4493 (1954)
[12] Moffitt, W, and Yang, J T, *Proc US Nat Acad Sci*, **42**, 596 (1956)
[13] Jardetzky, O, *First Biopolymers Symposium*, 501 (Interscience Publishers, 1964)
[14] Bradbury, E M, Crane-Robinson, C, Rattle, H W E, and Stephens, R M, in *Conformation of Biopolymers* (edit by Ramachandran, G N) (Academic Press, 1967)
[15] Moffitt, W, *J Chem Phys*, **25**, 467 (1956)
[16] Yang, J T, in *Poly-α-Amino Acids* (edit by Fasman G D), 270 (Arnold, London, and Dekker, New York, 1967)
[17] Zimm, B H, Doty, P, and Iso, K, *Proc US Nat Acad Sci*, **45**, 1601 (1959)
[18] Sternlicht, H, and Wilson, D, *Biochemistry*, **6**, 288 (1967)
[19] Bradbury, E M, Crane-Robinson, C, and Rattle, H W E, *Symposium on Magnetic Resonance Techniques in Biological Systems*, Tokyo (1967)

Closed Continuous-flow Centrifuge

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The first closed centrifuge has been developed in which it is possible to subject a mixture to an uninterrupted flow and a constant force field while collections of all components are being made. Practical applications of this device are considered

SITUATIONS often arise in the biological sciences when it is desirable to centrifuge large volumes to obtain relatively small fractions For example, the efficient separation of many blood components requires a system which permits removal of selected elements while returning most of the fractions to the donor In a continuous-flow centrifuge of small volume, large amounts of blood or other mixtures could be processed rapidly if it were not necessary to alter the force field or flow during the collection process

A recent collaborative effort of the National Cancer Institute and the Systems Development Division of the International Business Machines Corporation, Endicott, New York, has resulted in an effective, reliable, closed continuous-flow centrifuge primarily designed to separate whole blood into its individual cellular and fluid portions. This machine is the first closed centrifuge in which it is possible to subject a mixture to an uninterrupted flow and a constant force field while collections of all components are in progress. There have been many earlier attempts to develop such a centrifuge The first and remarkably effective approach was the development of the cream separator. In 1878 Carl Patrik Gustaf De Laval[1] of Sweden patented the first open, continuous-flow centrifuge, which not only revolutionized the dairy industry[2,3] but also represented the only completely continuous machine up to the present time

At the beginning of the Second World War there was a need for large quantities of human blood components In this emergency a 'De Laval' model *E*-19 cream separator was adapted by a group at Harvard, under the leadership of J Edwin Cohn, for the separation of plasma from whole blood[4] Subsequently, this machine has been modified and refined for the collection of plasma proteins and cells In making it a closed sterile system, however, the facility for continuous collection was lost[5] Other batch processing centrifuges have been described recently in which large volumes may be handled (Helixtractor Unit, International Equipment Co., Boston, Massachusetts, Continuous Action Rotor, Measuring and Scientific Equipment, Ltd, London), but to collect one of the components the centrifuge must be stopped One machine, a high speed density gradient centrifuge, deserves particular comment because it is capable of continuous flow processing[6] This machine permits high speed centrifugation but must be slowed down to unload the concentrated virus particles, hence the criterion of a continuous collection without alteration of the centrifugal field has not been fully achieved A simple lower speed batch separation centrifuge is reported by Barrett[7] of a method for the concentration of bilharzial meta-cercariae from infected water sources Although these batch separators are very useful, they are inefficient when the components to be separated, such as red blood cells and plasma, are of nearly equal volumes, or if a fraction intermediate in density between two principal components is to be collected

The NCI–IBM blood cell separator was designed to collect or return to the donor platelets, leucocytes, plasma and packed red cells The initial experience has been previously reported[8–10] and since then there have been many refinements in the design and materials

In 1962, shortly after the joint effort between the National Cancer Institute and International Business Machines Corp , Endicott, New York, was undertaken, a prototype centrifuge bowl was designed which consisted of meshed concentric cones This evolved into a device composed of three pieces fashioned of 'Delrin' (E I Dupont de Nemours and Co , Wilmington, Delaware) plastic in a truncated cone configuration The rotating face seal first used was modified from a design already in use by Anderson[11] Anderson's design consisted of a lower rotating half of 'Rulon' (Dixon Corporation, Bristol, Rhode Island), which is a 'Teflon' (E I Dupont de Nemours and Co , Wilmington, Delaware) TFE fluorocarbon compound, and an upper stationary half of stainless steel with a flame-plated tungsten carbide surface Both halves were lapped to a high degree of flatness and were spring-loaded against each other This design was modified by increasing the diameter and adding extra channels The stationary half was stainless steel which was hardened but not flame-plated Flatness was held to 30×10^{-6} in or less This design was not entirely successful and seal failure with resultant haemolysis occurred This problem was ultimately eliminated by the constant infusion of saline under pressure through tiny jets in the raised lands which separated the collecting channels (Fig 1) The 'Rulon' portion of the seal was temperature sensitive and changed shape with use The useful life of the steel–'Rulon' combination was found to be a maximum of 40 h and recently the 'Rulon' portion of the seal has been replaced by an extremely hard ceramic compound In contrast to the 'Rulon', the ceramic seal retains its flatness and has not shown wear after many hundreds of hours of use It is not sensitive to temperature changes.

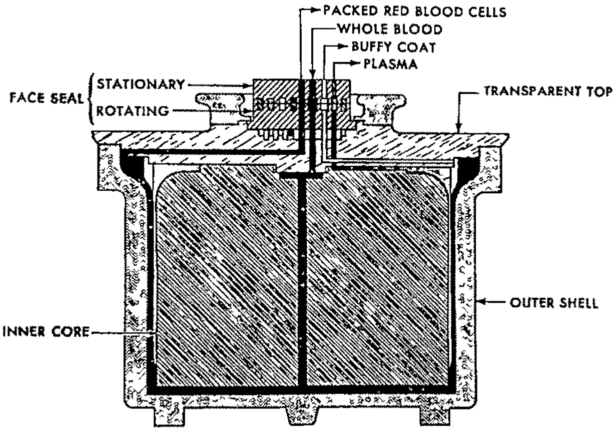

Fig 1

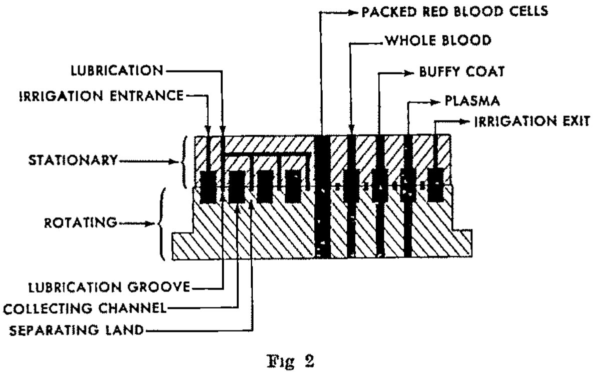

Fig 2

Various centrifuge bowl designs have been evaluated and the most successful, and the one currently used, is shown in Fig 2 This bowl, made of polycarbonate plastic, has a flow path 4 in long and 0 04 in wide with an effective radius of 2 5 in The effect of the length of the flow path on the ability of the centrifuge to separate was evaluated by processing 1-day-old ACD blood in bowls with 2, 3, 4 and 5 in flow paths As the flow was increased from 2–5 in at 50*g* and 40 ml /min flow rate the haematocrit in the red cell line increased from 54 per cent to 66 per cent and the plasma yield increased from 33 per cent to 56 per cent At *g* forces greater than 200, 85 per cent of the plasma was removed using the 4 in flow path bowl It was found that there was very little difference in separation efficiency among bowls with flow path widths of 0 020, 0 040 and 0 080 in

The present machine operates in the following manner blood from a reservoir, artery or vein enters the bowl through a stainless steel fitting in the stationary top half of the rotating face seal Blood then passes through the rotating face seal and down through a central passage in the solid inner core Blood moves peripherally at the bottom and then up the sides of the bowl into the flared portion of the top During this ascent up the sides of the bowl, separation of the blood components occurs Three exit ports, located in the clear bowl cover, permit collection from the bottom of the packed red cell layer, from the top of the plasma layer and from the plasma–red blood cell interface The flow of each separated fraction is controlled by peristaltic roller pumps which can be individually regulated by the operator The position of the buffy coat layer is controlled visually by varying the relative plasma and packed red cell flows The transparent cover allows the operator to observe the separation process and make appropriate adjustments in flow rate and *g* force to obtain the desired separation

Several biological problems are currently being investigated at the National Institutes of Health using the NCI–IBM blood cell separator The first is a study in dogs of the efficiency of buffy coat collection and the effect of centrifugation on the viability of cells[12] The second involves the collection of granulocytes from donors on a continuous flow basis *in vivo* for use in the supportive care of leucopenic recipients The third, an entirely different application, involves the infusion of large amounts of long chain free acids into dogs to study their effects on metabolism

A total of 160 centrifugations ranging from 1–12 h have been performed in dogs, using indwelling arteriovenous shunts[12] A maximum volume of 50 l of blood has been processed in a single centrifugation and a maximum flow rate of 12 l /h has been achieved. Leucocytes and platelets can be concentrated and collected in this system Within 2 h of centrifugation approximately one blood volume of leucocytes and platelets (24×10^9 and 197×10^9, respectively) can be collected in small volume (100 ml) with some red blood cell contamination (haematocrit of 10–15) Leucocyte and platelet concentrations of

$430 \cdot 0 \times 10^3$/mm^3 and $5{,}000 \times 10^3$/mm^3, respectively, have been obtained Up to 55 per cent of the leucocytes and 87 per cent of the platelets entering the centrifuge have been extracted Buffy coat preparations with a preponderance of either lymphocytes or granulocytes can be collected. Detectable damage to erythrocytes and lymphocytes has not been found to result from the centrifugation procedure Platelets seem to be sensitive, however, to mechanical and chemical factors associated with contacting surfaces and aggregation[12]

Preliminary studies in donors with chronic myelocytic leukaemia indicate that 30–60 per cent of the leucocytes entering the centrifuge can be collected in a small volume Donors have tolerated repeated centrifugation of up to 5 h without difficulty Leucocytes collected in this manner result in a rise in white blood count when transfused into acute leukaemia patients with drug induced leucopenia Recovery rates are comparable with those obtained with leucocytes processed by conventional plastic bag techniques

Fatty acid infusion studies in dogs have shown that long chain fatty acids, which result in haemolysis, thrombosis and death when introduced into the blood in the unbound state, are tolerated in large amounts when infused into plasma[13]. Bound to albumin, unesterified fatty acids are the chief form of lipid transport of the body When added to the plasma line of the cell separator before the plasma recombines with the red blood cells, a large quantity of concentrated free fatty acid may be infused into dogs without haemolysis The metabolic consequences of physiologically important quantities of fatty acids are being studied in this way. In addition, this technique provides a potential method for administering fat calories, including essential fatty acids, parenterally Up to 12 g of oleic acid, or nearly 100 calories/h, have been administered without difficulty to dogs of 12 kg

There are many areas of medicine and biological science in which an efficient continuous flow centrifuge could be useful Cells or large particles may be collected at low *g* forces as exemplified in the current use of the machine for the separation of the various cellular components in blood Other applications include the concentration of protozoa or parasites from water, blood or body fluids and the collection of bacteria from fluid culture medium The separation and collection of virus particles, chylomicrons and macromolecules are possible provided the machine is modified to operate at higher speeds The machine could be used for plasma exchange in a variety of clinical situations including hepatic coma, macroglobulinaemia, cryoglobulinaemia and so on, without the need for red blood cell exchange Preliminary work has already begun on a continuous-flow two stage procedure for the collection of platelet concentrates and the precipitation and removal of cryoglobulins Plasma dialysis could be carried out at conditions of pressure, *p*H or osmolarity that would be toxic to cellular elements in the blood

In summary, the evolution of an efficient reliable, continuous-flow centrifuge and its current use in the separation of blood cells in dogs and humans and in the study of fatty acid metabolism are described Possible practical applications of this device in other areas of investigation are outlined

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[1] McDowell, J C , *Ten Master Minds of Dairying*, 29 (Meredith Publishing Co , Des Moines, Iowa, 1930)

[2] Lampard, E E , *The Rise of the Dairy Industry in Wisconsin 1820–1920*, 204 (The State Historical Society of Wisconsin, Madison, Wisconsin, 1963)

[3] Pirtle, T R , *History of the Dairy Industry*, 83 (Mojonnier Bros Co , Chicago, Illinois, 1926)

[4] Cohn, E S , *Advances in Military Medicine*, chap 28, 364 (Little, Brown and Co , 1948)

[5] Tullis, J L , Tinch, R J , Gibson, J G , II, and Baudanza, P , *Transfusion*, 7, 232 (1967)

[6] Cline, G B., Nunley, C E , and Anderson, N G , *Nature*, 212, 487 (1966)

[7] Barrett, P D , and Ellison, I R , *Central African J Med* , 11, 338 (1965)

[8] Freireich, E J , Judson, G , and Levin, R H , *Cancer Res* , 25, 1516 (1965)

[9] Perry, S , Judson, G , and Vogel, J , *Exper Haematol* , 9, 38 (1966).

[10] Vogel, J M , Buckner, C D , and Perry, S , *Conf on Plasmapheresis*, Twentieth Scientific Meeting of Protein Foundation, Inc (1966).

[11] Barringer, H P , Anderson, N G , and Nunley, C E , *NCI Monograph 21, The Development of Zonal Centrifuges*, 191 (1966)

[12] Buckner, D , Eisel, R , and Perry, S , *Blood* (in the press)

[13] Greenough, W B , III, and Steinberg, D , *Clin Res* , 15, 319 (1967)

Counts of Radio Sources at 2,700 MHz

by

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Counts of radio sources from a 2,700 MHz survey made with the Parkes telescope for regions away from the galactic plane have shown a slope for the log *N*–log *S* relationship of –1 4. This is in strong contrast to the slope of –1 8 usually found in counts of radio sources at lower frequencies This low slope at 2,700 MHz throws considerable doubt on the often accepted hypothesis that the high slope for source counts at low frequencies is associated with an evolutionary decrease of the number density or of the level of emission of the radio sources or both.

PART of the motivation of the present survey came from the suggestion by one of us[1] that an alternative explanation of the high slope of the log *N*–log *S* curve may be found in the effects of the red-shift and the wide dispersion in radio source spectra

The survey was made with the 210 ft radio telescope and a new 2,700 MHz receiver[2] which has an input temperature of 80° K and an overall bandwidth of 200 MHz Peak-to-peak noise fluctuations with an output time constant of 2 s are 0 04° K, equivalent to 0 07 flux units The measured beamwidth of the telescope is 7 5 minutes of arc at 2,700 MHz The system can be operated with a backward-looking reference horn or an offset feed horn producing another 7·5 minute arc beam 18 5 minutes of arc off axis The latter mode was used in this survey and the difference between the signals in the on-axis and off-axis beams was recorded

The survey was made in two parts. The first covered an area of about nine hours of right ascension between declinations +4° and –4° and was surveyed by making scans at 2° per min in declination at intervals of 6 minutes of arc in right ascension. Within this region of 0.343 sr, all sources estimated to be stronger than 0.35 flux units were listed and subsequently re-observed for accurate position and flux density. The second part of the survey covered five selected areas of 40 square degrees each where there are two-colour (blue and ultraviolet) 48 in Schmidt plates to help in the identification of quasi-stellar objects. The total area covered by the five plates is 0.067 sr. Areas of 50 square degrees centred on the plate centres were scanned at 1° per min with scan intervals of 4.5 minutes of arc. The areas were scanned both in right ascension and in declination in order to reduce the possibility of confusion and to improve the signal-to-noise ratio. All sources estimated to be greater than 0.06 flux units were listed and subsequently re-observed for accurate position and flux density. As there is a bias in favour of detecting the stronger sources near the edge of any survey area, only sources within the smaller area of the two colour plates were used in constructing the log N–log S curve.

The purpose of making the survey in two parts with different levels of sensitivity was to provide source counts of similar statistical significance over a wide range of the log N–log S diagram. In the large survey area, 135 sources of flux density $\geqslant 0.4$ flux units were found and in the small survey area 210 sources of flux density $\geqslant 0.08$ flux units were found. The corresponding numbers per sr are 390 for the large and 3,100 for the small survey area. The composite log N–log S curve from the two areas is shown in Fig. 1. The straight line drawn through the observed points has a slope of -1.38, the statistical fluctuation errors would, however, allow limiting slopes of -1.30 and -1.50 to be fitted to the observations.

Confusion errors are believed to be negligibly small, because even for the five selected areas the number of effective beam areas per source is greater than 50. It is thought that errors due to angular extent and polarization of the sources are also negligible. Random errors of flux densities due to noise fluctuations are less than 0.02 flux units. Random errors of any kind will tend to increase rather than decrease the slope of the log N–log S curve.

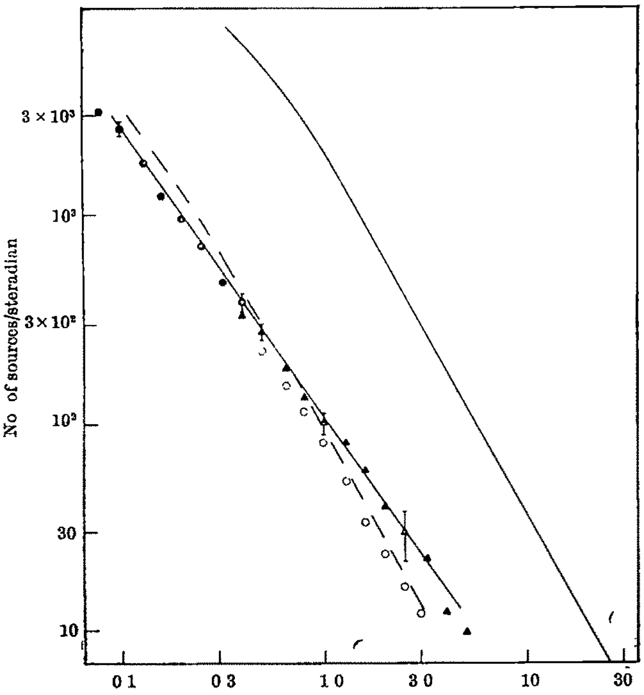

Fig. 1. Cumulative counts of radio sources as a function of flux density. ▲, 2,700 MHz survey of 0.34 sr; ●, 2,700 MHz survey of 0.067 sr; ○, counts of sources from the Parkes catalogue measured at 2,700 MHz. Solid line to right—counts from *3CR*, *4C* and *5C* surveys at 178 MHz after Gower. Dashed line is the expected count at 2,700 MHz obtained by convolving Gower's curve with the spectral index distribution for strong sources at 178 MHz.

In Fig. 1, the 2,700 MHz survey results are compared with source counts from the Parkes catalogues and the Cambridge catalogues. Data from the former, which were compiled at 408 MHz, have been restricted to about half the area of the 0° to +20° and 0° to −20° zones by excluding those regions where galactic features such as the North Polar Spur and the Cetus Arc influence the detection of weak sources. The apparent slope of the source counts at 408 MHz is -1.95, corrections for random errors in the measured flux densities and possible over-estimation of the flux densities of weaker sources would reduce the slope to ~ -1.85. For these sources, flux densities at 2,650 MHz are available, and their log N–log S relationship at this frequency is shown by the open circles in Fig. 1. Its slope of ~ -1.6 is significantly lower than the value at 408 MHz, the important result, however, is that the counts fall well below the counts from the 2,700 MHz survey, particularly at the higher flux densities. Most of the sources which are present in the 2,700 MHz survey, but missing from the Parkes catalogue, must have relatively flat spectra.

A similar result is apparent from a comparison of the results of the 2,700 MHz survey and the Cambridge surveys at 178 MHz. The composite curve due to Gower[3] for the source counts from the *3C* revised, *4C* and *5C* surveys is shown on the right-hand side of Fig. 1. Unfortunately, flux densities near 2,700 MHz are not available for these sources. We have therefore computed the expected source counts at 2,700 MHz by convolving Gower's results with the distribution of spectral indices given by Long *et al.*[4] for sources in the Cambridge catalogues with flux densities $\geqslant 5.0$ flux units. This is shown as a dashed line in Fig. 1. Relative to the observed counts at 2,700 MHz, the predicted counts show a marked deficiency for high flux densities and a slight excess at low flux densities.

We have, then, slopes for the log N–log S relationship down to a source density of 200 sr of -1.8 at 178 MHz, -1.85 ± 0.1 at 408 MHz, and -1.4 ± 0.1 at 2,700 MHz. Implicit in these differing slopes is a change in mean effective spectral index and/or the dispersion in spectral indices with flux density. For example, we can reproduce the 178 MHz counts from the 2,700 MHz counts by assuming a Gaussian distribution of spectral indices of standard deviation 0.17 (as found by Long *et al.* for *4C* catalogue sources stronger than 5 flux units (ref. 4)) with the mean effective spectral index given by

$$\bar{\alpha}_{2,700/178} = 0.56 - 0.179 \log S_{2,700} \qquad (1)$$

The 408 MHz counts may be reproduced from a similar expression.

Fig. 2 shows the 2,700 MHz survey sources on a diagram of $S_{2,700}$ against S_{178}. Here the 45° dashed lines are lines of constant effective spectral index over this frequency range. Sources which are not in the *4C* catalogue have been shown as having an upper limit of $S_{178} = 2.5$ flux units. Equation (1) is plotted as a dotted line, and detailed inspection indicates that it gives a reasonable representation of the trend in mean effective spectral index between 2,700 MHz and 178 MHz for these sources.

The transformation represented by (1), although effective, does not properly take into account the observed distribution of source spectra. Preliminary results from measurements at 468 MHz and 1,402 MHz with the Parkes interferometer indicate that as many as one-third of the 2,700 MHz survey sources which are not in the *4C* catalogue have inverted spectra. One example is Parkes 2,134+004, which has a flux density of 7·5 flux units at 2,700 MHz but for which the extrapolated flux density at 178 MHz is $\leqslant 0.2$ flux units—that is, below the limit of the fifth Cambridge catalogue.

In deriving the 2,700 MHz counts from the 178 MHz log N–log S curve, an apparent difficulty is encountered

The requisite changes with flux density in mean spectral index (for example, in accord with the relation reciprocal to (1)) and/or dispersion in spectral indices seem to be excluded by spectral measures of the 4*C* sources. Long *et al.*[4] have measured spectra in the frequency range 38 MHz to 1,410 MHz for 4*C* catalogue sources of $S_{178} \geqslant 5$ flux units, and Stewart and Long[5] have carried out the same measurements for a sample of 4*C* sources for which 2 flux units $\geqslant S_{178} \geqslant 5$ flux units. Both find the same mean spectral index of 0·75. Long *et al.* obtain a standard deviation for the spectral index distribution of 0·17, whereas Stewart and Long obtain the somewhat higher value of 0·25. Stewart and Long claim that the increase may be the result of experimental errors due to the lower flux densities involved. An examination of their Fig. 2, however, reveals a markedly different distribution of the spectral indices from that given in Fig. 1 of Long *et al.*, and we feel that the higher dispersion is likely to be real. A more important point, however, is that spectral indices in the range 38 to 1,410 MHz are not characteristic of effective spectral indices in the range 178 MHz to 2,700 MHz. A study of a large sample of sources in the Parkes catalogue has shown that the straight power law spectrum below 1,400 MHz exhibited by the majority of sources frequently does not continue to higher frequencies. For many radio galaxies the spectrum steepens, for many quasi-stellar objects it flattens, and not infrequently the slope reverses.

The reconciliation of the differing slopes for the log *N*–log *S* relationships at 178 and 2,700 MHz in terms of the spectral content of the surveys must therefore await complete investigation of the spectra of sources in both catalogues over this frequency range. It is evident from our preliminary investigations that the first requirement for this reconciliation—a difference in spectral content for the two catalogues—is indeed present.

The difference in spectral content is paralleled by a difference in identification content. The positions of all sources in the 2,700 MHz survey have been examined on the Palomar Sky Survey prints, and suggested identifications of optical counterparts for many of the sources have been made (unpublished results, J. V. Wall, J. K. Merkelijn and A. J. Shimmins). In Fig. 2, different symbols are used for identified galaxies, confirmed and possible quasi-stellar objects, and unidentified sources. It is apparent from this diagram that the identification content of the stronger 2,700 MHz sources is quite different from that of the stronger 178 MHz sources. For sources common to the 2,700 MHz and 178 MHz catalogues the ratio of galaxies to QSOs is 5 : 4. For sources not in the 4*C* catalogue the ratio is 1 : 10.

A further result from Fig. 2 is the clear distinction between the spectra of the radio galaxies and the QSOs which has previously been pointed out by two of us[6]. For sources with mean spectral index less than 0·4 the identifications are exclusively QSOs, between 0·4 and 0·6 the ratio of QSOs to galaxies is 2 : 1. Objects with spectral indices greater than 0·8 are almost exclusively galaxies. It seems that the unidentified sources lie in the region of the diagram occupied primarily by galaxies, which substantiates the argument[6] that the majority of unidentified sources are galaxies beyond the Palomar Sky Survey plate limit.

The question naturally arises as to which log *N*–log *S* slope is to be used in cosmological discussions. It has often been suggested that the evolutionary sequence for radio sources is such that the spectra steepen with age. On this basis, the more distant sources should have flatter spectra, and high frequency surveys with a selection bias in favour of flat spectrum objects might be more likely to reveal evolutionary effects. Another point in favour of high frequency source counts is that the flux density at a high frequency is more representative of a bolometric magnitude or integrated power than that at a low frequency. It is now known that there is a very large range in the form of radio source spectra, and, consequently, source counts in terms of flux density may not be valid substitutes for counts in terms of source power. Furthermore, the same source selection effects that produce the log *N*–log *S* slope at a particular frequency will also influence the identification content and the deductions from it. For example, from the quasi-stellar objects in the revised 3*C* catalogue, Schmidt[7] finds that the space density of QSOs at $z = 1$ is approximately a hundred times that of their local space density. A similar variation with epoch of the luminosity of one class of radio source is required by Longair's investigation[8] of the revised 3*C* sources. It has been shown here that the ratio of different types of radio sources in the 2,700 MHz survey departs radically from that of the revised 3*C* catalogue. It will be of great interest to see whether Schmidt's result for the 3*C* QSOs persists in the present sample when their red-shifts become available.

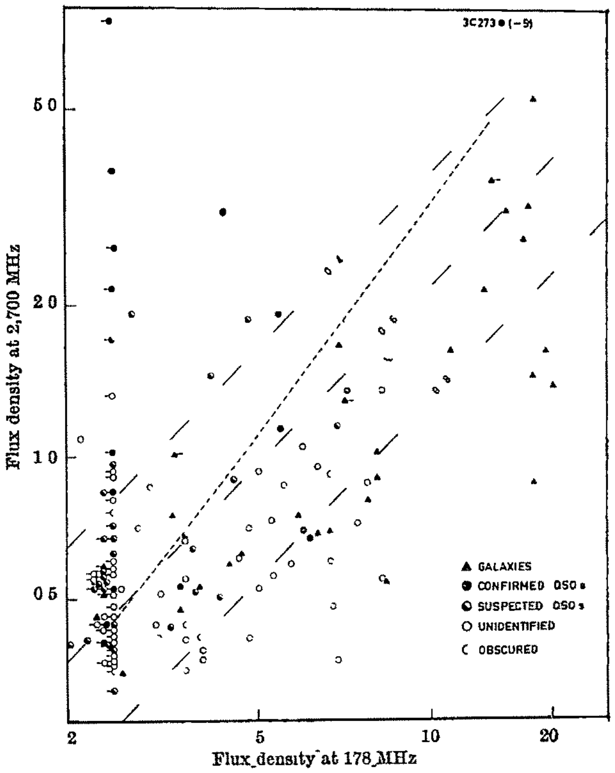

Fig. 2. The flux density at 2,700 MHz against the flux density at 178 MHz for sources from the 2,700 MHz survey of 0·34 sr. Different symbols indicate the identification class. 45° lines are lines of constant spectral index and the dotted line represents $\bar{\alpha}_{2\,700/178} = 0{\cdot}56 - 0{\cdot}197 \log S_{2,700}$. Sources not in the 4*C* catalogue are indicated as if they had an upper limit of 2·5 flux units at 178 MHz. Galaxies which are probably resolved in the 4*C* observations are indicated with the 178 MHz flux as a lower limit.

Finally, in view of the doubts raised on the interpretation of the slopes of the number–flux density relationships for the radio sources, their value as evidence against the steady state cosmology may be questioned.

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[1] Bolton, J. G., Texas Conference on Relativistic Astrophysics, New York (1967).
[2] Batchelor, R. A., Brooks, J. W., and Cooper, B. F. C., *IEEE Trans. on Antennas and Propagation* (in the press).
[3] Gower, J. F. R., *Mon. Not. Roy. Astro. Soc.*, 133, 151 (1966).
[4] Long, R. J., Smith, M. A., Stewart, P., and Williams, P. J. S., *Mon. Not. Roy. Astro. Soc.*, 134, 371 (1966).
[5] Stewart, P., and Long, R. J., *Mon. Not. Roy. Astro. Soc.*, 135, 361 (1967).
[6] Bolton, J. G., *Nature*, 211, 917 (1966).
[7] Schmidt, M., Intern. Astro. Union General Assembly, Prague (1967).
[8] Longair, M. S., *Mon. Not. Roy. Astro. Soc.*, 133, 401 (1966).

Purification of Antilymphocytic Antibody

by

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By elution from human lymphocytes it has been possible to obtain antilymphocytic antibody of much greater purity than before

NEARLY all the antilymphocytic activity of horse-anti-human antilymphocytic serum (ALS) prepared by chronic immunization is concentrated in the IgG fraction but, as reported previously[1], in our preparations of antilymphocytic IgG (ALS IgG) only a small proportion (usually less than 1 per cent) of the molecules are effectively antilymphocytic. In the experiments reported here, antilymphocytic antibody of much greater purity has been prepared by elution from human lymphocytes previously incubated *in vitro* with either ALG or crude ALS.

Several methods of elution, including exposure for various times to buffered solutions of differing *p*H at 37° C and 56° C, and also to high concentrations of urea, were investigated in preliminary trials, and it seemed that the most promising method was exposure for about 20 min to acid media in the *p*H range 2·5 to 4·0.

In the first of three experiments, 250 million human peripheral blood lymphocytes, prepared as described before[2], were incubated for 1·5 h at 37° C in 25 ml of medium (tissue culture medium 199 with 20 per cent autologous inactivated plasma) to which was added 4 ml of a solution containing 35 mg of ALS IgG which had been labelled with iodine-131 by the method of Hunter and Greenwood[3]. The cells were spun down, washed once with McIlvain citric acid–phosphate buffer at *p*H 7·2, resuspended, distributed equally between four counting tubes, spun down again and, after the supernatants had been separated, counted for iodine-131 in a well-type scintillation spectrometer (Nuclear Enterprises 'Gammamatic'). The sum of the four cell counts was 2,432 c.p.s. and a sample of the ALS IgG counted at the same time yielded a count of 14,500 c.p.s./mg. If all the IgG molecules were labelled the mean number of molecules taken up per cell was thus

$$\frac{2{,}432\ N}{14{,}500\ 10^3\ 250\ 10^6\ m} = 2\cdot51 \text{ million}$$

where N is Avogadro's number $\simeq$ $6\ 10^{23}$ and m is the molecular weight of IgG $\simeq$ 160,000.

The cells in each tube were then resuspended in 3 ml of eluting medium, consisting of McIlvain citric acid–phosphate buffer at *p*H 2·5, 3, 3·5 and 3 in tubes 1–4, respectively, and transferred to miniature (10 ml) Erlenmeyer flasks. Flasks 1–3 were placed on a mechanical shaker in a water bath at 37° C, flask 4 was placed on a similar shaker in a water bath at 56° C. After 20 min the suspensions were transferred to tubes and centrifuged; cells and eluates were then separated and counted for iodine-131, after which the eluates were brought to *p*H 7·2 by the addition of 1 N sodium hydroxide and dialysed overnight at 4° C against medium 199. The volume of each eluate was then made up to 4 ml with medium 199.

The proportion of adsorbed protein eluted in each tube and the protein content of each eluate were calculated from the iodine-131 counts. The agglutinin and cytotoxic titres^{-1} of a solution of the original labelled ALS IgG containing 0·875 g/100 ml were determined in the usual way and found to be 1,024 and 512, respectively. The titres^{-1} of each eluate were similarly determined (Table 1). The lymphocyte binding and lymphocyte stimulating activity of the eluted antibody was investigated by adding 1·4 ml of each eluate to human lymphocyte cultures[1,2] containing tritiated uridine and measuring the amount of iodine-131 and tritium taken up by the cells. Control cultures were set up, some containing various amounts of the original labelled ALS IgG, others without the addition of any antilymphocytic antibody. After 24 h the cells were prepared for liquid scintillation counting and counted with a Packard 'Tricarb' scintillometer using two channels, one adjusted for iodine-131 and the other for tritium. The iodine-131 and tritium activity in each culture was then calculated as described before[1], due allowance being made for the influence of iodine-131 on the count in the tritium channel. All cultures were in duplicate and the ratio of the mean tritium activity in a pair of cultures with added ALG or eluate to the mean tritium activity of the controls is referred to hereafter as the mean relative tritium activity. The control cultures were used to establish dose response curves (Fig. 1) relating the mean number of IgG molecules taken up per cell and the mean relative tritium activity to the amount of ^{131}I-labelled ALS IgG in the culture. The measured uptake of IgG per cell and mean relative tritium activity in the cultures containing eluted antibody are set out in Table 1. The amounts (E_I and E_H) of the original labelled ALS IgG which would produce the observed values of iodine-131 uptake and mean relative tritium activity have been read off from Fig. 1 and are shown in the same table. This table also shows values, calculated as described later, for the proportions of agglutinating, cytotoxic, lymphocyte binding and

Table 1 ELUTION OF ANTILYMPHOCYTIC ANTIBODY FROM LYMPHOCYTES EXPOSED TO ^{131}I-LABELLED ALS IgG

	Eluate				Agglutinating activity				Lymphocyte binding activity*				Lymphocyte stimulating activity*			
No	Conditions of elution *p*H	°C	Protein content (mg/100 ml)	Proportion of adsorbed antibody eluted (%)	Titre^{-1}	Proportion recovered (%)	Relative activity of eluted antibody	Cytotoxic titre^{-1}	Mean No molecules IgG taken up per lymphocyte (millions)	E_I	Proportion recovered (%)	Relative activity of eluted antibody	Mean relative tritium activity	E_H	Proportion recovered (%)	Relative activity of eluted antibody
1	2·5	37	0·76	81	64	12	36	<4	0·31	0·10	3·2	37	1·33	0·07	2·3	27
2	3·0	37	0·49	49	64	12	56	<4	0·23	0·08	2·6	47	2·78	0·36	11·7	209
3	3·5	37	0·43	49	32	12	64	<4	0·34	0·11	3·6	73	4·35	0·46	15·0	304
4	3·0	56	0·57	49	16	6	24	<4	0·43	0·14	4·5	69	0·82	0	0	0

* Based on observations with cultures containing 1·4 ml eluate obtained from 3 g of labelled ALS IgG.

E_I and E_H denote the amounts of the original labelled ALS IgG which, from Fig. 1, would produce the observed uptake of iodine-131 and the observed mean relative tritium activity, respectively.

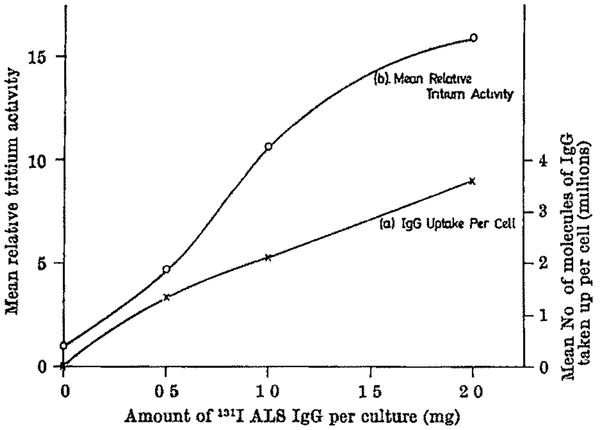

Fig 1 Dose response curves for human lymphocyte cultures containing tritiated uridine and ^{131}I-labelled ALS IgG relating (*a*) the number of IgG molecules taken up per cell and (*b*) the relative tritium activity to the amount of labelled IgG per culture

lymphocyte stimulating activity recovered by the combined adsorption–elution procedures, and the relative activity of each eluate as compared with the original labelled ALS IgG in respect of the same four properties

The proportions of agglutinating and cytotoxic activity recovered are clearly given by $\frac{T_A'}{T_A}$ V' and $\frac{T_C'}{T_C}.V'$, respectively, and the corresponding relative activities by $\frac{T_A'}{T_A}.\frac{P}{P'}$ and $\frac{T_C'}{T_C}$ $\frac{P}{P'}$, where T_A', T_C', P' denote, respectively, the reciprocal agglutinin and cytotoxic titres and the protein concentration of the eluate, T_A, T_C, P the reciprocal agglutinin and cytotoxic titres and the protein concentration of the original labelled ALS IgG, V' the volume of eluate per unit volume of the original labelled ALS IgG solution The proportions of lymphocyte binding and lymphocyte stimulating activity recovered might be defined, by analogy, as $\frac{E_I}{G}$ and $\frac{E_H}{G}$, respectively, and the corresponding relative activities by $\frac{E_I}{G}$ $\frac{P}{P'}$ and $\frac{E_H}{G}$ $\frac{P}{P'}$, where E_I and E_H have the meanings already ascribed, and G ($=3$ mg) denotes the amount of original labelled ALS IgG from which the eluted antibody in one culture was obtained These definitions must, however, be regarded as purely operational because, as will be seen later, different values are obtained if the conditions of assay are changed by altering the amount of eluted protein per culture

It will be seen that, of the procedures tried, elution at 37° C in a medium of *p*H 3 0 or 3 5 gave the biggest yield of antibody as judged by agglutinating activity, lymphocyte binding activity and lymphocyte stimulating activity At *p*H 2 5 (37° C) more protein (strictly speaking, more label) was eluted, but this gain did not compensate for the loss of activity, at 56° C (*p*H 3 0) the amount of protein eluted was the same as at 37° C (*p*H 3 0) and its capacity to bind to lymphocytes was relatively high, but stimulating activity was completely lost

The object of the second experiment was to investigate the possibility of obtaining purified antilymphocytic antibody by using crude ALS instead of ALS IgG as the starting point for the adsorption–elution procedure The ALS was obtained from the same chronically immunized horse which provided the serum from which the ALS IgG used in the first experiment was prepared It was inactivated by heating to 56° C for 30 min and then absorbed with one-third its volume of packed human AB red cell stroma, as a result of which the haemagglutinin titre^{-1} was reduced from 2,048 to 8. The agglutinin and cytotoxin titres^{-1} of the serum were both 1,024, and the IgG content was approximately 1 g/100 ml

Two hundred million human peripheral blood lymphocytes suspended in 20 ml medium 199 were mixed with 20 ml of ALS and incubated for 1 5 h at 37° C in a 500 ml Erlenmeyer flask, with gentle shaking for 2 min every 10 min The suspension was then distributed equally between four tubes, after which the cells were spun down, washed once and resuspended in 3 ml of eluting medium consisting of McIlvain citric acid–phosphate buffer of *p*H 2 5, 3 0, 3 5 and 4 0 in tubes 1–4, respectively Elution was carried out for 20 min in miniature Erlenmeyer flasks as in the first experiment, the only difference being that all the flasks were kept at 37° C After separation of the cells the eluates were brought to *p*H 7 2 by the addition of 1 N sodium hydroxide and dialysed overnight at 4° C against medium 199. The volume of each eluate was then made up to 5 ml with medium 199

Lymphocyte cultures containing tritiated uridine were set up in duplicate to provide dose response curves relating lymphocyte stimulating activity (as judged by uridine uptake) to the amount of ALS or eluate added to the culture, using doses of 0 05, 0 1 and 0 2 ml. of ALS, and 0 3, 0 6 and 1 2 ml of eluate The lymphocyte agglutinin and cytotoxin titres^{-1} of each eluate were determined in the usual way, and the proportions of agglutinating and cytotoxic activity recovered by the combined adsorption–elution procedure were calculated as in the first experiment Values have also been calculated for $\frac{E_H}{G}$, which was defined earlier as the proportion of lymphocyte stimulating activity recovered, as already mentioned, however, the values obtained for each eluate depend on the amount added to each culture The results are summarized in Fig 2 and in Table 2, which shows for each eluate the proportions of agglutinating and cytotoxic activity recovered, and the values of $\frac{E_H}{G}$ at two doses of eluate per culture, namely, 1 2 and 0 6 ml , corresponding to the amounts obtained from quantities of ALS containing 12 mg and 6 mg of IgG, respectively

Once again, of the procedures tried, elution at 37° C in a medium of *p*H 3 0 or 3 5 gave the biggest yield of antibody as judged by agglutinating and stimulating activity As in the first experiment, very little cytotoxic activity was recovered in any of the eluates

A third experiment was performed to determine whether the recovery of antibody from ALS at a *p*H between 3 0 and 3 5 might be improved (*a*) by carrying out the elution in two stages and (*b*) by adding human serum protein to the eluate before dialysis to protect against denaturation Three hundred and twenty million human peripheral blood lymphocytes suspended in 30 ml of medium 199 were mixed with 32 ml of ALS and incubated

Table 2 ELUTION OF ANTILYMPHOCYTIC ANTIBODY AT VARIOUS *p*H FROM LYMPHOCYTES EXPOSED TO ALS

No	Eluate		Agglutinating activity		Cytotoxic activity		E_H/G (Proportion of lymphocyte stimulating activity recovered)	
	Conditions of elution *p*H	°C	Titre^{-1}	Proportion recovered (%)	Titre^{-1}	Proportion recovered (%)	Values obtained in tests with 1 2 ml of eluate per culture	Values obtained in tests with 0 6 ml of eluate per culture
1	2 5	37	32	3	4	1	4 0	1 7
2	3 0	37	128	12	4	1	25 0	4 2
3	3 5	37	128	12	8	2	26 7	3 7
4	4 0	37	4	<1	4	1	2 0	2 3

as before for 1·5 h at 37° C. The suspension was then transferred to a large (100 ml.) tube and centrifuged The supernatant was separated and stored overnight at 4° C The cells were washed once, resuspended in 5 ml of McIlvain citric acid–phosphate buffer, *p*H 3·3, and transferred to a small Erlenmeyer flask which was shaken in a water bath at 37° C After 10 min, the suspension was transferred to a small tube and centrifuged The eluate was recovered, brought to a *p*H of approximately 6·0 by the addition of an equal volume of 0·2 M disodium phosphate and cooled to just above 0° C The cells were resuspended in 5 ml. of buffer at *p*H 3·3, and transferred back to the small Erlenmeyer flask in the water bath at 37° C After a further 15 min the eluate was again separated, brought to *p*H 6·0 and cooled, and the cells were resuspended in 5 ml of fresh buffer The two lots of eluate were thoroughly mixed Half of the composite eluate was mixed with 4 ml of inactivated human plasma and then simultaneously concentrated by ultrafiltration and dialysed overnight at 4° C against phosphate buffer, *p*H 7·2, to yield preparation *A*, the other half was similarly dialysed without previous addition of plasma to yield preparation *B* Both preparations were thus reduced in volume to slightly less than 4 ml and later made up to 4·0 ml by the addition of phosphate buffer, so that the volume of each preparation was one-quarter the volume of ALS from which it was derived, and preparation *A* was approximately equivalent to human plasma in respect to the content of human protein The nitrogen content of each sample of preparation *B* was determined by a micro-Kjeldahl procedure and found to be 52·6 µg/ml, corresponding to approximately 33 mg of protein/100 ml The lymphocyte agglutinating, cytotoxic and stimulating activity of these preparations, and also of the ALS before and after absorption with human lymphocytes, were measured in the usual way and the results are shown in Table 3 and Fig 3. It would seem that the two stage elution procedure has improved the yield of agglutinating antibody. This is most apparent in eluate *A* containing human plasma, but the difference between the two eluates depends on one tube in the agglutination test and cannot be regarded as significant Little or no cytotoxic activity was demonstrated in either eluate The uridine uptake curves are virtually identical for both, and are similar in form to those obtained with eluates 2 and 3 of the previous experiment, the recovery of stimulating activity also appears to be much the same as before if allowance is made for the four-fold concentration of eluates *A* and *B*.

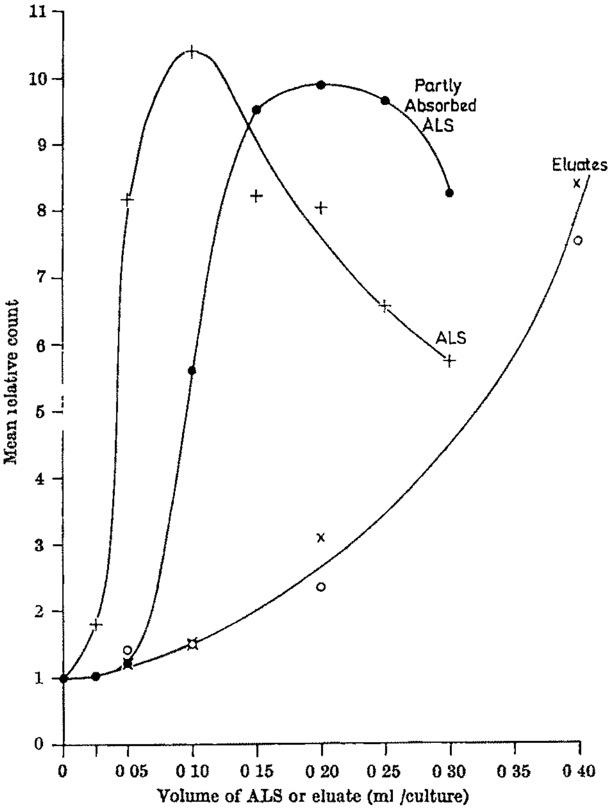

Fig 3 Dose response curves for human lymphocyte cultures containing tritiated uridine and either unabsorbed ALS, ALS partly absorbed with human lymphocytes or eluted antibody The curves relate the mean relative tritium count to the amount of ALS or eluate per culture See text (third experiment) for conditions of elution Because the points (× for eluate *A* and ○ for eluate *B*) for the two eluates fall so closely together, a single curve has been drawn to fit them

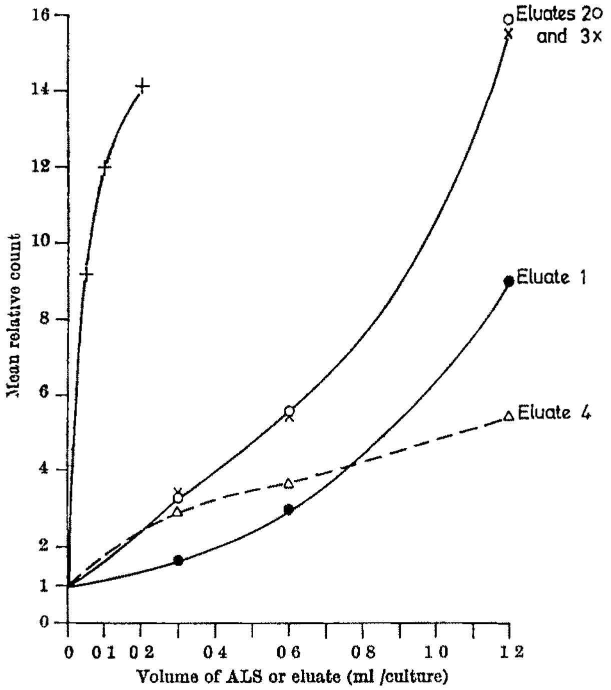

Fig 2 Dose response curves for human lymphocyte cultures containing tritiated uridine and either ALS or eluted antibody relating the mean relative tritium count to the amount of ALS or eluate per culture See text (second experiment) for conditions of elution Because the points for eluates 2 and 3 fall so closely together, a single curve has been drawn to fit them

There are two features of the results which would seem to merit special comment First, it is noteworthy that preparations have been obtained which are extraordinarily potent in respect of lymphocyte agglutinating and stimulating activity but which have little or no demonstrable cytotoxic activity In these respects, the purified antibody resembles[2] the divalent antibody fragment F(ab′)$_2$ which is obtained when the complement binding part of the IgG molecule is destroyed by digestion with pepsin It seemed possible that in the present elution experiments exposure to acid might have caused the loss of cytotoxic activity either by selectively destroying a subpopulation of antibody molecules responsible for such activity or by damaging the complement binding sites of IgG molecules in general

To test this a solution of ALS IgG was acidified to *p*H 3

Table 3 ELUTION OF ANTILYMPHOCYTIC ANTIBODY (TWO STAGE PROCEDURE) AT *p*H 3·3

Preparation	Protein content: Horse IgG (mg/100 ml.)	Protein content: Human plasma proteins	Agglutinating activity: Titre^{-1}	Agglutinating activity: Proportion recovered (%)	Cytotoxic titre^{-1}
Original ALS	Approx 1,000	Nil	1,024		1,024
Absorbed ALS	Not measured	Nil	512		512
Eluate *A*	33	As normal human plasma	2,048	50	< 4
Eluate *B*	33*	Nil	1,024	25	< 4

* The N content by micro-Kjeldahl was 52·6 µg./ml

with 0.1 M citric acid and incubated at 37° C for 20 min. The solution was then brought to pH 6 by the addition of 0.2 M disodium hydrogen phosphate and dialysed overnight against phosphate buffer, pH 7.2, at 4° C. During the course of the dialysis the volume was reduced to that of the original ALS IgG solution by ultrafiltration. This "acid-treated IgG" was found to possess the same lymphocyte agglutinating activity as the IgG from which it was prepared ($\text{titre}^{-1} = 4{,}096$ in each case), but the cytotoxic titre^{-1} was reduced by the treatment from 2,048 to 64. The lymphocyte stimulating activity as judged by uridine uptake in lymphocyte cultures devoid of complement was only slightly reduced by the treatment (Fig. 4*a* and *b*); on the other hand, in cultures containing complement[2] the treated IgG caused greater stimulation than the untreated IgG. It therefore seems clear that cytotoxic activity is selectively reduced, as compared with agglutinating or lymphocyte stimulating activity, by exposure to pH 3 at 37° C. Loss of cytotoxic activity thus seems inevitable with the method of elution used in the present experiments.

Because, as already reported[4], the immunosuppressive capacity of $F(ab')_2$ is small in comparison with that of the ALS IgG from which it is prepared, it would be interesting to measure the immunosuppressive capacity of acid-treated or eluted antibody. This is being undertaken in rats with purified antibody prepared from horse-anti-rat ALS.

Second, the dose response curves relating uridine uptake by lymphocytes in culture to the amount of antibody in the medium given by antibody eluted at optimal pH are markedly different in form from those obtained with either crude ALS or ALS IgG. Thus at a level of uridine uptake which is maximal or near-maximal for cells exposed to ALS or ALS IgG, the slope of the curve for eluted antibody is both steep and increasing.

Some light on possible reasons for this difference is provided by the data obtained with ^{131}I-labelled antibody. In previous observations[1] with human lymphocyte cultures containing tritiated uridine and ^{131}I-labelled ALS IgG it was found that there was an approximately linear relationship between the relative tritium activity and the number of IgG molecules taken up per cell, in so far as there was any departure from linearity a sigmoid curve appeared to give the best fit. It will be seen from Fig. 5, plotted from the data presented more fully in Fig. 1, that in the first experiment of those now reported there is the same sort of relationship between tritium activity and IgG uptake in cultures treated with unpurified ALS IgG, that is, a straight line fits the data approximately but a sigmoid curve is better. The effect of the eluates was observed at only one dose level for each, it will be seen, however, that the points for the active eluates (2 and 3) lie well to the left of the straight line fitted to the points obtained with unpurified IgG and *a fortiori* to the left of the sigmoid curve. While it would be unwise to seem to draw far-reaching conclusions from these two observations, they do at least suggest that it may be necessary to make a further revision of our hypothesis concerning the heterogeneity of antilymphocytic IgG molecules. We began by postulating that ALS IgG contains only two classes of molecule, namely, antilymphocytic molecules which bind to and stimulate lymphocytes and non-antilymphocytic molecules which neither bind nor stimulate. It became apparent that this was an over-simplification and that antilymphocytic IgG molecules formed a heterogeneous population, the previous experimental findings could, however, be accounted for by postulating heterogeneity in respect of avidity only and retaining the assumption that all IgG molecules which become attached to a lymphocyte exert an equal stimulating effect. We have now at least a suggestion that individual antilymphocytic IgG molecules may differ also in respect of their stimulating capacity.

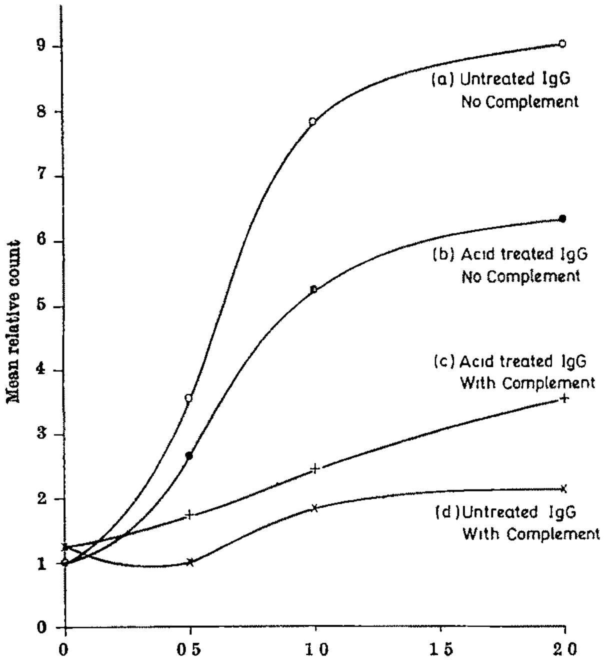

Fig. 4. Dose response curves for human lymphocyte cultures containing either untreated ALS IgG (*a*, *d*) or ALS IgG which had been exposed to pH 3.0 for 20 min at 37° C (*b*, *c*). Curves *a* and *b* relate to cultures set up in the usual way in a medium consisting of medium 199 with 20 per cent heat-inactivated autologous plasma. Curves *c* and *d* relate to cultures containing 20 per cent unheated autologous plasma. This was added after the ALS IgG.

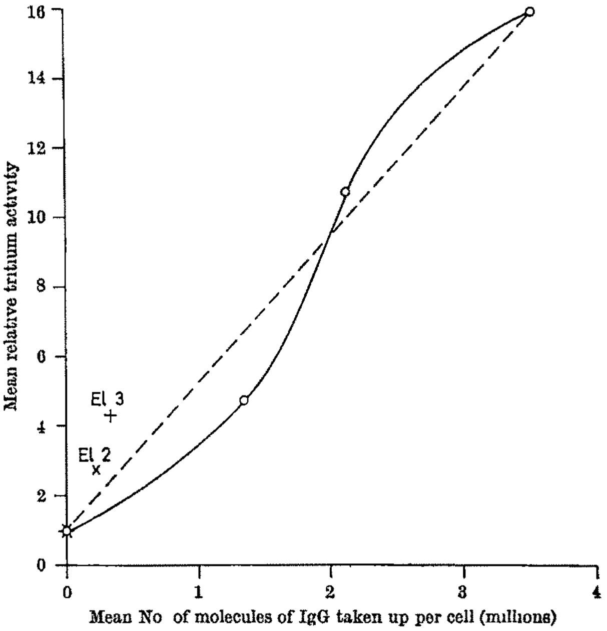

Fig. 5. Relationship between mean relative tritium activity and the number of molecules of ^{131}I-labelled antibody taken up per cell. The points connected by the sigmoid curve are based on data presented more fully in Fig. 1 and relate to cultures containing unpurified ALS IgG. The isolated points marked *El* 2 and *El* 3 relate to cultures containing eluted labelled antibody (eluates 2 and 3 of Table 1).

I thank Dr Keith James for helpful suggestions and for preparing and labelling the IgG used in the first experiment. I also thank W. McBride for performing the micro-Kjeldahl determinations and Mr George Brooks for technical assistance.

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[1] Woodruff, M. F. A., Reid, B. L., and James, K., *Nature*, 216, 758 (1967).
[2] Woodruff, M. F. A., Reid, B. L., and James, K., *Nature*, 215, 591 (1967).
[3] Hunter, W. M., and Greenwood, F. C., *Nature*, 194, 495 (1962).
[4] Anderson, N. F., James, K., and Woodruff, M. F. A., *Lancet*, i, 1126 (1967).

New Controlling Element in the *Lac* Operon of *E. coli*

by
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The *lac* promoter maps between the repressor *i* gene and the operator *o* gene, so that the operator gene is probably transcribed.

THE genetic elements (*lac*) determining lactose metabolism in *E. coli* map very close together in a small region of the chromosome (Fig. 1)[1]. Three structural genes, *z* (for β-galactosidase), *y* (for galactoside permease) and *a* (for thiogalactoside transacetylase), are regulated by the repressor product of the *i* gene. In the absence of β-galactoside inducers of the system, the repressor interacts with the *lac* operator (*o*) to prevent synthesis of the three proteins. Jacob and Monod have suggested that repression involves an inhibition of transcription of *lac* operon DNA into a messenger-RNA (*m*RNA) copy[1]. This model for regulation has recently received strong support from the experiments of Gilbert and Muller-Hill, who have isolated the repressor protein and have found that it binds specifically to *lac* operator double-stranded DNA[2]. Thus it seems likely that this repressor–operator complex prevents transcription of the *lac* operon.

There is genetic evidence indicating that it is not the operator itself which serves as a transcription initiation site. Whereas one would expect mutations in an initiation site to alter the potential for operon expression, operator-constitutive (o^c) mutations, including some well characterized deletions (unpublished results of Davies and Jacob), do not affect the rate at which the operon can be expressed[3]. Such an initiation site must therefore lie outside the operator, either between *i* and *o* or between *o* and *z*. Jacob, Ullman and Monod[3] have proposed that such a site, the promoter (*p*), maps between *o* and *z*.

We have reported the isolation and characterization of mutants which have all the properties of mutants in an initiation site of the *lac* operon[4]. These mutants, which reduce the maximum rate of *lac* operon expression, do not alter either the operator or the *i* gene. On the basis of genetic mapping, we had concluded that these mutants altered a site between *o* and *z*, possibly identical to the promoter. This conclusion was based on the use of certain i-o^c mutants thought to be deletions extending from the operator into or beyond the *i* gene[3]. Davies and Jacob have now shown, however, that these mutants are not overlapping deletions (unpublished results). In fact, it now seems likely that most or all of them behave as either negatively complementing i^- mutations (personal communication from J. Davies and W. Gilbert) or as double mutants (unpublished results). The former class would be mutations in the *i* gene, which produce a defective subunit of the repressor, impairing the normal function of wild-type repressor sub-units. The findings of Davies and Jacob have led us to re-examine the mapping of our mutants.

We present here conclusive evidence that the genetic site altered in our mutant maps between *i* and *o*. This evidence thus identifies a new controlling element determining the expression of the *lac* operon. We propose that it is in this region that the initiation of transcription takes place. Because, in the original formulation of the promoter concept, it was considered most likely that the site defined as promoter was an initiation point for *m*RNA transcription[3], we prefer to retain the term promoter to describe this new region (Jacob and Monod, personal communication, agree with this use of the term promoter). It now seems probable that, in fact, the region between *o* and *z* corresponds only to an initiation site for translation.

We have characterized four ultraviolet-induced mutants which co-ordinately reduce the rate of expression of the *z*, *y* and *a* genes. Some of their properties are described in Table 1. Three of these, although independently isolated, are almost identical in their characteristics, and at least two, *L*8 and *L*37, do not recombine with each other (unpublished results of J. Beckwith). *L*8 and *L*37 do, however, seem to be point mutants[9]. The lesion in the fourth mutant, *L*1, confers an additional property on the strain, and, although the operator in *L*1 is normal, the activity of its *i* gene is reduced.

Table 1

	Induced levels of *lac* enzymes as percentage of wild-type	Regulation	*cis*-Dominant
*L*1	2	Partly constitutive	Yes
*L*8, *L*37	6	Normally inducible	Yes
*L*29	4	Normally inducible	Yes

Three of these mutants, *L*1, *L*8 and *L*37, have been described before[4]. The fourth, *L*29, was isolated as one of two lac^- mutants found after ultraviolet mutagenesis of a lac^+ strain. The *o* and *i* characters of *L*1 were determined in diploid studies.

These mutations are *cis*-dominant in that they only reduce the rate of expression of the operon on the same chromosome and these effects are not relieved by the introduction of a second *lac* region into the cell[4]. An explanation for the properties of these mutants is that they have altered a site essential for the initiation of either transcription or translation of the operon. The result is a reduced efficiency of reading. In order to determine conclusively the position of the mutants relative to the operator and the *i* and *z* genes, we have employed a deletion analysis of this region. To avoid confusion we shall call these promoter mutants.

We have described a genetic system in which it is possible to isolate deletions removing the distal end (*a*

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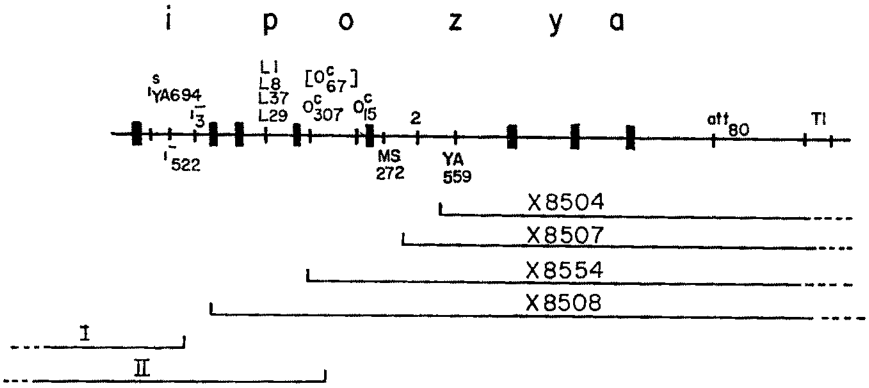

Fig 1 The *lac* operon transposed (in $\varphi 80dlac$) to the $\varphi 80$ site on the bacterial chromosome The $T1^R$ mutations were selected as before[7] All *i* gene and o^c mutations were isolated by Jacob, Monod and co-workers Lac^-_{MS272} is an extremely polar mutant isolated by Malamy[8] Lac^-_2 and lac^-_{YA559} have been described before[9] The orders of *i* and *o* mutations which are not established by deletion mapping have been determined by Davies and Jacob (results in preparation)

end) of the operon and extending varying distances toward and past the operator (Fig 1)[5] These deletions are isolated in a strain in which a $\varphi 80dlac$ transducing phage is integrated in the chromosome near the locus ($T1$) determining sensitivity to the bacteriophages $T1$ and $\varphi 80$ In the strain described in Fig 1, the *lac* region is inverted from its normal orientation on the chromosome so that the *i* gene is furthest from the $T1$ locus By selecting $T1^R$ mutants of such a strain, it is possible to isolate deletions extending from the $T1$ locus or beyond into the defective prophage[5,6] These deletions are presumed to include all the genetic material between the $T1$ locus and the determined deletion end, for they remove all point mutant sites in the *y* gene and in the *z* gene as indicated

The deletions isolated in this way, which we have used in our mapping studies, are described in Fig 1 Table 2 shows the frequency of recombination of these deletions with mutants affecting various components of the *lac* operon and the *i* gene These include an i^s mutation, two i^- mutants, four promoter mutants, three o^c mutants and three z^- mutants One of the mutants, o^c_{307}, was shown by Davies and Jacob (unpublished results) to map the furthest to the left of any known o^c mutant In most cases, the deletion strains were infected with an F' factor (*F-lac-pro*) carrying the mutation to be mapped Cultures of these partial diploids were then screened for recombinants. It should be noted therefore that the frequencies reported in Table 2 do not correspond to recombination frequencies measured in the standard way

The crucial deletion for determining the position of the promoter mutants relative to the operator is $X8554$ Two promoter mutants, $L8$ and $L37$, give frequencies of 0 065–0 086 per cent recombinants with this deletion In contrast, no recombinants have been detected between this deletion and any of the o^c mutants as indicated in Table 2 Either this deletion covers the entire operator region, or else its terminus lies very close to the end of the operator nearest the *i* gene In general, the other frequencies presented in Table 2 are entirely consistent with the order of mutations as determined by their ability to recombine with a series of other point mutations and deletions The map order is also in complete agreement with respect to the order of *i* and *o* mutations determined by Davies and Jacob (unpublished results) Furthermore, the results unambiguously show that the position of all four promoter mutations is between *i* and *o*

We have presented genetic evidence showing that mutants affecting the maximum level of *lac* operon expression map in the region between *i* and *o* The properties of these mutants can be accounted for by two different general explanations (1) These mutants define a site necessary for expression of the *lac* operon, which can be altered by mutation to reduce operon activity. (2) The mutants map outside all controlling sites for the operon, but in some undefined way affect operon expression negatively Although it is very difficult to conceive of any simple mechanisms for the second explanation, it is, of course, imperative to show directly that these mutants define an essential site

There is some evidence indicating that the region between *i* and *o* is essential to operon expression It is possible to isolate *lac*-constitutive mutations which are caused by deletions (Table 2) cutting into either the *i* gene (class I) or the *o* region (class II) from a point outside the *i* end of the *lac* region[5] In such a system we have isolated a large number of i^- lac^+ deletions In addition,

Table 2 PERCENTAGE OF RECOMBINANTS AMONG TOTAL COLONIES

Deletion Mutant	$X8504$	$X8507$	$X8554$	$X8508$
z^-_{YA559}	0	0	0	0
z^-_2	+	0	0	0
z^-_{MS272}	+	+	0	0
o^c_{15}	0 26	0 037	< 0 001	n t
o^c_{307}	n t	n t	< 0 001	n t
o^c_{67}	n t	n t	< 0 002	n t
$L1$, $L29$	+	+	+	0
$L8$	–	+	0 065	0
$L37$	0 79	0 34	0 086	0
i^-_3	n t	n t	0 19	0 069
i^-_{522}	n t	n t	0 16	0 094
i^s_{YA694}	+	+	0 24	+

The deletions are isolated in a strain carrying the $\varphi 80lac$ as described in the text and also carrying a *lac-proA,B* deletion To estimate the percentage of recombinants we constructed strains in which the mutant to be mapped against the deletion was carried on an *F-lac-proA,B*$^+$ episome With the dominant i^- mutation (i^-_{522}) and the o^c mutants, aliquots of a culture of a strain partially diploid in this way, for the *lac* region, were spread on to minimal medium (M63) containing glucose as carbon source, 5-bromo-4-chloro-indoxyl-β-D-galactoside (Xg, 0 004 per cent) and 0 004 M sodium citrate, necessary for the growth of $T1^R$ strains on this medium Because Xg is not an inducer[6], the constitutive diploid strains were blue and any i^+o^+ recombinants were white on these plates White colonies appearing on such medium were purified and assayed for β-galactosidase to ensure that they carried a wild-type *lac* region The same method was used to map the i^- deletion $X8508$ against i^-_3 The frequency of the *i* gene mutations with $X8554$ is actually the frequency of homogenotization of the i^+ or i^- allele To map the promoter mutants, $L8$ and $L37$, cultures of partially diploid strains were spread on *lac*-EMB plates and pure lac^+ (wild-type) colonies were picked and verified by assay The percentage of recombinants is based on samples from many independent diploid cultures, the frequencies are not seriously biased by "jackpots" caused by recombination events occurring early in the growth of the culture Some mutations (in F' donors) were mapped by crossing against the F^- *lac* S^r strains and selecting lac^+S^r recombinants (spot tests) Positive results are indicated as + and negative as 0 These spot tests detect recombinants at a much higher efficiency than the scoring techniques described here Some combinations were not tested (n t) we have isolated one deletion of class II. This deletion removes the *i* gene and the promoter region, but leaves part of the operator intact. As expected, the expression of the operon is impaired by the deletion of the promoter region. The properties of this strain will be described elsewhere (unpublished results of J. H. Miller, J. R. Beckwith and E. R. Signer). In addition to confirming the mapping results described here, the properties of this deletion also point to the importance of the promoter region in the expression of the *lac* operon. Thus it seems most likely that the mutants, *L*1, *L*8, *L*37 and *L*29, do lie in an essential region (the promoter) determining the initiation of operon expression.

The evidence pointing to a new site essential to *lac* operon expression which maps between *i* and *o* provides strong indication that the transcription process is initiated before the operator region. This conclusion does not exclude the possibility that the initiation of operon translation also occurs in this region. If protein synthesis does begin before *o*, however, then the operator should be translated; there is no evidence to support this prediction. We therefore favour the view that there are two sites essential to the expression of the *lac* operon. One would be the promoter, lying between *i* and *o*, which is the site of initiation of *m*RNA synthesis. Initiation of translation may then take place at a second site in the *o*–*z* boundary region, defined by Jacob, Ullman and Monod[3].

With this picture of the organization of the controlling sites of the *lac* operon, it is possible to visualize a fairly straightforward mechanism for the expression and regulation of operon activity. According to this scheme, the promoter region serves as the initiation point for transcription, possibly by acting as a binding site for RNA polymerase. Promoter mutants would reduce the site's affinity for the polymerase. While it is possible that the repressor hinders binding of RNA polymerase, the results presented here suggest a very simple alternative. In binding to the operator, the repressor could directly block the progress of the RNA polymerase into the structural genes of the *lac* operon.

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[1] Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).

[2] Gilbert, W., and Muller-Hill, B., *Proc. US Nat. Acad. Sci.*, **56**, 1891 (1966); Gilbert, W. and Muller-Hill, B., *Proc. US Nat. Acad. Sci.* (in the press).

[3] Jacob, F., Ullman, A., and Monod, J., *J. Mol. Biol.*, **13**, 704 (1965).

[4] Scaife, J., and Beckwith, J. R., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 403 (1967).

[5] Beckwith, J. R., Signer, E. R., and Epstein, W., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 393 (1967).

[6] Franklin, N. C., Dove, W. F., and Yanofsky, C., *Biochem. Biophys. Res. Commun.*, **18**, 910 (1965).

[7] Beckwith, J. R., and Signer, E. R., *J. Mol. Biol.*, **19**, 254 (1966).

[8] Malamy, M., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 89 (1967).

[9] Newton, W. A., Beckwith, J. R., Zipser, D., and Brenner, S., *J. Mol. Biol.*, **14**, 290 (1965).

Lower Dentition of *Kenyapithecus africanus*

by
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A mandible of *Kenyapithecus africanus*, with many of the teeth preserved, has been found at Kathwanga, Rusinga Island, Kenya. Previously the lower dentition of this species was unknown—except by inference from the mandible fragments with the crowns of the teeth broken off. The age of this member of the Hominidae is early Miocene.

SINCE the report of the discovery of early Miocene Hominidae on Rusinga Island[1] detailed geological studies of the deposits on the island have been carried out by Mr John van Couvering.

While van Couvering was engaged on this work, his wife, Judy, assisted by Erastus Ndere, undertook palaeontological work with a view to obtaining additional material of the fossil reptiles and fishes that occur in the Rusinga deposits. During this work, however, they noticed some primate teeth, on the surface, at Kathwanga (Site 5 of Rusinga Island)[2]. Following this up, they located the anterior region of a mandible *in situ*. The surface specimens and the *in situ* fragment have now been fitted together. The result provides the first opportunity to study the nature of the lower dentition of *Kenyapithecus africanus*.

Site and Geological Age

The specimen which will be described has been catalogued as Rusinga 1967, No. 394, and is housed at the Centre for Prehistory and Palaeontology, Nairobi. It comes from the site which was recorded as *R*.5 (ref. 2) in 1932. This is at Kathwanga on the west side of the island. The geological horizon from which the mandible was obtained is a grey tuffaceous deposit which forms part of the Kathwanga series. This series is at present regarded as the youngest element of the fossiliferous deposits of the Lower Miocene sequence on Rusinga[3]. As a result of the new geological work which has been carried out by van Couvering it is hoped that further evidence for dating by potassium–argon methods will soon be available from specimens which he has collected. There will also be a much more detailed picture of the overall stratigraphic sequence of the deposits exposed on the island and the relative age of the strata, visible at different sites.

The description of the specimen is: Family, Hominidae; genus, *Kenyapithecus*, Leakey 1961; species, *africanus* (Le Gros Clark and Leakey, 1951). The detailed diagnosis was given in ref. 1.

The new specimen consists of the greater part of a well-preserved mandible with many of the teeth preserved. The whole of the symphyseal region is in perfect condition anteriorly, except for very slight damage to the alveolar margins of the incisor sockets. All the incisor teeth had been lost before fossilization and the sockets are completely filled with matrix. The left canine is intact, while the crown of the right canine has been broken off about halfway up its length. On the right side the corpus of the mandible is reasonably intact to a point just behind the third molar, but the whole of the ascending ramus is

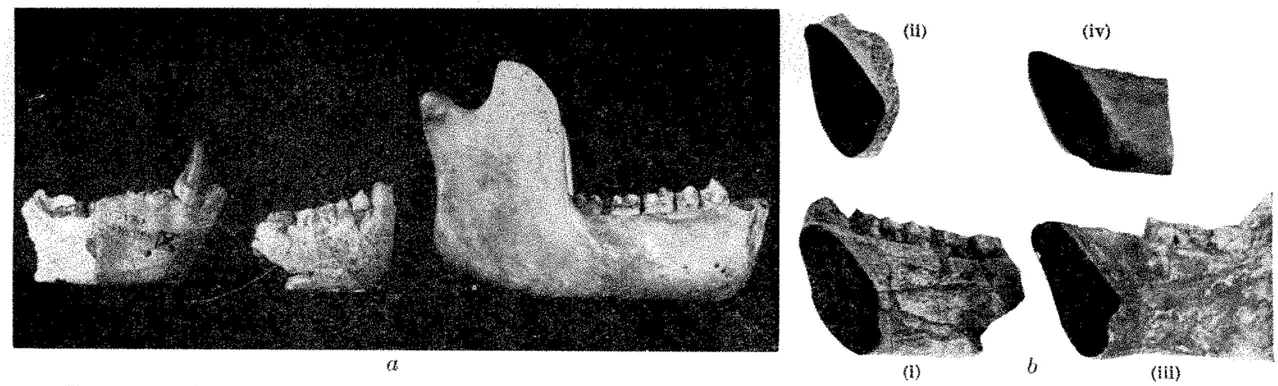


Fig. 1. *a*, Profile view of the new *Kenyapithecus africanus* mandible (centre) compared with *Proconsul major* (left) and gorilla (right). *b*, Cross-sections through the symphysis of the mandible of *Kenyapithecus africanus* (i), the earlier specimen *CMH* 142 (ii), *Proconsul nyanzae* (iii) and chimpanzee (iv).

missing as well as small parts of the inner wall of the corpus. On the left side, the corpus has broken off diagonally in a line starting just behind the second molar, at the alveolar margin, extending forwards to a point below the first molar. In addition to the loss of the incisors, the left fourth premolar, part of the left first molar and the left third molar are missing.

The morphology of the specimen compares very closely with that of the two mandibular fragments of *Kenyapithecus africanus*, from Rusinga Island, which were described before[1]. The chin region in the present specimen is rounded, as in the previously published specimens, and not triangular as in all examples of *Proconsul*. There is a slight depression on either side of the mid-line just below the alveolar margin. The sockets of the incisors lie in a straight line between the crowns of the canines—exactly as in the previously described specimens[1]. The corpus of the mandible is relatively high at the third premolar and narrows rapidly backwards towards the area of the third molars (Fig. 1*a*). In this respect, there is a measure of similarity with *Proconsul major*, but not with other species of *Proconsul*. The body of the corpus is not massive. At the fourth premolar where the height is 34 mm the bilateral thickness is only 13·5 mm; on the earlier specimen *CMH* 142 (ref. 1) the corresponding figures are 39·5 and 13 mm, while in *Proconsul major* they are 39 mm and 21 mm, and in a female specimen of *Proconsul nyanzae*, 32·25 mm and 16 mm.

The cross-section through the symphyseal region is similar to that already described[1] (Fig. 1*b*). This illustration is based on cutting through a plaster cast of the new mandible along the mid-line. From the incisural, lingual, alveolar margin the wall of the mandible, in the intercanine area, slopes rapidly downwards and slightly backwards, to well beyond the midway point of the total height and then forwards again. In *Proconsul* the initial slope is less steep and more backwards.

A noticeable feature of the morphology is the relatively great width between the canines, compared with any species of *Proconsul*. The measurement between the inner alveolar margins of the canines of the new *Kenyapithecus* specimen is 20·5 mm. In a *Proconsul nyanzae*, of comparable size, it is only 16·5 mm and even in the very much larger *Proconsul major* only 20 mm. In the specimen of *Kenyapithecus africanus* from Rusinga[1] the corresponding figure was 17·5 mm.

Linked with this large inter-canine width is the marked backward divergence of the molar–premolar series (Fig. 2). In the living members of the Pongidae, the cheek teeth tend to be set in parallel rows or even to converge backwards, so that the third molars are closer to each other than are the third premolars. In all species of *Proconsul*,

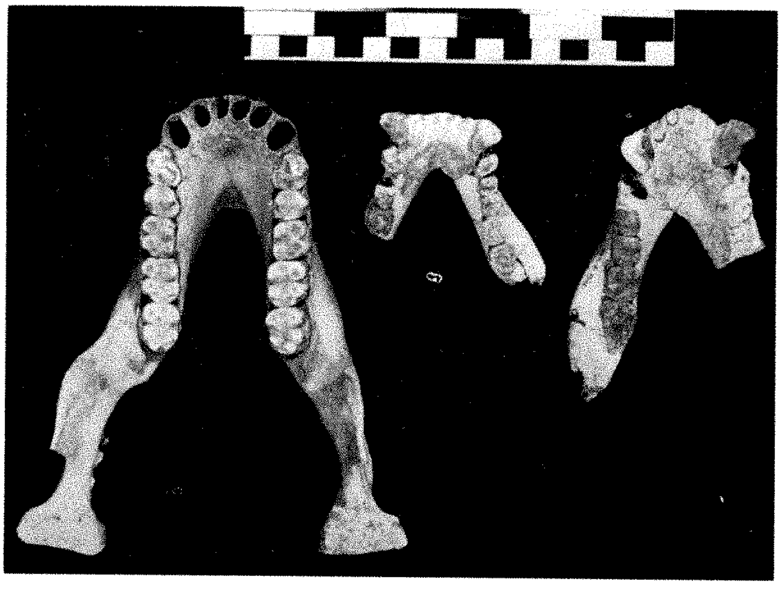


Fig. 2. Occlusal view of *Kenyapithecus africanus* mandible (centre) with *Proconsul major* (right) and gorilla (left).

there is some degree of backward divergence. In the specimen now being described, the divergence is much greater. As a result of this, although the length from the point of the canine to the back of the third molar is almost the same as in a *Proconsul nyanzae*, the overall length of the dental area is shorter and the width greater (Fig. 2). It must therefore be assumed that the face was noticeably less prognathous.

The difference in the arrangement of the teeth in the mandible as between the modern apes (Pongidae) and man, and his nearer relatives such as the Australopithecines (Hominidae), can be conveniently and graphically demonstrated by means of what I term the "basic rectangle of the mandible". This is obtained by drawing a line tangential to the anterior alveolar margins of the two central incisors, and another tangential to the two most posterior points of the third molars. When this has been done, perpendiculars are dropped from the two most posterior points of the third molars, to meet the line tangential to the incisors.

Naturally, there is some degree of individual variation within any genus or species, but, nevertheless, experiment has shown that, in spite of these minor variations, the shape of the basic rectangle is remarkably consistent for modern Pongidae on the one hand, and modern man on the other. In the Pongidae it has sides which are nearly twice as long (or in some cases more than twice as long) as the upper and lower ends. In *Homo sapiens*, however, as well as in *Homo erectus*, and in such specifically uncertain members of the genus *Homo* as the Heidelburg jaw, the basic rectangle is seen to be nearly a square, with equal, or almost equal, sides and ends.

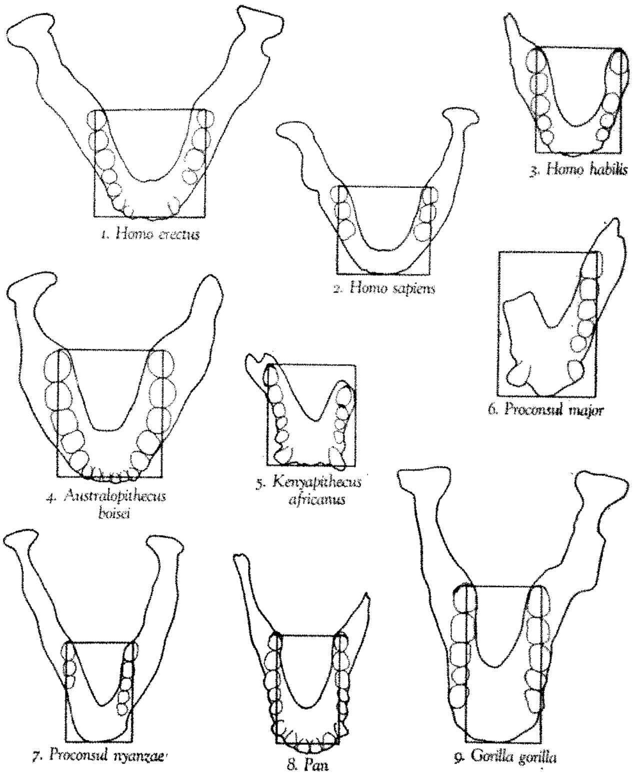
When the basic rectangle for the three species of the genus *Proconsul* is drawn, it is seen to lie somewhat intermediate between Hominidae and Pongidae. In the same way, the basic rectangle for *Homo habilis* and for the Australopithecines is not nearly so equal sided as in the more advanced Hominidae, but the shape is nevertheless clearly distinct from that which is characteristic of the Pongidae and from that of *Proconsul*.

The point of interest in Fig. 3 therefore lies in the fact that, when the basic rectangle for *Kenyapithecus africanus* is drawn, it is found to stand very close to the shape seen in *Homo habilis* and *Australopithecus*, and links it clearly with the Family Hominidae, rather than the Pongidae.

The anterior region of the mandible, on either side of the symphysis, is rounded in contour and differs very strongly from that of all known specimens of *Proconsul*, in which the structure, as a whole, suggests an inverted triangle. The mental foramina are situated a little below the half-way line on the wall of the corpus, below the fourth premolars, rather than beneath the third premolars as in *Proconsul*.

The Teeth

The incisors, as already noted, are missing. The left canine is almost perfectly preserved, but the upper half of the crown has been broken off on the right side. The first feature of importance is the angle at which the canines are set in the mandibular corpus. The buccolingual axis of the crown of the tooth is aligned much more antero-posteriorly than in any *Proconsul*, in which genus the canine teeth are usually set somewhat diagonally across the line of the molar–premolar series (Fig. 2). The alignment in *Kenyapithecus* is, indeed, remarkably like that seen in *Homo*. Next it must be noted that although the crown of the canine is high and large relative to the other teeth, it differs in several morphological respects from the corresponding teeth of *Proconsul*, and still more so from those of fossil and living Pongidae. First, the whole crown is considerably compressed bilaterally, while the anterior face is relatively much narrower than in the case of any recorded *Proconsul*. Second, the cross-section through the canines, near the base of the crown, is entirely different, with almost parallel sides. This feature has already been noted in connexion with the published mandibular fragments, where a cross-section of the root is visible in the alveolus although the crowns are broken off. This difference in the cross-section is now seen to be a basic character, associated with a wholly different type of canine crown structure in the crowns of *Kenyapithecus* which are even less conical and less like those of the pongids than in the case of *Proconsul*.



1. *Homo erectus* 2. *Homo sapiens* 3. *Homo habilis* 4. *Australopithecus boisei* 5. *Kenyapithecus africanus* 6. *Proconsul major* 7. *Proconsul nyanzae* 8. *Pan* 9. *Gorilla gorilla*

Fig. 3. Diagrammatic illustration of the difference between the "basic rectangle" of different higher primates and man (all to the same scale).

A third difference is to be seen in the relative length of the canine root. X-ray photographs show that the root of the canine is short relative to crown length, a feature which was also noted in connexion with specimen *CMH* 142 from *R*.106 in the earlier report. Fourth, the wear on the crown of the canine is quite unlike that of any *Proconsul*. There is an attrition facet down the lingual aspect, instead of along the buccal face. This feature is clearly linked with the lack of a diastema between canine and premolar, resulting in a quite different type of canine occlusion.

Both the third premolars are preserved and the following important points should be noted. The teeth are set forward in the corpus of the mandible in such a way that there is no diastema between the premolar and the canine. The anterior root of the premolars lies well in front and to the buccal side of the posterior margin of the root of the canine. This character has already been described for the *Kenyapithecus africanus* mandibles in the earlier report.

The third premolars are, moreover, rotated and set in the corpus so that they are aligned somewhat in the same general direction as the molar series, not diagonally across the corpus of the mandible, as in *Proconsul* or in the pongids. The posterior root of the third premolar is set slightly to the lingual side and not completely distal to the anterior root, but is less laterally displaced than in *Proconsul* and the living Pongidae. The crowns are composed of a single major cusp, in front of which there is a convex anterior face sloping steeply downwards. This is wholly unlike the situation in *Proconsul*, in which there is a long, low incline anterior to the cusp with a minor

Table 1. COMPARATIVE DENTAL MEASUREMENTS OF MANDIBULAR TEETH

	Kenyapithecus africanus		*Proconsul major*		*Proconsul nyanzae* ♂		*Proconsul nyanzae* ♀	
	Antero-posterior L	Bucco-lingual B	Antero-posterior L	Bucco-lingual B	Antero-posterior L	Bucco-lingual B	Antero-posterior L	Bucco-lingual B
Canine	8·5	11·5	11·0	15·0	10·0	12·0	8·5	11·5
Third premolar	6·5	11·0	—	—	7·0	12·5	7·0	8·5
Fourth premolar	6·25	7·0	9·5	9·5	8·75	7·0	7·75	6·5
First molar	9·0	8·0	12·5	10·5	10·25	8·5	9·0	7·5
Second molar	11·5	9·5	13·5	12·5	12·0	10·5	10·25	9·0
Third molar	12·25	10·25	17·75	14·0	14·5	11·25	12·0	9·5
P_3–M_3 length		50·00		62·5		54·0		45·5
Canine–M_3 length		58·5		79·0		66·5		57·0

cuspule on the forward edge. Posteriorly, a small fovea is present but is not very strongly developed. In all species of *Proconsul* the third premolar is rather markedly elongate with the main single cusp lying between a backward and a forward projecting region. *Proconsul* usually also has a marked wear facet developed on the buccal face of the third premolar as a result of contact with the upper canine.

Only the right fourth premolar is preserved. It is aligned in the same general direction as the molar teeth, so that its two roots are set one behind the other, a feature that was also clearly visible in *CMH* 142, described before[1]. There are two paired cusps in front of which there is a small anterior fovea, while posteriorly there is a relatively strongly marked basin, somewhat like that seen in the corresponding tooth of *Proconsul*.

The right first molar is almost perfect, but has a small area of enamel missing from its buccal face. On the left side only the posterior half of this tooth is preserved. In common with the three known *Proconsul* species, as well as many other primitive members of the Hominoidea, the first molar is markedly smaller than the second, which in turn is smaller than the third. In addition to the four principal cusps, a small hypoconulid is present. There is a rather deep pit on the lingual half of the tooth forming a valley between the metaconid and entoconid.

It is not possible to determine for certain whether a buccal cingulum was present on this tooth, because of the damage to the enamel in this region.

Both right and left second molars are preserved. The tooth on the left side is in perfect condition, but the enamel of the crown of the right second molar is somewhat pitted by weathering. The left tooth will therefore be used for description. The four principal cusps are strongly developed, while there is also a strongly marked and more or less conical hypoconulid which is set slightly to the buccal side of the mid-line. A well marked posterior fovea lies between the hypoconulid and the entoconid. As in the case of the first molar, there is a rather deep pit lying between the metaconid and the entoconid. Slight traces of an external cingulum can be seen.

The third molar has lost part of the enamel from the talonid and from part of the lingual face posteriorly. It is also somewhat pitted by weathering. The general morphology can be clearly seen in spite of the damage. The tooth is, moreover, wider anteriorly than posteriorly as in all species of *Proconsul*. It tapers towards the talonid, so that the overall appearance (viewed from above) is that of an almost triangular tooth (Fig. 2). The hypoconulid is well defined and lies slightly on the lingual side of the mid-line, with a well marked posterior fovea also on the lingual side. There are slight traces of a cingulum between the protoconid and the hypoconulid.

The length of the molar–premolar series is 50 mm measured on the right side, while the length of the series, including the canine, is 58·5 mm. The corresponding measurements on a female *Proconsul nyanzae* are 45·5 mm and 57 mm, and on a male *P. nyanzae*, 54 mm and 66·5 mm. In general dental size, therefore, this specimen lies somewhat intermediate between male and female examples of *Proconsul nyanzae*. These data are summarized in Table 1.

The new specimen of *Kenyapithecus africanus* is believed to represent a male, but it is slightly smaller in size than specimen *CMH* 142, described before[1]. It is, however, much more massive than the Songhor specimen[1] which was considered to represent a female.

Value of the New Specimen

While a detailed study remains to be carried out, it is already clear that the newly discovered mandible from Rusinga considerably enlarges our knowledge of the early Miocene members of the Hominidae. Well preserved examples of the lower teeth, with the exception of the incisors, are now available. It was already known that traces of a cingulum were present in the upper molars; and this feature is now also known to be present in the lower molars. In both upper and lower molars, however, the cinguli are very much less developed and less beaded than in any species of *Proconsul*.

It was evident from the earlier specimens that the lower canines must have had moderately large crowns, and also clear (from the cross-sections of the roots visible in the alveolus) that when the crowns became known they would probably differ from *Proconsul*, both in morphology and cross-section. This has now been shown to be the case, especially in the shape of the anterior face of the canines and their cross-section at the alveolar margin, and in the nature of the wear facets due to a different type of occlusion with the upper canines.

The lower third premolars are found to be monocuspid and morphologically not unlike those of the Pongidae. Nevertheless, this tooth differs, in several important features, from all those of any species of *Proconsul* species as well as from known *Dryopithecus* specimens. It is also set at a different angle in the mandible, with the crown more antero-posteriorly aligned and the roots set more one behind the other, instead of transversely, more as in man.

The third molar is the largest and the first molar the smallest in the molar series in which there is a resemblance to *Proconsul*. A marked character, which had been suspected from the earlier specimens but not proved, is the very strong backward divergence of the cheek teeth on either side, which must have been associated with a correspondingly shorter, less prognathous face.

Origins of *Kenyapithecus*

With the additional specimen now available, it becomes increasingly probable that *Kenyapithecus africanus* may have been derived from some earlier, but so far unknown, member of the genus *Proconsul*, possibly in Oligocene times. There are a number of characters that seem to link it with *Proconsul*, although it differs markedly in others. Because, however, it was contemporary with the earliest, so far known, *Proconsul* specimens, it seems to be a reasonable conclusion that a pre-Miocene *Proconsul*-like stock may have divided into two branches, resulting, respectively, in *Proconsul* and *Kenyapithecus*.

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[1] Leakey, L. S. B., *Nature*, **213**, 155 (1967).

[2] Le Gros Clark, W., and Leakey, L. S. B., *Fossil Mammals of Africa*, No. 1, 1951 (Brit. Mus. Nat. Hist., 1951).

[3] Shackleton, R. M., *Quart. J. Geol. Soc.*, **107** (1951).

LETTERS TO THE EDITOR

ASTRONOMY

Polarization of Galactic Radio Emission at λ 1·25 m

SYNCHROTRON radiation from the Milky Way at radio wavelengths is linearly polarized if the magnetic field in the emitting region is in the same direction throughout the line of sight, and if the Faraday rotation is small within the emitting region Observations at λ0 21 m show polarization up to 25 per cent in a direction somewhat inclined from the perpendicular to the spiral arm, at λ0 73 m the percentage polarization is less, and the polarized area is also less, indicating that Faraday rotation is becoming important The polarization at longer wavelengths has so far remained unknown, in spite of several attempts to measure it[1-4]

The reduction of polarization at longer wavelengths might be attributed to Faraday rotation within a region of highly organized field, generally running along the spiral arm Bingham and Shakeshaft[5] show that a diverging field fits the data well, but their model, along with any other involving a smooth field, does not easily fit the data in a region of maximum polarization near $l=135°$, $b=5°$, where the high degree of polarization indicates that the line of sight traverses a large region of constant Faraday depth[5], that is inside which the rotation is zero Here the polarization falls with increasing wavelength without a corresponding rotation of the plane of polarization, and it is therefore necessary to postulate in addition that there is a less regular component of the variation of Faraday depth through the emitting region This "random" component must comprise reversals of the line-of-sight component of the magnetic field

Irregularities in Faraday depth would also show up as irregularities in the angular distribution of polarization, possibly on a small angular scale which would give an average polarization near zero over a telescope beamwidth of a few degrees Such irregularities have been found in a recent survey using the Mark I 250 ft telescope at λ0 73 m, with a beamwidth of 45′ arc (These observations will be reported separately)

Further confirmation of this physical picture has now been sought by using the telescope as a polarimeter at λ1 25 m, to survey the area $02h<\alpha<05h$, $55°<\delta<65°$, which contains the highly polarized region at $l=135°$, $b=5°$ At the longer wavelength the internal depolarization of irregularities dispersed through the emitting region would be expected to remove most of the polarization, a further reduction was expected because the irregularities would not be completely resolved by the beamwidth of 75′ arc, so that the observed polarization would be appreciably less than the actual polarization brightness temperature

In this survey, the polarimeter only detected the difference between vertical and horizontally polarized components The sidelobes of the aerial feed, which was a simple crossed dipole system, interacted with the large scale structure of unpolarized emission to give a baseline uncertain to about 3° K near the galactic plane, although small-scale polarized structure could be detected with certainty down to a polarization brightness temperature T_{pb} of about 2° K

Most of the region near $l=135°$, $b=5°$, now seems to be unexpectedly highly polarized at λ1 25 m Regions where T_{pb} exceeded 3° K are shown in Fig 1

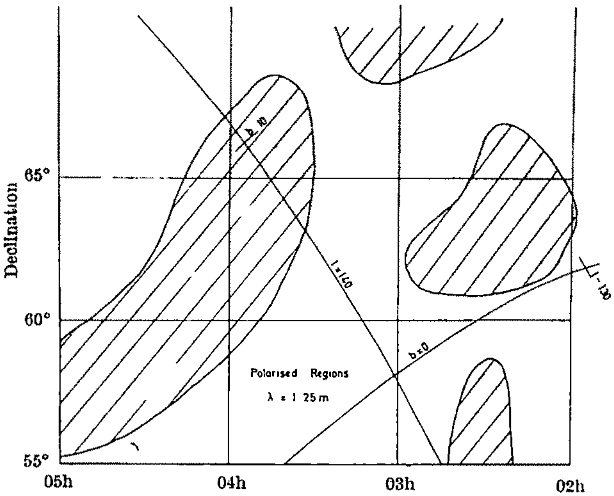

Fig 1

Inside these regions, the polarization varied rapidly with angle, often with structure unresolved by the telescope beam Nevertheless, the recorded values of T_{pb} often exceeded 8° K, while at λ0 73 m the corresponding value of T_{pb} is typically only 4° K Over all other regions of sky covered by this brief experiment at λ1 25 m, including a large region covering the North Galactic Spur, T_{pb} was found to be less than 2° K

The high degree of polarization remaining at λ1 25 m, amounting to at least 5 per cent as compared with a theoretical maximum of 70 per cent in a perfectly aligned field, shows that even for this long wavelength there is no rotation greater than about one radian within a substantial proportion of the line of sight The spiral arm is at least 500 pc thick in this direction, so that the smooth region is at least 40 pc deep Any irregularities which cause the depolarization between λ0 21 m and λ0 73 m must lie outside the uniform region, in which the product of the average electron density N_e and the line of sight component of H must be less than about $1\ 5\times 10^{-8}$ gauss cm^{-3} The observation that adjacent lines of sight, separated by 1 25°, often have different net polarizations is probably a consequence of a variable Faraday rotation in front of the smooth region

If we suppose that the field strength is about 3×10^{-6} gauss and that it is aligned along the spiral arm in the direction $l=70°$, then the line of sight through the polarized region crosses the field at angles up to 30° The electron density must then be less than 0 01 cm^{-3} Even if the field follows the direction $l=50°$, the wide angle of sky covered by the polarized regions suggests that the electron density must still be less than 0 03 cm^{-3} in a considerable region of the spiral arm

These averages of electron density refer to averages through a line of sight extending for about 50 pc, one may infer that the values around 0 1 cm^{-3} deduced from the absorption of lower frequency radio waves[6] refer to an average through discrete concentrations rather than a uniform value throughout the spiral arm

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[1] Thomson, J M , *Nature*, **180**, 495 (1957)
[2] Razin, V A , *Astro J Moscow*, **35**, 241 (1958)
[3] Pawsey, J L , and Harting, E , *Aust J Phys* , **13**, 740 (1960)
[4] Matthewson, D S , Broten, N W , and Cole, D J , *Aust. J Phys* , **19**, 93 (1966)
[5] Bingham, R G , and Shakeshaft, J R , *Mon Not. Roy. Astr Soc* , **136**, 347 (1967)
[6] Smith, F G , *Mon. Not Roy Astro Soc* ,131, 145 (1965)

Spectrum of the Radio Source 4C 50.11/NRAO 150

MOST extra-galactic radio sources have a spectrum which over a limited frequency range is of the form $S\propto\nu^{-\alpha}$, where S is the flux density and ν is the frequency. The spectral index α is usually constant over a considerable frequency range, which implies a straight spectrum when plotted logarithmically, the average value over all sources being 0.75 with a range from about 0.2 to 1.3. For some sources, however, α changes slowly with frequency with the result that the spectra are concave or convex[1-3]. It is believed that the radiation is produced by the synchrotron mechanism, where energy losses by the electrons can lead in time to an increase in the slope[4,5] and where, in addition, different component sources may contribute to the overall spectrum. High frequency observations by Dent and Haddock[6] showed that two sources, *3C* 84 (NGC 1275, a Seyfert galaxy) and *3C* 279 (a quasi-stellar source), have spectra which decrease in flux density from about 38 MHz towards higher frequencies, but beyond 3,000 MHz the slope of the spectrum is positive. This suggested the presence of a small diameter component which had a low frequency cut-off due to synchrotron self-absorption[7] and stimulated a search for other sources with similar spectra. Kellermann (personal communication) has recently observed fourteen sources with irregular spectra, of which some have a tendency towards these latter characteristics, but so far there have been no published spectra similar to *3C* 84 and *3C* 279. Recent observations at the frequencies of 3,200, 6,600 and 10,600 MHz, however, using the 150 foot telescope of the Algonquin Radio Observatory, when combined with the observations by other observers[8-11], have shown that the source *4C* 50.11/NRAO 150 is of this form but with the addition of two apparent peaks at frequencies of about 350 and 9,000 MHz. The source is included in the list by Kellermann, but details of the characteristics failed to show up because of the omission of low frequency observations and the new measurements between 5,000 and 15,000 MHz.

If it is assumed that the spectrum is the result of the superposition of non-thermal components and that the peaks are the result of components which have an optical depth sufficiently great to produce synchrotron self-absorption, then it is possible to distinguish three components, designated *A*, *B* and *C*. The resulting spectrum is shown in Fig. 1, which also shows the spectra of *3C* 84 and *3C* 279. At low frequencies, there is some evidence for absorption resulting from ionized hydrogen in the galactic plane, amounting to 2 dbar or more, obtained by assuming the single component *A* with a straight spectrum, and taking the upper limit of 2 flux units at the frequency of 22 MHz (Costain, private communication). This seems to be reasonable for the direction of the source, which has galactic co-ordinates $l^{II}=150°$, $b^{II}=-2°$. The relative flux densities of *B* and *C* and the slope of *A* were deduced from the long baseline measurements of Palmer *et al.*[12] at 2,600 MHz, where the observation of fringes from the source is taken to imply that *B* has an appreciably greater flux density than *C* at this frequency and therefore that *A* must also decrease rapidly with an index of about 1.3.

Although the peaks in the spectrum may suggest that the source is variable, no variations greater than the random errors of about 10 per cent were observed during the period February–August 1967, and the spectrum as shown is believed to be correct for the present epoch. Unfortunately, the source is situated close to the galactic plane so that obscuration may make optical identification difficult, and there is no apparent correlation with any object on the Palomar Sky Survey. In addition, the proximity of the source to the galactic plane could produce some confusion affecting the accuracy in determination of the flux density at 38 MHz even though the nearest source in the *4C* survey (*4C* 51.11) is more than 1° away.

The observation of fringes in the long baseline observations also indicates that *B* has a diameter less than about 5×10^{-2} s of arc. This is consistent with the assumed spectrum, which for a cut-off frequency of 200 MHz implies that, even if the magnetic field is taken to be as high as 10^{-2} gauss, the diameter is less than 2×10^{-2} s of arc. The diameter of component *C* is probably even less, as evidenced by the much higher cut-off frequency. The small diameter for *B*, the comparatively low flux density and the high cut-off frequency are also evidence that it cannot be an H II region. The same is probably also true for *C*, and confirms the assumption that the components are a result of a non-thermal mechanism. The same arguments can also be used to suggest that the source is probably not situated in the galaxy. The position of component *B* as determined from the NRAO observations at 1,400 MHz (ref. 10), and that determined for *C* from the present observations at 10,600 MHz, seem to be separated by 45 s of arc, although the combined errors amount to 33 s of arc. These are shown in Fig. 2, where there is also plotted the source position from the *4C* survey at 178 MHz (ref. 9). The steeper spectrum for component *A* suggests an older and possibly a more extended source than the two apparently more recent components with small diameter. It is tempting to suggest a model for the source, consistent with the observations, which consists of an older and comparatively extended region or halo, corresponding to component *A* together with two small diameter components *B* and *C* which are probably very recent ejections.

If the general interpretation of these results is accepted, it suggests that the source may be a radio galaxy or quasar in the early stages of development, and leads to the following comments.

First, if components *B* and *C* are young sources, then the spectral index of 0.5 is very much steeper than the value of 0.25 assumed by Kellermann[5] to be the initial spectrum for a young radio source. The value of 0.25 was previously chosen, because it appeared to be the smallest value for the sources considered, but could possibly have been due to the presence in the source of a number of components. It is possible that sources do not develop in the comparatively simple way that has been suggested. For example, acceleration mechanisms, resulting in the development of less steep spectra, may be active following

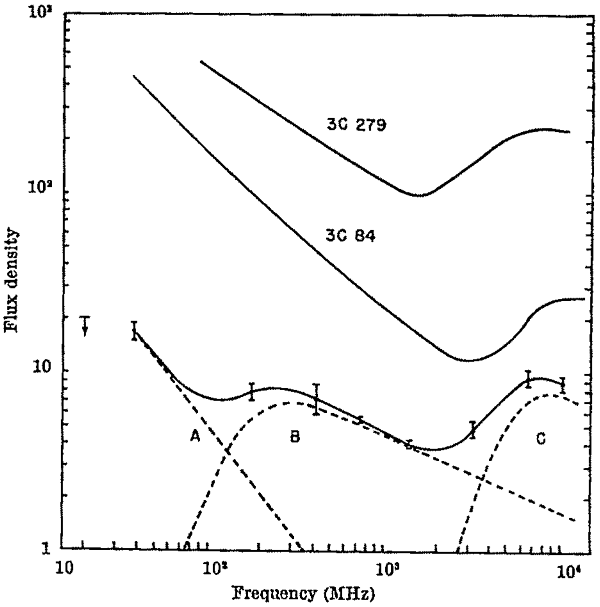

Fig. 1. The spectrum of the radio source *4C* 50.11/NRAO 150 showing the suggested three components *A*, *B* and *C*. The spectra of *3C* 84 and *3C* 279, displaced along the vertical axis, are included for comparison purposes.

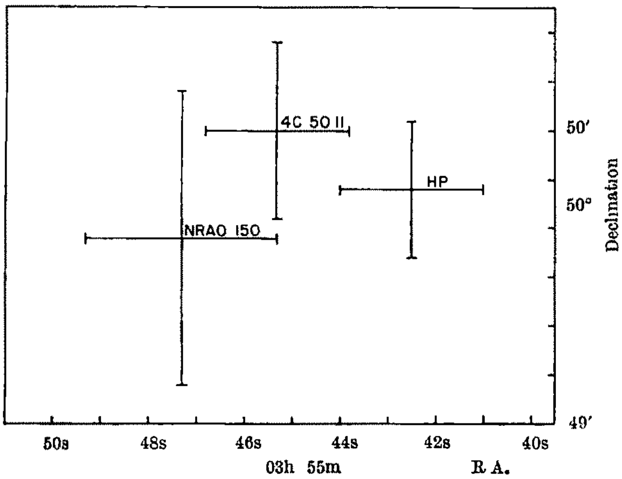

Fig 2 The position of the source 4*C* 50 11/NRAO 150 as determined from the 4*C* survey at a frequency of 178 MHz, the NRAO survey at 1,400 MHz and the present results (HP) at 10,600 MHz The arms of the cross represent error limits

the initial ejection as has been suggested by Ryle and Longair[13] In any case, there is no evidence that the energy spectrum of electrons produced in the initial ejections is the same for all sources There remains the alternative that the source may be older and have a large linear size, but the distance would have to be very great to account for the smallness of the angular size

Second, the results demonstrate the importance of long baseline observations, when combined with detailed knowledge of spectra, for resolving the structure of radio sources This is demonstrated in the present case where it is possible to separate with some precision the components *B* and *C* And, as a corollary, statistics involving the classification of sources into single, double, multiple, core and halo and so on which are derived from interferometer measurements at a single frequency may not be significant when applied to radio sources in general, and in particular to those with an apparently flat spectrum or where observations are not taken at sufficiently close frequency intervals to define the spectrum accurately In fact, for the model proposed, the present source may well be classified under any one of these headings depending on the frequency at which observations are made For example, at a frequency of 3,700 MHz the source might appear to have two components of equal intensity, on either side of this frequency to have two unequal components, at near to 1,000 and 10,000 MHz to be a single source, and at a few hundred MHz to be a point source with a more extended region If the source ages according to current theories, the points where synchrotron self-absorption occurs are expected to move to lower frequencies so that in time the source may appear as triple over a large frequency range

The source is suggested as one worthy of further investigation with long baseline radio interferometers, and it is still possible that the source could be observed at optical wavelengths

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[1] Conway, R C , Kellermann, K I , and Long, R J , *Mon Not. Roy Astro. Soc* , 125, 261 (1963)
[2] Kellermann, K I , *Ap J* , 140 969 (1964)
[3] Dent, W A , and Haddock, F T , *Ap J* , 144, 568 (1966)
[4] Kardashev, N S , *Sov Ast A-J* , 8, 217 (1962)
[5] Kellermann, K I , *Ap J* , 146, 621 (1966)
[6] Dent, W A , and Haddock, F T , *Nature*, 205, 487 (1965)
[7] Slish, V I , *Nature*, 199, 682 (1963)
[8] Williams, P J S , Kenderdine, S , and Baldwin, J E , *Mem Roy Astro Soc* , 70, 53 (1966)
[9] Gower, J F R , Scott, P. F , and Wills, D , *Mem Roy. Astro Soc* , 71, 49 (1967)
[10] Howard, W E , and Moran, S P , *Ap J Suppl* , 10, 1 (1965)
[11] Pauliny-Toth, I I K , Wade, C M , and Heeschen, D S , *Ap J* Suppl , 13, 65 (1966)
[12] Palmer, H P , Rowson, B , Anderson, B , Donaldson, W , Miley, G K , Gent, H , Adgie, R L , Slee, O B , and Crowther, J H , *Nature*, 213, 789 (1967)
[13] Ryle, M , and Longair, M S , *Mon Not Roy Astro Soc* , 136, 123 (1967)

PLANETARY SCIENCE

Semi-annual Variation in Upper Atmosphere Density

I RECENTLY reported[1] the results of an analysis that indicated a semi-annual variation in air density at a height of 190 km in 1966–67, with the unexpectedly large maximum to minimum ratio of 1 45 The maxima were in April and October, the minima in January and June, as usual Newell[2] has subsequently discussed these results, and has left two open questions on which I should like to comment

First, Newell remarks that the enhanced semi-annual variation at a height of 190 km cannot be accounted for by Cook's explanation[3] of the semi-annual variation at a height of 1.100 km, in terms of variations in the concentration of helium at heights near the turbopause (about 120 km) In one respect Newell's remark is quite true. there is very little helium at 190 km—certainly not enough to give rise to a variation by a factor of 1 45 However, the essence of Cook's suggestion, namely, that variations at heights as great as 1,100 km may be attributable to effects at heights as low as 120 km, is strengthened by the finding that an important semi-annual variation extends to a lower level than previously known—190 km It seems quite likely that the semi-annual variation in 1966–67 was appreciable down to 150 km, where synoptic measurements are very scarce, and possibly even to 120 km.

The second question left open in Newell's communication concerns Anderson's controversial hypothesis[4] that the semi-annual variation can be ascribed to the influence of latitude and is not a true time-variation Anderson's hypothesis can be disproved if analysis of the orbits of satellites at several different heights and latitudes yields similar variations of density with time Such evidence is now available Recent results[5] from Midas 2, which has a circular orbit at a height of about 480 km and an inclination to the equator of 32°, show time-variations that are in excellent agreement with those obtained[1,6] from the near-polar orbits of Secor 6 at a height of 190 km and Echo 2 at a height of 1,100 km

Fig 1 gives the values of air density obtained[5] from Midas 2, corrected to a level of solar activity corresponding to a 10 7 cm radiation flux of 125×10^{-22} W m^{-2} Hz^{-1} The values are averages over latitudes between 32° N. and 32° S The two "spikes" indicate times of strong geomagnetic disturbance, when values were excluded. It should be noted that the absolute values of density in Fig 1 are subject to a possible bias error because the mass of the satellite is not exactly known; but this does not affect the validity of the variations Fig 1 shows that, if the maximum and minimum densities in the semi-annual variation are defined as values sustained over 30 days, the maximum to minimum density ratio was 2 1 at a height of 480 km in 1966–67 It is also of interest that on all occasions when the time resolution is adequate (that is, after April 1966) the minima are double; the "main" minima in January and July are accompanied by subsidiary minima in March and May It remains to be

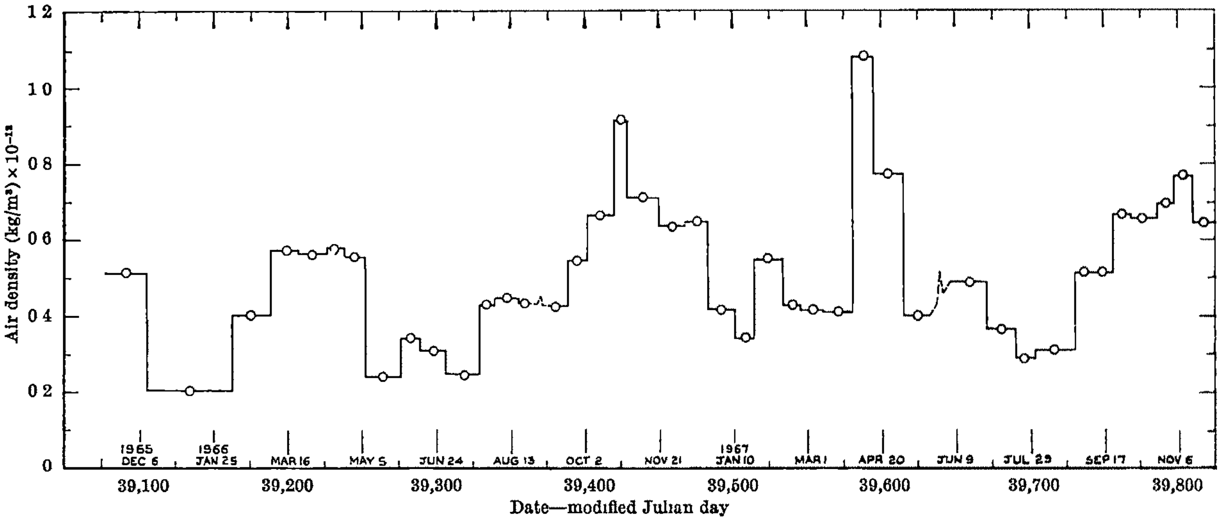

Fig. 1. Values of air density at a height of 480 km, obtained from the orbit of Midas 2, corrected to a mean level of solar activity

seen whether this is a regular pattern or merely a chance effect.

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[1] King-Hele, D. G., *Nature*, **216**, 880 (1967).
[2] Newell, R. E., *Nature*, **217**, 150 (1968).
[3] Cook, G. E., *Planet. Space Sci.*, **15**, 627 (1967).
[4] Anderson, A. D., *LMSC-6-77-67-40* (Lockheed Missiles and Space Company, Palo Alto, California, 1967).
[5] King-Hele, D. G., and Hingston, J., *RAE Tech. Rep.* 67321 (1967).
[6] Cook, G. E., and Scott, D. W., *Planet. Space Sci.*, **15**, 1933 (1967).

Solubility of Barium Sulphate in Sea Water

Most of the elements in solution in sea water are present in smaller amounts than would be expected on the basis of solubility equilibria. The concentrations of many of these elements are controlled by other processes such as ion exchange, adsorption and biological removal. There are, nevertheless, several elements for which solubility may be an important factor affecting abundance and distribution in parts of the ocean. These generalizations are based on comparisons[1,2] of the observed concentrations of elements in sea water with those which would be expected from a consideration of their solubility. Even when reliable abundance data are available, however, exact comparisons are precluded by the lack of accurate, experimentally determined values for the solubility in sea water of all but a few compounds and the approximate nature of theoretical calculations which must usually be made using assumed values for the activity coefficients of ions in this complex solution.

There is thus considerable interest in obtaining values for solubilities in sea water by direct measurement, especially for the less soluble compounds which may in some circumstances limit the concentrations of certain elements. This interest extends to a more diverse range of compounds when atypical environments are considered, whether natural, as in some isolated basins, or artificial, as in polluted waters. The presence of dissolved ions in natural waters precludes the use of methods such as those based on conductance measurements, which have been most widely used to measure the solubility of sparingly soluble compounds in pure water. We describe here a radiochemical method for determining the solubility of barium sulphate in sea water, the technique, although well known to chemists, does not seem to have been applied previously to sea water. We chose barium sulphate for the initial investigation because in studies of barium in the ocean there has been some discussion[3,4] of the possibility that the limited solubility of its sulphate (the least soluble compound formed with a major anion in sea water) might play an important part in influencing its distribution. This communication gives the results of preliminary experiments which indicate the scope and value of the method.

Initially, experiments were carried out using barium sulphate labelled with barium-140. The results were unsatisfactory in that there was no clear indication that equilibrium between the solid phase and the solution was attained and control experiments using pure water gave low results. Anomalous results for the solubility of similarly labelled barium sulphate in water have been reported by Spitsyn *et al.*[5] although their findings were not confirmed by other workers[6-7].

Experiments using barium sulphate labelled with barium-133, prepared by a method similar to that described by Rosseinsky[8], have given more satisfactory results. An amount of the solid in considerable excess of that needed to give a saturated solution was stirred with sea water in a closed glass flask at 20° C. The sea water was surface water ($S = 35‰$) from the English Channel, collected 22 miles from Plymouth and filtered through a membrane filter of retentivity about 0·5μ. Aliquots of the supernatant solution were removed through membrane filters at intervals and the radioactivity of barium-133 in a known volume was measured and compared with that of a known amount of the labelled compound dissolved and counted in the same conditions.

In three separate experiments, the radioactivity of the supernatant solution reached an essentially constant level after a time which varied from 5 to more than 10 days, apart from a few irregular high values attributable to failure of the filtering arrangements. On the basis of sixteen separate measurements made during these experiments during the period when steady state conditions were established, the mean value for the solubility of barium sulphate in sea water at 20° C was found to be 81 μg/l. (48 μg of barium/l.) with a standard deviation of 5·8 μg/l. In another experiment, a steady state was not established, however, the apparent solubility at the end of the experiment was similar to this value. To check the validity of the method, duplicate experiments were made in a similar way using water which had been deionized and then distilled in a silica still. The solubility found was 2·30 ± 0·22 mg of $BaSO_4$/l. at 20° C, in satisfactory agreement with accepted values such as that of Rosseinsky[8] (2·42 mg/l. at 25° C).

In interpreting the results for sea water it has been assumed that any barium originally present in solution (probably less than 10 μg/l.) would substantially exchange with the labelled solid phase during the experiment so that the amount of radioactive barium present in solution in steady state conditions gave a measure of the total barium present in solution. Experiments in which unlabelled barium sulphate was shaken with sea water saturated with labelled barium support this assumption.

The solubility of barium sulphate in sea water at 20° C and 1 atm found in these experiments is almost identical to that calculated by Chow and Goldberg[3] (52 μg of barium/l.) and appreciably higher than that calculated by Krauskopf[1] (about 30 μg of barium/l.). This gives some support to the values of activity coefficients assumed by Chow and Goldberg[3], although too much weight should not be placed on the closeness of agreement until the work has been extended to samples of sea water from other sources. Work on these lines will be continued and experiments will be made at the lower temperatures which are more characteristic of ocean waters. The results reported here, in conjunction with determinations of barium in sea water[3,4,9], confirm that barium is undersaturated in the surface waters of the ocean.

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[1] Krauskopf, K. B., *Geochim. Cosmochim. Acta*, **9**, 1 (1956).
[2] Goldberg, E. D., in *Chemical Oceanography* (edit. by Riley, J. P., and Skirrow, G.), **1**, 163 (Academic Press, London, 1965).
[3] Chow, J. T., and Goldberg, E. D., *Geochim. Cosmochim. Acta*, **20**, 192 (1960).
[4] Turekian, K. K., and Johnson, D. G., *Geochim. Cosmochim. Acta*, **30**, 1153 (1966).
[5] Spitsyn, V. I., Torchenkova, E. A., and Glazkova, I. N., *Dokl. Akad. Nauk SSSR*, **133**, 1111 (1960).
[6] Ramette, R. W., and Anderson, O., *J. Inorg. Nucl. Chem.*, **25**, 763 (1963).
[7] Bovington, C. H., *J. Inorg. Nucl. Chem.*, **27**, 1975 (1965).
[8] Rosseinsky, D. R., *Trans. Faraday Soc.*, **104**, 116 (1958).
[9] Bowen, H. J. M., *J. Mar. Biol. Assoc. UK*, **35**, 451 (1956).

Significance of Stylolites in Dolomitic Limestones

STUDIES of Western Australian stylolitic limestones reported here strongly support the widely held view that stylolites form by pressure-solution[1-3], and agree with suggestions that pressure-solution can significantly reduce the volume of carbonate formations. Furthermore, these results indicate that selective pressure-solution of calcite in dolomitic limestone can cause relative enrichment in dolomite, and this allows speculation that the process can form pure dolomite rocks (dolostones) from some dolomitic limestones. Calcite is likely to be released for the cementation of nearby formations, and thinning in the limestone bodies may induce new structures in overlying strata.

Many data relevant to this study were provided by cores of the Ordovician Thangoo Limestone, made available by West Australian Petroleum Pty., Ltd., from their Roebuck Bay No. 1 Well in the Canning Basin (lat. 18° 09′ 30″ S., long. 122° 27′ 30″ E.). The rocks come from the interval between 3,354 and 4,000 ft and range from limestones (micrites and intrasparites with less than 10 per cent replacement dolomite), through dolomitic limestones (micrites and pelsparites with 10 per cent or more replacement dolomite), to fine-grained dolostones (entirely dolomite, without palimpsest textures to demonstrate replacement).

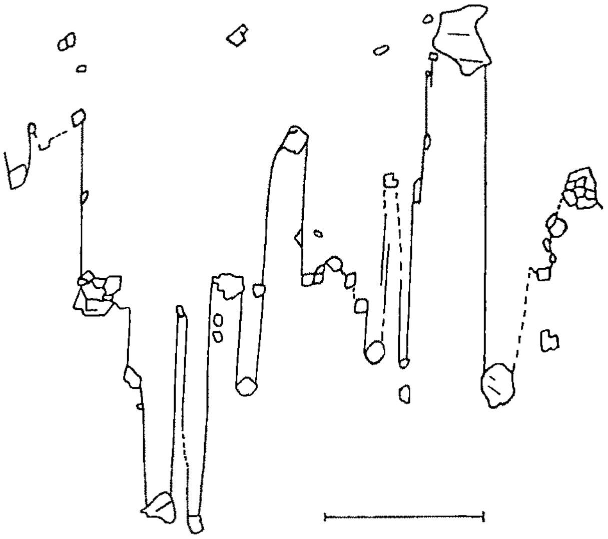

Fig. 1. Stylolite from intrasparite between 3,515 and 3,522 ft. Dolomite grains or aggregates occur at the ends of most columns, but are sparse elsewhere. A few grains show cleavage. Drawn to scale from thin section. The horizontal line represents 1 mm.

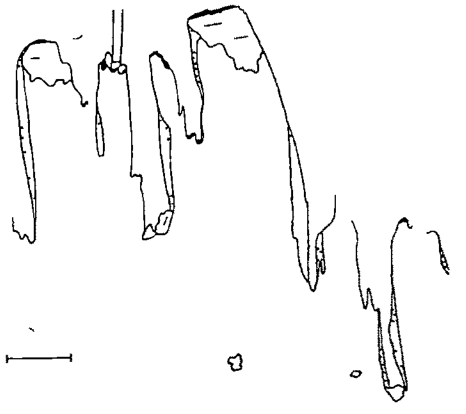

Fig. 2. Stylolite from intrasparite between 3,420 and 3,426 ft. Sparry calcite (stippled) occurs along columns, dolomite grains (some with cleavage) at ends of columns. Clay is shown black. The column of a second stylolite interferes near top left. Drawn to scale from thin section. The horizontal line represents 1 mm.

Small stylolites, usually with an amplitude of 1–5 mm, are numerous in the limestone and dolomitic limestones, but have not been recognized in the dolostones. The stylolites are partly defined by argillaceous material and commonly have dolomite grains at the ends of columns, even if dolomite is sparse elsewhere in the rock (Fig. 1). A few stylolites also contain narrow calcite veins along the sides of columns (Fig. 2).

The interrelationships of the calcite, dolomite and stylolites in the Thangoo Limestone are important, for two interpretations of fabric in stylolite-bearing dolomitic limestones are current. One view is that stylolites act as migration channels for subsequent dolomitizing solutions[4,5], and the other is that the dolomite precedes the stylolites and is concentrated along them by pressure-solution of the calcite matrix[6]. The latter view is indirectly supported by discoveries of Recent and contemporary dolomitization of limestones near the surface[7], for these discoveries suggest that dolomite formed before stylolites in many of the old rocks. The dolomite grains in Figs. 1 and 2 evidently preceded the stylolitic columns and formed protective caps at their ends. By contrast the sparry calcite along the sides of columns in Fig. 2 was emplaced during or after their development, and may have come from calcite dissolved at the ends. The original composition and texture is therefore retained only in rock between the stylolites.

The minimum section loss of Thangoo rocks resulting from pressure-solution was estimated by Stockdale's method[2] of summing the amplitudes of the stylolites. The resulting figures are approximate, for measurement is complicated by coalescence of stylolites, and the section loss represented by a stylolite may be several times its amplitude No loss from pressure-solution can be recorded for the dolostones because they lack recognizable stylolites, but the minimum loss for dolomitic limestone varies from 10 per cent to 20 per cent, and for other limestones from 30 per cent to 50 per cent Thus rocks which were originally highly dolomitic apparently lost less volume than those which were more calcitic, confirming the interrelationships indicated in Figs 1 and 2 The thickness of Thangoo Limestone intersected by Roebuck Bay No 1 (646 ft.) probably represents a loss of at least 130 ft, perhaps far more These figures are not excessive when compared with those accepted for other stylolitic limestones[1-3,8,9]

It is clearly important to seek the destination of the large volumes of dissolved calcite Initially, the calcite may precipitate elsewhere in the limestones themselves, but after all pores have been filled it must be expelled and may enter porous, less soluble rocks in the column, such as sandstones The expulsion of pore fluids and dissolved calcite in these conditions has already been commented on[3,9]

Pressure-solution is certainly not restricted to calcite, for it has been reported elsewhere in dolomite, and in siderite, gypsum, sandstone, quartzite and volcanic rock[1,10] Stylolitic boundaries, however, are found between dolomitic and calcareous portions of many Palaeozoic dolomitic limestones, and the physico-chemical conditions which allowed dolomite to persist while calcite dissolved may therefore have been widespread If so, the consequences are likely to have been both mineralogically and structurally significant The evidence indicates that selective pressure-solution of calcite can increase the proportion of dolomite in dolomitic limestones, and we can speculate that some dolostones form in this way Selective pressure-solution can also release calcite for the cementation of nearby formations It follows that sedimentary bodies derived by partial or complete removal of calcite from patchily dolomitized limestone beds will not only be thinner than their precursors, but very different in shape Faulting and folding in overlying strata may well accompany the thinning

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[1] Pettijohn, F J, *Sedimentary Rocks* (Harper, New York, 1957)
[2] Stockdale, P B, *J Geol*, 34, 399 (1926)
[3] Dunnington, H V, *J Sediment Petrol*, 24, 27 (1954)
[4] Ohle, E L, *Econ Geol*, 46, 871 (1951)
[5] Towse, D F, *Bull Amer Assoc Petrol Geol*, 41, 2493 (1957)
[6] Brown, C W, *J Sediment Petrol*, 29, 260 (1959)
[7] Murray, R C, and Pray, L C, *Spec Publ Soc Econ Paleonto Mineral*, 13, 1 (1965)
[8] Weller, J M, *Stratigraphic Principles and Practice* (Harper, New York, 1960)
[9] Ramsden, R M, *Bull Amer Assoc Petrol Geol*, 36, 2185 (1952)
[10] Golding, H G, and Conolly J R, *J Sediment Petrol* 32, 534 (1962)

Origin of the Lake Constance Basin

Lake Constance is one of the largest freshwater lakes of Central Europe and is located in south-western Germany, bordering Switzerland and Austria (Fig. 1)

The lake has a surface of 540 km^2 and a maximum depth of 252 m; its longest axis is 69 km and the maximum

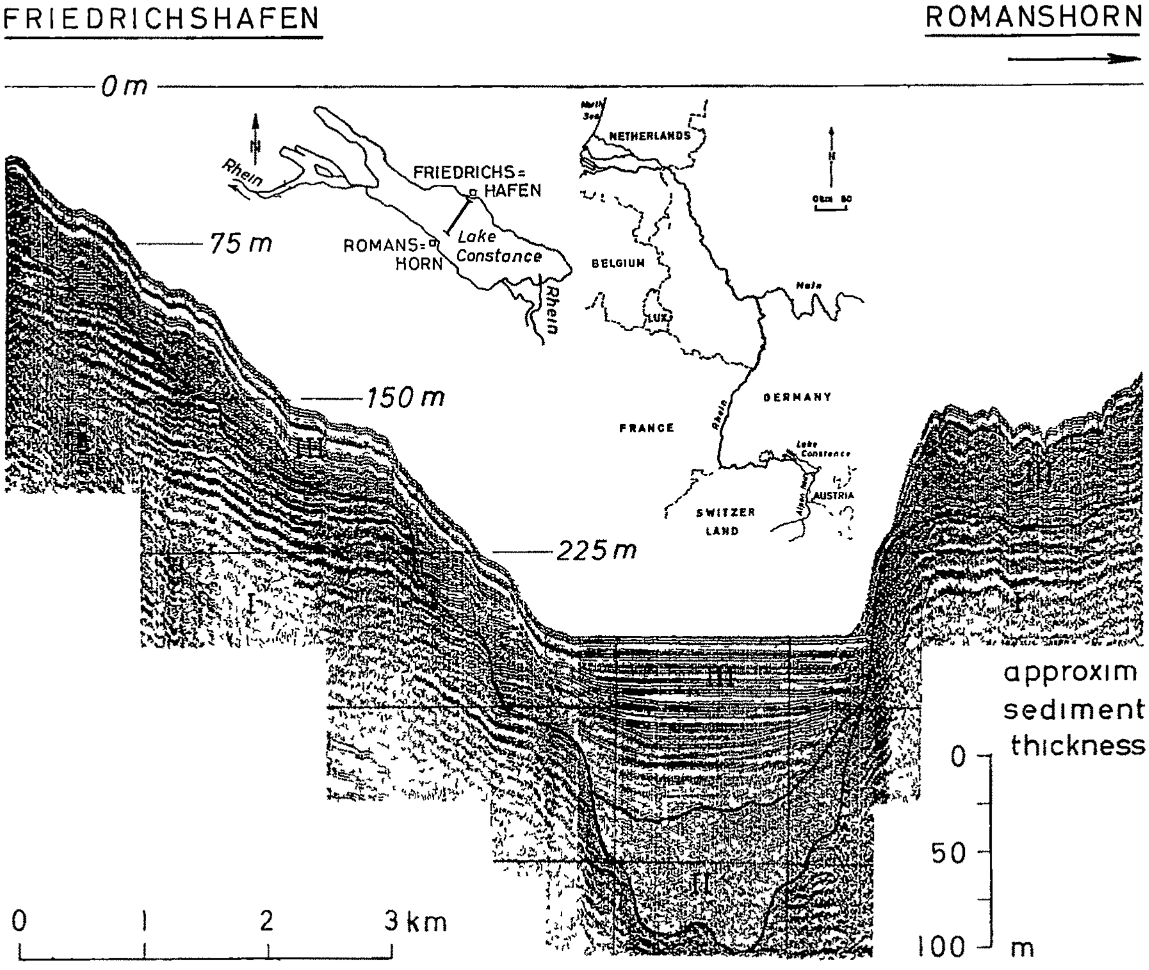

Fig 1 Continuous seismic profile (cross-section) between Friedrichshafen and Romanshorn

width about 15 km. There have been two principal theories of the origin of the lake and its basin (a) the structural theory, and (b) the glacial theory There is also (c), a combination of (a) and (b)

In connexion with a large scale research project concerned with the sediments of Lake Constance[1-4], an extensive continuous seismic survey of the sub-bottom structures was carried out during November and December 1967 Thirty-five cross-sections and two longitudinal sections were surveyed Measurements were made using a Bolt air gun with a 1 in ^{3} chamber at a pressure of about 2,000 lb /in ^{2} The air gun was triggered every 1 7 s and was towed near the surface mounted in a rubber inner tube A series of hydrophones mounted as an eal was towed about 100 ft behind the vessel The cruising speed of the vessel was 8 km/h

A typical cross-section of the central part of the lake between the towns of Friedrichshafen and Romanshorn is shown in Fig 1 On this profile, three chief principal geological units can be recognized III, a well-defined horizontally bedded youngest unit which reaches its maximum thickness in the deep parts of the lake basin, II, an older unit, underlying unit III with diffused ill-defined reflexions, and I, the oldest unit with well-defined somewhat undulatory reflexions forming the framework of the lake basin

Unit I, in which the lake basin was eroded, represents the Tertiary molasse This unit can be correlated directly with many Tertiary outcrops along the shoreline of the lake. Unit II probably consists of Late Pleistocene heterogeneous glacial material (till), which in turn is overlain by unit III representing postglacial (Holocene) fine-grained, well-bedded lake sediments The three lithological units can be traced throughout the central and western parts of the lake In the eastern part, however, the relations are less clear, probably because of the considerable amount of deltaic sediment deposited by the River Rhine[2]

This seismic survey seems to indicate clearly that the Lake Constance basin is the result of glacial erosion rather than of structural deformation The results of the survey confirm the glacial theory of formation of the lake basin postulated by Wagner[5] Recent seismic surveys in the vicinity of the lake evaluated by A Schreiner, in a lecture given at Heidelberg in 1966, have also ruled out the possibility that tectonic movements were being involved in the formation of the lake basin

This work was supported by a grant from the Deutsche Forschungsgemeinschaft Seismic equipment and personnel were made available by the Bundesanstalt fur Bodenforschung in Hanover The Anstalt fur Bodenseeforschung der Stadt Konstanz put the research vessel August Thienemann at our disposal

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[1] Müller, G , *Naturwissenschaften*, **53**, 237 (1966)
[2] Muller, G , in *Deltas in their Geologic Framework* (edit by Shirley, M L), 107 (Houston Geol Soc , Houston, 1966)
[3] Muller, G , *Naturwissenschaften*, **54**, 454 (1967)
[4] Muller, G , Schreiner, A , and Staesche, W , *Naturwissenschaften*, **54**, 87 (1967)
[5] Wagner, G , *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und -Tier e V* , **27** (1962)

Late Palaeozoic Glacial Facies and the Origin of the South Atlantic Basin

IN an attempt to add detail to the established picture of glaciation in the southern hemisphere during the Late Palaeozoic, we have studied tillites and associated rocks in Antarctica, Africa, South America and the Falkland Islands in the past few years[1-4] As well as determining the direction of ice movement from directional properties of the rocks, we have subdivided the strata into several environmentally significant facies These distinct lithological types are further restricted geographically in such a way that their distributions add information on palaeogeography For example, linear sand bodies interpreted as glacial eskers are interbedded with tillite in the western Falkland Islands[5] These deposits pass laterally into a thick, massive envelope of tillite and associated turbidites in the eastern portion of the Falklands, which suggests that terrestrial ice occupied the west while, farther east, glacial-marine accumulation took place in deeper water. Similar approximate palaeogeographic reconstructions from tillite facies can now be made for widely scattered parts of the glaciated continent of Gondwanaland

Marine fossils are not extensively known from the glacial sequences, but they have been described from Brazil, Argentina and South West Africa[6-8] Usually these sequences are considered to be of shallow marine origin A marine interpretation is also preferred for very thick accumulations (up to more than 2,000 m) known from several areas in the South Atlantic region A catchment area of considerable depth is suggested by the great thicknesses, and, moreover, strata associated with the tillite in this facies are those commonly associated with deep-marine downwarps—turbidites and deposits from related kinds of mass-movement processes These thick sequences are referred to here as being of deep-water glacial-marine origin, but they do not contain fossils and it remains to be proved that they are truly marine

Fig 1 shows a geographic reconstruction of a portion of Gondwanaland in Late Carboniferous to Early Permian time The locations and orientations of Africa and South America are based largely on the geodetic fit at the 500 fathom isobath[9], and Antarctica is fitted using the same feature The effects of post-Aptian disruption of the linear Andean–West Antarctic trend to form the present Scotia Arc[10] have been removed from the map Extensive right-slip faulting, here tentatively suggested to explain relative movement between East and West Antarctica since the end of the Palaeozoic, has also been removed Any reconstruction of this kind for South America, Antarctica and Africa leaves a broad oceanic region at the join area The present attempt minimizes the size of this region at about 5×10^6 km^2

Distribution of three glacial facies in Gondwanaland is also shown in Fig 1 Facies represented are terrestrial till, shallow-water glacial sediments, interbedded with continental deposits, and deeper-water strata, probably of glacial-marine origin The most striking characteristic of the map is the rough matching of facies boundaries across continental boundaries This provides evidence on a detailed scale in support of continental drift The present wide separation of these rocks may have resulted from drift of the continents away from each other

It can also be observed that the probable deep-marine facies occurs only in a belt around the margin of the present South Atlantic basin Glacial sediments laid down in shallow water and terrestrial tillites are located progressively farther from the basin margins The generalized palaeogeographic interpretation is that of ice bodies flowing toward and discharging into a deep, centrally located sea or series of seas on the site of the southern Atlantic basin Evidence from these rocks thus suggests that a portion of the South Atlantic basin was already in existence in Late Palaeozoic time The deep-water portion of this basin is succeeded to the north by deposits of the shallow-water and terrestrial regimes, suggesting that the basin did not extend north of about 32° S latitude, and that Africa and South America were still attached

The suite of glacial rocks referred to here constitute part of the well known southern hemisphere Gondwana Series This is a thick, regressive sequence ranging in age

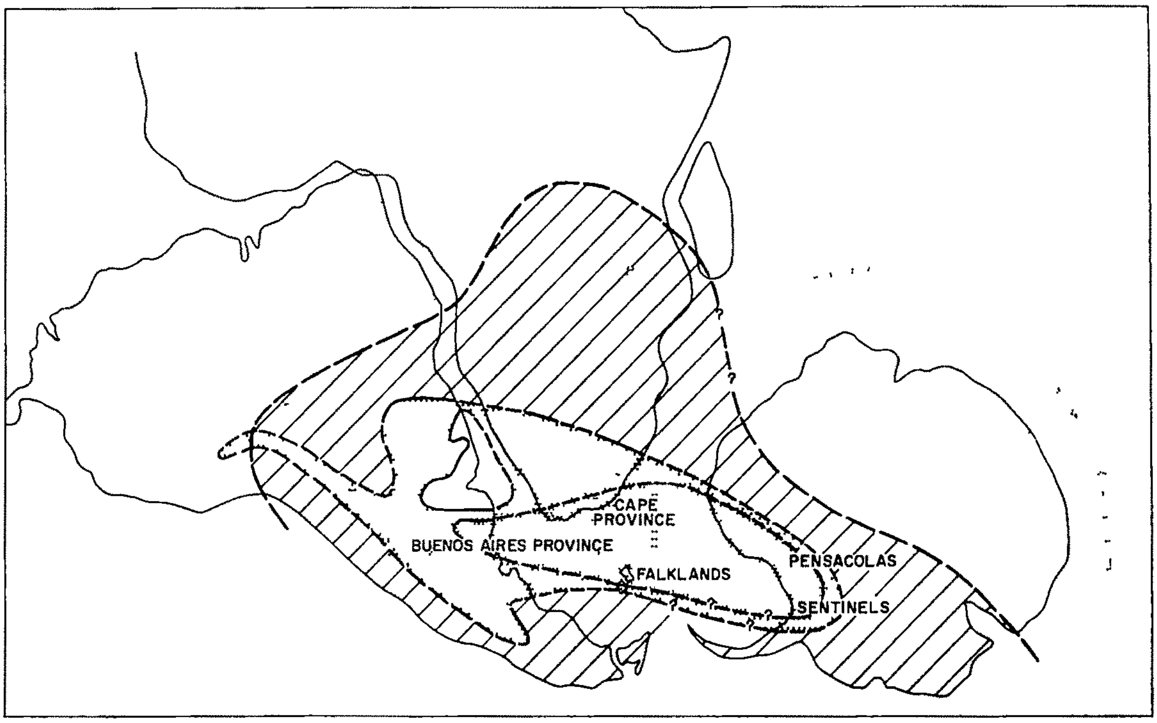

Fig. 1. Reconstruction of a portion of Gondwanaland in Late Palaeozoic time. The diagonally lined area represents the distribution of terrestrial tillites and related deposits, the open stippled area represents the region of shallow-water glacial and associated continental deposits, and the close stippled region represents the area of deep-water deposits, in part glacial-marine and marine. The unpatterned areas are presumed upland source regions

from Early Devonian (possibly Silurian) at the base, to Triassic sedimentary and Jurassic intrusive and extrusive rocks at the top. Basal marine strata lie on crystalline basement rocks and pass gradually upward into continental beds in the regions of continuous accumulation. Elsewhere, unconformities are developed, indicating intermittent uplift and subaerial erosion in marginal areas of the basin. In the areas studied, the earliest marine incursion is judged from the nature of the strata laid down to have been epicontinental, most of these deposits are probably not of deep-marine origin. Deep-water deposition marginal to the South Atlantic basin seems only to have begun with sedimentation of the glacial sequences in Late Carboniferous and Early Permian time, but timing cannot yet be determined precisely in most cases.

The presence of such a sizable waterbody in this region may have contributed to the onset of Late Palaeozoic glaciation. In order for glaciation of continental dimensions to begin in the Late Palaeozoic there must have been abundant precipitation, and thus evaporation from a large source. In a fully reconstructed Gondwanaland, the most centrally located portions of the supercontinent would lie at distances of thousands of kilometres from the peripheral ocean basins. Glaciation would therefore probably be confined to coastal areas. Development during the Middle and Late Palaeozoic of first an epicontinental and then a deeper basin penetrating to the interior of the continent would, however, provide abundant moisture to even the most remote parts of the supercontinent. Even so, Late Palaeozoic glaciation would probably not have occurred if a vast Gondwanaland had not been insulated at the time in high latitudes. The data of palaeomagnetism[11] indicate that the supercontinent drifted across the polar region at approximately the same time that the South Atlantic began to form. Without such a fortuitous arrangement, Late Palaeozoic continental glaciation might not have occurred at all.

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[1] Frakes, L. A., Matthews, J. L., Neder, I. R., and Crowell, J. C., *Science*, **153**, 746 (1966).
[2] Frakes, L. A., and Crowell, J. C., *Geol. Soc. Amer. Bull.*, **78**, 37 (1967).
[3] Crowell, J. C., and Frakes, L. A., *IUGS Symp. on Continental Drift*, Montevideo, October 1967.
[4] Frakes, L. A., Amos, A. J., and Crowell, J. C., *IUGS Symp. on Gondwana Stratigraphy*, Mar del Plata, September 1967.
[5] Frakes, L. A., and Crowell, J. C., *Geol. Soc. Amer. Bull.*, **78**, 37 (1967).
[6] Rocha-Campos, A. C., in *Problems in Brazilian Gondwana Geology* (edit. by Bigarella, J. J., *et al.*), 27 (1967).
[7] Harrington, H. J., *J. Paleontol.*, **29**, 113 (1955).
[8] Dickins, J. M., *Palaeontol.*, **4**, 138 (1961).
[9] Bullard, E., Everett, J. E., and Smith, A. G., *Phil. Trans. Royal Soc.*, A, **258**, 41 (1965).
[10] Frakes, L. A., *Geol. Soc. Amer. Bull.*, **77**, 1463 (1966).
[11] Creer, K. M., *Phil. Trans. Roy. Soc.*, A, **258**, 27 (1965).
[12] Irving, E., *J. Geophys. Res.*, **71**, 6025 (1966).

PHYSICS

Motion of a Fluid through a Vibrating Pipe

I HAVE shown[1] that for the laminar flow of a fluid through a pipe the friction factor is increased when the pipe is vibrated in a direction perpendicular to the flow, the magnitude of the increase depending on the vibration amplitude and frequency, pipe diameter, kinematic viscosity and mean flow velocity. The purpose of this communication is to investigate the cause of this effect for small vibrations.

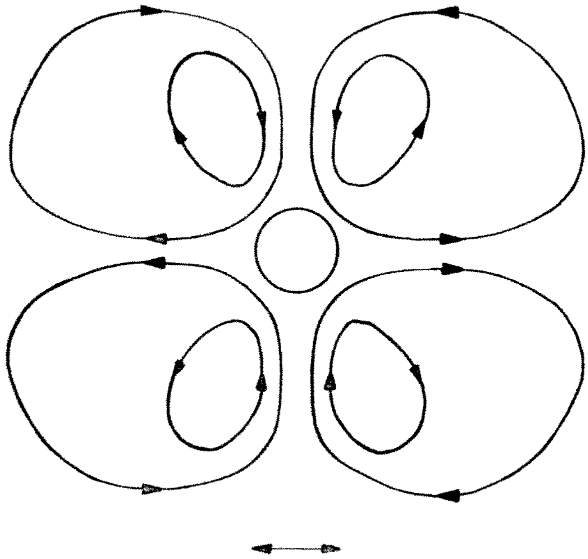

Fig. 1.

Andrade[2] and others have shown that when a stationary cylinder is subject to sound waves of sufficient intensity a system of vortices is formed around the cylinder according to Fig. 1. Schlichting[3] has analysed the case of two coaxial cylinders where the inner one vibrates in a direction at right angles to its axis. A system of vortices similar to those obtained by Andrade is formed.

F. G. McLaren and myself have subjected a cylinder containing air to transverse vibrations. A similar system of vortices was seen with the aid of smoke, and also with aluminium powder on water (Fig. 2). Such a secondary flow could give rise to an increase in friction factor when a fluid flows through a pipe.

For the case of a vibrating cylinder coaxial within another, Schlichting[3] obtained in polar co-ordinates (r,φ) the expression

$$\psi = A\left[\frac{C_1}{r^2} + C_2 + C_3r^2 + C_4r^4\right] \sin . 2\varphi$$

where

$$A = -\frac{3}{2}\frac{S^2n}{R}$$

for the stream function of the steady flow between the cylinders, where the vibrating cylinder of radius R had an amplitude S and angular frequency n.

Vibrating the outer cylinder and making the radius of the inner cylinder tend to zero gives the expression for the stream function

$$\psi = \frac{AR}{2}(x^4-x^2) . \sin . 2\varphi$$

where the radius of the outer cylinder is R and the dimensionless radius x is equal to r/R.

We can write the Navier Stokes equation in cylinder polar co-ordinates (r, φ, z) for the z direction (velocity W) in dimensionless form where we substitute

$$u = -\frac{1}{r}\frac{\partial\psi}{\partial\varphi}, v = \frac{\partial\psi}{\partial r}, \psi = \nu . \psi a, \ W = W_0w, r = xR$$

to give

$$\frac{1}{x}\left[\frac{\partial\psi a}{\partial x}\frac{\partial w}{\partial\varphi} - \frac{\partial\psi a}{\partial\varphi} . \frac{\partial w}{\partial x}\right] = -\frac{R^2}{\mu W_0}\left(\frac{\partial P}{\partial z}\right) + \nabla^2 w$$

where

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{1}{x}\frac{\partial}{\partial x} + \frac{1}{x^2}\frac{\partial^2}{\partial\varphi^2}$$

and ν is the kinematic viscosity.

To solve this equation we assume that the flow is described by the following equations, where w_0 refers to the velocity in an unvibrated pipe in the laminar region.

$$\psi a = 0 + K\psi_1$$

$$w = w_0 + Kw_1 + K^2w_2 + \ldots .$$

where

$$K = \frac{AR}{\nu}, \psi_1 = \frac{1}{2}(x^4-x^2) \sin 2\varphi, \ w_0 = 1-x^2$$

Following the general reasoning adopted by Dean[4] for a similar type of solution for flow in curved pipes, the second term of the series for w is given by

$$w_1 = \left[\frac{5x^2}{48} - \frac{x^4}{6} + \frac{x^6}{16}\right] \cos . 2\varphi$$

To determine the rest of the terms is a lengthy process, but the problem is to determine the effect of vibrations on the friction factor, such that this series is convergent. A measure of this effect can be obtained from the flow rates for vibrating and non-vibrating conditions.

The rate of flow through a pipe is proportional to

$$\int_0^1 dx \int_0^{2\pi} xw d\varphi$$

so we see that further terms in the w series are required, for the ones that effect the flow are those where w is a function of x only. We find for the next term of the series that the part that is independent of φ is given by

$$w_2 = \frac{5x^4}{384} - \frac{13x^6}{576} + \frac{11x^8}{768} - \frac{x^{10}}{320} - \frac{19}{11520}$$

Using this term and neglecting further terms in the series gives the rate of flow through a pipe with a constant pressure gradient as

$$\frac{\pi R^2 W_0}{2}[1 - 1{\cdot}2 \times 10^{-3} K^2 \ldots\ldots]$$

When no vibration is present the rate of flow is given by $\frac{1}{2}\pi R^2 W_0$, but when vibration is present the rate of flow

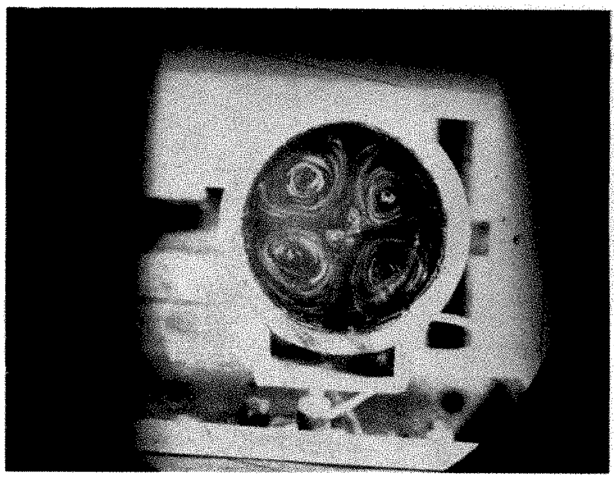

Fig. 2. Photograph of the secondary flow within a vibrating cylinder obtained with aluminium powder on the surface of water. The pipe diameter was 2·5 in. and the frequency of the weak vibration was 18 Hz. Subjecting the cylinder over a wide frequency range to additional vibrations and to intense vibrations gave very complex secondary flows, some of which appeared to be quite irregular. Very high streaming velocities were associated with some of the flows.

is reduced for the same applied pressure gradient. Consequently, the friction factor is increased and the effect depends on the value of K. It can be seen that for the equation to be valid then K must not be large. The equation gives then the effect of small vibrations on the laminar flow through a pipe.

The layer near the vibrating wall in which viscosity effects are large can be assumed to have a thickness $\sqrt{2\nu/n}$. This dimension must be small compared with the radius of the pipe if the analysis described is to be satisfactory.

In view of the limitations due to K and $\sqrt{2\nu/n}$ the theory would not be expected to describe the experimental investigation mentioned earlier[1], because K was of the order of 1,300–21,000 and $\sqrt{2\nu/n}$ was equivalent to a range of 6–28 per cent of the pipe radius.

The theory does indicate, however, that the friction factor will be increased in vibrating condition and when the vibrations are small this effect depends on K and

$$\frac{1}{R}\sqrt{\frac{2\nu}{n}}$$

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[1] Morton, A. S., *Nature*, **217**, 43 (1968).
[2] Andrade, E. N. Da C., *Proc. Roy. Soc.*, A, **134**, 445 (1931).
[3] Schlichting, H., *Physik. Z.*, XXXIII, 327 (1932).
[4] Dean, W. R., *Phil. Mag.*, S.7.5, 673 (1928).

THE SOLID STATE

Stress Dependence of Tensile Creep Rate in Carbons and Graphites

DESPITE the importance of carbons and graphites for very high temperature structural applications, very little is understood about the mechanisms of deformation and failure in these materials[1]. In principle, the stress dependence of the creep rate offers a simple method to obtain information on which of several possible deformation mechanisms operate at high temperatures. Using the empirical relationship $\dot{\varepsilon}=A\sigma^n$, where $\dot{\varepsilon}$ is the creep rate, σ is the stress and A is a proportionality constant, the stress exponent n is expected to equal one for Nabarro–Herring diffusion mass-transport creep; a much larger value of n, typically between 4 and 5, is expected for an edge dislocation climb mechanism such as occurs in many metals.

Stress exponent values of 5·5–8 have been reported for tensile creep of polycrystalline coke–pitch synthetic graphites[2-5]. This has been interpreted as evidence that climb of edge dislocation segments in crystallite tilt boundaries may be the rate-controlling mechanism[3]. Such graphites, however, usually show little or no reduction in area, which suggests that void growth (for example, by crack propagation or separation) is a principal source of macroscopic plastic strain and the deformation is highly localized in these materials. Recent investigations of the deformation behaviour of a high-density, stress-recrystallized coke-pitch graphite (ZTA) have supported this hypothesis[4-6]. Evidently, such "conventional" binder-filler synthetic graphites are not very suitable materials on which to investigate the deformation behaviour of graphite crystallites.

There are several other types of synthetic carbon and graphite in which void growth does not dominate the deformation. Pyrolytic carbons can be elongated more than 25 per cent parallel to the substrate plane at constant volume[1], and glassy (vitreous) carbons show considerable reduction in area in tensile creep (unpublished results), as does (reportedly) a commercially available fine-grained isotropic graphite ('Poco'). In such carbons, considerable crystallite deformation must occur, and a careful study of the macroscopic deformation behaviour should give information about the important fundamental mechanisms. An investigation of the stress dependence of the creep rate in pyrolytic and glassy carbons has been carried out and some results are reported here. Detailed accounts of these studies will be published elsewhere.

The pyrolytic carbon used was deposited at 2,200° C from methane by a commercial producer (Super Temp Corp.) and had a substrate-nucleated microstructure. Dog-bone type specimens of as-deposited material were deformed in tension parallel to the substrate plane under constant-load stresses of 5,000–30,000 lb./in.^{2}. Two grades of glassy carbon, *GC*-20 and *GC*-30, heat treated to temperatures of 2,000° C and 3,000° C, respectively, according to the manufacturer (Tokai Electrode Mfg. Co. Ltd.), were similarly tested at stresses ranging from 500–18,000 lb./in.^{2}. The creep tests were performed in a resistively heated graphite tube furnace in a helium atmosphere at temperatures of 2,500°–2,900° C. In common with most other carbons and graphites, neither pyrolytic nor glassy carbons exhibited a minimum or steady state creep rate. A stress-change technique was therefore developed to determine the stress dependence for a range of stresses in a single test on a single specimen. In all, nine glassy carbon and thirteen pyrolytic carbon tests were run.

In the earliest stages of the elongation of pyrolytic carbon, when layer straightening is the dominant process, $n\sim 4$ was found. In the range of elongation $1<\varepsilon<4$ per cent, however, n decreased rapidly to a value of about 1·5 where it remained until at least 26 per cent elongation was reached. After about 8 per cent elongation, the gauge section material was found to be well graphitized and very highly oriented with the layer planes aligned closely parallel to the stress axis[1]. No dependence of n on stress or temperature was observed. The creep activation energy was about 250 kcalories/mole, independent of elongation, in agreement with values found by Kotlensky[7] for pyrolytic carbon creep in the range $\varepsilon<10$ per cent and by Zukas and Green[5] for ZTA creep. This is an appropriate value for diffusion by a vacancy mechanism parallel to the layer planes[1]. It seems that in pyrolytic carbon, dislocation climb may be important initially, but after breakdown of the as-deposited structure has begun, a viscous process such as Nabarro–Herring creep predominates. The small excess of n over unity may be an experimental artefact, or may indicate simultaneous operation of a subsidiary deformation process with $1<n<3$.

The deformation behaviour of glassy carbon was quite different. The value of n increased approximately linearly with stress from a value near unity for $\sigma\leq 1,000$ lb./in.^{2} to ≥ 6 for $\sigma\geq 13,000$ lb./in.^{2}. No dependence of n on temperature or elongation (0–11 per cent) was observed, and the creep behaviour of the two grades was similar. A very high effective activation energy value of about 350 kcalories/mole was found in the medium stress range in which temperature dependence of the creep rate was investigated. Although Nabarro–Herring creep may be significant in glassy carbon at very low stresses, other mechanisms of uncertain nature must operate in the medium and high stress ranges. Because glassy carbon is a non-graphitizing carbon with small apparent crystallite size (~ 100 *A*) and a disordered structure, dislocation mechanisms seem unlikely. On the other hand, the appreciable reduction in area (nearly the constant volume value in *GC*-30) suggests that the deformation mechanism differs from that in conventional binder-filler synthetic graphites although the values of n are similar. More detailed knowledge of the structure of glassy carbons will probably be required before explanations of the interesting properties of these materials can be ventured with confidence.

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[1] Fischbach, D. B., and Kotlensky, W. V., *Electrochem. Tech.*, **5**, 207 (1967).
[2] Green, W. V., *Carbon*, **4**, 81 (1966).
[3] Green, W. V., and Zukas, E. G., *Electrochem. Tech.*, **5**, 203 (1967).
[4] Zukas, E. G., and Green, W. V., *Nature*, **212**, 1454 (1966).
[5] Zukas, E. G., and Green, W. V., *Paper MI 45, Eighth Conf. on Carbon*, Buffalo, New York (June 1967).
[6] Green, W. V., Levenson, L. S., Reiswig, R. D., and Zukas, E. G., *Paper MI 44, Eighth Conf. on Carbon*, Buffalo, New York (June, 1967).
[7] Kotlensky, W. V., *Carbon*, **4**, 209 (1966).

Structure corresponding to the Ideal Composition $3Bi_2O_3.17Nb_2O_5$

As part of a study[1] of the structures of compounds in the Bi_2O_3–Nb_2O_5 binary system, we have determined the crystal structure of $3Bi_2O_3.17Nb_2O_5$, which we believe to be the ideal formula of the compound prepared by Roth and Waring[2] and referred to as $Bi_2O_3.6Nb_2O_5$.

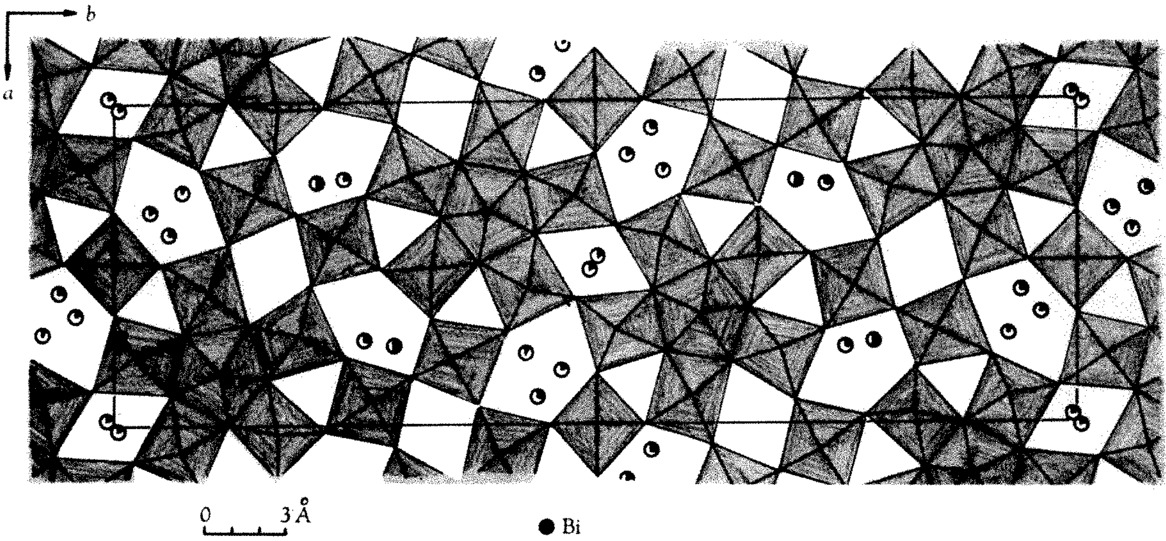

Fig. 1. The {001} projection of the structure of $3Bi_2O_3.17Nb_2O_5$ showing the $Me_{34}O_{94}$ matrix (shaded) and the occupancies of the bismuth sites.

The pale yellow plates of this material are orthorhombic with $a = 12{\cdot}516 \pm 0{\cdot}005$, $b = 37{\cdot}14 \pm 0{\cdot}02$, $c = 3{\cdot}922 \pm 0{\cdot}002$ Å, $U = 1828{\cdot}3$ Å^3. Using this unit cell we have been able to index fully the X-ray powder pattern published by Roth and Waring[2]. Single crystal photographs suggested that the structure was of the tetragonal bronze type[3] but with one cell edge tripled, and the intensities showed a remarkable similarity to those found by Sleight[4] for $Nb_{16}W_{18}O_{94}$.

The structure has been determined from some 1,300 independent reflexions, obtained with Cu–$K\alpha$ radiation and visually estimated. Least-squares refinement, in space group *Pba*2, has now reached $R = 0{\cdot}10$ and is being continued.

All atoms in $3Bi_2O_3.17Nb_2O_5$ are situated near two levels approximately $c/2$ apart. The structure is based on an $Me_{34}O_{94}$ matrix in which four of the twelve pentagonal tunnels are regular (in contrast with the other eight) and contain niobium atoms in pentagonal bipyramidal co-ordination. All the other niobium atoms have an octahedral co-ordination. The remaining eight pentagonal and six diamond-shaped tunnels, which are empty in $Nb_{16}W_{18}O_{94}$, contain all the bismuth atoms. We have found that these are statistically distributed among a number of possible sites, and have used the occupancy at each site as a parameter in the least-squares refinement. The resulting structure is shown schematically in Fig. 1.

The apparent chaos among the bismuth atoms is not unreasonable. The pentagonal and even the diamond-shaped tunnels are too large for the bismuth atom (ionic radius 0·96 Å) and, consequently, these atoms are located away from the centre of the hole. Furthermore, the bismuth atoms show a very strong tendency towards a one-sided four-fold co-ordination similar to that found, for example, in $BiSbO_4$ (ref. 5) and a number of other complex oxides and oxyhalides of bismuth[6-9]. This kind of co-ordination, a consequence of a lone pair of electrons, is more often associated with tervalent antimony[10,11] and is even found for arsenic[12]. The bismuth atoms could achieve this co-ordination at a number of sites. The bismuth occupancies of these sites are uneven (see Fig. 1) but add up to 5·7 ($2\sigma = 0{\cdot}2$) per unit cell, whereas 6 would be expected from the formula. It seems likely that the remaining 0·3 atoms are spread out thinly among some or all of the other possible sites. If, however, the true formula is $Bi_2O_3.6Nb_2O_5$, there would be $5\frac{5}{7}$ bismuth atoms per unit cell, but electrical neutrality would require the random removal of half an oxygen atom per unit cell.

It is difficult to reach a clear-cut decision between the two compositions, the difference between which is very slight (15·0 per cent Bi_2O_3, 85·0 per cent Nb_2O_5, $D_{\text{calc}} = 5{\cdot}37$ g cm^{-3} for 3 : 17, and 14·3 per cent Bi_2O_3, 85·7 per cent Nb_2O_5, $D_{\text{calc}} = 5{\cdot}30$ g cm^{-3} for 1 : 6). We consider, however, that $3Bi_2O_3.17Nb_2O_5$ represents the ideal structural formula.

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[1] Keve, E. T., and Skapski, A. C., *Chem. Commun.*, 281 (1967).
[2] Roth, R. S., and Waring, J. L., *J. Res. Nat. Bur. Stand., Sect. A*, **66**, 451 (1962).
[3] Magnéli, A., *Arkiv Kemi*, **1**, 213 (1949).
[4] Sleight, A. W., *Acta Chem. Scand.*, **20**, 1102 (1966).
[5] Aurivillius, B., *Arkiv Kemi*, **3**, 153 (1951).
[6] Aurivillius, B., *Arkiv Kemi*, **1**, 463 (1949).
[7] Aurivillius, B., *Arkiv Kemi*, **1**, 499 (1949).
[8] Aurivillius, B., *Arkiv Kemi*, **2**, 519 (1950).
[9] Aurivillius, B., *Arkiv Kemi*, **5**, 39 (1952).
[10] Edstrand, M., *Svensk. Kem. Tidskr.*, **67**, 230 (1955).
[11] Rogers, D., and Skapski, A. C., *Proc. Chem. Soc.*, 400 (1964).
[12] Skapski, A. C., *Chem. Commun.*, 10 (1966).

CHEMISTRY

Nuclear Hyperfine Interactions in Diatomic Molecules and the Ground State of VO

THE identification of the ground states of the diatomic oxides of the transition metals presents interesting experimental[1] and theoretical[2] problems, and the VO molecule is one example. Analogy with TaO suggests[3] that the lowest state should be $\sigma^2\delta\ {}^2\Delta$, but theoretical calculations[4] indicate that it is $\sigma\delta^2\ {}^4\Sigma^-$. This is supported by unpublished observations of P. H. Kasai and W. Weltner of the electron spin resonance spectrum of matrix isolated VO. A rotational analysis[5] of several bands of the green system ($\nu_{00} = 17{,}419$ cm^{-1}) led to values of the internuclear distances in the two states, but the transition was not identified: it was suggested that it might be spin-forbidden.

We now report preliminary results of the rotational analysis of bands of the green and red[6,7] (ν_{00}, $F_4 = 12{,}707$ cm^{-1}) systems. We have found that both systems may be observed in absorption at $t \sim 1{,}650°$ C in a carbon tube furnace with short paths: both systems are therefore most probably allowed. The bands of the green system consist of the four branches analysed correctly by Lagerqvist and Selin[5] as R and P branches and, in addition, four broad line branches, two R and two P. The lines of these branches are about 0·4 cm^{-1} wide, and the widths are independent of N, at least for $15 \leqslant N \leqslant 70$. The energy levels in all states are determined by an integral quantum number N rather than half integral J, so that the transition is ${}^4\Sigma$ (case b)–${}^4\Sigma$ (case b), with four R and four P branches.

Analysis of the red system is incomplete, but parts of the two shortest wavelength components of the 1–0 and 0–0 bands have been analysed. These bands show many branches, broad and sharp, and the upper state energy levels are determined by a half integral quantum number J. Thus the upper state is a case a state. Lower state combination differences are found in excellent agreement with those observed in the green bands. In the shortest wavelength components of the 0–0 and 1–0 bands, six sharp branches have been analysed which may be assigned as the ${}^TR_{42}$, ${}^SR_{43}$, ${}^SQ_{42}$, ${}^RQ_{43}$, ${}^RP_{42}$, and ${}^QP_{43}$ branches in ${}^4\Pi_{5/2}$–$X{}^4\Sigma^-$.

Line widths in the green bands are unaffected by the many upper state perturbations: this fact, together with the observation of broad lines in the red system, suggests that the cause of the broadening, or unresolved splitting, is to be sought in the behaviour of two of the four components of the ${}^4\Sigma^-$ ground state. Analysis of the red bands shows that the F_2'' ($J = N + \frac{1}{2}$) and F_3'' ($J = N - \frac{1}{2}$) components are sharp and it is therefore the F_1'' ($J = N + \frac{3}{2}$) and F_4'' ($J = N - \frac{3}{2}$) components which are broadened, by a factor of about three.

The theoretical calculations[4] suggest that the σ electron in the $\sigma\delta^2$ ground configuration is predominantly $4s\sigma$ on V. The splitting of the levels may be understood on the basis of a magnetic hyperfine interaction of this electron with the ^{51}V nucleus of spin $\frac{7}{2}$ and magnetic moment 5·14 nm. The δ electrons will not contribute significantly to the hyperfine energy which may be written[8]

$$W_{hfs} \simeq b\,\bar{I}.\bar{s},$$

with

$$b = \frac{2\,\mu_0\mu_I}{I}\left[\frac{8\pi}{3}\,\psi^2(0) - \frac{3\cos^2\theta - 1}{2r^3}\right]_{av} :$$

for a near spherical distribution of charge, only the term in $\psi^2(0)$ will be important. If the magnetic interaction is weak compared with the coupling of the other angular momenta the overall coupling will be $b_{\beta J}$ and the hyperfine energy will be given by

$$W_{hfs} = \frac{b}{3}\,\frac{[J(J+1) - N(N+1) + S(S+1)]}{2\,J(J+1)}\;\bar{I}.\bar{J}$$

where

$$\bar{I}.\bar{J} = \tfrac{1}{2}\,[F(F+1) - J(J+1) - I(I+1)].$$

If this hyperfine splitting is not resolved, the line width will be given by $(W_{F\max} - W_{F\min})$, where $F_{\max} = J + I$ and $F_{\min} = J - I$, as in BiO (ref. 9). This leads to calculated widths of lines originating from F_1'' and F_4'' which are about three times those arising from F_2'' and F_3'' for $N \gtrsim 15$ when the widths become constant, in good agreement with the observed spectrum.

We conclude that the ground state of VO is $\sigma\delta^2\ {}^4\Sigma^-$, and that this state provides a further example of a situation in which nuclear hyperfine splittings contribute significantly to the appearance of the optical spectra[10].

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[1] Cheetham, C. J., and Barrow, R. F., *Adv. High Temp. Chem.*, **1**, 7 (1967).
[2] Carlson, K. D., and Claydon, C. R., *Adv. High Temp. Chem.*, **1**, 43 (1967).
[3] Cheetham, C. J., and Barrow, R. F., *Trans. Faraday Soc.*, **63**, 1835 (1967).
[4] Carlson, K. D., and Moser, C., *J. Chem. Phys.*, **44**, 3259 (1966).
[5] Lagerqvist, A., and Selin, L. E., *Ark. Fys.*, **12**, 553 (1957).
[6] Keenan, P. C., and Schroeder, L. W., *Astrophys. J.*, **115**, 82 (1952).
[7] Gatterer, A., Junkes, J., Salpeter, E. W., and Rosen, B., *Molecular Spectra of Metallic Oxides* (Specola Vaticana, Città del Vaticano, 1957).
[8] Frosch, R. A., and Foley, H. M., *Phys. Rev.*, **88**, 1337 (1952).
[9] Barrow, R. F., Gissane, W. J. M., and Richards, D., *Proc. Roy. Soc.*, A, **300**, 469 (1967).
[10] Jorgensen, C. K., *Mol. Phys.*, **7**, 417 (1964).

Electron Spin Resonance Study of Nitroxide Radicals from Diethylhydroxylamine, Dioctylhydroxylamine and *N*-Hydroxypyrrolidine

WE present here the results of an electron spin resonance investigation of the free radicals formed from diethylhydroxylamine, dioctylhydroxylamine and N-hydroxypyrrolidine. Dioctylhydroxylamine was prepared by the method of Harris and Olcott[1]. Diethylhydroxylamine was obtained from Pennsalt Chemicals (85 per cent and 100 per cent) and from K and K Laboratories (85 per cent). N-Hydroxypyrrolidine was prepared by the method Wolfenstein[2] used for N-hydroxypiperidine and by the method described by Blout[3].

Purity of compounds was determined by thin-layer chromatography using solvent systems of acetone, methanol and acetone : methanol 1 : 1 (v/v). The K and K diethylhydroxylamine (85 per cent) gave three spots and the 100 per cent material gave two spots. N-Hydroxypyrrolidine also gave two spots. As a result of these tests, the samples were purified as oxalate derivatives and the hydroxylamine was recovered by treatment with liquid ammonia. When standard solutions of the purified materials were spotted immediately after release from the oxalate salt, each gave only a single spot.

The tertiary butylhydroperoxide used to oxidize the hydroxylamines was a product of K and K Laboratories. The molar ratios of hydroperoxide to hydroxylamine were 1 : 1 or 2 : 1. Spectra were obtained with a Varian E-3 EPR spectrometer.

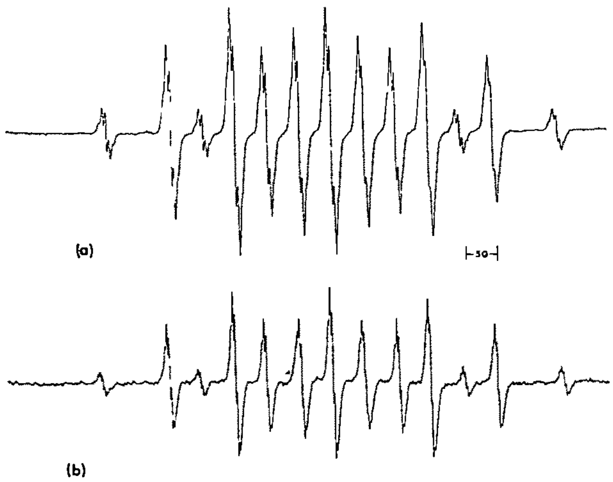
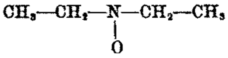
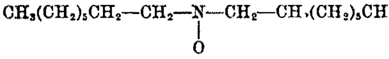
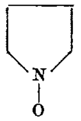


Fig 1 Room temperature electron spin resonance spectra for (*a*) dioctylhydroxylamine and (*b*) diethylhydroxylamine showing hyperfine coupling to β and γ protons In (*a*) there are four equivalent γ protons giving a hf splitting of five lines with intensity ratios 1 4 6 4 1 In (*b*) there are six equivalent γ protons giving seven lines with intensity ratios 1 6 15 20 15 6 1

The electron spin resonance spectra show that the free radicals are nitroxides resulting from abstraction of hydrogen from the hydroxyl group Similar spectra have been obtained by others from related amines oxidized to nitroxide free radicals[4–6] The three compounds gave similar electron spin resonance spectra consisting of fifteen lines resulting from coupling to nitrogen and to the four equivalent protons β to the nitrogen The differences in the observed spectra result from different values for the nitrogen and β proton coupling constants In conditions of high resolution, coupling to γ protons could be resolved for diethyl- and dioctyl-hydroxylamines purged of oxygen (Fig 1) This coupling has not so far been detected For *N*-hydroxypyrrolidine, however, the γ splitting, while observable, could not be resolved The results are summarized in Table 1

Table 1 HYPERFINE COUPLING CONSTANTS FOR THE NITROXIDE RADICALS OF DIETHYLHYDROXYLAMINE, DIOCTYLHYDROXYLAMINE AND *N*-HYDROXYPYRROLIDINE

	Coupling constants (gauss)		
Radical	A_N	A_N^{β}	A_H^{γ}
Diethylhydroxylamine  $CH_3—CH_2—\underset{\overset{\mid}{O}}{N}—CH_2—CH_3$	15 9	10 9	0 33
Dioctylhydroxylamine  $CH_3(CH_2)_5CH_2—CH_2—\underset{\overset{\mid}{O}}{N}—CH_2—CH_2(CH_2)_5CH_3$	15 4	10 2	0 50
N-Hydroxypyrrolidine 	15 0	19 2	~0 3

The commercial samples of diethylhydroxylamine were frequently found to exhibit nitroxide electron spin resonance spectra as received and the nitroxide radical concentration in these samples was observed to vary with exposure to ambient light This suggested that nitroxide free radicals are produced in hydroxylamines photochemically To investigate this possibility, samples, both as received and after purification as the oxalate, were prepared in quartz sample tubes with and without oxygen and illuminated in the cavity by a high intensity, *AH*-6 mercury lamp The response of these samples to light was found to be quite different before and after purification and to be independent of oxygen content

For the unpurified samples, the light produced a marked increase in the electron spin resonance signal which decayed when the lamp was extinguished The light also increased the yield of free radicals in samples to which peroxide had been added When wavelengths below 280 mμ were filtered out, the *AH*-6 lamp still produced a substantial electron spin resonance signal, indicating that long wavelength ultraviolet and visible light readily causes these effects Attempts to establish the rates of growth and decay of the free radicals against light intensity were unsuccessful because reproducible results could not be obtained

The purified samples, on the other hand, showed quite different behaviour Before illumination, the pure samples exhibited little or no initial nitroxide electron spin resonance signals, and illumination with wavelengths greater than 280 mμ produced no detectable electron spin resonance spectra in these samples The effect of short wavelength ultraviolet was investigated by irradiating the purified samples for 5 min at 254 mμ by means of two 15 W germicidal ultraviolet lamps The samples were immediately placed in the cavity and a significant nitroxide signal was observed which decayed slowly on standing Kinetic data could not be obtained at this wavelength because the output from these lamps could not be directed into the cavity

Ultraviolet spectra of the hydroxylamines were in accord with these findings The purified hydroxylamines showed no characteristic absorption above 220 mμ whereas the unpurified samples showed weak absorption bands at 240 mμ and 335 mμ After the purified samples had been irradiated at 254 mμ, a band appeared at 240 mμ This band increased with irradiation and is probably caused by the formation of oxidation products, such as nitroxide radicals which show absorbance at 240 mμ

These results show that nitroxide radicals can be produced directly from hydroxylamines by irradiation at 254 mμ, but not by light of longer wavelength The production of nitroxide radicals by long wavelength ultraviolet and visible light in unpurified or commercial samples is attributed to small amounts of light-sensitive impurities present in these materials Although these impurities have not been identified, traces of peroxides, for example, could cause these effects and account for the non-reproducible kinetic results

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[1] Harris, L A , and Olcott, H S , *J Amer Oil Chem Soc* , **43**, 11 (1966)
[2] Wolfenstein, R , *Chem Ber* , **25**, 2777 (1892)
[3] Blout, E R , Cohen, S G , and Green, M , *US Patent* 2,843,481, July 15, 1958
[4] Coppinger, G M , and Swalen, J D , *J Amer Chem Soc* , **83**, 4900 (1961)
[5] Chapelet-Letourneurx, G , Lemaire, H , and Rassat, A , *Bull Soc Chim* , 3283 (1965)
[6] Adams J Q , Nicksic, S W , and Thomas, J R , *J Chem Phys* , **45**, 654 (1966)

Molecular Interactions between Tryptophan and Nucleic Acid Components in Frozen Aqueous Solutions

NUCLEIC acids exhibit specific interactions with nucleoproteins and enzymes at different stages of the replication and expression of the cellular genome, and it seemed worthwhile to investigate the nature of such molecular interactions between nucleic acid components and amino-acids. This article deals with a study of complexes of tryptophan and nucleosides in frozen aqueous solutions We have already shown that, in frozen aqueous solutions, nucleic acid derivatives and aromatic amino-acids form aggregates[1,2] Thus freezing aqueous solutions may induce the formation of molecular complexes as a result of a close contact between two kinds of molecules

We have used two techniques in this investigation Emission measurements were made at the temperature of liquid nitrogen (77° K) with an Aminco–Bowman spectrophotofluorimeter Aqueous solutions were frozen in quartz tubes of 2 mm inner diameter Absorption spectra were deduced from reflectance spectra (the opacity of the frozen samples precludes the use of standard absorption spectroscopy) Reflectance spectra were obtained with a Cary 14 spectrophotometer equipped with the total diffuse reflectance accessory adapted for low temperature measurements (our unpublished work). All spectra were recorded at 253° K, for no modification was observed between 253° and 77° K

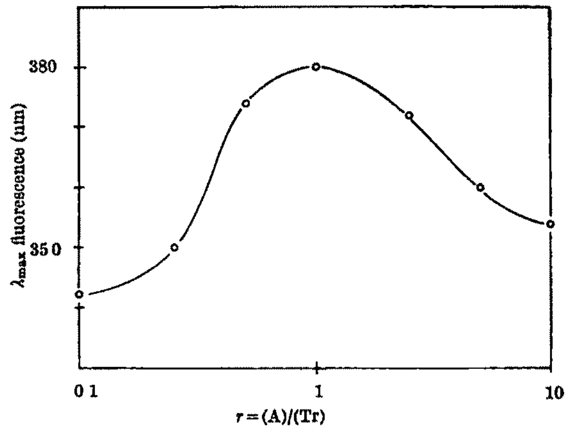

Fig 1 Variation of the fluorescence λ_{max} of tryptophan (Tr) and adenosine (A) mixtures in frozen aqueous solutions (77° K) as a function of the concentration ratio r

Table 1 FLUORESCENCE λ_{max} OF TRYPTOPHAN, NUCLEOSIDES AND EQUIMOLECULAR MIXTURES OF TRYPTOPHAN AND NUCLEOSIDES IN FROZEN AQUEOUS SOLUTIONS AT 77° K ($c=10^{-3}$ M)

Fluorescence λ_{max} (aggregates, nm)		Fluorescence λ_{max} of equimolecular mixtures with tryptophan (nm)
340	Tryptophan	
345	Guanosine	360
340	Adenosine	380
340	Cytidine	420
330	Thymidine	420
—	Uridine	420

In frozen aqueous solutions at 77° K, the fluorescence spectrum of 10^{-3} M equimolar mixtures of tryptophan and nucleosides is red-shifted as compared with that of the separated components (Table 1) The red-shift is larger with pyrimidine than with purine nucleosides, but is not modified when the concentration of both components of the mixture increases from 10^{-4} to 10^{-2} M When the ratio, r, of the nucleoside and tryptophan concentrations differs from unity, the fluorescence spectrum is the superposition of the emission from the component in excess and from the complex The apparent λ_{max} of the fluorescence spectrum goes through a maximum for $r \simeq 1$ (Fig 1), thus indicating that a 1 1 complex is probably formed The phosphorescence of the molecular mixtures is very weak, and its spectrum and decay characteristics are very close to those observed in pure tryptophan solutions in the same conditions.

The addition of increasing amounts of tryptophan to a 10^{-3} M solution of cytidine (or uridine) produces, after freezing, a new absorption band on the long-wavelength side of the component spectra (Fig 2*b*) The variation of the intensity of this band at 310 nm with tryptophan concentration (Fig 2*a*) confirms that a one to one complex is formed

The formation of aggregates in frozen aqueous solutions is inhibited by addition of small amounts of organic solvents like ethanol or propylene glycol[1-4] In such conditions, the fluorescence and absorption bands, characteristic of the complex, are not observed It must also be noted that no complex is formed in fluid solution at 293° K The appearance in mixtures of electronic transitions different from those of the components has been ascribed to molecular complexes in which charge-transfer forces play an important part[5] Indole derivatives (including tryptophan) form molecular complexes with biologically important compounds[6] Charge-transfer complexes of indoles with several electron-acceptor molecules have also been reported[7] The energy values of the lowest empty molecular orbitals of nucleic acid bases, calculated in the SCF–LCAO–MO approximation, suggest that pyrimidines are better electron acceptors than purines[8] In agreement

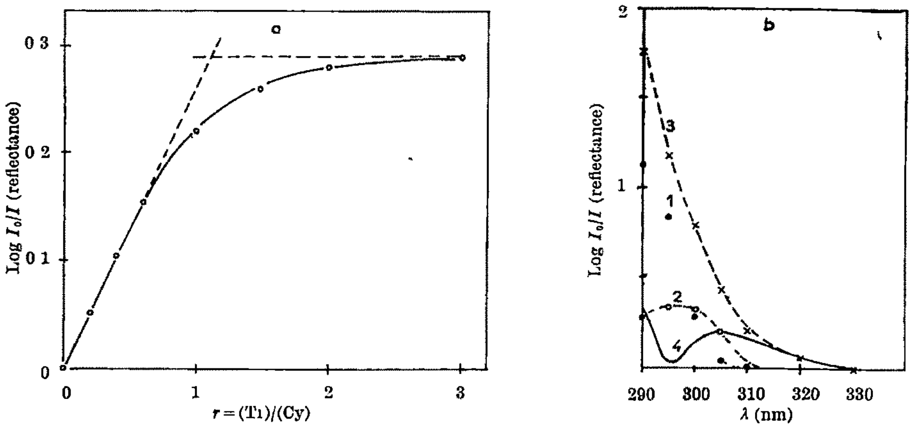

Fig 2 *a*, Variation of the band intensity at 310 nm in cytidine (Cy) and tryptophan (Tr) frozen aqueous mixtures when the concentration of tryptophan increases from 0 to 3×10^{-3} M I and I_0 are the light intensities reflected by the frozen sample with and without solute molecules, respectively {Cy}$=10^{-3}$ M *b*, Long wavelength side of the apparent absorption spectra deduced from the reflectance spectra of 10^{-3} M frozen aqueous solutions of (1) cytidine ( ), (2) tryptophan (– – –), and (3) their equimolecular mixture (– – –) Spectrum (4) (——) is the difference between (3) and the sum (1)+(2)

with this, the transition energies corresponding to their complexes with tryptophan are lower for pyrimidine than for purine nucleosides We did not observe any new electronic transitions when nucleosides were mixed with tyrosine or phenylalanine, which are poorer electron donors than tryptophan Thus charge-transfer forces are probably involved in stabilizing the ground electronic states of nucleoside–tryptophan complexes

Such molecular complexes may be important in the interactions between nucleic acids and enzymes This work is now being extended to tryptophan derivatives, oligopeptides and oligonucleotides

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[1] Hélène, C , *Biochem Biophys Res Commun* , **22**, 237 (1966)
[2] Hélène, C , Santus, R , and Ptak, M , *CR Acad Sci* , **262** C, 1349 (1966)
[3] Wang, S Y , *Fed Proc* , **24**, 5 (1965)
[4] Hélène, C , and Michelson, A M , *Biochim Biophys Acta*, **142**, 12 (1967)
[5] Mulliken, R S , *J Amer Chem Soc* , **74**, 811 (1952)
[6] Pereira J F , and Tollin, G , *Biochim Biophys Acta*, **143**, 79 (1967)
[7] Foster, R , and Hanson, P , *Trans Faraday Soc* , **60**, 2189 (1964)
[8] Berthod, H , Giessner-Prettre, C and Pullman A , *Theoret Chim Acta*, **5**, 53 (1966)

Tryptophan Emission from Trypsin and Polymer Films

THE sensitivity of the fluorescence and phosphorescence of the aromatic amino-acids to variations in their immediate environment[1–6] provides one of the few tools for probing the interior of proteins in a wide range of conditions The present studies show that for the interval 200°–300° K the phosphorescence from the tryptophans in the enzyme trypsin more closely resembles that from tryptophan in polyvinyl alcohol films (PVA) than that from tryptophan in aqueous systems This conclusion is based on comparisons of the wavelengths at which the maxima for fluorescence and phosphorescence are observed as well as the quantum yields for emission in the various systems Two of the most significant observations leading to this conclusion are that · (*a*) phosphorescence is observed at room temperature from tryptophan in either PVA or trypsin, and (*b*) temperature has many similar effects on the phosphorescence from tryptophan in PVA and from trypsin In the range 77°–170° K, however, the trypsin interior seems to be less "rigid" than either a water or PVA matrix

Previous work has shown that, even though the radiative quantum yield (fluorescence plus phosphorescence) of aromatic amino-acids in frozen aqueous media (77° K) remains essentially 1 0, the ratio of phosphorescence to fluorescence (P/F) varies markedly with *p*H and added solutes[6]. Because a variety of added solutes produce approximately comparable changes, these effects must primarily reflect alterations in the crystalline structure and rigidity, or both, of the surrounding matrix, some of the observed effects, it seems, can, however, also be produced by direct interaction of the added solute molecules with the aromatic amino-acids Experiments have thus been undertaken in our laboratory using simplified model systems in which the aromatic amino-acids are embedded in polymers containing hydroxyl, amino or carboxyl side-chains to test the effects of these groups on the aromatic residues in relatively fixed spatial environments such as are thought to comprise the interior of proteins

For the present studies, thin films of tryptophan in PVA were prepared such that the amino-acid molecules should be sufficiently separated so as to minimize self-quenching The water was evaporated from 1 mm layers of 10 per cent PVA solutions containing 1×10^{-4} M tryptophan (final thickness was about 0 1 mm and average separation corresponded to that of molecules in a 10^{-3} M solution). Thin layers of dried trypsin were prepared by evaporating solutions containing 1 mg/ml. trypsin dissolved in distilled water which had been spread on to 'Visking' dialysis membrane

Table 1 EMISSION CHARACTERISTICS OF TRYPTOPHAN IN DIFFERENT ENVIRONMENTS

	λ_F (nm)	Q_{F1}	λ_P (nm)	Q_P	P/F	τ_{37P} (s)	Q_{F1+P}
Room temperature							
PVA film	335	0 43	440	0 02	0 05	0 3 and 1 3	0 42
H_2O	350	0 20	—	0	—	—	0 20
0 55 per cent glucose	350	0 20	—	0	—	—	0 20
Trypsin in water	335	0 08	—	0	—	—	0 08
Trypsin film	335	0 15	440	~0 001	0 007	0 5*	0 15
77° K							
PVA film	330	0 63	430	0 28	0 44	5 0	0 89
H_2O	325	0 10	—	0	—	—	0 10
0 55 per cent glucose	325	0 72	425	0 17	0 24	5 8	0 89
Trypsin film	335	0 35	435	0 06	0 18	4 4	0 41

* Temperature intensity curve suggests that the long τ_{37} component disappears at 1–2 s, leaving only the short λ component

The intensity of emission arising from these preparations on excitation with 270 nm wavelength light was compared with that from a 1 mm layer of 1×10^{-4} M tryptophan dissolved in water (in order to observe emission from comparable numbers of tryptophan molecules) The determinations of fluorescence at room temperature were made in a "front face" apparatus[7] at 45° to the incident light Corrections for light scattering were made according to the method previously described[8] and the effect of polarization on the emission from these films was minimized by techniques to be discussed elsewhere (submitted for publication) The behaviour of fluorescence and phosphorescence as a function of temperature (down to 77° K) was studied in a 'Dewar' cooled by liquid nitrogen with the films mounted slightly off 45° to the exciting and emitting monochromators to minimize surface reflectance The temperature was monitored with an iron-constantan thermocouple

The feeble phosphorescence persisting at room temperature is normally obscured by scattered light and can also be confused with emission from the PVA A rotating shutter was used to minimize the scattered light Furthermore, it was established that the emission at room temperature had fine structure characteristic of tryptophan phosphorescence and also had an excitation spectrum corresponding to the absorption spectrum of tryptophan By contrast, the excitation spectrum of emission from PVA alone extends to considerably longer wavelengths

The values of λ_F and λ_P for the various preparations vary by only 10 nm at 77° K (Table 1) From previous results, it was concluded that frozen aqueous matrices are so rigid at this temperature that they are incapable of effective collisional interaction with the excited amino-acids; the temperature studies discussed here indicate, however, that this may not be so for tryptophan in trypsin There is clearly a slight increase in the interactions between tryptophan and PVA at room temperature as the fluorescence maximum is shifted 5 nm toward lower energies, these interactions must, however, be much less than those in the aqueous solutions because in the latter there is a red-shift of 25 nm on warming This difference in the extent of interaction at room temperature is further reflected in the observation that the fluorescence quantum yield in PVA is 0 3–0 56 compared with 0 2 in water† It is difficult to ascribe unequivocally the larger (compared with glucose–water) values of P/F in the PVA films to enhanced intersystem crossing alone, because in both

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† The variation in the Q_P values in PVA is probably not simple variance because eight determinations clustered around either 0 3 or 0 56 Investigations are now under way to determine whether this reflects hygroscopic behaviour of the polymer which changes either its rigidity or the mutual tryptophan–PVA orientation differences observed between yields in ices of distilled water and of glucose have been attributed previously to alterations in the structure of the ice[6]

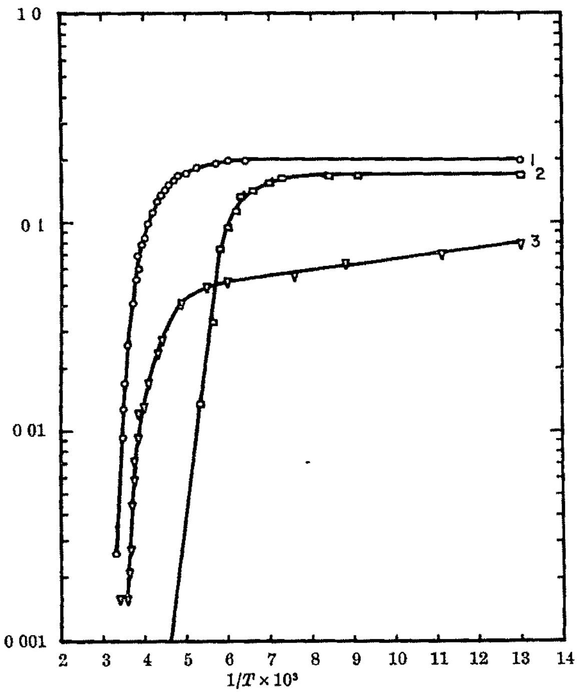

Fig 1 Arrhenius plots of phosphorescence quantum yields as a function of temperature for (1) tryptophan in PVA film, (2) tryptophan in 0 55 per cent glucose–H_2O, (3) trypsin film

systems the total quantum yield of tryptophan is not unity even at 77° K

Comparison of the temperature plots in Fig 1 indicates that both the onset and extent of quenching of phosphorescence in the PVA films are shifted toward higher temperatures relative to the data for glucose–water In fact, this shift is so pronounced that tryptophan phosphorescence persists even up to room temperature in some of the PVA films the tryptophan phosphorescence can be distinguished from the weak emission from PVA itself (having a single peak), by its fine structure with a maximum at 445 nm and secondary peaks at 425 and 475 nm as well as by differences in their excitation spectra Because the slopes of the Arrhenius plots are the same, presumably the interactions causing quenching are the same or have the same activation energies in the two systems, but the number of unperturbed molecules differs significantly

It is evident in Fig 1 that there is a great similarity between the effects of temperature on emission in tryptophan in the PVA films and in trypsin This includes the positions of the break point (c 170° K) and the almost identical slopes in the range 200°–300° K There are differences, however, when the samples are cooled from 170° K to 77° K for example, phosphorescence intensity may remain almost constant in some PVA preparations (Fig 1) and increase up to about 30 per cent in other PVA preparations, whereas there is always an increase of about 30 per cent in trypsin In those PVA preparations which exhibit an increase in intensity in the low temperature range, the high temperature slope is displaced slightly towards lower temperatures so that phosphorescence may disappear somewhat below room temperature This presumably indicates a less rigidly bonded PVA structure which could arise from differences in configuration, molecular weight or water content The overall similarities in the emission behaviour of trypsin and of tryptophan in PVA indicate, however, that the rigidity of the two systems with consequent probability of quenching interactions is very similar Furthermore, at least some of the entities in the interior of trypsin near some or all of the tryptophans are not completely "frozen in" even at 77° K, whereas it is possible to vary the extent of this "freezing in" in the PVA matrix

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[1] White, A , *Biochem J* , 71, 217 (1959)
[2] Cowgill, R W , *Arch Biochem Biophys* , 100, 36 (1963)
[3] Feitelson, J , *J Phys Chem* , 68, 391 (1964)
[4] Cowgill, R W , *Biochim Biophys Acta*, 133, 6 (1967)
[5] Cowgill, R W , *Biochim Biophys Acta*, 140, 37 (1967)
[6] Bishai, F , Kuntz, E , and Augenstein L , *Biochim Biophys Acta*, 140, 381 (1967)
[7] Drobnik, J , and Augenstein, L , *Photochem Photobiol* , 5, 13 (1966)
[8] Kuntz, E , Bishai, F , and Augenstein, L , *Nature*, 212, 980 (1966)

BIOCHEMISTRY

Inhibition by Drugs of the Accumulation *in vitro* of 5-Hydroxytryptamine in Guinea-pig Vas Deferens

AFTER an injection of ^{14}C-5-hydroxytryptophan, autoradiographic grains were found to accumulate in the sympathetic neurones of the pineal gland and vas deferens[1] These observations suggested that the sympathetic nerves of these organs might take up or retain 5-hydroxytryptamine Subsequent work has shown that 5-hydroxytryptamine can accumulate in the guinea-pig vas deferens *in vitro* by an active transport process[2] Subcellular distribution studies also indicated that the site of accumulation of the 5-hydroxytryptamine is the storage vesicle present in the sympathetic neurone[2] Certain drugs such as imipramine[3] and cocaine[4] are potent inhibitors of the uptake of noradrenaline into sympathetic neurones, and these drugs also block the uptake of 5-hydroxytryptamine in platelets[5] and some tissues in the rat[6] Subsequent work has shown that the site of blockade of noradrenaline uptake is the neuronal membrane[7] These observations prompted us to investigate the ability of these drugs to block the uptake in the vas deferens of 5-hydroxytryptamine labelled with carbon-14

The vas deferens were obtained from guinea-pigs weighing 250–300 g The animals were killed by a blow on the head, bled and the vas deferens rapidly removed and chilled They were cut into segments weighing approximately 20 mg and four such segments were incubated for 30 min at 37° C in 4 ml of Krebs–Ringer phosphate containing 0 4 μCi of ^{14}C-5-hydroxytryptamine (specific activity 40 mCi/mmole)

On removal from the medium they were washed, blotted and homogenized separately in 0 4 N perchloric acid and the homogenate was centrifuged at 15,000*g* for 15 min A 1 ml sample of the supernatant fluid was adjusted to *p*H 10 with sodium hydroxide and 1 5 ml of 0 5 M borate buffer *p*H 10 and the 5-hydroxytryptamine was extracted into 6 ml of isoamyl alcohol by shaking for 5 min After centrifugation a 4 ml sample of the isoamyl alcohol extract was transferred to a counting vial containing 4 ml of ethanol and 10 ml of phosphor 10 mg of ascorbic acid was added to a second 1 ml sample of the supernatant and the *p*H was adjusted to 1 0 by the addition of concentrated hydrochloric acid The 5-HIAA was extracted into 5 ml of diethyl ether by shaking for 3 min After standing, a 4 5 ml sample of the ether phase was shaken for 3 min with 1 ml of 0 5 M phosphate buffer *p*H 7 9 An 0 4 ml sample of the buffer extract was added to the phosphor containing 5 ml of a mixture of ethanol methanol (3 1) These methods had been shown to be specific by paper chromatography

Guinea-pig vas deferens incubated with ^{14}C-5-hydroxytryptamine (2 5 × 10^{-6} M) for 30 min was shown to give

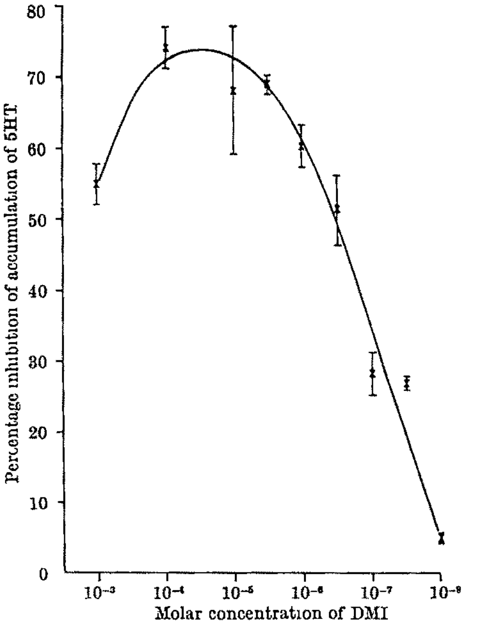

Fig 1 Inhibition of uptake of ^{14}C-5-hydroxytryptamine (5HT) in guinea-pig vas deferens by desmethylimipramine (DMI) Each group contained vas deferens from four guinea-pigs Results are expressed as mean ±*S E M*

a two-fold increase in concentration in the tissue above the medium In addition, approximately equimolar amounts of the deaminated metabolite 5-HIAA were also present in the vas deferens In the presence of cocaine 1×10^{-4} M and imipramine 1×10^{-4} M, this accumulation was reduced to 22 per cent and 38 per cent of controls, respectively The conversion to 5-HIAA, however, was unchanged at these concentrations Desmethylimipramine was found to inhibit uptake in concentrations as low as 3×10^{-8} M From 3×10^{-5} M to 1×10^{-8} M the inhibition was linear with the logarithm of the concentration for the drug (Fig 1) With very high concentrations of desmethylimipramine (10^{-3} M) the formation of 5-HIAA was inhibited, but at lower concentrations the formation was unaffected

In a previous study, it was shown that the antidepressant drug imipramine and a variety of structurally related compounds blocked the uptake of tritiated noradrenaline into the intact brain[8] It was found that those drugs with antidepressant activity blocked the uptake into brain, but those structurally related drugs which were not antidepressant did not A similar study was undertaken to determine the effect of tricyclic compounds with and without antidepressant activity on the blockade of accumulation of 5-hydroxytryptamine by the guinea-pig vas deferens For this study, the concentrations were chosen at which desmethylimipramine produced approximately 50 per cent inhibition (5×10^{-7} M). There seemed to be a consistent relationship between the ability of tricyclic compounds to block the accumulation of 5-hydroxytryptamine in vas deferens and their antidepressant activity (Table 1) There was no change in the formation of 5-HIAA

Table 1 INHIBITION OF ACCUMULATION OF ^{14}C-5-HYDROXYTRYPTAMINE IN GUINEA-PIG VAS DEFERENS BY ANTIDEPRESSANT DRUGS

Drug	Inhibition of accumulation (%)	Clinical activity†
Imipramine	57*	+
Desmethylimipramine	62*	+
Amitriptyline	43*	+
Compound 2	−2	−
Compound 3	47*	+
Compound 4	−3	−
Compound 6	43*	+
Chlorpromazine	10	−

Groups of vas deferens obtained from five guinea-pigs were incubated with ^{14}C-5-hydroxytryptamine Drugs were added at a concentration of 5×10^{-7} M After 1 h the uptake of ^{14}C-hydroxytryptamine and the formation of HIAA were measured in the tissue Results are expressed as the percentage of inhibition of accumulation of 5-hydroxytryptamine as compared with the control value Compound 2 is the isopropyl analogue of imipramine Compound 3 is the ethyl analogue of desmethylimipramine Compound 4 is the 2 chlor 10 (dimethylamino ethoxy ethyl) derivative of phenothiazine Compound 6 is the 2 chloro derivative of desmethylimipramine

* $P < 0.01$

† Clinical observations by experienced psychiatrists carried out on a limited number of patients Details of the tests can be obtained from Dr Murray Weiner, Geigy Research Laboratories, Yonkers, New York

The process of biogenic amine uptake by the vas deferens seems to be more closely related to the brain than other peripheral tissues The uptake of noradrenaline[8] in the brain and 5-hydroxytryptamine in the vas deferens is blocked by imipramine and its clinically active congeners Furthermore, chlorpromazine inhibits the uptake of biogenic amines in peripheral tissues[3] but not in brain[8] or vas deferens In view of these observations the vas deferens could serve as a useful model for brain biogenic amine containing neurones in studying the effect of certain drugs on amine uptake processes

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[1] Taxi, J, and Droz, B, *CR Acad Sci*, Ser D, **263**, 1237 (1966)
[2] Thoa, N G, Eccleston, D, and Axelrod, J, *The Pharmacologist*, **9**, 347 (1967)
[3] Axelrod, J, Whitby, L G, and Hertting, G, *Science*, **133**, 383 (1961)
[4] Whitby, L G, Hertting, G, and Axelrod, J, *Nature*, **187**, 604 (1960)
[5] Stacey, R S, *Brit J Pharmacol Chemother*, **16**, 284 (1961)
[6] Axelrod, J, and Inscoe, J K, *J Pharmacol Exp Therap*, **141**, 161 (1963)
[7] Hillarp, N A, and Malmfors, T, *Life Sci*, **3**, 703 (1964)
[8] Glowinski, J, and Axelrod, J, *Nature*, **204**, 1318 (1964)

Concentrations of Metabolic Intermediates in Kidneys of Rats with Metabolic Acidosis

GOODMAN *et al* [1] have shown that there is enhanced renal gluconeogenesis in the rat with chronic metabolic acidosis The enhancement of renal gluconeogenesis with glutamine, glutamate, α-ketoglutarate and oxaloacetate, but not with fructose or glycerol as substrates, led to the tentative conclusions that in acidosis there is acceleration of one of the steps in the gluconeogenesis pathway distal to oxaloacetate The results presented here represent an attempt to define that step

Two types of experiments were carried out on locally bred albino rats weighing 200–300 g Five or six rats were used in each experiment They were deprived of all solid food during the experiment but allowed free access to 0 2 per cent sodium chloride in water The control rats were fed 10 ml of 20 per cent dextrose in water by stomach tube every 12 h, while the experimental rats were fed 10 ml of 20 per cent dextrose containing 200 mM ammonium chloride In a total of six such experiments, fourteen control and sixteen experimental rats were used

In the second type of experiment, rats were also deprived of solid food and allowed free access to 0 2 per cent sodium chloride, and both control and experimental rats were fed 10 ml of 20 per cent dextrose in water by tube every 12 h As an acidifying agent, acetazoleamide ('Diamox') was injected intraperitoneally in a dose of 75 mg/kg every 12 h The controls were injected with an equal volume of saline Fourteen control and eighteen experimental rats were used in six experiments All experiments were concluded after 48 h

Rats were anaesthetized with intraperitoneal pentobarbital 50 mg/kg, both kidneys were then rapidly

Table 1 MEANS OF CONCENTRATIONS OF METABOLIC INTERMEDIATES IN RAT KIDNEY

Treatment		N	Pyruvate	Citrate	α-Kg	Malate	Aspartate	PEP
NH_4Cl	Control	14	120 5	427 0	114 6	452 9	1,118 3	97 5
			9 2*	30 0	10 6	40 9	21 7	8 6
	Acidotic	16	117 4	375 5	71 4	258 1	1,035 5	143 3
			18 2	21 1	3 0	24 1	49 1	6 2
	P		N S	< 0 05	< 0 01	< 0 001	N S	< 0 01
'Diamox'	Control	14	115 2	342 6	186 2	376 8	1,055 0	79 5
			4 0	20 6	17 6	32 1	61 1	5 8
	Acidotic	18	126 7	241 0	118 0	268 2	995 5	103 7
			11 1	13 0	8 6	13 4	52 0	8 0
	P		N S.	< 0 001	< 0 005	< 0 005	N S	< 0 05

Values are in μmoles/kg of wet weight α-Kg, α-Ketoglutarate PEP Phosphoenol-pyruvate N, Number of rats * Standard error

removed, dropped into a stainless steel mortar containing liquid air and squashed flat immediately There was never a delay of more than 5 s between removal of kidneys and complete freezing Tissue from each rat was processed separately The tissue was ground to a powder under liquid air, and then transferred frozen to weighed 'Duall' all-glass homogenizers containing ice cold 6 per cent perchloric acid The further handling of the tissue and the enzymatic measurements of the metabolic intermediates, except citrate, were by standard methods[2] Citrate was measured with the enzyme citrate-lyase as described by Moellering and Gruber[3]

The results are shown in Table 1 With both acidifying agents, there were significant reductions in the concentrations of citrate, α-ketoglutarate and malate as compared with controls There was no statistically significant change in the concentrations of pyruvate or aspartate In four of the six experiments with ammonium chloride and four of the six with acetazoleamide, however, there was a fall in aspartate in the experimental rats There was consistently a significant rise in phosphoenol-pyruvate The fact that identical results are obtained with both ammonium chloride and acetazoleamide implies that the effect resulted from the acidosis rather than from the agent which produced it Similar protocols have previously been shown to cause metabolic acidosis[1,4] in rats

The first important site of possible control in the gluconeogenetic pathway involves carboxylation of pyruvate to oxaloacetate by the enzyme pyruvate carboxylase with further conversion of oxaloacetate to phosphoenolpyruvate by the enzyme phosphoenol pyruvate carboxykinase (PEPCK) (ref 5) Aspartate was measured in these experiments as a guide to the concentrations of oxaloacetate, which usually exists in very small concentrations in tissues The fact that the concentration of aspartate was not significantly changed or at least fell in most experiments would indicate that there was no change, or possibly a reduction, in concentrations of oxaloacetate The rise of phosphoenol-pyruvate indicates therefore an increased activity of PEPCK This supports the conclusion of Goodman *et al* that the acceleration of gluconeogenesis is at a site distal to oxaloacetate

In chronic metabolic acidosis with an increased production of ammonia, there is increased activity of the enzymes glutaminase 1 and glutamine transaminase[6,7] The product of the former reaction is glutamate and of the latter α-ketoglutaramate and subsequently α-ketoglutarate α-Ketoglutarate is also the deamination product of glutamate It might therefore be expected that there would be a rise in the concentrations of these two intermediates In fact, a reduction in glutamate concentrations in similar conditions has already been reported[4] and in these experiments there is a fall also in α-ketoglutarate The decreased concentrations of glutamate have been shown to be important because glutamate itself inhibits the enzyme glutaminase 1 (ref 8). This is another example of feed-back inhibition It is possible that α-ketoglutarate acts similarly on the glutamine transaminase It is very likely that in metabolic acidosis the enhanced gluconeogenesis which occurs serves to remove products such as glutamate and α-ketoglutarate and thereby allows the necessary increased production of ammonia The data presented here indicate that the site of enhancement of the gluconeogenesis pathway in acidosis lies at the step between oxaloacetate and phosphoenol pyruvate, mediated by the enzyme PEPCK The reduction in concentrations of citrate and malate is also indicative of increased diversion of oxaloacetate along the gluconeogenesis pathway

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[1] Goodman, A D, Fuisz, R E, and Cahill, G F, *J Clin Invest*, **45**, 612 (1966)

[2] Bergmeyer, H U, *Methods of Enzymatic Analysis* (Academic Press, New York and London, 1963)

[3] Moellering, H, and Gruber, W, *Anal Biochem*, **17**, 369 (1966)

[4] Goldstein, L, *Amer J Physiol*, **210**, 661 (1966)

[5] Utter, M F, *Ann NY Acad Sci*, **72**, 451 (1958)

[6] Rector, F C, Seldin, D W, and Copenhaver, J H, *J Clin Invest* **34**, 20 (1954)

[7] Goldstein, L, *Nature*, **201**, 1229 (1966)

Regulatory Effect of N⁶-Benzyladenine and Pseudothymine (6-Methyluracyl) on the Synthesis of Nucleic Acids

CYTOKININS have been shown to have a hormone-like regulatory effect on the synthesis of DNA-dependent RNA (refs 1 and 2) McCalla *et al*[3] demonstrated a very low level of incorporation of benzyladenine into the insoluble RNA fraction We confirmed these findings and showed (unpublished work of G Matolcsy, K. Bojthe and B I Pozsár) that benzyladenine was incorporated without degradation Fox[4,5] showed that labelled benzyladenine accumulates to a large extent in the soluble RNA fraction, and Letham and Ralph[6] hydrolysed the isolated RNA from plant tissues, which liberated basic compounds with cytokinin-like activity We have examined the effect of several pyrimidine base-analogues on nucleic acid metabolism The synthesis of RNA was increased most by pseudothymine (6-methyluracyl) This compound is a structural isomer of thymine and can be regarded as a potential antagonist of both uracyl and thymine Its biological effect has been little studied, however[7]. This finding led us to compare this compound with 6-benzyladenine, known to be a substance with the biological activity of a cytokinin.

The method of Whitehead *et al*[8] was used to prepare both 2′-^{14}C- and 8-^{14}C-benzyladenine Labelled 2-^{14}C-6-methyluracyl was prepared by the method of Donleavy and Kise[9] Insoluble RNA was isolated from the leaf tissues using a modification by Fletcher and Osborne[10] of the Schmidt and Thannhauser[11] method

Neither the N⁶-benzyladenines nor ^{14}C-pseudothymine (^{14}C-2-6-methyluracil, unpublished work of G Matolcsy, I Pintér and B I Pozsár) incorporated into the insoluble RNA as well as adenine and uracil, respectively (Table 1) After the benzyladenine treatment, the

Table 1 INCORPORATION OF LABELLED PURINE AND PYRIMIDINE BASES INTO INSOLUBLE RNA IN THE PRIMARY LEAVES OF PINTO BEAN

Labelled bases	Incorporation into the RNA fraction (c p m / 100 mg fresh weight)	Mean error of mean value	mg/100 mg fresh weight		Specific activity (c p m / mg)	Incorporation of purine and pyrimidine bases and analogues (per cent)
			N	RNA		
8-^{14}C-Adenine	16,842	245	0 84	—	20,050	100
2′ ^{14}C-Benzyladenine	114	28	0 84	—	135	6 75
8-^{14}C-Benzyladenine	98	22	0 84		116	5 80
2-^{14}C-Uracyl	31,890	547	—	6 85	4,656	100
2-^{14}C-Methyluracyl	2,615	52	—	6 85	382	8 20

Exposure to labelled compounds lasted 4 h

Table 2 EFFECT OF BENZYLADENINE ON THE INCORPORATION OF LABELLED ADENINE AND OROTIC ACID INTO INSOLUBLE RNA, COMPARED WITH THE WATER CONTROL, IN THE PRIMARY BEAN LEAVES

Labelled bases	Effect of water treatment			Effect of benzyladenine floating			Effect of treatment on the stimulation compared with the water control (per cent)
	Incorporation (c p m /100 mg fresh weight)	Mean error of mean value	Specific activity (c p m /mg of ribose)	Incorporation (c p m /100 mg fresh weight)	Mean error of mean value	Specific activity (c p m /mg of ribose)	
8-^{14}C-Adenine	34,840	572	67,000	82,820	1,371	159,200	237
2-^{14}C-Orotic acid	42,810	653	82,320	97 420	1,642	187,300	227

In 100 mg of fresh weight of leaves was 0 52 mg of ribose.

Table 3 EFFECT OF PSEUDOTHYMINE ON THE INCORPORATION OF LABELLED ADENINE AND URACYL INTO INSOLUBLE RNA, COMPARED WITH THE WATER CONTROL, IN THE PRIMARY BEAN LEAVES

Labelled bases	Effect of water treatment			Effect of 6-methyluracyl floating			Effect of treatment compared with the water control (per cent)
	Incorporation (c p m /100 mg fresh weight)	Mean error of mean value	Specific activity (c p m /mg of ribose)	Incorporation (c p m /100 mg fresh weight)	Mean error of mean value	Specific activity (c p m /mg of ribose)	
8-^{14}C-Adenine	12,290	187	23,630	19,590	312	37,670	159
2-^{14}C-Uracyl	10,440	163	20,070	13,250	206	25,480	127

In 100 mg fresh weight of leaves was 0 52 mg of ribose

incorporation of the labelled adenine was nearly as great as that of the orotic acid (Table 2) Our results indicate that pseudothymine stimulates the incorporation of labelled adenine and uracil into the insoluble RNA fraction compared with the water control (Table 3) The effect of pseudothymine, which has a pyrimidine structure, is analogous to the hormone-like activity of benzyladenine This may indicate that both purine and pyrimidine derivatives have the same biological effectiveness

The purine and pyrimidine derivatives which we investigated were incorporated in very small amounts into the insoluble RNA, but they were characteristically accumulated in the soluble RNA (*t*RNA ?) Assuming the Watson–Crick model, it is evident that the base-analogues act on the stimulation of protein synthesis by increasing the level of *t*RNA. We could, however, demonstrate that the purine and pyrimidine derivatives have a stimulating and regulating effect on the incorporation of natural nucleobases into the insoluble RNA, so that it is impossible to interpret the stimulating effect on the basis of the Watson–Crick model It is interesting, however, that base-analogues are accumulated only in the soluble RNA fraction (*s*RNA, *t*RNA), but we do not know the form in which they are involved in the stimulation of incorporation of natural nucleobases into the RNA We finally conclude that not only purine but also pyrimidine derivatives, such as pseudothymine, have a hormone-like effect, by stimulating nucleic acid synthesis

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[1] Osborne, D J , *J Sci Food Agric* ,16, 1 (1965).
[2] van Overbeck, J , *Science*,152, 721 (1966)
[3] McCalla, D R , Morre, D J , and Osborne, D J , *Biochim Biophys Acta*, 55, 522 (1962)
[4] Fox, J E , *Plant Physiol* , 39, 31 (1964).
[5] Fox, J E , *Plant Physiol* , 41, 75 (1966)
[6] Letham, D S , and Ralph, R K , *Life Sci* , 6, 387 (1967)
[7] Schlegel, D E , and Rawlins, T E , *J Bact* , 67, 103 (1954)
[8] Whitehead, C W , and Traverso, J J , *J Amer Chem Soc* , 82, 3971 (1960)
[9] Donleavy, J J , and Kise M A , in *Organic Synthesis* (edit by Blatt, A H), 2, 1422 (Wiley, New York, 1963)
[10] Fletcher, R A , and Osborne D J , *Nature*, 207, 1176 (1965)
[11] Schmidt, G , and Thannhauser, S J , *J Biol Chem* ,161, 83 (1945)

L-2-Amino-3-phenyl-1-propanol (L-Phenylalaninol) as a Constituent of a Fungal Metabolite

WE have investigated the structure of a biologically active polypeptide compound, antiamoebin, isolated from the mycelium of the organism *Emericellopsis poonensis* Thirum by Thirumalachar (personal communication) On acid hydrolysis this substance gave an organic base which was identified as L-2-amino-3-phenyl-1-propanol (L-phenylalaninol)

When refluxed with 6 N hydrochloric acid for 28 h, antiamoebin gave a basic compound, which gave a positive ninhydrin reaction and R_F value of 0 72 in the solvent system *n*-BuOH AcOH H_2O, 4 1 5 The base was purified by distillation and recrystallization from benzene The melting point was 95° C, and $[\alpha]_D^{24^\circ}$ − 25 76 (C 3 3, ethanol) analysis gave C 71 78 per cent, H 8 52 per cent, N 8 93 per cent The molecular weight (by the Rast method) was 153 From the analysis and the molecular weight the molecular formula was calculated as $C_9H_{13}NO$ (calculated values for $C_9H_{13}NO$, C 71 52 per cent, H 8 61 per cent, N 9 27 per cent and a molecular weight of 151)

Characteristics of derivatives were as follows. (1) Dibenzoyl derivative, melting point 169° C, analysis gave C . 76 9 per cent, H 5 82 per cent, N 3 62 per cent and a molecular weight (by the Rast method) of 359. The calculated values for $C_{23}H_{21}NO_3$ are C 76 88 per cent, H 5 85 per cent, N 3 89 per cent and molecular weight 359. (2) Acid oxalate, melting point 180° C, analysis gave C 55 00 per cent, H 6 16 per cent, N . 5 80 per cent The calculated values for $C_{11}H_{15}NO_5$ are C 54 77 per cent, H 6 22 per cent, N · 5 81 per cent

These properties identified the base as L-2-amino-3-phenyl-1-propanol The reported values for this compound are melting point 95° C, $[\alpha]_D^{24^\circ}$ − 23 3 (C 2·01, methanol)[1] and the melting point for acid oxalate 177° C (ref 2) The identity was further confirmed by the mixed melting point, infrared spectrum and paper chromatographic behaviour of the authentic sample

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[1] Kapil, R S , Gautam, B C , Vohra, M M , and Nitya Nand, *Indian J Chem* , 4, 177 (1966)
[2] Hunt, J H , and McHale, D , *J Chem Soc* , 2073 (1957)

RNA in the Elementary Bodies of Trachoma Agent

TRACHOMA agent, a member of the psittacosis–lymphogranuloma–trachoma (PLT) group, is an obligate parasite of the conjunctival cells in the human eye Some strains, at least, retain their infectivity for the human eye after adaptation to growth and several passages in human cell cultures[1,2]. The infectious particles of trachoma agent are 0·3 μm in diameter and contain both DNA and RNA[3]

After entry into the host cell cytoplasm, the elementary bodies develop into large compact inclusion bodies containing glycogen[4,5] These contain non-infectious structures in which the infectious elementary bodies are formed towards the end of the growth cycle. In this respect, the trachoma agent differs from the psittacosis agent The latter forms diffuse cytoplasmic inclusions[4], consisting of large reticulated forms which replicate by binary fission to produce elementary bodies similar to those of trachoma agent[6] Both the large forms and the elementary bodies of psittacosis agent contain DNA and RNA[7], and the large forms contain soluble RNA species[8,9], ribosomal subunits and ribosomes[10] This investigation deals with the nature of the RNA species and the ribosomal subunits in the infectious elementary bodies of trachoma agent

We used the T'ang strain of trachoma agent, propagated in *FL* cells[11] for 350–400 passages Two-day-old *FL* cells in tube cultures (about 2×10^5 cells/culture) were transferred to a chemically defined medium[12] and infected with enough agent to ensure the development of one inclusion body in each cell Each of the infected cultures contained 1 μCi of ^{3}H-cytidine (specific activity, 2 2 Ci/mmole, ICN Corp , USA) and was incubated at 37° C To label proteins, the concentration of leucine was reduced to one-fortieth of that in the defined medium and 1 μCi ^{14}C-leucine (specific activity 165 mCi/mmole, Radiochemical Centre, Amersham) was added to each *FL* culture Forty-five hours after inoculation with trachoma the medium was removed and the infected cells were washed at room temperature with an 8 per cent (w/w) sucrose solution made in 10^{-2} M *tris* hydrochloric acid buffer at *p*H 7 5 and immediately adjusted to 4° C The cells were scraped into about 0 1 ml of the sucrose solution and disrupted by pipetting through a narrow capillary The nuclei, which are not damaged by this process, were centrifuged for 5 min at 1,500 r p m at 4° C, and the cytoplasmic fraction was removed The elementary bodies were purified by zone centrifugation in sucrose gradients (15–30 per cent w/w) formed on top of a cushion of 0 4 ml of 45 per cent (w/w) sucrose, prepared in *tris* buffer The gradients were centrifuged for 30 min at 10,000 r p m in the *SW* 39 rotor of the Spinco model *L*2 preparative ultracentrifuge at 7° C The material in the cushion was removed, diluted and banded in a sucrose gradient (30–45 per cent w/w) for 45 min at 10,000 r p m at 7° C About 99 per cent of the particles which band in the middle of the gradient are elementary bodies as judged by electron microscopy[3] When necessary, the infected cells were homogenized in 10^{-2} M *tris* buffer containing 1 5×10^{-3} M Mg^{++} and 10^{-2} M KCl (*tris*+Mg^{++} buffer) The elementary bodies were purified in sucrose gradients (30–45 per cent w/w) made in the same buffer They were then removed from the gradients and the optical density (*O D*) of the particle suspension was determined at 260 mμ wavelength The yield of elementary bodies from about 2×10^6 infected *FL* cells is 4×10^8 plaque forming units as determined by the plaque assay developed by Bernkopf[13] Such a purified preparation had an absorption of 1 *O D* unit at 260 mμ wavelength Direct isolation of RNA from the elementary bodies of trachoma agent was achieved by dissolving the particles in 1 per cent solution of sodium dodecyl sulphate (SDS)[14] The dissolved particle suspension was diluted three-fold with SDS buffer and carefully layered with a plastic spoon on top of a sucrose gradient (15–30 per cent w/w) made in SDS buffer Care was taken to prevent shearing of the DNA of this agent, which contains about 40 per cent of the radioactivity of the incorporated ^{3}H-cytidine The gradients were spun in the *SW* 25 1 rotor for 18 h at 24,000 r p m. at 25° C The gradients were collected through a Gilford spectrophotometer and each fraction was divided one portion was precipitated with cold 20 per cent trichloroacetic acid (TCA) and the other was treated with 1 N potassium hydroxide for 18 h at 37° C before treatment with TCA. The alkali was used to determine the amount of radioactive isotope which was incorporated into DNA, which is resistant to treatment with alkali The acid-precipitable radioactivity was collected on 'Millipore' filters and counted in a Packard liquid scintillation counter In the experimental conditions, all the DNA sedimented to the bottom of the centrifuge tube When necessary, the cytoplasmic fractions of non-infected HeLa, *FL* or *BSC*$_1$ cells were used as markers for ribosomes and as a source of ribosomal RNA

To obtain ribosomal subunits from the elementary bodies of trachoma agent, the particles were disrupted by two methods. (*a*) To a suspension of elementary bodies, Mg^{++} was added to a final concentration of 3×10^{-3} M, the suspension was diluted threefold with *tris* buffer containing 3×10^{-3} M Mg^{++} and disrupted with 1 per cent sodium deoxycholate (DOC, N B Co , USA) (*b*) To the suspension of elementary bodies, Mg^{++} was added to a final concentration of 10^{-2} M, and the particles were treated in the MSE ultrasonic disintegrator for 3 min at 1 5 A The suspension was diluted three-fold in *tris* buffer containing 10^{-2} M Mg^{++} and layered with a plastic spoon on top of a sucrose gradient (15–30 per cent w/w) prepared in the same buffer The gradients were centrifuged for 18 h at 18,000 r p.m in the *SW* 25 1 rotor at 7° C, and collected through a Gilford spectrophotometer Each fraction was divided into two portions, one of which was treated with TCA and the other first with alkali and then TCA

The results (Fig 1) demonstrate the nature of the RNA species isolated from the elementary bodies of trachoma agent Three RNA species with sedimentation coefficients of 21*S*, 16*S* and 4*S* were obtained The sedimentation coefficients were determined by comparison with the sedimentation of animal cell ribosomal RNA (28*S* and 16*S*) centrifuged either together with trachoma RNA or on separate gradients About 70–75 per cent of the radioactive RNA is found in the 21*S* and 16*S* RNA species present in the elementary bodies and the rest in the 4*S* RNA This indicates that no cellular 28*S* RNA is present in the purified preparations of the trachoma elementary bodies as contamination from the host cells

The localization of RNA within the infectious elementary bodies was also investigated The particles were disrupted with DOC (method *a*) and centrifuged in sucrose gradients in the conditions used for the isolation of ribosomal and ribosomal subunits from HeLa cells[15] Fig 2*A* shows that trachoma RNA sediments in the sucrose gradient as two subunits with sedimentation constants of 53*S* and 30*S*, respectively Ribosomal subunits are obtained when Mg^{++} is present in the *tris* buffer at concentrations of 10^{-2} M or 3×10^{-3} M (Fig 2*A*)

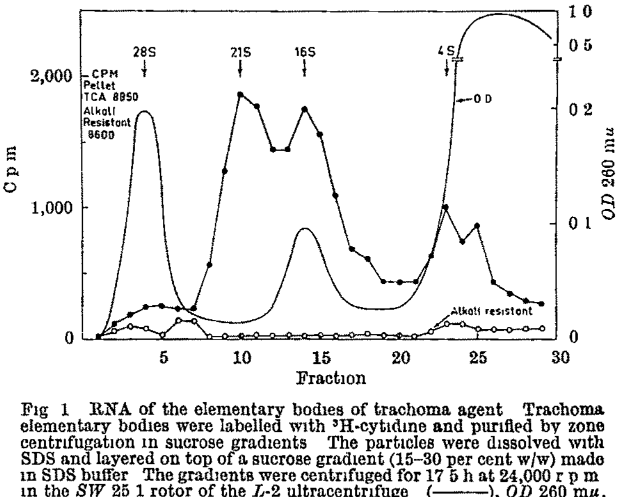

Fig 1 RNA of the elementary bodies of trachoma agent Trachoma elementary bodies were labelled with ^{3}H-cytidine and purified by zone centrifugation in sucrose gradients The particles were dissolved with SDS and layered on top of a sucrose gradient (15–30 per cent w/w) made in SDS buffer The gradients were centrifuged for 17 5 h at 24,000 r p m in the *SW* 25 1 rotor of the *L*-2 ultracentrifuge (———), *OD* 260 mμ, (—●—), TCA precipitable radioactivity, (—○—), alkali resistant radioactivity

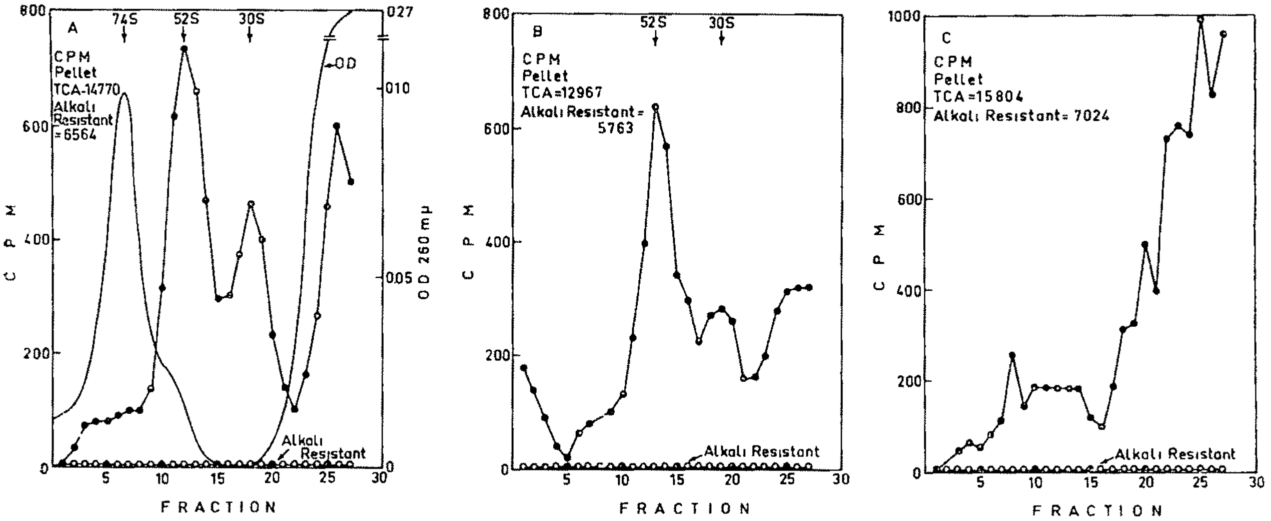

Fig 2 Effect of Mg++ concentration on the isolation of ribosomal subunits from the elementary bodies of trachoma agent Elementary bodies, purified by zone centrifugation in sucrose gradients, were divided into three portions to which different Mg++ concentrations were added *A* 3×10^{-3} M Mg++, *B*, 1 5 $\times 10^{-3}$ M Mg++, and *C*, no Mg++ added. The samples were treated with DOC and centrifuged in sucrose gradients (15–30 per cent) made in *tris* 10^{-2} M and supplemented with Mg++ at concentrations of *A*, 3×10^{-3} M, *B*, 1 5 $\times 10^{-3}$ M, and *C*, 3×10^{-3} M The gradients were centrifuged for 17 h at 17,000 r p m at 7° C (———), *OD* 260 mμ, (—●—), TCA precipitable radioactivity, (—○—), alkali resistant radioactivity

and also 1 5 $\times 10^{-3}$ M (Fig 2*B*) Without Mg++, all the RNA is found at the top of the sucrose gradient which shows the lability of ribosomal subunits in the absence of Mg++ (Fig 2*C*). Attempts were made to isolate the RNA from the different ribosomal subunits and to determine the *S* value by zone centrifugation in SDS sucrose gradients From the 52*S* and 30*S* ribosomal subunits, 20*S* and 16*S* RNA molecules, respectively, were isolated and identified

To characterize further the ribosomal subunits, the elementary bodies of trachoma agent, labelled in the RNA with ^{3}H-cytidine and in the proteins with ^{14}C-leucine, were purified by zone centrifugation in sucrose gradients The particles were disrupted with DOC and the ribosomal subunits were isolated in sucrose gradients Fig 3 shows that radioactive RNA is found in the 52*S* and 30*S* bands Radioactive proteins can be clearly detected in the 52*S* subunits The large amount of labelled proteins which sediment into the gradient masks the 30*S* subunit

In these conditions, there was no evidence for intact ribosomes in the elementary bodies of trachoma agent Sonic treatment (method *b*), which releases about 80 per cent of the RNA, was used to break the elementary bodies and 52*S* and 30*S* ribosomal subunits—not ribosomes—were obtained Furthermore, when the elementary bodies of trachoma agent were prepared and purified in *tris* and Mg++ buffer and dissolved in 1 per cent DOC, ribosomal subunits but not ribosomes were obtained

This study of the nucleic acids of the infectious elementary bodies of trachoma agent has demonstrated the presence of three RNA species with sedimentation constants of 21*S*, 16*S* and 4*S*, respectively The trachoma RNA species resemble in their behaviour the ribosomal RNA species isolated from the elementary bodies and the reticulated forms of psittacosis agent[8] Using the present techniques, 52*S* and 30*S* ribosomal subunits and 21*S* and 16*S* ribosomal RNA were isolated from purified elementary bodies of the 6 *BC* strain of psittacosis agent (unpublished results of Sarov and Becker) Similarly, ribosomal subunits with sedimentation constants of 50*S* and 30*S*, but not ribosomes, were isolated from the purified developing reticulated forms of psittacosis agent[10]

The ribosomal RNA and subunits of both trachoma and psittacosis agents resemble those of bacterial cells[16], which also have sedimentation constants of 50*S* and 30*S* by contrast with the 60*S* and 40*S* ribosomal subunits of animal cells[15] The absence of ribosomes in the sucrose gradient analyses of the elementary bodies of trachoma agent may be caused by (*a*) lability of ribosomes which dissociate into subunits, (*b*) attachment of ribosomes to membranes with consequent failure of release from the disrupted elementary bodies or (*c*) the assembly of ribosomal subunits into ribosomes only in the presence of newly synthesized messenger RNA molecules[15,17] which are formed only after the entry of the infecting elementary bodies into the host cell cytoplasm Further experiments are in progress to define the nature and synthesis of trachoma ribosomes and ribosomal subunits

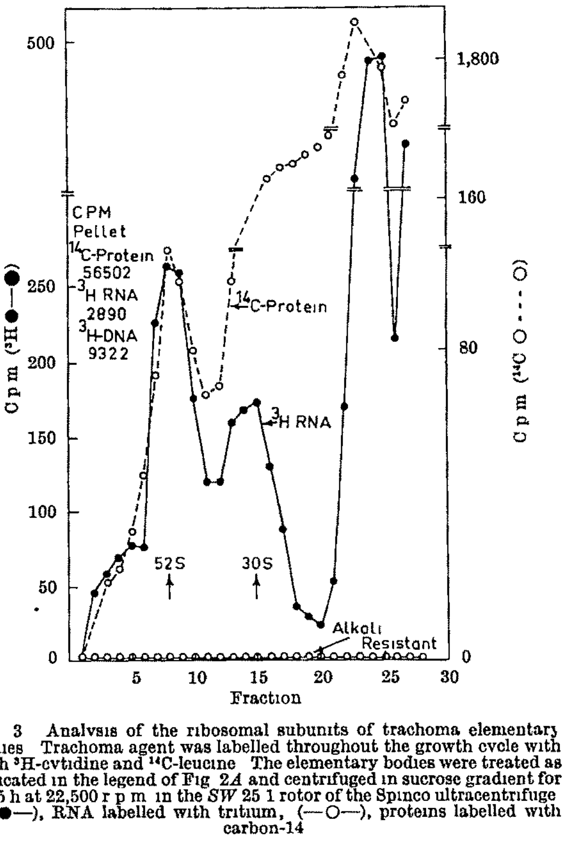

Fig 3 Analysis of the ribosomal subunits of trachoma elementary bodies Trachoma agent was labelled throughout the growth cycle with both ^{3}H-cytidine and ^{14}C-leucine The elementary bodies were treated as indicated in the legend of Fig 2*A* and centrifuged in sucrose gradient for 15 5 h at 22,500 r p m in the *SW* 25 1 rotor of the Spinco ultracentrifuge (—●—), RNA labelled with tritium, (—○—), proteins labelled with carbon-14

The presence of ribosomal RNA and ribosomal subunits in the elementary bodies of trachoma and psittacosis agents demonstrates the cellular nature of these agents, which clearly differ from viruses[6] The taxonomic position of the PLT group, and the exact relationship between trachoma and psittacosis agents, are yet to be established

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[1] Becker, Y , Mashiah, P , and Bernkopf, H , *Bull Res Council, Israel*, **10E**, 160 (1963)
[2] Bernkopf, H , Treu, G , and Maythar, B , *Arch Ophthal* , **71**, 693 (1964)
[3] Sarov, I , Becker Y , and Bernkopf, H , *Israel J Med Sci* , **2**, 661 (1966)
[4] Gordon, F B , and Quan A L , *J Infect Dis* , **115**, 186 (1965)
[5] Bernkopf, H , Mashiah, P , and Becker, Y , *Ann NY Acad Sci* , **98**, 62 (1962)
[6] Moulder, J W , *Ann Rev Microbiol* , **20**, 107 (1966)
[7] Tamura, A , and Higashi, N , *Virology*, **20**, 596 (1963)
[8] Tamura, A , and Iwanaga, M , *J Mol Biol* , **11**, 97 (1965)
[9] Tamura, A , Matsemoto, A , and Higashi, N , *J Bact* , **93**, 2003 (1967)
[10] Tamura, A , *J Bacteriol* , **93**, 2009 (1967)
[11] Bernkopf, H , Mashiah, P , and Maythar, B , *Bull Res Council, Israel*, 8E, 121 (1960)
[12] Ossowski, L , Becker, Y , and Bernkopf, H , *Israel J Med Sci* , **1**, 186 (1965)
[13] Bernkopf, H , *Amer J Ophthalmol* , **63**, 1206 (1967)
[14] Gilbert, W , *J Mol Biol* , **6**, 384 (1963)
[15] Joklik, W K , and Becker, Y , *J Mol Biol* , **13**, 496 (1965)
[16] Osawa, S , *Progress in Nucleic Acid Research and Molecular Biology* (edit by Davison, J N , and Cohn, W E), **4**, 161 (Academic Press, 1965)
[17] Nakada, D , and Kaji, A , *Proc US Nat Acad Sci* , **57**, 128 (1967)

Temperature Dependence of Cortisol Binding to Plasma Proteins

THE binding of cortisol to plasma proteins increases with a decrease in temperature No direct measurements have been reported of the effect of temperature on the binding constants of transcortin and albumin, but it has been suggested that as the temperature decreases new binding sites appear on transcortin[1]

The cortisol-binding characteristics of two pools of normal human plasma have been determined at different temperatures, using ultrafiltration of plasma with precautions against adsorptive loss and ethanol denaturation as described earlier[2] The unbound cortisol concentration in plasma water was determined in each plasma pool before and after the addition of known amounts of cortisol The cortisol additions were sufficient to increase the plasma cortisol concentration by 5, 15, 30 and 400 µg/100 ml Plasma 11-OH corticosteroids were determined by the method of Mattingly[3]

If transcortin and albumin each have a single species of binding sites for cortisol with capacities, respectively, P_t and P_a and equilibrium constants K_t and K_a, Tait and Burstein[4] have shown from mass action considerations that with total cortisol concentration T, free concentration F, amount bound to transcortin B_t, and to albumin B_a, then

$$K_t(P_t - B_t) = B_t/F \qquad (1)$$

$$(P_aK_a) = B_a/F \qquad (2)$$

$$T = F + B_a + B_t \qquad (3)$$

Because P_a is very much larger than the cortisol additions used, the binding sites on albumin never approach saturation and P_a and K_a cannot be determined independently Their product (P_aK_a) can however, be determined with good precision

Substitution of equations (1) and (2) into (3) yields

$$T - F = P_t + (P_aK_a)/K_t + 1/K_t + (P_aK_a)\,F - T/F\,K_t \qquad (4)$$

with known values T and F and unknowns K_t, P_t and (P_aK_a) The addition of cortisol to a plasma sample provides further values of T and F with the binding constants unchanged Three such results for T and F (T_1, T_2, T_3 and F_1, F_2, F_3) permit the three unknown constants to be determined, thus

$$(P_aK_a) = \frac{(B_3 - B_1)(a_1 - a_2) - (B_2 - B_1)(a_1 - a_3)}{(F_3 - F_1)(a_1 - a_2) - (F_2 - F_1)(a_1 - a_3)}$$

$$K_t = \frac{(F_3 - F_1)(a_1 - a_2) - (F_2 - F_1)(a_1 - a_3)}{(B_2 - B_1)(F_3 - F_1) - (B_3 - B_1)(F_2 - F_1)}$$

and

$$P_t = B_2 + (a_2 - P_aK_a - 1)/K_t - (P_aK_a)\,F_2$$

where

$$B_1 = T_1 - F_1,\ B_2 = T_2 - F_2,\ B_3 = T_3 - F_3,\ \text{and } a_1 = T_1/F_1,\ a_2 = T_2/F_2,\ a_3 = T_3/F_3$$

The values selected for T critically affect the precision with which the binding constants can be measured The lowest T_1 possible leads to the best estimate of K_t, a value of T_2 approximately equal to P_t is necessary for accurate determination of P_t and the best estimate of (P_aK_a) is given by a value of T_3 at least three times greater than P_t Using cortisol additions of zero, 15 and 400 µg/100 ml , the precision of estimates of P_t and (P_aK_a) is about ± 5 per cent (1 *S D*) and of K_t approximately ± 10 per cent (1 *S D*)

Table 1 EFFECT OF TEMPERATURE ON BINDING CONSTANTS OF TRANSCORTIN (P_t, K_t) AND ALBUMIN (P_aK_a)

Temperature (degrees C)	Plasma I P_t M/1 × 10⁷	 K_t 1/M × 10⁻⁷	 (P_aK_a) —	Plasma II P_t M/1 × 10⁷	 K_t 1/M × 10⁻⁷	 (P_aK_a) —
10	4 6	44	3 7	7 5	26	3 7
25	4 9	26	3 1	—	—	—
37	5 1	11	2 6	6 7	9	2 8

The results obtained are shown in Table 1 The small changes of P_t with temperature are probably not significant The most marked effect of decreasing temperature is an increase in K_t with a lesser increase in (P_aK_a) The calculation of these constants is based on the assumption that transcortin retains at low temperature its characteristic of having only a single species of binding site. A Scatchard plot[5] of the full data from one of the plasmas is shown in Fig 1 Deviations from linearity on such a plot provide a sensitive indication of inhomogeneity of binding sites[6] The figure shows good linearity, and so

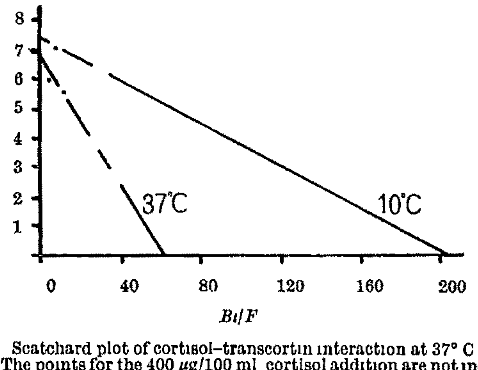

Fig 1 Scatchard plot of cortisol–transcortin interaction at 37° C and at 10° C The points for the 400 µg/100 ml cortisol addition are not included because they were used to calculate P_aK_a

it may be concluded that a single species of binding site is predominant on transcortin both at 37° C and at 10° C

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[1] Mills, I H , *Brit Med Bull* , 18, 127 (1962)
[2] Pearson, J , Keane, P M , and Walker, W H C , *Nature*, 216, 1334 (1967)
[3] Mattingly, D , *J Clin Pathol* , 15, 274 (1962)
[4] Tait, J F , and Burstein, S S , in *The Hormones*, 5, 441 (edit by Pincus, G , Thimann, K V , and Astwood, E B) (Academic Press, 1964)
[5] Scatchard, G , *Ann NY Acad Sci* , 51, 660 (1949)
[6] Bull, H B , *An Introduction to Physical Biochemistry*, 120 (Davis, Philadelphia, 1964)

Regulation of Insulin Secretion by Short Chain Fatty Acids

Evidence is accumulating that in some mammalian species there may be a direct effect of short chain fatty acids on the secretion of insulin from the pancreas Thus it has been shown that octanoate causes a marked release of insulin from slices of rat pancreas[1] Furthermore, butyrate was found to evoke a rapid and sustained elevation of serum insulin when infused into adult sheep[2] These results contrast with the lack of effect on insulin secretion when longer chain fatty acids, such as palmitate, are incubated with pancreas slices[3]

With the development of methods for the isolation of islets of Langerhans in large numbers[4,5] it has become possible to study the mechanism of insulin secretion by the measurement of concentrations of metabolic intermediates. We have measured intracellular concentrations of glucose-6-phosphate following incubation of rat islets with butyrate, octanoate and citrate The results suggest that short chain fatty acids are important regulators of glucose-6-phosphate levels in pancreatic islet cells, and in this way they may influence insulin secretion.

Islets of Langerhans were isolated from pancreas by collagenase digestion of tissue taken from male albino Wistar rats which had been fasted overnight The separated islets were divided into groups of ten and incubated in bicarbonate-buffered medium containing albumin together with either glucose, octanoate, butyrate or citrate In each experiment, the effects of low and high glucose were compared with those of low glucose plus organic acid After 30 min of incubation at 37° C the medium was sampled for determination of its insulin content by the immunoassay of Hales and Randle[6]. Rat insulin provided standards for the assay The islets from each group of incubation flasks were then pooled in pointed centrifuge tubes, the medium removed and the islets quickly frozen to $-76°$ C by immersion of the tube in a bath of solid carbon dioxide and ethyl alcohol They were then subjected to sonication for 20 s, after the addition of perchloric acid (0 1 ml /ten islets) The supernatants from the perchloric extracts were then neutralized with potassium carbonate, before the estimation of glucose-6-phosphate by the enzymatic cycling procedure described by Lowry *et al* [7] This gave us reproducible results in good agreement with those already reported by Matchinsky[8] for rat islets Similar concentrations of glucose-6-phosphate have been reported by Idahl and Hellman[9] for islets taken from obese hyperglycaemic mice, in which β-cells constitute more than 90 per cent of the islet tissue

The results obtained are shown in Table 1, which shows that butyrate, octanoate and citrate all stimulate the release of insulin from isolated rat islets of Langerhans, and that paralleling the secretion there is an increase in the intracellular concentrations of glucose-6-phosphate in the islets

These results can be explained as an effect of short chain fatty acids on the intracellular accumulation of citrate which may then affect phosphofructokinase, resulting in an inhibition of glycolysis and accumulation of glucose-6-phosphate, as has been suggested by Randle *et al* for other tissues[10]. Furthermore, the results do not support the idea that the release of insulin brought about by sugars is mediated by glycolysis, for, in the presence of fatty acids, glycolysis is inhibited, but rather they suggest that glucose has to be metabolized by some other pathway in order to stimulate insulin secretion We have already indicated that the pentose phosphate pathway may be important in this respect[11]

Table 1 SECRETION OF INSULIN AND THE INTRACELLULAR CONCENTRATIONS OF GLUCOSE-6-PHOSPHATE IN ISOLATED RAT ISLETS OF LANGERHANS INCUBATED IN THE PRESENCE OF OCTANOATE, BUTYRATE AND CITRATE

Addition to medium	Insulin secretion (μ units/10 islets/min, mean ± *S D*)	Glucose-6-phosphate levels ($\mu\mu$moles/40 islets, mean ± *S D*)
2 5 mM glucose	18 ± 4 (20)	25 5 ± 7 0 (8)
20 0 mM glucose	39 ± 6 (20)	59 5 ± 10 0 (8)
2 5 mM glucose + 5 0 mM sodium citrate	37 ± 5 (20)	49 0 ± 12 0 (8)
2 5 mM glucose	18 ± 4 (20)	26 5 ± 5 0 (8)
20 0 mM glucose	38 ± 5 (20)	65 5 ± 11 0 (8)
2 5 mM glucose + 5 0 mM sodium butyrate	33 ± 4 (20)	47 5 ± 10 0 (8)
2 5 mM glucose	19 ± 3 (20)	22 5 ± 5 0 (4)
20 0 mM glucose	40 ± 5 (20)	51 0 ± 5 0 (4)
2 5 mM glucose + 5 0 mM sodium octanoate	38 ± 4 (20)	48 5 ± 7 0 (4)

It is not known whether longer chain fatty acids can also exercise a regulatory effect by this means, because so far it has been difficult to obtain effects of albumin-bound long chain fatty acids on insulin secretion In addition, ketone bodies have also not been shown to be effective in releasing insulin in most species, whether used *in vitro*[3,12] or *in vivo*[13] Thus we found that 5 mM β-hydroxybutyrate did not alter rates of secretion when incubated with isolated rat islets. Nevertheless, ketone bodies and long chain fatty acids may be important in modifying insulin release in certain other species, for Madison *et al.* have obtained effects of these agents in inducing insulin secretion in the dog[14,15]. By contrast, a much more consistent effect of short chain fatty acids on release of insulin has been obtained. Our results indicate that the "glucose fatty acid cycle"[10] may be operative as a means of metabolic regulation in pancreatic islet cells

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[1] Sanbar, S S , and Martin, J M , *Metabolism*, 16, 482 (1967)
[2] Manns, J G , and Boda, J M , *Amer J Physiol* , 212, 747 (1967)
[3] Howell, S L , thesis, Univ London (1967)
[4] Lacy, P E , and Kostianovsky, M , *Diabetes*, 16, 35 (1967)
[5] Howell, S L , and Taylor, K W , *Biochim Biophys. Acta*, 130, 519 (1966)
[6] Hales, C N , and Randle, P J , *Biochem J* , 88, 137 (1963)
[7] Lowry, O H , Passonneau, J V , Hasselberger, F. X , and Schulz, D W , *J Biol Chem* , 239, 18 (1964)
[8] Matchinsky, F M , *Fed Proc* , 26, 257 (1967)
[9] Coore, H G , Hellman, B , Idahl, L A , and Taljedal, I B , *Opusc Med* , 12, 285 (1967)
[10] Randle, P J , Garland, P B , Newsholme, E. A , and Hales, C N , *Ann NY Acad Sci* , 131, 324 (1965)
[11] Montague, W , Howell, S L , and Taylor, K W , *Nature*, 215, 1088 (1967)
[12] Coore, H G , and Randle, P J , *Biochem J* , 93, 66 (1964)
[13] Fajans, S S , Floyd, J C , Knopf, R F , and Conn, J. W , *Ciba Found Colloq Endocrinol* , 15, 99 (1964)
[14] Madison, L L , Mebane, D , Unger, R H , and Lochner, A , *J Clin Invest* , 43, 408 (1964)
[15] Seyffert, W A , and Madison, L L , *Diabetes*, 16, 765 (1967)

Clinical and Pharmacological Effects of Monoamine Precursors or Haloperidol in Chronic Schizophrenia

MANY psychotropic drugs have been shown to interfere with monoamine metabolism in the brain[1,2] Recently, interest has been focused on tryptophan and 5-hydroxytryptamine metabolism in mental depression[3,4] During combined treatment with tryptophan and a monoamine oxidase inhibitor (MAOI) in healthy volunteers, mental effects described as drunkenness and hyperreflexia appeared[5] In schizophrenic patients, the same combination of drugs caused central nervous stimulation and varying effects on the disease symptoms[6-8] (reviewed in ref 9) In contrast, 5-hydroxytryptophan also in combination with a monoamine oxidase inhibitor is reported to have no such effects on schizophrenic patients[7,10] This discrepancy may depend on an accumulation of tryptamine after treatment with MAOI and tryptophan but not 5-hydroxytryptophan Because of these findings we have made therapeutic investigations of schizophrenic patients given single doses of monoamine precursors and at the same time made repeated estimations of acid monoamine metabolites in the lumbar cerebrospinal fluid Ashcroft *et al* have pointed out that ventricular CSF rich in acid monoamine metabolites might reach the lumbar tap site if large amounts of cerebrospinal fluid were tapped[11] In our study, however, the concentration of the acid monoamine metabolite which was uninfluenced by the treatments was unaffected by repeated tapping of the lumbar fluid 5-Hydroxyindole acetic acid (5-HIAA) and homovanillic acid (HVA) in the cerebrospinal fluid were estimated by the methods of Roos[12] and Andén, Roos and Werdinius[13] Our patients had been in hospital for many years, and 14 days before these studies were undertaken all treatment was stopped

One patient was given 50 mg of DL-5-hydroxytryptophan intravenously. No mental effects could be observed and there seemed to be no discomfort On the other hand there were considerable changes in the concentration of 5-hydroxyindole acetic acid in the cerebrospinal fluid (Table 1)

Table 1

Patient No	Intravenous injection of	5-HIAA in CSF (μg/ml) Before injection	5-HIAA in CSF (μg/ml) 2 h after injection	HVA in CSF (μg/ml) Before injection	HVA in CSF (μg/ml) 2 h after injection	HVA in CSF (μg/ml) 6 h after injection	HVA in CSF (μg/ml) 24 h after injection
1	5-Hydroxytryptophan, 50 mg	0 047	0 085	0 058	0 059	—	—
2	L-DOPA, 50 mg	0 026	0 038	0 031	0 041	0 077	—
3	Haloperidol, 20 mg	0 026	0 029	0 031	0 048	0 042	0 034
4	Haloperidol, 40 mg	0 041	—	0 044	0 080	0 089	0 10

5-HIAA, 5-Hydroxyindole acetic acid, CSF, cerebrospinal fluid, HVA, homovanillic acid

Another patient was given 50 mg of L-DOPA with no side effects nor apparent influence on the mental state The concentration of homovanillic acid in the cerebrospinal fluid was, however, increased (Table 1) Bernheimer *et al* [14] gave 50 mg of L-DOPA intravenously to both normal subjects and patients with various organic brain diseases but did not observe any changes of the concentration of homovanillic acid in the cerebrospinal fluid

In two schizophrenic patients who, by mistake, happened to receive intravenous haloperidol, 20 and 40 mg, respectively, we could measure the concentrations of homovanillic acid and 5-hydroxyindole acetic acid in the cerebrospinal fluid at intervals up to 24 h after injection These patients had previously been treated with large doses of other neuroleptics but without effect After the injections of haloperidol—they were free of other drugs—the patients were moderately sedated and showed mild orthostatic symptoms Patient No 4 (who received 40 mg) had a moderate hand and foot tremor for 4 days but showed no rigidity or motor unrest There were no changes in the mental state despite mild sedation. The concentration of homovanillic acid in cerebrospinal fluid in this patient doubled within 6 h of the injection The patient who received 20 mg of haloperidol showed no change in the concentration of homovanillic acid in the cerebrospinal fluid (Table 1) It has been shown experimentally that after doses of haloperidol or chlorpromazine comparable with those used in psychiatric therapy, homovanillic acid in the corpus striatum increases significantly[15-17]. It was therefore surprising that only after doses of 40 mg was there a noticeable change in the concentration of homovanillic acid in the cerebrospinal fluid In dogs, however, a similar phenomenon has been encountered A dose of 0 3 mg/kg of haloperidol increased the homovanillic acid in the corpus striatum more than 100 per cent but had no influence on the homovanillic acid in the cerebrospinal fluid (unpublished results of H Andersson).

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[1] Schildkraut, J J , *Amer J Psychiat* , 122, 509 (1965)

[2] Bunney W E , and Davis, J M , *Arch Gen Psychiat* , 13, 483 (1965)

[3] Persson, T , and Roos, B -E , *Lancet*, ii, 987 (1967)

[4] Coppen, A , Shaw, D M , Herzberg, B , and Maggs, R , *Lancet*, ii, 1178 (1967)

[5] Sjoerdsma, A , Oates, J A , Zaltzman, P O , and Udenfriend, S , *J Pharmacol Exp Ther* ,126, 217 (1959)

[6] Lauer, J W , Inskip, W M , Bernsohn, J , and Zeller, E A , *Arch Neurol Psychiat* , 80, 122 (1958)

[7] Pollin, W , Cardone, P , and Kety, S S , *Science*, 133, 104 (1961)

[8] Alexander, T , Curtis, C G , Sprince, H , and Crosley, A P , *J Nerv Ment Dis* , 137, 135 (1963)

[9] *Psychopharmacology Bulletin*, 4, 19 (1967)

[10] Feldstein, A , Freeman, H , Hope, J M , Dibner, I M , and Hoagland, H , *Amer J Psychiat* ,116 219 (1959)

[11] Ashcroft, G W , Crawford, T B B Eccleston, D , Sharman, D F , MacDougall, E J , Stanton, J B , and Binns, J K , *Lancet*, ii, 1049 (1966)

[12] Roos, B -E , *Life Sci* ,2, 1 (1963)

[13] Andén, N -E , Roos B -E , and Werdinius, B , *Life Sci* , 2, 448 (1963)

[14] Bernheimer, H , Birkmayer, W , and Hornykiewics, O , *Wien Klin Wschr* , 78, 417 (1966)

[15] Andén, N -E , Roos, B -E , and Werdinius, B , *Life Sci* , 3, 149 (1964)

[16] Laverty, R , and Sharman, D F , *Brit J Pharmacol Chemother* , 24, 759 (1965)

[17] Juorio, A V , Sharman, D F , and Trajkov, T , *Brit J Pharmacol Chemother* , 26, 385 (1966)

MICROBIOLOGY

Isolation of *Salmonella typhimurium* Mutants with Increased Recipient Ability by the Use of *R* Factor

SEXUAL recombination occurs only at very low frequencies between *Escherichia coli* Hfr and *Salmonella typhimurium* recipients[1-5] Frequencies of transfer of *R* factors[6] from *E coli R*+ to *S typhimurium R*- are also very low (10-7/ donor)[7] Thus a characteristic feature of the mating of *E coli* with *S typhimurium* is the low recipient ability of *Salmonella* strains The low recipient ability of *Salmonella* was overcome by isolating fertile mutants by an indirect selection method in *S typhimurium LT-7 mut*[5] We reported that these fertile mutants are restrictionless and modificationless for foreign deoxyribonucleic acids (DNA) such as phage *P*22 DNA, *R* factors, *F* factor and bacterial chromosomes[8]. Eisenstark[9] found that the frequencies of hybridization between *E. coli* Hfr and *S. typhimurium* *LT*-2 are increased by treatment of *LT*-2 recipients with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG). We have found that two types of mutants of *LT*-2 selected by the transfer of an *R* factor from *E. coli* *K*-12 behaved, after elimination of the *R* factor with acridine orange, as good recipients of *E. coli* chromosomes, in the same way as was shown for *K*-12 (*P*1) by means of *F*-prime factor transfer[10].

For the isolation of mutants with high recipient abilities for the transfer of *R* factor from *E. coli* about 5×10^8 cells of a logarithmic phase culture of *LT*-2 *cys D*-36 (cysteine-requiring) were washed and suspended in 20 ml of saline containing 40 µg/ml of NG. These cells were incubated without aeration at 37° C for 1 h. After washing the cells by centrifugation, they were suspended in 10 ml of fresh 'Penassay' broth (Difco) and incubated overnight without aeration at 37° C. A sample (5 ml.) of this culture was added to an equal volume of fresh 'Penassay' broth and incubated with aeration until the cell titre reached about 5×10^8/ml. Of this culture, 5 ml was mixed with an equal volume of a culture of *E. coli* *CSH*-2(222) (a methionineless, F^- substrain of *K*-12 with an *R* factor 222 carrying the markers of resistance to sulphonamide, streptomycin, chloramphenicol and tetracycline) grown to about 5×10^8 cells/ml in a 200 ml Erlenmeyer flask. The mixture was incubated without aeration at 37° C for 6–8 h. Then 0.1 ml samples of the mixture were plated on minimal agar containing 50 µg/ml of *l*-cysteine and 25 µg/ml of chloramphenicol or 8 µg/ml of tetracycline hydrochloride.

Samples without the treatment with NG gave none or few colonies per plate and those treated with NG gave more than ten colonies per plate. After successive single colony isolations from those colonies, *R* factor was eliminated by the treatment with 20 µg/ml of acridine orange[11]. It was shown that the drug resistance markers of the *R* factor were eliminated as a whole. Most clones of *LT*-2 *cys D*-36 thus obtained from the samples treated with NG gave increased recipient abilities for the transfer of *R* factor from *K*-12 R^+, whereas all clones, as far as studied, from the samples without NG treatment gave low recipient abilities comparable with the original *LT*-2 *cys D*-36. We refer to those mutants as *LT*-2 *Rfer*1 (*fer* = fertile) and the original *LT*-2 *cys D*-36 as *LT*-2 *Rinfer* (*infer* = infertile).

In the next step, it was attempted to isolate mutants with better recipient abilities from *LT*-2 *Rfer*1. About 10^8 cells of a logarithmic phase culture of *LT*-2 *Rfer*1 were suspended in 10 ml of 'Penassay' broth containing 40 µg/ml of NG. These cells were incubated with aeration at 37° C overnight (it was found that treatment with NG in saline for 1 h did not give satisfactory results). After washing the cells by centrifugation, they were suspended in 'Penassay' broth and grown to about 5×10^8 cells/ml by aerating at 37° C. The procedure thereafter was identical to that in the first step. Mutants isolated by the second step procedure had higher recipient abilities than *Rfer*1. These mutants are referred to as *LT*-2 *Rfer*2. The donation of *R* factor from *E. coli* to *Salmonella* did not result in the transfer of chromosomal markers to a measurable extent when studied for lactose and cysteine markers.

The frequencies of transfer of *R* factor from *CSH*-2-(222) to various *Salmonella* mutants and the frequencies of recombinants between *K*-12 Hfr and various *Salmonella* mutants are shown in Tables 1 and 2, respectively. Table 3 shows the efficiencies of plating (eop) on various *Salmonella* mutants of phage *P*22 (a clear mutant) grown on various *Salmonella* derivatives. *LT*-2 *Rfer*1 and *Rfer*2 as well as *LT*-7 *fer* gave the highest eop of phage *P*22 grown on any strain, while the eop of phage *P*22 grown on these strains were about 10^{-4} on *LT*-2 *Rinfer* and *LT*-7 mut^+ as compared with the eop on *Rfer*1 and *Rfer*2 strains. The eop of phage *P*22 grown on *LT*-2 *Rinfer* and *LT*-7 mut^+ were about equal on all the strains. The modification of phage *P*22 grown on *LT*-2 *Rinfer* and *LT*-7 mut^+ was completely lost within one cycle of growth of the phage in *LT*-2 *Rfer*1 or *Rfer*2. There was no difference in the adsorption to various *Salmonella* mutants of phage *P*22 grown on any of these strains. Degradation of DNA of ^{32}P-labelled phage *P*22 grown on *LT*-7 *fer* occurred up to 40 per cent in the infection of *LT*-7 mut^+ or *LT*-2 *Rinfer*[8]. These findings indicate that the mutations leading to *Rfer*1 are modificationless and restrictionless as to phage *P*22.

In the transfer of *R* factor 222 from *CSH*-2(222), *LT*-2 *Rfer*1(222) or *LT*-2 *Rfer*2(222), *LT*-2 *Rinfer* was a very poor recipient (the frequency of transfer was about 10^{-7}/donor). When *LT*- 2 *Rinfer* (222) was used as a donor, *LT*-2 *Rinfer* was a better recipient (the frequency of transfer was about 10^{-4}/donor). These results indicate that *Rfer*2 as well as *Rfer*1 and *E. coli* *K*-12 is restrictionless and modificationless in contrast to *Rinfer* which is considered to be positive in host-controlled restriction and modification.

Table 1. FREQUENCIES OF TRANSFER OF AN *R* FACTOR 222 FROM *E. coli* *CSH*-2(222) TO VARIOUS MUTANTS OF *S. typhimurium* *LT*-2

Fertility of recipient	No. of strain	Frequency of *R* transfer
Rinfer	*LT*-2 *cys D*-36	1.0×10^{1}/ml
*Rfer*1	40–4	2.9×10^{3}
*Rfer*2	1–11	1.4×10^{5}
*Rfer*2	2–41	3.5×10^{4}
*Rfer*1	3–41	2.3×10^{3}
*Rfer*2	4–51	2.4×10^{4}
*Rfer*2	5–51	6.0×10^{4}
F^- (wild type)	$W3102/\lambda^r$	2.6×10^{6}

Mating conditions are described in the text. Duration of mating was 8 h. $W3102/\lambda^r$ is a galactose-negative, λ-resistant, $\lambda^- F^-$ substrain of *E. coli* *K*-12.

Table 2. FREQUENCIES OF RECOMBINANTS IN THE CROSSES OF *E. coli* *W*2252 (Hfr) WITH VARIOUS SUBSTRAINS OF *S. typhimurium*

	Recipient					
	LT-2 *Rinfer*	*LT*-2 *Rfer*1	*LT*-2 *Rfer*2	*LT*-2 *Rfer*2	*LT*-7 *fer*	*W*677/*Sm* F^-
Selected marker	Lactose$^+$	Lactose$^+$	Lactose$^+$	Proline$^+$	Lactose$^+$	Proline$^+$
Frequency per donor cell	$<6.0\times10^{-10}$	1.0×10^{-8}	1.0×10^{-7}	2.8×10^{-7}	3.0×10^{-3}	2.0×10^{-4}

The procedure for conjugation was that of Sanderson and Demerec[13]. *W*677/*Sm* is a threonine, leucine, and thiamine-requiring, mannitol, xylose, maltose, galactose, and lactose-non-fermenting, streptomycin-resistant, F^- derivative of *E. coli* *K*-12.

Table 3. EFFICIENCIES OF PLATING OF A CLEAR MUTANT OF PHAGE *P*22 ON VARIOUS SUBSTRAINS OF *S. typhimurium*

	Efficiency of plating of phage *P*22 grown on					
Indicator	*LT*-7 *fer*	*LT*-7 *mut*	*LT*-7 mut^+	*LT*-2 *Rinfer*	*LT*-2 *Rfer*1	*LT*-2 *Rfer*2
LT-7 *fer*	1.0	1.0	1.0	1.0	1.0	1.0
LT-7 *mut*	1.0×10^{-4}	4.0×10^{-1}	7.3×10^{-1}	2.1	5.6×10^{-3}	1.2×10^{-2}
LT-7 mut^+	3.0×10^{-5}	1.3×10^{-2}	7.2×10^{-1}	1.4	6.1×10^{-5}	1.1×10^{-4}
LT-2 *Rinfer*	6.0×10^{-5}	2.0×10^{-3}	1.1	1.4	7.8×10^{-5}	1.1×10^{-4}
LT-2 *Rfer*1	1.2×10^{-1}	3.4×10^{-1}	1.2	6.5×10^{-1}	7.5×10^{-1}	1.0
LT-2 *Rfer*2	5.0×10^{-1}	3.6×10^{-1}	2.5×10^{-1}	1.0	3.3×10^{-1}	4.4×10^{-1}

During this investigation, we have found that *Rfer*2 mutants are sensitive to coliphages *T*3, *T*7, *W*-31 (ref. 12) and *P*1 to all of which *Rinfer* and *Rfer*1 strains are resistant. Most *Rfer*2 strains were resistant to phage *P*22 and rough in their colonial morphology. These coliphage-sensitive mutants could also be isolated in a single step from *LT*-2 *Rinfer* by the second method referred to. The sensitivity to phage *P*1 of these mutants has made possible the transduction with *P*1 in *S. typhimurium* and between *E. coli* and *S. typhimurium*. The transduction in *S. typhimurium* as well as the phage sensitivity patterns of these mutants will be reported in a later paper.

*Rfer*1 mutants, after transfer of *R* factor and before its elimination, could be easily screened with a spot test by their sensitivity to phage *P*22 grown on *Rinfer* (modified *P*22) and by the absence of their restrictions of *P*22 grown on *Rfer*1 (non-modified phage). *Rfer*2 mutants could be easily screened by their sensitivity to the coliphages

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[1] Baron, L S , Carey, W F , and Spilman, W M , *Intern Cong Microbiol* , Stockholm, 50 (1958)
[2] Baron, L S , Carey, W F , and Spilman, W M , *Proc US Nat Acad Sci* , **45**, 976 (1959)
[3] Baron, L S , Spilman, W M , and Carey, W F , *Science*, **130**, 566 (1959)
[4] Zinder, N D , *Science*, **131**, 813 (1960)
[5] Miyake, T , *Genetics*, **47**, 1043 (1962)
[6] Watanabe, T , *Bact Rev* , **27**, 87 (1963)
[7] Watanabe, T , and Fukasawa, T , *J Bacteriol* , **81**, 669 (1961)
[8] Okada, M , Watanabe, T , and Miyake, T , *J Gen Microbiol* , **49**, 888 (1968)
[9] Eisenstark, A , *Proc US Nat Acad Sci* , **54**, 117 (1965)
[10] Glover, S W , Schell, U , Symonds, N , and Stacey, K A , *Genet Res Camb* , **4**, 480 (1963)
[11] Watanabe, T , and Fukasawa, T , *J Bact* , **81**, 679 (1961)
[12] Sanderson, K E , and Demerec, M , *Genetics*, **51**, 897 (1965)
[13] Watanabe, T , and Okada, M , *J Bact* , **87**, 727 (1964)

Combined Inhibition of Influenza Virus Reproduction in Cell Culture using Interferon and Amantadine

AMANTADINE hydrochloride is the first synthetic anti-influenza compound which has been successfully tested in human beings[1,2] Results of an investigation showed that none of its derivatives was more effective than amantadine itself Thus attempts to increase the antiviral prophylactic activity of amantadine and to reduce its dosage in order to diminish the toxicity[3] are necessary

The simultaneous administration of amantadine with the other virus inhibitors which interfere with intracellular stages of virus–cell interaction can help to solve both the problems Our experiments have shown that the combination of amantadine with an inhibitor of nucleic acid synthesis (5-fluorouracil) used against influenza *A/WSN* virus[4], and with a moderate inhibitor of protein synthesis (D,L-ethionine) used against fowl plague virus, could produce an additive effect on the reproduction of the viruses in chick embryo cells

A further experiment on combination was undertaken using amantadine and interferon Amantadine has been shown to slow down or block the penetration of influenza A virus particles inside the cells[5], interferon induces the synthesis of hypothetical "translation inhibitor protein"[6] and finally reduces the number of newly formed viral particles[7] The addition of these two activities was expected to produce a mutual enhancement of the antiviral effect Furthermore, prophylactic anti-influenza properties of human interferon in volunteers[8] indicated that such a combination was of practical interest. The results of experiments with the two inhibitors on chick embryo cells infected with influenza *A/WSN* virus are presented here

The assay of multiplication of *WSN* virus in chick embryo cells, and the procedure of preparation and titration of crude allantoic interferon from chick embryos infected with the same virus have been described[9]

When cultures treated with interferon were inoculated with 10^{-3} plaque forming units (PFU)/cell, the replication of virus was inhibited to a certain extent, especially during the first 48 h after inoculation (Table 1) The simultaneous introduction of amantadine with the inoculum also decreased the yields of virus In the presence of both inhibitors the infectious virus was not detected in cells or medium The addition of interferon and amantadine to the medium 1 h after inoculation did not prevent weak virus multiplication during the first 48 h, but practically stopped it after 72 h The inhibitors produced only a marginal provisional effect when added in combination 24 h after inoculation In these conditions, the separate use of inhibitors was not effective (Table 1) In reduced doses the inhibitors were less effective, but their additive effect was well expressed in spite of some rise in the multiplicity of infection (Table 1, experiment 3)

An additive effect of interferon and amantadine on the cumulation of haemagglutinins was also observed in cultures infected at a high input multiplicity (Table 2)

Table 3 presents the proportion of infected cells in the cultures infected at the multiplicity of 10^{-2} PFU/cell The "infectious centres" technique was used The rate of cumulative inhibition was almost ten times higher than could be expected from the product of separate effects of interferon and amantadine The sum of the inhibitors also prevented more effectively the development of cytopathic changes (Table 3)

Table 1 INFLUENCE OF INTERFERON AND AMANTADINE ON THE YIELDS OF INFLUENZA *A/WSN* VIRUS IN CHICK EMBRYO CELLS

Time of introduction of inhibitor	Dose of interferon (units of activity)*	Concentration of amantadine (μg/ml)	Experiment No	Virus titre (log PFU/ml) Time after inoculation (h)			
				1	24	48	72
Control	0	0	1	2 2	3 3	6 4	6 1
			2	<1 0	4 9	6 7	6 9
Before adsorption†	8	0	1	<1 0	2 1	2 9	4 5
			2	<1 0	2 8	2 6	3 6
	0	25	1	<1 0	3 2	4 5	5 6
			2	<1 0	2 5	4 9	5 3
	8	25	1	<1 0	<1 0	<1 0	<1 0
			2	<1 0	<1 0	<1 0	<1 0
1 h after inoculation ‡	8	0	2	—	—	4 0	3 9
	0	25	2	—	3 2	4 8	5 1
	8	25	2	—	1 7	2 7	<1 0
24 h after inoculation ‡	8	0	2	—	—	>6 3	>6 3
	0	25	2	—	—	>6 3	>6 3
	8	25	2	—	—	5 3	>6 3
Before adsorption†	0	0	3	1 3	6 3	7 1	—
	2	0	3	—	5 0	5 5	—
	0	5	3	—	5 5	6 6	—
	0	2	3	—	5 7	7 1	—
	0	1	3	—	5 5	6 8	—
	2	5	3	—	4 3	4 4	—
	2	2	3	—	4 7	4 7	—
	2	1	3	—	4 9	5 0	—

Multiplicity of infection experiments (1) and (2) 10^{-3} PFU/cell, experiment (3) 5×10^{-3} PFU/cell

* One unit of interferon activity is the dilution necessary to inhibit 50 per cent PFU of *WSN* in chick embryo cells

† Interferon was introduced 24 h before inoculation of virus removed during the virus adsorption period, and then added again so that it was present in the maintenance medium until the end of the experiment Amantadine was introduced simultaneously with the virus inoculum, removed with it after 1 h of adsorption at 37° C, and then a new dose was added to the maintenance medium

‡ Interferon and amantadine were added to the maintenance medium

Table 2 CUMULATION OF VIRAL HAEMAGGLUTININS IN THE PRESENCE OF INTERFERON AND AMANTADINE

Inhibitor	Haemagglutinins	Haemagglutination titre Time after inoculation (h)		
		1	9	24
None	Extracellular	—	256	128
	Intracellular	—	256	64
Interferon	Extracellular	—	32	32
	Intracellular	—	32	32
Amantadine	Extracellular	—	64	64
	Intracellular	—	32	32
Interferon plus amantadine	Extracellular	—	8	16
	Intracellular	—	16	16

Multiplicity of infection 2×10^2 PFU/cell
The doses and introductions of inhibitors as in experiment 1 of Table 1

Table 3. THE PROPORTION OF INFECTED CELLS IN THE PRESENCE OF INTERFERON AND AMANTADINE

Inhibitor	Ratio of the infectious centres number the number of cells in chick embryo cell culture (per cent)*			Cytopathogenic effect	
	Time after inoculation (h)				
	1	24	48	24	48
None	0 04	100 0	100 0	1+	2+
Interferon	0 04	1 5	1 7	—	1+
Amantadine	0 004	8 5	77 0	—	1+
Interferon plus amantadine	0 005	0 013	0 031	—	—

Multiplicity of infection 10^{-2} PFU/cell.
Doses and introductions of the inhibitors as in experiment 1 of Table 1
* Average of two cultures

Cessation of the process of infection (Table 1, experiments 1 and 2) and the degree of reduction of the proportion of infected cells in the presence of the two inhibitors indicate a synergistic action of interferon and amantadine This can be explained by the nature of the inhibitory action of the preparations Interferon reduces the quantity of virions released from the infected cells, the number of which has already been reduced by the action of amantadine (Table 3) The released particles after adsorption on intact cells are subjected to the action of amantadine, and inhibition of the two phases of virus–cell interaction again occurs It seems likely that if the dosage of inhibitors is sufficient to reduce the number of released and adsorbed viral particles to less than the initial number, the constant presence of the two inhibitory activities and a low multiplicity at the start will lead practically to cessation of the infectious process The inhibitors could not stop the infection when most of the cells were infected or when the doses were low and produced only an additive effect

The method of approach described here can be examined in human volunteers experimentally infected with influenza A2 or else it can be tested during an influenza A2 epidemic Furthermore, in combination with interferon, amantadine can become active against infections caused by viruses which are sensitive to it only *in vitro* such as rubella and parainfluenza[10,11]

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[1] Wood, T R , *Ann NY Acad Sci* ,130, 419 (1965)
[2] Stanley, E D , Muldoon, L W , Akers, L W , and Jackson, G G , *Ann NY Acad Sci* ,130, 44 (1965)
[3] Sabin, A B , *J Amer Med Assoc* ,200, 943 (1967)
[4] Lavrov, S V , *Vop Virus* (in the press)
[5] Davies, W L , Grunert, R R , Haff, R F , McGahen, J W , Neumayer, E M , Paulshock, M , Walts, J C , Wood, T R , Hermann, E C , and Hoffman, C E , *Science*, 144 862 (1964)
[6] Marcus, P J , and Salb, J M , *Virology*, 30, 502 (1966)
[7] Hallum, J , and Youngner J , *J Bact* , 92 1047 (1966)
[8] Soloviev V D , Bektemirov, T A , Porubel, L A , and Rapoport, R I , *Vop Virus*, 3, 311 (1966)
[9] Orlova, T G , Mentkevich, L M , Tatarinova, Yu N , and Stakhanova, V M , *Acta Virol* ,11, 183 (1967)
[10] Oxford, J S and Schild, G C , *Brit J Exp Pathol* , 48, 235 (1967)
[11] Smith, C B , Purcell, R H , and Chanock, R M , *Amer Rev Resp Dis* , 95, 689 (1967)

IMMUNOLOGY

Regeneration of Bone Marrow Cells and Thymus induced by 19S Alpha-2 Globulin in Irradiated Mice

THE 19*S* alpha-2 globulin isolated by us from the alpha-2 globulin fraction of human serum (obtained from Mann Res Lab , New York) by the procedure of Schonenberger *et al* [1] and identified by electrophoresis on polyacrylamide gel[2] and ultracentrifugation was found to stimulate the regeneration of bone marrow cells in X-irradiated mice (*C*57*Bl*/6) A sample (0 5 mg) of the 19*S* alpha-2 globulin injected intraperitoneally into 33–35 day old mice within an hour of exposure to 170 r produced a significant speeding up in the incorporation of ^{3}H-thymidine into bone marrow cells, as compared with that in irradiated animals injected with saline (Fig 1) Seven days after irradiation 19*S* alpha-2 globulin-injected animals showed an actual overshoot in number of cells of femoral and tibial bone marrow (55 × 10^6/mouse), as compared with unirradiated controls (42 × 10^6/mouse) and irradiated but saline-injected controls (41 × 10^6/mouse), respectively The total DNA content of bone marrow cells at this stage showed a similar picture, the femoral and tibial bone marrow cells of mice injected with 19*S* alpha-2 globulin containing 527 μg of DNA, while the irradiated (saline-injected) controls contained 410 μg and the non-irradiated controls contained 414 μg of DNA

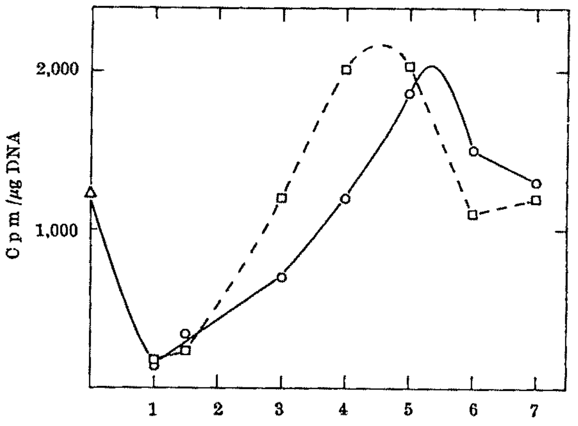

Fig 1 Effect of human serum 19*S* alpha-2 globulin on incorporation of ^{3}H-thymidine into DNA of bone marrow cells of *C*57*Bl*/6 mice after a single exposure to 170 r of X-rays Cells from the tibia and femur of five mice from each group were collected daily and pooled in 4 ml of Tyrode solution and 1 ml samples in duplicate were incubated with 18 mCi/mmole of ^{3}H-thymidine at 38° C for 3 h Nucleic acids were extracted and incorporation measured with a 'Tri-Carb' scintillation counter[3] Solid lines and circles represent the results of the irradiated and saline-treated control animals, and broken lines and squares represent 19*S* alpha-2 globulin-injected, irradiated mice The incorporation of ^{3}H-thymidine in the unirradiated controls is presented at day 0 (triangle) Each point is the mean of the duplicate samples whose values differed by less than 10 per cent

With four irradiations (170 r once a week), and 0 5 mg of the 19*S* alpha-2 globulin injected 1 h after each irradiation, the regeneration effect of the protein fraction was enhanced as compared with a single treatment (Fig 2). Other protein fractions isolated from the alpha-2 globulins of human serum seemed to have marginal or no effect on the regeneration of bone marrow cells after similar radiation treatment.

The same 19*S* alpha-2 globulin also had a stimulating effect on the regeneration of the thymus In mice exposed to 170 r once a week for 4 weeks, injection of 5 μg or more of the globulin after each exposure produced, 3 weeks after the last injection, a significant increase in thymus weight Here again, other fractions isolated from the alpha-2 globulins of human serum had less or no effect. The results of two experiments are presented in Fig 3, in which the effect of 0 5 and 0 1 mg of 19*S* alpha-2 globulin on the regeneration of the thymus is compared with two other protein fractions These two fractions (*B* and *C*) represent a mixture of various proteins which remained in the solution after precipitation of 19*S* alpha-2 globulin at *p*H 5 1 at low ionic strength[1] To this solution ammonium sulphate was added until 60 per cent saturation was reached Precipitate *B* was still found to contain some 19*S* alpha-2 globulin, detectable on electrophoreograms The higher concentration (0 5 mg/injection) therefore still had a stimulating effect on thymus regeneration At the lower concentration (0 1 mg/injection) at which the 19*S* alpha-2 globulin fraction had an effect on thymus regeneration, fraction *B* was ineffective The supernatant (*C*), obtained after treatment with ammonium sulphate, had no significant effect on thymus regeneration at either concentration tested.

Extracts of sheep spleen had previously been shown to have a stimulating effect on thymus regeneration in mice treated as already described[4] By comparing different fractions isolated from sheep spleen, it was found that only those which contained 19*S* alpha-2 globulin (detectable by gel electrophoresis) had a stimulating effect on the regeneration of thymus in irradiated mice These preparations also hastened bone marrow regeneration, preceding the thymus regeneration

This investigation arose indirectly from previous studies on the inhibitory effect of RLP—a protein factor derived from sheep spleen—on radiation leukaemogenesis in *C57Bl* mice[5-7], and from subsequent indications that alpha-2 globulin fractions of human serum had similar effects[8] The effect of 19*S* alpha-2 globulin on radiation leukaemogenesis in *C57Bl* mice is now being investigated Preliminary results indicate that radiation leukaemogenesis is inhibited by 19*S* alpha-2 globulin, as by RLP derived from sheep spleen

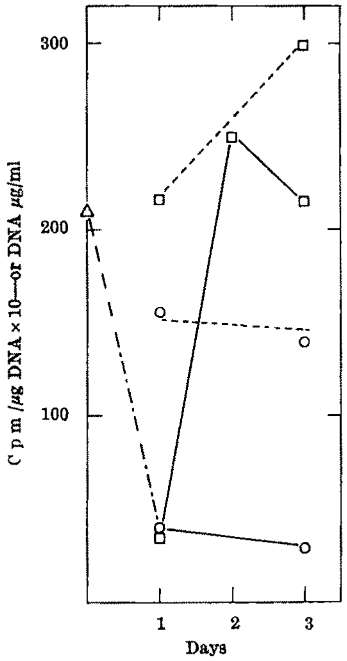

Fig 2 Effect of human serum 19*S* alpha-2 globulin on incorporation of ^{3}H-thymidine into DNA of bone marrow cells of *C57Bl/6* mice after four X-ray exposures (170 r once a week) Experimental procedure as described in Fig 1 The incorporation of ^{3}H-thymidine (unbroken line) and DNA content (dashed line) of aliquots of tibial and femoral cells from the 19*S* alpha-2 globulin injected mice (squares) and saline injected mice (circles) is shown during 3 days after the fourth irradiation The incorporation of ^{3}H-thymidine in unirradiated controls is represented by a triangle at day 0 Each point is the mean of the duplicate samples whose values differed by less than 10 per cent

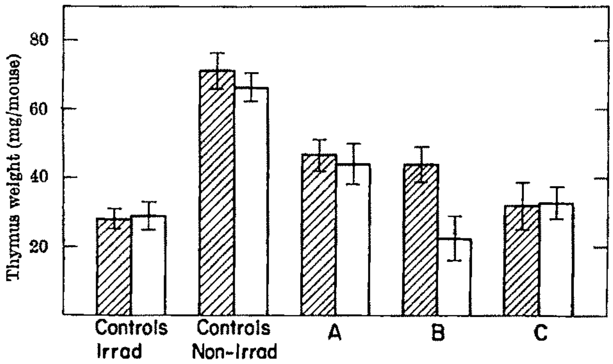

Fig 3 Effect of various fractions isolated from human serum alpha-2 globulins on the weight of the thymus of irradiated mice 3 weeks after the fourth X-ray exposure. Each column represents the mean value of the thymus weight of seventeen mice Confidence intervals (95 per cent) for the means of each group are presented. 0 5 mg (shaded columns) and 0 1 mg (blank columns) of proteins were injected after each irradiation 19*S* alpha-2 globulin (*A*) and two other fractions described in the text (*B* and *C*) were tested The controls were injected with saline

Recently, Hanna *et al* [9] demonstrated a protective effect of 19*S* alpha globulin, isolated from mouse and rat serum, against the lethal action of X-irradiation in mice If this will prove to belong to the alpha-2 globulin group, their results, together with those presented here and elsewhere[8], will support the idea of the special physiological role of 19*S* alpha-2 globulin

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[1] Schönenberger, M , Schmidtberger, R , and Schultze, H E , *Z Naturforschung*, 13B, 761 (1958)

[2] Davis, B J , *Ann NY Acad Sci* ,121, 404 (1964)

[3] Trainin, N , Burger, M , and Kaye, A M , *Biochem Pharmacol* , 16, 711 (1967)

[4] Cividalli, G , and Knyszynski, A , *Radiat Res* , 30, 148 (1967)

[5] Berenblum, I , Cividalli, G , and Hodes, M E , *Nature*, 202, 973 (1964)

[6] Berenblum, I , Cividalli, G , Trainin, N , and Hodes, M E , *Blood*, 26, 8 (1965)

[7] Berenblum, I , Trainin, N , Cividalli, G , Boiato-Chen, L , and Knyszynski, A , *Tumori*, 53, 5 (1967)

[8] Berenblum, I , Burger, M , Cividalli, G , Knyszynski, A , and Trainin, N , *Abstr Proc Symp on Perspectives in Leukemia*, New Orleans, Louisiana, December 1966 (in the press)

[9] Hanna, jun , M G , Nettesheim, P , Fisher, W D , Peters, L C , and Francis, M W , *Science*, 157, 1458 (1967)

Failure to induce Transplantation Resistance against Polyoma Tumour Cells with Syngeneic Embryonic Tissues

MANY tumours contain new transplantation antigens not present in the host tissue of animals in which they arise This applies particularly to those tumours induced by certain oncogenic viruses and chemical carcinogens[1] It is not known whether the factor determining these antigens originates in new nucleic acid derived by viral infection or induced mutation or whether the antigens arise through some type of derepression of normal cellular genes The latter hypothesis suggests that tumour and embryo may share antigens Preliminary data[2] have shown that some of the so-called tumour-specific transplantation antigens of sarcomas induced by 3-methylcholanthrene may be found in embryomas, thus lending support to the derepression hypothesis

To investigate the relationship of embryonic antigens and tumour specific antigens induced by oncogenic viruses, we have chosen two tumour cell lines induced by polyoma virus The origins, properties and tumour specific transplantation antigens of polyoma tumour cells 4198 (originated in *C3H$_f$/HeN* mouse) and polyoma tumour cells 89 (originated in *C57Bl/Ka* mouse) were reported previously[3].

Adult mice (1–2 months old) were given syngeneic 10 day old minced embryos or homografts of polyoma tumours (4198 tumour to *C57Bl* mice or 89 tumour to *C3H* mice) three times within an interval of 2 weeks A third group of mice was given polyoma virus Two weeks after the last inoculation, all mice were challenged subcutaneously with syngeneic polyoma tumour cells.

The results in Table 1 show that mice immunized with syngeneic embryos failed to develop transplantation resistance against syngeneic polyoma tumour cells Strong resistance was induced in mice inoculated with virus The results concerning resistance induced by homografts or polyoma tumours were similar to those reported previously, that is, a moderate degree of resistance was induced in *C57Bl* mice and weaker resistance in *C3H* recipients[3]

Table 1. GROWTH OF SYNGENEIC POLYOMA TUMOUR CELLS IN MICE IMMUNIZED WITH SYNGENEIC EMBRYOS, HOMOGRAFTS OF POLYOMA TUMOUR CELLS AND POLYOMA VIRUS

Recipient and treatment	No of mice developing tumours / No of mice challenged Challenging dose of syngeneic polyoma tumour cells 5 × 10⁵	 5 × 10⁴	 5 × 10³
C57Bl	6/6 (48 31–56)	6/6 (69 58–72)	3/6 (84 58–107)
C57Bl + embryo	6/6 (51 43–52)	5/5 (62 48–70)	4/5 (89 70–110)
C57Bl + homograft	5/5 (54 45–66)	3/5 (65 57–72)	1/6 (86)
C57Bl + virus	3/5 (57 44–86)	1/6 (78)	0/5
C3H	5/5 (24 16–36)	5/5 (32 23–50)	5/5 (42 36–48)
C3H + embryo	4/4 (23 17–24)	5/5 (39 25–51)	5/5 (46 38–60)
C3H + homograft	5/5 (32 25–40)	4/5 (38 25–49)	2/5 (48 43, 54)
C3H + virus	2/5 (48 47, 50)	0/5	0/5

Figures in parentheses are average time of death and range in days

In the discussion of Prehn's paper, Defendi has stated that he has tested two hamster embryo lines transformed by polyoma and one rat polyoma tumour by Prehn's method and obtained negative results, however, no data were presented Our results also failed to confirm the notion that tumour-specific transplantation antigens induced by polyoma virus may arise through derepression of antigens present in embryonic tissue

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[1] Sjögren, H O, *Prog Exp Tumor Res*, 6, 289 (1965)

[2] Prehn, R T, *Cross-reacting Antigens and Neoantigens* (edit by Trentin, J J), 105 (The Williams and Wilkins Co, Baltimore, 1967)

[3] Ting, R C, *Nature*, 211, 1000 (1966)

Role of *X*, *Y* and *Z* Cells in Secondary Immunological Response

ACCORDING to many authors[1–4], the secondary immunological response should consist of responses of cells endowed with immunological memory (*Y* cells in the scheme of Sercarz and Coons[3]) acquired during contact with antigen administered in the previous immunization, and of cells which had come into contact with the antigen for the first time (*X* and *Z* cells according to Sercarz and Coons)

Recently the term "net excess secondary response" has been introduced for the determination of that part of the response which arises out of the reaction to the antigen of cells endowed with immunological memory (*Y* cells) The "net excess secondary response" is calculated by subtracting from the total secondary response the quantity of antibody, which is obtained in control animals when the antigen is administered for the first time[1] This calculation is based on the assumption that the dose used for secondary immunization will produce, on a particular day, the same reaction arising from the inclusion of *X* cells in animals immunized for the first or second time This assumption, however, involves the risk of error, because the antigen, when introduced into the organism for the second time, encounters antibodies which were absent after the first treatment or present in a negligible amount (natural antibodies) These antibodies probably have a marked influence on the fate of the administered antigen and consequently on the course of immunization Their effect is probably similar to that of passively introduced antibodies

To explain the influence of antibodies on the course of immunization, experiments have been carried out with adult rabbits 1 h after an intravenous injection of sheep red blood cells as antigen—serum from rabbits immunized with sheep red blood cells (SRBC) was injected One group of rabbits received injections of serum drawn from a rabbit on the seventh day after a single immunization, this serum contained mainly 19*S* antibodies In another group, serum from a rabbit which had been immunized once a week for 7 months was injected; this serum contained only 7*S* antibodies Antigen only was given to the control group The haemolytic and haemagglutination titres of the two injected sera were identical.

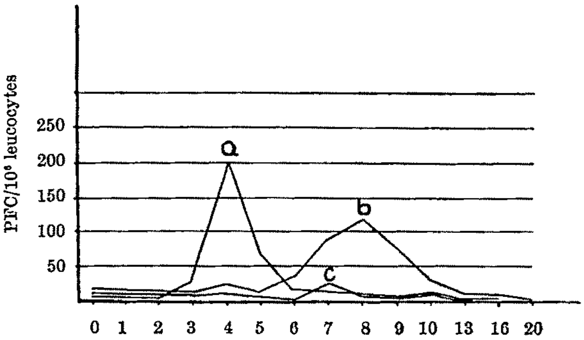

Fig 1 Dynamics of PFC formation in the blood of rabbits *a*, Injected antigen only (3 ml of 10 per cent SRBC), *b*, injected antigen and homologous serum 19*S*, *c*, injected antigen and homologous serum 7*S* Each point represents the average of two rabbits.

Plaque forming cells (PFC) were determined by Šterzl's modification[5] of the Jernes's method[6] (direct haemolytic agar–plaque technique), and antibodies were determined by the method described by Adler[7]

Plaque forming cells were examined using the buffy coat of the peripheral blood, because previous observations (partially described in ref 8) of more than fifty rabbits have shown that the appearance of PFC, their peak and disappearance run parallel to the time course of the PFC in the spleen

Results are shown in Fig 1 The peak of PFC in control rabbits was on the fourth day, and in rabbits which received antigen and homologous antibodies the peak was after 7–8 days—it took nearly twice as long. The peak was also lower, although to a different degree with different antibodies It was markedly reduced by a serum containing 7*S* antibodies, in agreement with the results of other investigators[9,10]

The mechanism of these phenomena probably depends on a decrease in the quantity of antigen caused by the added homologous serum The two sera used contained different types of antibodies, although they were equal titres Thus they decreased the amount of active sheep red blood cells to different degrees[11] Experiments to examine the influence of different doses of antigen on immunization have shown a decreased and delayed reaction of the formation of antibodies when the doses had been reduced[2]

The results in Fig 1 suggest that, when antibodies produced actively act similarly to those produced passively, the peak of cells producing antibodies after a second introduction of antigen should also be delayed by 7–8 days This has been confirmed in experiments with rabbits from which blood samples were taken daily

Table 1 PLAQUE FORMING CELLS IN RABBITS AFTER SECOND IMMUNIZATION

Rabbit No	Days after second immunization 0*	 1	 2	 3	 4	 5	 6	 7	 8	 9	 10	 11
1	5†	3	143	1,523	96	9	0	35	0	0		
2	3	37	512	2,984	270	52	38	165	8	8		
3	0	0	42	211	55	63	65	121	37	7	3	
4	6	2	180	1,360		5	181	24	9	3	0	
5	312	280	40	1,800	680	33	39	51	21	8	5	
6	5	31	49	707	97	219	0	24	0	4		
7	0	42	153	590	116	70	2	130	7	5		
8	0	3	213	420	50	127	0	43	4	7		
9	24	2	73	910	31	27	36	59		3		
10	152	66	82	3,405	77	121	62	90	7	9		
11	33	129	675	2,154	73	43	1,479	173		77		
12	141	0	222	2 286	79	31	6	0				
13	194	40	143	1,408	1,243	229	50	90				
14	0	0	115	940	320	563	26	20	0	0		
15	0	0	5	367	54	20	87	3	0	0		
16	0	0	46	43	0		43		120	32	85	0
17	0	0	0	0	0		13	0	0	0	2	
18			240	30	21	2	2	64	3	4		

Only eighteen of the twenty-five rabbits examined are included in the table, because the remaining seven had received various doses of antigen

* 5–15 min before second immunization

† Numbers of plaque forming cells/10⁶ of blood leucocytes

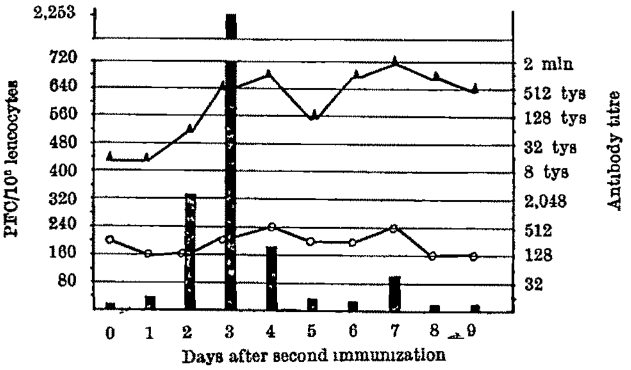

Fig 2 Dynamic of PFC in the blood and of antibody formation in the serum of two rabbits Columns, PFC, ○, agglutinins, ▲, haemolysins

for 9–10 days after the second injection of antigen. Most of the twenty-five rabbits examined showed, as well as a high peak of PFC on the third day after the second injection of antigen, a second peak usually smaller than the first peak, on the seventh (seldom on the sixth or eighth) day (Table 1) Antigen was administered again several days (1–7, sometimes 14) after the first injection An early injection of antigen elicits a secondary response producing chiefly IgM antibody, and so the direct method of detecting PFC can be used here[2] Results of experiments with two peaks of PFC are illustrated in Fig 2.

In the next experiment, 6-mercaptopurine was given to rabbits immunized once and twice with SRBC. 6-Mercaptopurine was given daily in doses of 6 mg/kg of body weight throughout the experimental period beginning on the day of injection of the antigen In rabbits immunized for the second time, the administration of 6-mercaptopurine began on the day of the second injection of antigen. In such a dose this compound has a much more inhibitory effect on the primary than on the secondary response[12,13]

The primary response in two rabbits which had received 6-mercaptopurine was quite low (one animal 8 per cent, the second 25 per cent of the average value for PFC) while rabbits being immunized for the second time showed the first peak only, on the third day, at a rather lower level than in the rabbits not given 6-mercaptopurine The second peak was not observed in any of the three rabbits examined

These experimental results suggest that the secondary response consists of two parts, which differ in the time of appearance of cells producing antibodies and in the height of the peak of these cells The first part is probably Nossal's "net excess secondary response", in which *Y* and *Z* cells are included, and this part is the true secondary response. On the other hand, *X*, *Y* and *Z* cells participate in the second part, which resembles the reaction taking place after the first injection of antigen

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[1] Nossal, G J V, Austin, C M, and Ada, G. L, *Immunology*, 9, 333 (1965)
[2] Sercarz, E E, and Byers, V S., *J. Immunol*, 98, 836 (1967)
[3] Sercarz, E E, and Coons, A H, *Mechanisms of Immunological Tolerance* (Czechoslovak Acad Sci, Prague, 1962)
[4] Šterzl, J, *Nature*, 209, 416 (1966)
[5] Šterzl, J, and Mandel, L, *Folia Microbiol*, 9, 173 (1964).
[6] Jerne, N K, Nordin, A A, and Henry, C, in Amos, B, and Koprowski, H, *Cell-Bound Antibodies*, 109 (The Wistar Institute Press, Philadelphia, 1963)
[7] Adler, F L, *J Immunol*, 95, 26 (1965)
[8] Halasa, J, *Folia Microbiol* (in the press)
[9] Moller, G, and Wigzell, H, *J Exp Med*, 121, 969 (1965)
[10] Rowley, D A, and Fitch, F W, *J Exp Med*, **120**, 987 (1964)
[11] Greenbury, C L, and Moore, D H, *Immunology*, 6, 421 (1963).
[12] Schwartz, R, Stock, J, and Dameshek, W, *Proc Soc Exp Biol, NY*, 99, 164 (1958)
[13] Šterzl, J, *Zurnal Higieny, Epidemiologii, Mikrobiologii i Immunologii*, 7, 323 (1963)

PATHOLOGY

Specificity of the Agglutinin in Extracts of Wheat Germ

Extracts of wheat germ prepared to contain lipase have been shown to have the additional property of agglutinating mammalian cells[1] This agglutinin is heat stable, and thus distinguishable from the lipase, which is destroyed by heating to 65° C Aub *et al* suggested that the agglutinin would agglutinate tumour cells more strongly than the equivalent normal cell[1-3] The implication that extracts of wheat germ contain an agglutinin specific for tumour cells is not upheld by the experiments described here, and which involve the ability of extracts of wheat germ to agglutinate red cells and to produce mixed cell agglutination, and the ability of various sugars and salivas to inhibit this agglutinin. Wheat germ lipase (10 mg/ml., Sigma and Calbiochem) was heated at 65° C for 15 min to destroy lipolytic activity[1], the preparation was centrifuged for 10 min at 3,500 r p m to remove the denatured protein, and kept frozen at −20° C

Siliconed glassware was used throughout. To prevent cells from sticking to the glass, they were suspended in a 0 02 per cent solution of crystalline bovine serum albumin in phosphate buffered saline. The cell lines used were kept frozen in liquid nitrogen. A mouse mammary tumour, which arose spontaneously in a *C3H* mouse in August 1962, was obtained from King's College Hospital, London

Wheat germ extract was titrated in two-fold dilutions (0 1 ml. amounts), and an equal volume of 2 per cent red blood cells was added. After thorough mixing, the cells were allowed to react for a minimum of 60 min at room temperature. Agglutination was read macroscopically. The mixed agglutination test was carried out as described by Swinburne, Frank and Coombs[4], except that 0 02 per cent crystalline bovine serum albumin in buffered saline was used as a diluent instead of diluted normal rabbit serum

Direct agglutination was inhibited by mixing equal amounts of wheat germ extract and inhibiting agent (molar sugar solution, salivas) and allowing the mixture to stand at room temperature for 10 min before adding red blood cells These tests were done as chequer board titrations. Mixed agglutination was inhibited by mixing equal amounts of wheat germ extract and inhibiting agent (molar sugar solution, salivas) for 10 min This mixture was then used for sensitizing buccal cells

Wheat germ lipase extract was found to agglutinate the most common types of human red blood cells (A, B, O, AB); also A_3 (one example), $A_{intermediate}$ (one example), Ai (two examples) to almost the same titre (1 · 4 or 1 : 8). Strong mixed agglutination (to a titre of 1 : 32) was observed with buccal epithelial cells from secretor and non-secretor individuals of blood groups A, B and O and human group O indicator red blood cells

Using cultured human diploid tissue cells and O indicator cells a different pattern was observed For no obvious reasons the strength of the reaction varied from completely negative to strongly positive Both of the established human cell lines (Detroit 98 and HeLa 229) gave a very strong mixed agglutination reaction up to a titre of 1 8 Normal mouse spleen and bone marrow preparations gave an extremely strong reaction up to a titre of 1 : 32 The reaction between normal mouse lymph node cells and wheat germ extract was much less strong but definitely positive in a dilution of 1 2. All mouse cell lines (*LM*, *C57*, *C3H*) and the Ehrlich ascites, *BP8*, and mammary mouse tumours gave a mixed agglutination reaction up to a titre of at least 1 . 8 Sarcoma I cells, however, although from a transplantable mouse tumour, failed to give a positive reaction on several occasions when mouse red blood cells were used as indicator cells. When human group O cells were used, the reaction was positive to a titre of 1 2.

Direct agglutination of human red blood cells (groups A, B and O) by wheat germ extract was completely inhibited by human secretor salivas (of blood groups A, B and O) The salivas of non-secretor individuals behaved differently; some inhibited the reaction completely whereas others inhibited it only partly. The agglutinin in wheat germ lipase extract (Sigma) was partly inhibited by D-galactose and L-fucose, it was completely inhibited by *N*-acetyl-D-glucosamine No inhibition was observed with *N*-acetyl-D-galactosamine

No inhibition of mixed agglutination was obtained with either wheat germ preparation using D-galactose and L-fucose as inhibiting agents *N*-Acetyl-D-galactosamine partially inhibited wheat germ extract (Sigma) and completely inhibited wheat germ extract (Calbiochem). *N*-Acetyl-D-glucosamine completely inhibited both wheat germ extracts

Table 1 REACTION OF WHEAT GERM AGGLUTININ WITH TISSUE CELLS DETECTED BY MIXED AGGLUTINATION

Tissue cells	Indicator cells	Reaction
Human buccal epithelial cells A B, O	Human O	+
Detroit 98 Human aneuploid line	,, O	(+ +) 1 8
HeLa 229 Human aneuploid line	,, O	+ + 1 8
MRC I Human diploid cell strain	,, O	+ 1 2
*HF*2 (human foetal skin)	,, O	Weak 1 2
*HF*2 (human foetal skin)	,, O	+ 1 8
*HF*24 (human foetal skin)	,, O	0 1 2
Kidney I (human adult kidney)	,, O	(+ +) 1 8
Normal mouse spleen cells	Mouse	+ 1 32
	Human O	+ 1 32
Normal mouse bone marrow cells	Mouse	+ 1 32
	Human O	+ 1 32
Normal mouse lymphoid cells	Mouse	+ 1 2
	Human O	+ 1 2
LM (mouse cell line)	Mouse	+ 1 32
	Human O	+ 1 8
C57S (mouse cell line)	Mouse	+ 1 32
	Human O	+ 1 8
C3HS (mouse cell line)	Mouse	+ 1 8
	Human O	+ 1 8
Ehrlich ascites (mouse tumour)	Mouse	+ + + 1 32
	Human O	+ + 1 8
KCH (mouse tumour)	Mouse	+ + 1 8
	Human O	(+) 1 8
Sarcoma I (mouse tumour)	Mouse	0 1 2
	Human O	(+ +) 1 2
*BP*8 mouse tumour	Mouse	+ + + 1 32
	Human O	+ + 1 8

Aub *et al.*[1–3] suggested that tumour cells were more readily clumped by an agglutinin present in the supernatant of heated wheat germ lipase extract than were the homologous normal tissue cells Our study of wheat germ agglutinin was undertaken in the hope that this might eventually be used as a reagent to detect specific cell surface components. The results, however, suggest that the wheat germ lectin is capable of reacting with a wide range of normal and abnormal, adult and foetal cells of human and murine origin The fact that it agglutinates normal red blood cells and normal tissue cells, and fails to agglutinate some tumour cells, is not consistent with the hypothesis of tumour specificity put forward by Aub *et al* In fact, they themselves showed that the lectin reacted with normal mouse red cells and spleen cells It should be pointed out that Aub *et al.* investigated not the combination of wheat germ lectin with cells but the direct agglutination of tissue and tumour cells by wheat germ extract Direct agglutination of cells will be affected by many factors such as distribution and amount of the combining site, dispersal and size of the electrocharge, and it is possible that the difference between tumour and normal cells described by Aub *et al* is governed by these factors, and that it is incorrect to interpret their results as demonstrating a combining site on tumour cells which is absent from the homologous normal cell. Extracts of wheat germ contain a lectin which reacts with a combining group which is very common, and which occurs erratically on a wide variety of cells. The fact that the reaction of the lectin is inhibited by *N*-acetyl-D-glucosamine suggests that this sugar or some similar sugar plays a part at the wheat germ lectin combining site This seems to be the first example of a 'non-specific'' lectin which is inhibited by *N*-acetyl-D-glucosamine[5].

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[1] Aub, J C , Tieslau, C , and Lankester, A , *Proc US Nat Acad Sci* , 50, 613 (1963)

[2] Aub, J C , Sanford, B H , and Cote, M N , *Proc US Nat Acad Sci* , 54, 396 (1965)

[3] Aub, J C , Sanford, B H , and Wang, L H , *Proc US Nat Acad Sci* , 54, 400 (1965)

[4] Swinburne, L M , Frank, B B , and Coombs, R R A , *Vox Sang*, 6, 274 (1961)

[5] Krupe, M , *Blutgruppenspezifische Pflanzliche Eiweisskorper* (Enke-Verlag, Stuttgart, 1956)

Human Case of Piroplasmosis (Babesiosis)

INFECTIONS with the protozoon *Babesia* occur frequently in wild and domestic animals, but human beings are apparently very rarely affected In the British Isles, cattle are the most commonly infected animals and may develop red water disease. The following case is only the third in the world recorded in a human and the first in the British Isles. It is significant that all three patients had previously undergone splenectomy[1,2].

The patient was a 48 year old deep-sea fisherman from Northern Ireland who had never been abroad. He was admitted to the Renal Unit, Belfast City Hospital, in a moribund state on September 2, 1967. On August 29 he had become vaguely unwell and had passed blood-stained urine By the following day he was jaundiced and a day later he was admitted to Downe Hospital, Downpatrick, in a very ill condition, now deeply jaundiced. He seems to have passed urine only once after arrival in hospital The specimen contained urobilin but there was no report of the presence of blood or haemoglobin He had a pyrexia of 103° F and marked conjunctival injection The haemoglobin level was 7 2 g/100 ml and the specimen was grossly haemolysed. A presumptive diagnosis of Weil's disease was reached and he was transferred to the Renal Unit because of the anuria.

On arrival he was too ill to give a history. He was deeply jaundiced and hypotensive There was a right paramedian abdominal scar with a transverse extension to the left

On September 3 the patient's haemoglobin level had fallen to 4 6 g/100 ml and 2 days later it was 4 0 g/100 ml. Both specimens were markedly haemolysed The blood urea was 312 mg/100 ml , CO_2 combining power 13, Na 120, K 5 3 and Cl 85 mequiv /l , respectively. The total bilirubin was 5 4 mg (direct 1·1 mg)/100 ml , thymol turbidity 2 U and serum transaminase 820 U. The agglutination test for Weil's disease was negative

At this point the diagnosis was thought to be serum hepatitis, because the patient had received several blood transfusions about 4 months earlier in connexion with a difficult gastroenterostomy and vagotomy, during which the spleen had inadvertently been ruptured and removed

The blood count findings on September 5 (haemoglobin, 4 0 g/100 ml.) were red cells $1{\cdot}0 \times 10^6/\text{mm}^3$, haematocrit 12 per cent, mean corpuscular haemoglobin concentration 32 per cent, mean corpuscular volume 120 μ^3, reticulocytes 25 per cent The white cells numbered $46{,}500/\text{mm}^3$, with a differential count of myelocytes 22 per cent, metamyelocytes 19 per cent, neutrophils 47 per cent, lymphocytes 12 per cent The direct antiglobulin (Coombs) test was negative There were thirty-six nucleated red cells per 100 leucocytes The red cells showed macrocytosis and polychromasia Fragmented forms were conspicuous, but the outstanding feature was the presence of numerous intra-erythrocytic organisms These were often circular and frequently vacuolated to form small rings measuring up to 2 5μ in diameter and contained one or sometimes two chromatin dots A variety of shapes, including some budding forms, was noted and occasionally extended loops were seen Infected red cells frequently contained more than one organism, up to eight being present in a single cell

The diagnosis was now considered to be a gross haemolytic anaemia secondary to *P falciparum* malaria, the infection probably having been transmitted in a blood transfusion He had been treated by peritoneal dialysis, massive doses of penicillin and hydrocortisone, and an exchange transfusion was in preparation The patient, however, died that day, the third after admission to the Renal Unit

At autopsy there was profound bile staining of all organs The spleen was absent and the gastroenterostomy was free of ulceration The kidneys were markedly swollen and the bladder contained 25 ml of black urine No malarial pigment was found in any of the usual sites The typical changes of a haemoglobinuric nephrosis were seen in the kidneys All vascular spaces, especially those in the brain, contained numerous immature granulocytes and nucleated red corpuscles

The intra-erythrocytic organisms were later identified as a piroplasm, ***Babesia divergens***. This tick-borne infection was possibly picked up during a caravan holiday in mid-August

We thank Professor P C C Garnham and Mr P G Shute who made the diagnosis We thank also Dr C F Brennan who referred the patient to us

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[1] Skrabalo, Z , and Deanovic, Z , *Documenta Med Geog. Trop* , 9, 11 (1957).

[2] Braff, E , and Condit, P , *Morbidity and Mortality Weekly Report* (US Dept. of Health, Education and Welfare, Bureau of Disease Prevention and Environmental Control, National Communicable Disease Center, Atlanta, Georgia, January 7, 1967)

RADIOBIOLOGY

Low Oxygen Enhancement Ratios for Radiosensitive Bacterial Strains, and the Probable Interaction of Two Types of Primary Lesion

WHEN exposed to ionizing radiation, bacterial strains which are deficient in ability to by-pass or repair damage to nucleic acid have lower oxygen enhancement ratios (OERs) than their more radioresistant parents[1] This was predicted from a model in which the principal responsibility for the oxygen effect is attributed to a primary lesion of "type *O*", whereas a different primary lesion, "type *N*", is more easily repaired or by-passed by some strains than others With certain strains, notably ***Escherichia coli B***, the lethal effects of type *N* lesions are readily modified by post-irradiation culture conditions The greater the extent of repair of type *N* damage, the higher the OER[2,3]

Results have been extended by the use of a number of radiosensitive mutants of *E coli B*, selected by Hill, who showed that there was no obvious correlation between sensitivity to ultraviolet light and inability to perform "host cell reactivation" (HCR) on ultraviolet irradiated bacteriophages[4] By using these and other mutants of the parent strain, we have found that the OER is negatively correlated with sensitivity to ultraviolet light, whether or not the strain is HCR⁻ (Table 1)

If variation in radiosensitivity within a group of strains, or with culture conditions for a single strain, depends only on the reparability of type *N* lesions, and if these operate independently of type *O* events, *m* (the OER) and *Do* (the dose to give an average of one lethal event per cell) are linearly related by the expression

$$m = m_N + (m_o - m_N)\lambda o Do \qquad (1)$$

where m_o and m_N are the OERs pertaining to type *O* and *N* events, respectively, and λo is the sensitivity to type *O* events The value of m_N was found to be 1 5 for *E coli B*, and this was supported by calculations, on these assumptions, from independent observations[2,3]

Table 1 OXYGEN ENHANCEMENT RATIOS, X-RAY DOSES (UNDER ANOXIA) TO GIVE AN AVERAGE OF ONE LETHAL EVENT PER BACTERIUM (THAT IS VALUES OF *Do*), AND DOSES OF ULTRAVIOLET LIGHT TO GIVE 10 PER CENT SURVIVAL FOR ELEVEN VARIANTS OF *E coli B*

Variant	HCR + or −	Oxygen enhancement ratio	*Do* (krads)	Ultraviolet dose for 10% survival (ergs/mm²)
Bs-12	−	1 17	2 61	1 8
Bs-1	−	1 77	2 78	4 9
Bs-11	+	2 22	3 29	31
Bs-2	+	2 33	3 94	52
Bs-3	−	2 33	10 4	81
B/rWP₂hcr⁻	−	2 42	12 6	37
Bs-8	−	2 52	11 8	35
B	+	2 74	9 3	236
B/r	+	3 19	15 4	580
B-H	+	3 34	14 9	460
B/rWP₂hcr⁺	+	3 27	17 9	980

Bacteria were cultured before irradiation in Difco broth with 5 g/l of sodium chloride, and plated on solid medium of the same composition Survival curves for X-rays allowed the fitting of slopes from which *Do* could be calculated For ultraviolet irradiation, however, survival curves differed greatly in shape In some cases (for example, *B/r*) a large fraction of the dose to give 10 per cent survival was accounted for by the shoulder, whereas in others (for example, *B*, *Bs*-12) survival curves were steepest at zero dose and became progressively less steep "Sensitivity" to ultraviolet is therefore not definable by a single parameter and the last column must be regarded as a rough guide

The data of Table 1 for a group of mutants of *E. coli B* are plotted in Fig 1, which shows that the data fit roughly with a straight line relationship, but the point for one strain in particular—the highly sensitive *Bs*-12—falls well below the line Furthermore, its oxygen enhancement ratio of only about 1 2 is significantly less than what should be the minimum possible value, 1 5, if the model and the inferred value for m_N were correct The model therefore evidently requires modification

The evidence of correlation between the sensitivity of a family of strains to ultraviolet light and to X-rays in

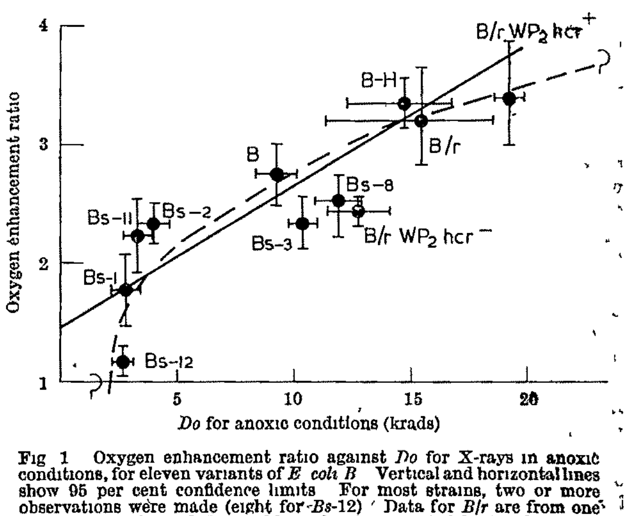

Fig 1 Oxygen enhancement ratio against *Do* for X-rays in anoxic conditions, for eleven variants of *E coli B* Vertical and horizontal lines show 95 per cent confidence limits For most strains, two or more observations were made (eight for *Bs*-12) Data for *B/r* are from one observation only, and the confidence limits computed for the data from that experiment

anoxic conditions, and the negative correlation between OER and radiosensitivity, add to the plausibility of inferring that type N primary lesions occur in nucleic acids[3], and that these primary events have only a minor responsibility for the oxygen effect No such clear inference can be made in respect of primary events of type O These are events which must have at least four to five times the probability of becoming lethal when oxygen is present during irradiation[3] Lipids are readily peroxidized under X-irradiation[5], and so it may well be that type O events are to be associated with damage to "unit membranes" in the cell The idea that radiation might, by its effect on membranes, cause cell death by the release of lytic enzymes is by no means new, and evidence of such action, direct[6] or circumstantial[7], is accumulating

A difficulty in attributing a "component of sensitivity" to membrane damage is, however, the nature of dose-effect relationships observed with ionizing radiation, which strongly suggest that damage to a unique "target" may be caused by one or a few energy loss events; and it is difficult to envisage that a "hit" in a membrane, or the release of a few enzyme molecules, could satisfy this requirement This difficulty would be resolved if the release of lytic enzymes resulted in attack on potentially lethal lesions in the most plausible "vital target" of the replicating cell, the DNA Thus it may be envisaged that there is interaction between the consequence of primary type O events and some of the type N events which would not be lethal without this interaction

If these primary events result in interaction the simple treatment set out here can be only a first approximation Various assumptions could be made from which relationships between m and Do could be predicted, but there are too many possibilities to make this adequately heuristic For example, it could be that the presence of oxygen affects the probability of lethal interaction, as well as the probabilities that type N and type O events are independently lethal It may, however, be interesting to derive an expression for change in m with a change in probability that type N events are independently lethal

In anoxic conditions, let λ_N and λ_o represent respectively the sensitivities to potentially lethal events of type N and type O In anoxic conditions, let p_1, p_2, p_3 and p_4 represent probabilities as follows·

p_1 type N events independently lethal
p_2 type N events lethal as a result of interaction
p_3 type O events independently lethal
p_4 type O events lethal as a result of interaction

In the presence of oxygen, these probabilities become $p_1\,m_N$; $p_2\,m_N$, $p_3\,m_o$ and $p_4\,m_o$ Thus the overall sensitivities in the absence and presence of oxygen are

$$\lambda=p_1\lambda_N+p_2p_4\lambda_N\lambda_O+p_3\lambda_O=1/D_o$$
$$m\lambda=m_np_1\lambda_N+m_Nm_op_2p_4\lambda_N\lambda_O+m_Op_3\lambda_O$$

In this case, even if p_3 and p_4 are constant, λ is not linearly related to p_1, because p_2 may increase as p_1 decreases For example, effective repair processes would reduce the probability that type N events could be independently lethal, but additional lesions might then be available for interaction If the two equations are combined, we have

$$m=m_nm_o\left\{1-\frac{p_1\lambda_N}{\lambda}-\frac{p_3\lambda_O}{\lambda}\right\}+\frac{p_1\lambda_Nm_N+p_3\lambda_Om_O}{\lambda}$$

If p_1 and p_3 are small, λ approaches $p_2p_4\lambda_N\lambda_O$, and m approaches m_Nm_O. If p_1 is near to 1, p_2 is small, and

$$\lambda=p_1\lambda_N+p_3\lambda_O \qquad m\lambda=p_1\lambda_Nm_N+p_3\lambda_Om_O$$

giving

$$m=m_N+p_3\lambda_O(m_O-m_N)D_O$$

Thus, as in the previous treatment, m cannot be less than m_N, though m_N can be less than 1 A possible relationship between m and Do is shown by the dotted line of Fig 1

Besides the evidence from strain *Bs*-12 that the value 1 5 for m_N is too high, Davies[9] has reported a strain of *Chlamydomonas* (*UVS*1) which has an OER of 1 (in terms of the slopes of the survival curves) To account for this observation, Davies put forward a model which he described as an alternative to the one based on two types of primary event with different intrinsic OERs He described this "alternative model" in terms of differential reparability of lesions incurred in the absence and presence of oxygen, and proposed that the *UVS*1 strain of *Chlamydomonas* was deficient in a repair system operating specifically on lesions incurred in the absence of oxygen (for example, cross-linking) but not in its presence Davies's model is, in effect, no different from the one described here, for differences from strain to strain in the reparability of lesions of different types are implicit in the values of the exponents· that is, λ_N, λ_O, $m_N\,\lambda_N$ and $m_O\,\lambda_O$ in my original model, or these parameters with their associated probabilities in the modified version Whichever way the model is described, it is a requirement that, to accommodate a strain which has an OER of 1 or less, there must be a form of damage which is less reparable after irradiation in the absence of oxygen than in its presence, which suggests a value of m_N less than 1 It may yet prove necessary to invoke comparison with the phenomenon long ago observed with bacteriophage, and the kappa particle of *Paramecium*, namely, that in aqueous suspension they are protected by oxygen against the lethal effects of radiation[10-12], so that presumably the overall lethal damage to the nucleic acid core is reduced in the presence of oxygen

I thank Dr J L Moore, Dr A J Forage and Mrs B Knowles for their help I also thank Dr Ruth Hill for sending me various radiosensitive mutants of *E. coli B*

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[1] Alper, T , *Mutation Res* , 4, 15 (1967)
[2] Alper, T , *Intern. J Radiat Biol* , 3, 369 (1961)
[3] Alper, T , *Phys Med Biol* , 8, 365 (1963)
[4] Hill, R F , and Feiner, R R , *J Gen Microbiol* , 35, 105 (1964)
[5] Wills, E D , and Wilkinson, A E , *Radiat Res* , 31, 732 (1967)
[6] Allison, A C , and Paton, G R , *Nature*, 207, 1170 (1965)
[7] Allison, A C , Magnus, I A , and Young, M R , *Nature*, 209, 874 (1966)
[8] Pollard, E C , and Weller, P K , *Radiat Res* , 31, 617 (1967)
[9] Davies, D R , *Nature*, 215, 829 (1967)
[10] Alper, T , *Brit J Radiol* , 27, 50 (1954)
[11] Bachofer, C S , and Pottinger, M A , *Science*, 119, 378 (1954)
[12] Kimball, R F , *Ann NY Acad Sci* , 59, 638 (1955)

PHYSIOLOGY

ABO Blood Groups, Secretor Status, and Intestinal Mucosal Concentrations of Alkaline Phosphatase

HUMAN serum alkaline phosphatase from some individuals can be separated by starch gel electrophoresis into two components The faster moving zone has the electrophoretic characteristics of liver and bone alkaline phosphatase[1] and, like liver alkaline phosphatase, it is only slightly inhibited by treatment with phenylalanine. By contrast, the slower moving zone has the electrophoretic characteristics of small intestinal alkaline phosphatase[2,3] and its activity is markedly inhibited by phenylalanine[4,5]

The occurrence of this intestinal band of alkaline phosphatase activity in the serum has been found to depend on the ABO blood group and ABH secretor status of the individuals[6-10] The zone is rarely found in the serum of those who are non-secretors, whatever their ABO blood group In secretors, activity is seldom found in those of blood group A, more often in those of group AB, and frequently in those of groups O or B

It is not known, however, whether the transfer of alkaline phosphatase activity from the mucosa to the blood reflects differences in the rate of formation of the enzyme in the mucosa by individuals of varying ABO blood group and secretor status or some other process, such as variations in the rate at which alkaline phosphatase leaks out of the intestinal cell To investigate this problem further, we have measured the concentration of alkaline phosphatase in small intestinal mucosal specimens obtained at operation from individuals of known ABO blood group and secretor status

Small strips of jejunal mucosa were obtained from patients undergoing operations, such as Polya partial gastrectomy, which involved the opening of the small intestine and formation of a gastrojejunostomy

The mucosal specimens were freed of any submucosal tissue and kept at -20° C until they were analysed, preliminary work having indicated that there was no loss of activity during storage After freeze-drying for 12 h, duplicate mucosal samples of about 40 mg were weighed exactly and the alkaline phosphatase was extracted by Ahmed and King's modification of Morton's butanol extraction procedure[11,12] The tissue was homogenized with 2 5 ml of *n*-butanol for 3–5 min, the tubes being surrounded by ice The butanol extract was then emulsified with water and centrifuged at 3,000 r p m for 15 min before the enzyme-rich water layer was removed.

Alkaline phosphatase activity in the samples was measured by the method of Kind and King[13] using phenyl phosphate as substrate, and the results were expressed as King–Armstrong (KA) units per g of dry weight of tissue ABO blood groups were determined by standard agglutination techniques and secretor status by the method of Boyd and Shapleigh[14]

Jejunal mucosal samples were obtained from thirty-five patients of known ABO blood group, and saliva samples for determination of secretor status were obtained in twenty-nine Of those remaining, four died after their operation before saliva samples could be collected and two were considered, from knowledge of their red cell Lewis groups, to be ABH secretors.

Table 1 MEAN MUCOSAL ALKALINE PHOSPHATASE CONCENTRATIONS IN INDIVIDUALS OF DIFFERING ABO BLOOD GROUP AND SECRETOR STATUS

Genetic category	No of samples obtained	Tissue alkaline phosphatase in KA units/g dry weight Mean	Range	*S E M*
Group O and B	21	645	111–995	52
A	13	534	164–828	54
AB	1	387	—	—
Group O and B secretors	14	725	347–995	52
A secretors	7	552	298–828	61
AB secretors	1	387	—	—
All non-secretors	9	425	111–818	81

Table 1 shows the distribution of the results according to ABO blood group and secretor status The same categories as have been found to be relevant to studies of serum intestinal alkaline phosphatase concentrations have been used in this investigation

Mucosal alkaline phosphatase concentrations tended to be higher in individuals of blood groups O and B than in those of group A (mean concentrations 645 and 534 KA units per g dry weight, respectively), but this difference could well have been due to chance (*t*, 1 41, *P*, 0 16) When secretor status was taken into account, however, a gradient was found with the highest mean value being obtained in O or B secretors (725 KA units), an intermediate value (552 KA units) in group A secretors, and the lowest in non-secretors (425 KA units) Fig 1 shows the distribution of the individual results This gradient of activity reflects the patterns previously delineated by starch gel electrophoresis of human serum The low mucosal alkaline phosphatase concentration (387 KA units) found in the single individual of blood group AB, which was judged by red cell Lewis grouping to be a secretor, was an exception to the trend, for serum intestinal alkaline phosphatase activity in group AB secretors tends to be intermediate between that of group O or B secretors and that of group A secretors Even when this anomalous result is taken into account, however, a non-parametric test for trend shows that the observed gradient is very unlikely to be due to chance ($P < 0\ 01$) (ref 15).

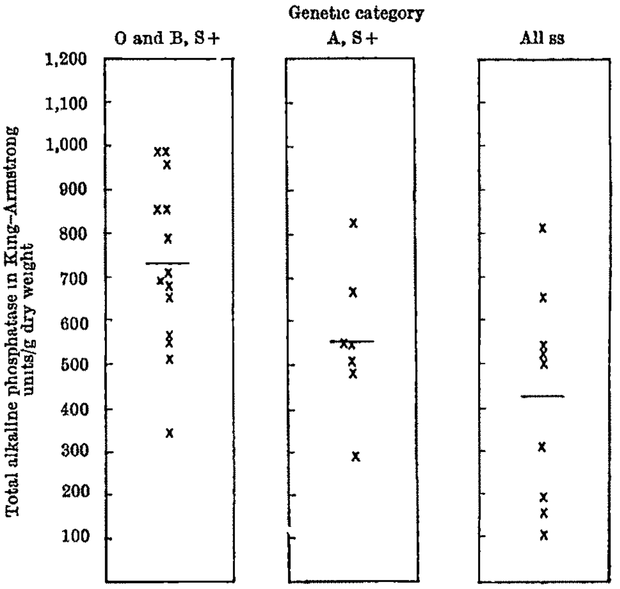

Fig 1 Distribution of individual measurements of tissue alkaline phosphatase concentration according to genetic category, mean concentrations in each group are indicated by horizontal bars

At least two factors apart from ABO blood group and secretor status could affect the jejunal alkaline phosphatase concentration; these are feeding and the anatomical site high or low in the intestine from which the mucosa was removed. Serum intestinal alkaline phosphatase activity is known to rise in the period following a fatty meal[16–18], the change being greatest in O and B secretors and least in non-secretors—changes which presumably could reflect alterations in mucosal phosphatase output. All the patients from whom our mucosal samples had been obtained had fasted for at least 12 h before operation and therefore it is unlikely that the patterns of enzyme activity found reflect differences in feeding

Alkaline phosphatase concentrations in human ileal mucosa are, as in the rat, much lower than those in the jejunum (our unpublished observations) All the mucosal specimens obtained in this study were taken during a standard operative procedure, gastrojejunostomy, and it is unlikely that the site of operation would have varied consistently in individuals of different genetic constitution.

The patterns of intestinal mucosal alkaline phosphatase activity found are therefore likely to reflect real variations in enzyme concentration between individuals of differing ABO blood group and secretor status In an earlier study, Shreffler[19] was unable to show any consistent variation in mucosal alkaline phosphatase concentration between individuals of different ABO blood group and secretor status His results, however, were obtained on mucosal specimens removed at post-mortem examination, and autolysis of the tissues could well have obscured any real variation present The finding of high mucosal enzyme concentrations in those individuals who by genetic constitution would be expected to show most intestinal alkaline phosphatase activity in their serum supports the hypothesis that ABO blood groups and secretor status affect the rate of production of alkaline phosphatase by the mucosal cell If blood groups and secretor status primarily affected the rate of enzyme leakage from the intestinal cell, then mucosal alkaline phosphatase concentrations might be expected to be highest in non-secretors and lowest in O and B secretors, the opposite of the gradient found. Another possible explanation of the serum enzyme variation is that blood group and secretor status affect the rate of removal of intestinal alkaline phosphatase from the circulation. If this were the case, however, then mucosal enzyme concentrations would be unlikely to show any gradient according to the genetic constitution of the individuals.

The precise metabolic role of intestinal alkaline phosphatase is not understood. The rise in the serum concentration of the enzyme after a fatty meal suggests that it may play a part in fat absorption or transport, which therefore might be affected by the ABO blood group and secretor status of the individuals. It has been claimed from studies of blood lipid concentrations in twins that day-to-day variation in free serum cholesterol levels is affected by ABO blood group[20]. On the other hand, the increase of serum turbidity after a fatty meal has not been found to differ with the ABO blood group or secretor status of the individuals. These observations could be reconciled if genetic characteristics affected transport of some specific class of lipid.

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[1] Hodson, A. W., Latner, A. L., and Raine, L., *Clin. Chim. Acta*, **7**, 255 (1962).

[2] Robinson, J. C., and Pierce, J. E., *Nature*, **204**, 472 (1964).

[3] Moss, D. W., Eaton, R. H., Smith, J. K., and Whitby, L. G., *Biochem. J.*, **98**, 320 (1966).

[4] Fishman, W. H., Green, S., and Inglis, N. I., *Nature*, **198**, 685 (1963).

[5] Kreisher, J. H., Close, V. A., and Fishman, W. H., *Clin. Chim. Acta*, **11**, 122 (1965).

[6] Arfors, K. E., Beckman, L., and Lundin, L. G., *Acta Genet.*, **13**, 89 (1963).

[7] Arfors, K. E., Beckman, L., and Lundin, L. G., *Acta Genet.*, **13**, 366 (1963).

[8] Beckman, L., *Acta Genet.*, **14**, 286 (1964).

[9] Bamford, K. F., Harris, H., Luffman, J. E., Robson, E. B., and Cleghorn, T., *Lancet*, i, 530 (1965).

[10] Shreffler, D. C., *Amer. J. Hum. Genet.*, **17**, 71 (1965).

[11] Ahmed, Z., and King, E. J., *Biochim. Biophys. Acta*, **40**, 320 (1960).

[12] Morton, R. K., *Nature*, **172**, 65 (1953).

[13] Kind, P. R. N., and King, E. J., *J. Clin. Pathol.*, **7**, 322 (1954).

[14] Boyd, W. C., and Shapleigh, E., *Blood*, **9**, 1195 (1954).

[15] Terpstra, T. J., *Proc. Kon. Ned. Akad. Wetenschap.* A55 (Indagationes Mathematicae, 14), 327 (1952), quoted by Noether, G. E., *Elements of Nonparametric Statistics* (J. Wiley, London, 1967).

[16] Langman, M. J. S., Leuthold, E., Robson, E. B., Harris, J., Luffman, J. E., and Harris, H., *Nature*, **212**, 41 (1966).

[17] Inglis, N. I., Krant, M. J., and Fishman, W. H., *Proc. Soc. Exp. Biol. and Med.*, **124**, 699 (1967).

[18] Klein, U. E., Drube, H. C., and Hansen, H. T., *Klin. Wschr.*, **45**, 95 (1967).

[19] Shreffler, D. C., *Proc. Soc. Exp. Biol. and Med.*, **123**, 423 (1966).

[20] Blankenhorn, D. H., Jensen, J., Sturgeon, P., Chin, H. P., Armstrong, B. W., and Engelman, L., *Nature*, **215**, 1499 (1967).

Storage of Carbon Dioxide in Muscle

WHEN ventilation is suddenly arrested, the carbon dioxide produced by the body is stored within the body tissues as bicarbonate and dissolved carbon dioxide. It has been suggested that this storage of carbon dioxide does not proceed uniformly in all tissues. Rather it has been proposed that the body can be conceived as being composed of multiple compartments which store carbon dioxide at different rates.

In the multicompartment model of carbon dioxide stores proposed by Fahri and Rahn, during apnoea, muscle—because it has a low ratio of perfusion per unit volume—acts as a repository for carbon dioxide produced by other organs[1]. When there is no ventilation, therefore, the carbon dioxide content of the venous blood should be less than the carbon dioxide content of the arterial blood—the reverse of the usual situation.

To test this prediction, we measured the carbon dioxide and oxygen tension, the hydrogen ion concentration and the haematocrit of blood from the femoral artery and vein, and calculated carbon dioxide contents in nine anaesthetized and paralysed dogs during apnoea.

After the paralysed dogs had been ventilated with 100 per cent oxygen for 30 min, arterial and venous blood was obtained from one hind limb, and carbon dioxide production was measured. Apnoea was produced by stopping artificial ventilation. During the apnoeic period, blood samples were obtained at intervals of 1 or 2 min while the trachea of the dog was connected to a spirometer which contained oxygen so that full arterial saturation could be maintained. In three dogs, blood flow in the opposite limb was measured with a rotameter.

Table 1. FEMORAL ARTERIAL AND VENOUS CARBON DIOXIDE TENSIONS AND CONCENTRATIONS DURING APNOEA IN NINE DOGS (MEAN ± STANDARD ERROR)

Time (min)	CO_2 tension (mm Hg)		CO_2 concentration difference (vol. per cent)
	Artery	Vein	Vein–artery
0	35 ± 4	40 ± 5	+2·1 ± 0·7
1	47 ± 4	44 ± 4	−1·4 ± 0·3
2	52 ± 5	47 ± 4	−2·5 ± 0·6
4	63 ± 6	53 ± 5	−3·3 ± 0·9
6	73 ± 6	60 ± 5	−3·3 ± 0·6

The carbon dioxide production of the dogs during the control period of artificial ventilation averaged 74 ml./min (standard error, ± 4). The mean blood flow in the femoral artery was 135 ml./min in the control period but decreased to an average of 92 ml./min during apnoea. Table 1 shows the carbon dioxide tensions and the venous minus the arterial carbon dioxide concentration differences observed during apnoea. During artificial ventilation the venous carbon dioxide tension exceeded the arterial by 5 mm of mercury while the carbon dioxide content of the vein was 2·1 vol. per cent greater than the artery. After 1 min of apnoea, these gradients were reversed and the venous carbon dioxide tension and concentration were less than those of the artery. At 6 min of apnoea, the average venous carbon dioxide tension was 13 mm of mercury less than that measured in the artery while the carbon dioxide concentration was 3·3 vol. per cent less.

The result of multiplying the average blood flow of the femoral artery by the mean venous–arterial carbon dioxide concentration differences shows that, during each minute of apnoea, muscle stores about 3 ml. of carbon dioxide produced elsewhere. This is about 4 per cent of the total production of carbon dioxide by the body each minute.

According to the multicompartment theory, carbon dioxide is stored in different tissue compartments, each with its own level of perfusion, metabolic rate and dissociation curve for carbon dioxide[2]. Ventilation changes produce rapid alterations in carbon dioxide content in richly perfused tissue such as the brain, but only slow changes in tissues with a large volume but a relatively poor perfusion such as muscle. The results of this study agree with the idea that muscle carbon dioxide reaches equilibrium sluggishly after a change in arterial carbon dioxide tension, thus producing the reversal of the usual venous–arterial concentration differences.

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[1] Fahri, L. E., and Rahn, H., *Anesthesiology*, **21**, 604 (1960).

[2] Cherniack, N. S., Longobardo, G., Staw, I., and Heymann, M., *J. Appl. Physiol.*, **21**, 785 (1966).

Induction of Hyperluteinization and Precocious Opening of the Vagina in Rats with a Transverse Cut in the Hypothalamus made shortly after Birth

THERE have been several reports that electrolytic lesions in the hypothalamus of infantile rats result in a precocious opening of the vagina and eventually in an early luteinization of the ovary[1-5]. It seems that in all cases when the ovaries of animals bearing hypothalamic lesions contained corpora lutea at puberty there was no more than the usual number of these bodies.

In a series of experiments, one transverse cut was made in the hypothalamus of thirty-six female random bred albino rats not more than 24 h old, and of six rats not more than 36 h old; a pointed, fine gauge steel needle (3·2 cm long and 0·5 mm in diameter) was used. (To make mechanical lesions in the hypothalamus of adult rats Halász and Gorski[6] used a double-edged knife fixed in the holder of a stereotaxic instrument.) The operation was performed by hand and without anaesthesia. The object of the experiment was to place the cut at about the level of the arcuate nucleus, transect all the antero-lateral connexions to the median basal hypothalamus and follow the effects of the lesion on the ovary for longer periods. In most of the animals the lesion involved the mammillary body.

Twelve sham operated rats and eighty-three normal animals served as controls. Into the sham operated rats we inserted the point of the needle far enough to perforate the tissues of the skull, carefully avoiding injury to the brain mass. Both experimental animals and controls were isolated from the males. The animals were killed at various times between 3·5 and 12 months old.

Thirteen rats had ovaries with scores of luteal bodies (Fig. 1). In eight of these the lesion extended posteriorly to include a portion of the mammillary body. The remaining five rats are still alive. In two animals, the lesion was made behind the origin of the pituitary stalk. The left ovary was removed from one of the latter on the day the vagina opened—the twenty-seventh day after birth. The ovary contained numerous medium sized follicles but no corpora lutea, and weighed 5·4 mg. When it was 10 months old this animal was killed. Its remaining ovary, which was enormously enlarged and weighed 200·8 mg, contained innumerable small and medium sized luteal bodies (Fig. 2*a* and *b*).

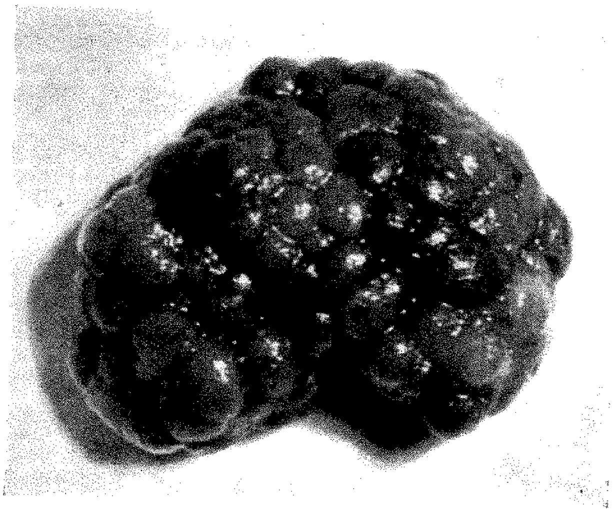

Fig. 1. Numerous corpora lutea in the ovary of a rat with a transverse cut through hypothalamus, killed 10 months after birth. The ovary at killing weighed 86·4 mg. (× 9.)

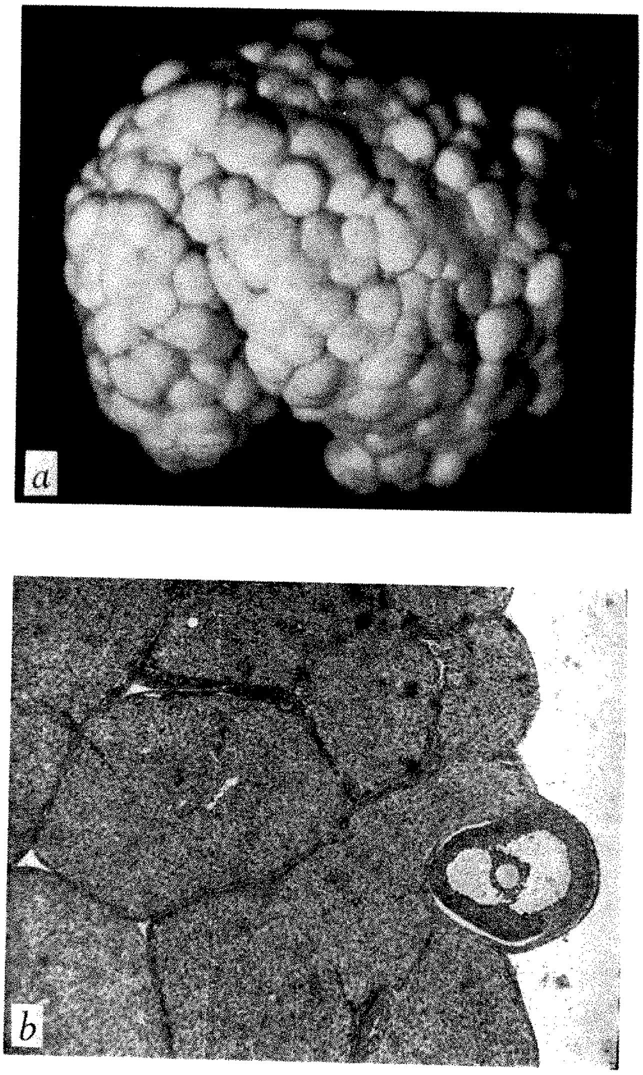

Fig. 2. *a*, "Innumerable" luteal bodies in the right ovary of a rat, operated as described, unilaterally ovariectomized on the day the vagina opened and killed when 10 months old. At death the ovary weighed 200·8 mg. (× 9.) *b*, Histological section through the same ovary, showing closely packed corpora lutea and one medium sized Graafian follicle. (× 42.)

The number of corpora lutea in the ovaries bearing a large number of them seems to remain constant for many months, although on the day of vaginal opening, if they were present, no more than the normal quota was to be found. This seems to be in agreement with the situation found by the authors already mentioned. The increase in number of corpora lutea seems therefore to be a result of repeated luteinization. Fresh corpora lutea had a normal histological structure.

Daily examinations of the vaginal smear for several weeks after the opening of the vagina revealed, in all the rats with supernumerary corpora lutea, repeated cycling periods, sometimes regular and sometimes irregular. The same can be said for the three rats from which daily vaginal smears were taken for 50 consecutive days beginning with the eighth month of their post-natal life. As well as numerous luteal bodies a few Graafian follicles, large and medium sized, were found in the ovaries of these rats. Burst follicles were also found in the ovaries of two animals. When confined with sexually mature males, these rats failed to mate.

Table 1 gives mean values (± standard deviation) for the age and body weight of experimental animals and for normal and sham operated controls on the day the vagina opened. As can be expected, there are individual varia-

Table 1

	No. of rats	Age (days)	Body weight (g)
With hypothalamic lesions	42	37·2 ± 5·98	75 ± 21·1
Sham operated	12	41·0 ± 2·38	100 ± 5·8
Normal controls	83	44·0 ± 4·75	108 ± 14·1

tions, but the precocity of the appearance of the phenomenon in the operated rats compared with normal controls is demonstrated both by the number of individuals (in six rats the vagina opened before they were 30 days old: in one on day 26, in one on day 27, and in four on day 29) and by the mean value for all rats. In normal rats we never observed this phenomenon earlier than day 32, regardless of season. Oddly enough, the opening of the vagina in sham operated rats was also significantly advanced (Table 1). A lag in the rate of gain of body weight regularly follows the injury to the hypothalamus, but this does not seem to affect seriously the precocity of the opening of the vagina. At death or after repeated laparotomies, that is, any time between 3 and 12 months after birth, the ovaries of all the rats in which the vagina opened precociously contained numerous corpora lutea, but this holds for an even larger number of rats in which the vagina opened within the interval characteristic for normal controls.

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[1] Donovan, B. T., and van der Werff ten Bosch, J. J., *Nature*, **178**, 745 (1956)
[2] Bogdanove, E. M., and Schoen. H. C., *Proc. Soc. Exp. Biol. and Med.*, **100**, 664 (1959).
[3] Elwers, M., and Critchlow, V., *Amer. J. Physiol.*, **198**, 381 (1960).
[4] Gellert, R. J., and Ganong, W. F., *Acta Endocrinol.*, **33**, 569 (1960).
[5] Horowitz, S., and van der Werff ten Bosch, J. J., *Acta Endocrinol.*, **41**, 301 (1962).
[6] Halász, B., and Gorski, R. A., *Endocrinology*, **80**, 608 (1967).

Continued Cleavage of Fertilized Bovine Ova in the Rabbit

FERTILIZED sheep ova have been shown to continue cleavage to the early blastocyst stage after transfer to the reproductive tract of the female rabbit[1]. This *in vivo* culture technique has facilitated the long distance transport of sheep ova[2]. This report suggests that a similar technique may be applied to cattle ova.

During 1967, cattle ova were recovered from the fallopian tubes of one hundred and forty cattle after superovulation treatment with placental gonadotrophins. Culture studies were made on five hundred of these ova. *In vitro* culture of ova in media based on cattle blood serum has not been successful. This agrees with previous reports[3]. Continued but limited cleavage to the 24-blastomere stage has been achieved in bovine follicular fluid. This supports the findings of other work[4].

In transferring cattle ova to the pseudo-pregnant rabbit, we have had limited success after using cattle serum as the recovery and transfer medium. We transferred one hundred and five fertilized ova using serum; fifty-one of these developed further. The maximum cleavage stage reached was nineteen blastomeres. We had better results after using bovine follicular fluid for recovery and transfer.

Seven sexually mature cattle were treated with 3,000 IU pregnant male serum gonadotrophin (PMSG) to induce superovulation on the sixteenth day of the oestrous cycle. The cattle were slaughtered 3 days after breeding and injection of 2,000 IU human chorionic gonadotrophin (HCG). Fifty-six of the fifty-nine ova recovered from these cattle were fertilized; thirty-two of these were used in transfers to seven pseudo-pregnant rabbits. Within 20 min of the slaughter of the cattle we began to recover the ova from their fallopian tubes using bovine follicular fluid. We then studied the ova within a temperature controlled cabinet (26·7° C).

Table 1. DEVELOPMENT OF CATTLE OVA IN THE RABBIT

Heifer identn.	Rabbit identn.	Fertilized ova Transferred	Fertilized ova Recovered	Initial cell stages	Final cell stages	Culture period (h)
98	*B*43	6	5	× 5 – × 10	4 × 80 + 1 × ruptured ovum	98
104	*B*7	6	1	× 4 – × 10	1 × 60 +	95·5
112	*B*9	4	2	× 6 – × 10	1 × 100 + 1 × 19	98
114	*B*5	4	3	× 8 – × 16	2 × 100 + 1 × degenerate ovum	94
124	*B*42	3	3	× 7 – × 8	3 × 90 +	94
129	*B*6	1	1	× 8	1 × 90 +	96
139	*B*28	8	4	× 5 – × 10	1 × 90 + 3 × 100 +	116·25

The fertilized ova were transferred in bovine follicular fluid to the ligated fallopian tubes of the rabbits, which had been injected 20–47 h previously with 30 IU of HCG. Four days later, the rabbit oviducts were removed and nineteen ova were recovered. Results of the transfers given are in Table 1. Seventeen ova had developed to the early blastocyst stage; in one instance the zona pellucida had ruptured and one ovum was degenerate. After recovery from the rabbits, the ova were fixed in acetic acid alcohol and stained with lacmoid solution. The high power of the microscope was used to estimate the number of nuclei in each blastocyst. On the basis of evidence for the early stages of the bovine ovum[5], the stages of cleavage reached by the cultured ova seem compatible with normal development.

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[1] Averill, R. L. W., Adams, C. E., and Rowson, L. E. A., *Nature*, **176**, 167 (1955).
[2] Adams, C. E., Rowson, L. E. A., Hunter, G. L., and Bishop, G. P., *Proc. Fourth Int. Congr. Anim. Reprod. (Hague) Physiol. Sect.*, 381 (1961).
[3] Brock, H., and Rowson, L. E., *J. Agr. Sci.*, **42**, 479 (1952). Dowling, D. F., *J. Agr. Sci.*, **39**, 374 (1949). Hafez, E. S. E., Sugie, T., and Gordon, I., *J. Reprod. Fert.*, **5**, 359 (1963). Pincus, G., *Proc. First Nat. Egg-Transfer Breed. Conf., Texas*, 18 (1951).
[4] Thibault, C., *Ann. Biol. Anim. Bioch. Biophys.*, **6** (2), 159 (1966).
[5] Hamilton, W. J., and Laing, J. E., *J. Anat.*, **80**, 194 (1946).

Effect on the Guinea-pig Taenia Coli of the Substitution of Strontium or Barium Ions for Calcium Ions

THE spontaneous discharge of the taenia coli of the guinea-pig is not inhibited by even a high concentration of tetrodotoxin (5×10^{-6} g/ml.), but it is blocked by a low concentration of ionic manganese (0·5 mM)[1], which is known to be a specific inhibitor of the calcium spikes in crustacean muscle[2] and also in barnacle muscle fibres[3]. These facts suggest that in the smooth muscle of taenia the membrane current during the action potential is carried principally by calcium ions. This would explain earlier findings that spike generation continues in solutions which are deficient in or free from sodium[4–6] and that

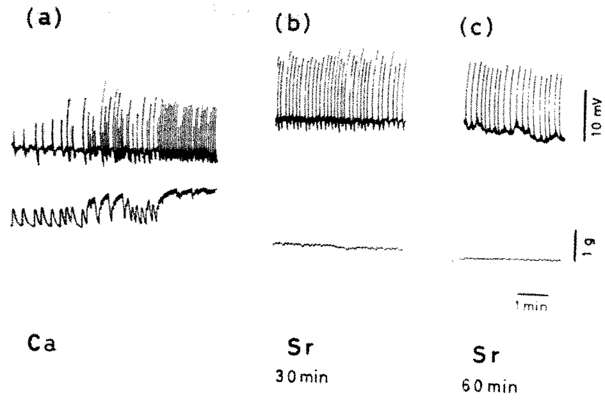

Fig. 1. Sucrose-gap records from the isolated taenia coli of the guinea-pig: spontaneous action potentials (upper tracings) and development of tension (lower tracings). Records were taken: (*a*) in normal Locke solution; (*b*) 30 min after replacement of calcium ions by an equivalent amount of strontium ions; (*c*) 60 min after the substitution. Note the decoupling of excitation and contraction in calcium-free medium which contains strontium.

spike height is almost independent of the external concentration of sodium ions[6]. Both the spontaneous activity and the potassium contracture are depressed by several transition metal ions such as manganese, nickel, cobalt or iron. The contraction induced by acetylcholine in normal Locke solution or in potassium-rich medium is also blocked by these divalent ions. The block is competitively antagonized by raising the external concentration of calcium, which supports the view that the entry of calcium ions across the membrane acts as a trigger for contraction[7].

We have examined the effect of substituting strontium or barium ions for calcium ions. Membrane potential and tension were recorded by a sucrose-gap method similar to that used by Bülbring and Burnstock[8]. The experiments were carried out on taenia coli of the guinea-pig at room temperature (23°–25° C). GEDTA (0·1 mM) was added to calcium-free solution to eliminate residual calcium ions.

Replacement of calcium ions in normal Locke solution (1·8 mM) with an equivalent amount of strontium or barium ions resulted in a depolarization of about 10 mV and an increase in spike frequency and tension. The tension, however, was not maintained for more than 20 min and it fell to less than one-tenth, in spite of the continuation of high frequency spike discharge (Fig. 1). Intracellular microelectrode measurements showed that a five-fold increase in the concentration of strontium or barium ions resulted in repolarization to almost the normal resting potential (stabilization) and in prolongation of the spike duration (probably because of a decrease of potassium permeability). In these media, the relation between spike frequency and tension was variable, but nearly normal tension was obtained in some cases.

The decoupling of excitation and contraction in calcium-free solution containing other alkaline-earth metal ions is understandable if it is assumed that the calcium ions which entered the cell during an action potential exert a direct influence on the contractile proteins and that other alkaline-earth metal ions have a relatively weak effect on the smooth muscle actomyosin system. Information on the sensitivity of intestinal smooth muscle proteins to divalent cations is not yet available, but according to S. Ebashi (personal communication), strontium and barium ions have very little effect on the superprecipitation of chicken gizzard actomyosin—one-twentieth and one-two-hundredth of that of calcium ions, respectively. This indicates that strontium and barium ions cannot be effective substitutes for calcium ions in triggering contraction. This is probably also true with the taenia coli of the guinea-pig, so the dissociation of spike discharge from the development of tension strongly supports the view that the calcium which carries the electrical current during the action potentials acts directly on the contractile mechanism. The possibility still remains, however, that the release of calcium from intracellular binding sites plays some part in contraction which has been demonstrated for the tonic phase of the potassium contracture[9].

In media containing strontium or barium ions, as in normal Locke solution, tetrodotoxin (10^{-5} g/ml.) had no inhibitory effect on the spikes, but the transition metal ions retained their suppressive effect on the spikes. The excitation mechanism in a calcium free medium containing strontium or barium seemed therefore to be essentially the same as that in normal Locke solution.

Acetylcholine (10^{-6} g/ml.), which in normal conditions invariably induces depolarization, increase in spike frequency and contraction, had surprisingly little effect on membrane potential and tension when calcium ions were substituted by strontium ions (Fig. 2). Similar results were observed with histamine (10^{-6} g/ml.) and angiotensin (10^{-7} g/ml.), and even ten to one hundred times the usual concentrations of these drugs had very little effect. The loss of the effect was reversed in proportion to the amount of calcium ions that was restored to the medium. The effect of these substances on potassium depolarized taenia coli also became negligible when the external calcium ions were replaced by an equivalent amount of strontium ions, and completely disappeared in a solution in which barium ions replaced calcium ions. The inability of the ileum of guinea-pig to respond to acetylcholine in a medium containing strontium ions is also suggested by the data given by Johnson during his study of the release of acetylcholine from nerve endings[10].

In the absence of calcium ions, noradrenaline (10^{-6} g/ml.), adrenaline (10^{-6} g/ml.) and isoprenaline (10^{-7} g/ml.) lost

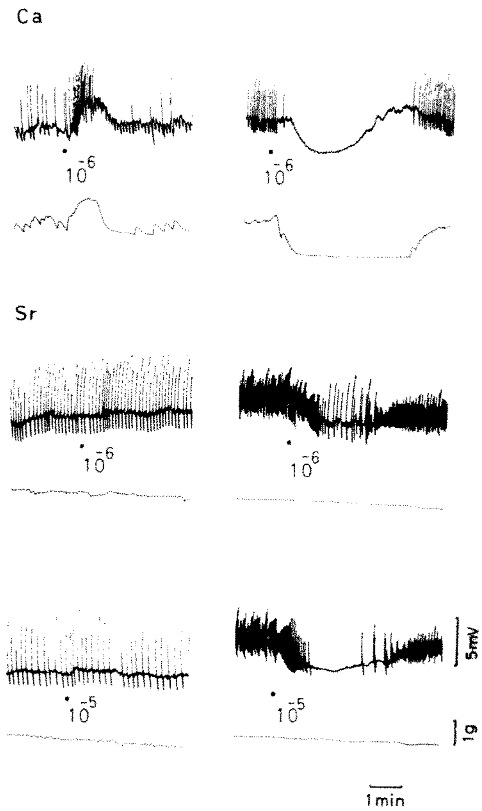

Fig. 2. Sucrose-gap records showing the effect of acetylcholine (left) and adrenaline (right) on the taenia coli of the guinea-pig. The dots indicate the time of drug applications for 30 s. The upper records show the effect of acetylcholine (10^{-6} g/ml.) and adrenaline (10^{-6} g/ml.) in normal Locke solution. The middle records show the ineffectiveness of both drugs after exposure for 20 min to calcium-free solution containing 1·8 mM strontium. The lower records were taken after an interval of 6 min, when both drugs were applied in a concentration of 10^{-5} g/ml. in solution containing strontium.

their ability both to hyperpolarize the membrane and to inhibit the spontaneous discharge of "barium ion-spikes". The effect of these catecholamines often remained in a solution containing strontium (Fig. 2), but if the tissue was washed by calcium ion-free Locke solution containing 1 mM GEDTA for several minutes beforehand, the effect almost disappeared in media containing strontium ions. Schild has recently demonstrated the special role of calcium in the relaxation of the depolarized rat uterus induced by isoprenaline[11].

The ineffectiveness of the drugs after the substitution of strontium or barium ions for calcium ions is the result not of depolarization nor of the presence of high frequency discharge for the following reasons: (1) the results are essentially the same even if the membrane potential is maintained at its normal value by increasing the concentration of strontium or barium ions to 7·2 or 18 mM; (2) the spike frequency is initially high in media containing strontium or barium ions, but it gradually declines to less than normal in an hour. In spite of this slowing, the ineffectiveness of drugs remains unchanged throughout the experiment.

These results suggest that calcium ions, as the charge carrier during the action potential in the taenia coli of the guinea-pig, can be replaced by other alkaline-earth metal ions, although strontium and barium ions have a weaker stabilizing action than calcium ions, and that barium and strontium ions are weak substitutes for calcium ions in triggering contraction. Calcium ions seem to play an essential part in the actions of certain drugs. Various normally excitatory or inhibitory drugs have little effect when calcium ions are replaced by other alkaline-earth metal ions, even though a normal excitation mechanism is maintained.

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[1] Nonomura, Y., Hotta, Y., and Ohashi. H., *Science*, **152**, 97 (1966).
[2] Fatt, P., and Ginsborg, B. L., *J. Physiol.*, **142**, 516 (1958).
[3] Hagiwara, S., and Nakajima, S., *J. Gen. Physiol.*, **49**, 793 (1966).
[4] Holman, M. E.. *J. Physiol.*, **136**, 569 (1957).
[5] Holman, M. E., *J. Physiol.*, **141**, 464 (1958).
[6] Bülbring, E., and Kuriyama, H., *J. Physiol.*, **166**, 29 (1963).
[7] Hotta, Y., *Jap. J. Smooth Muscle Res.*, **2**, 147 (1966).
[8] Bülbring, E., and Burnstock, G., *Brit. J. Pharmacol. Chemother.*, **15**, 611 (1960).
[9] Imai, S., and Takeda, K., *J. Physiol.*, **190**, 155 (1967).
[10] Johnson, E. S., *Brit. J. Pharmacol. Chemother.*, **21**, 555 (1963).
[11] Schild, H. O., *Nature*, **215**, 650 (1967).

Amphetamine Anorexia by Direct Action on the Adrenergic Feeding System of Rat Hypothalamus

It has been suggested that amphetamine causes loss of appetite by direct chemical interaction with the hypothalamic feeding system. This is based on the observation that intraperitoneally injected amphetamine is less effective in depressing food intake in rats when they have lateral hypothalamic lesions and have recovered from the post-operative aphagia[1]. Such evidence is not conclusive, however, because of the possibility that anorexia arises from the action of amphetamine on a part of the system which is necessary to feeding in the normal rat, but which is not necessary to the elicitation of eating by food deprivation in rats which have overcome the aphagia resulting from the lesions. Amphetamine raises the current threshold for elicitation of eating by electrical stimulation of the lateral hypothalamus[2,3]; this indicates, also indirectly, that it acts there or on efferent systems. Determination of the behavioural effect of administering amphetamine directly into the lateral hypothalamus is a necessary part of establishing the suggestion that anorexia arises primarily from its action there.

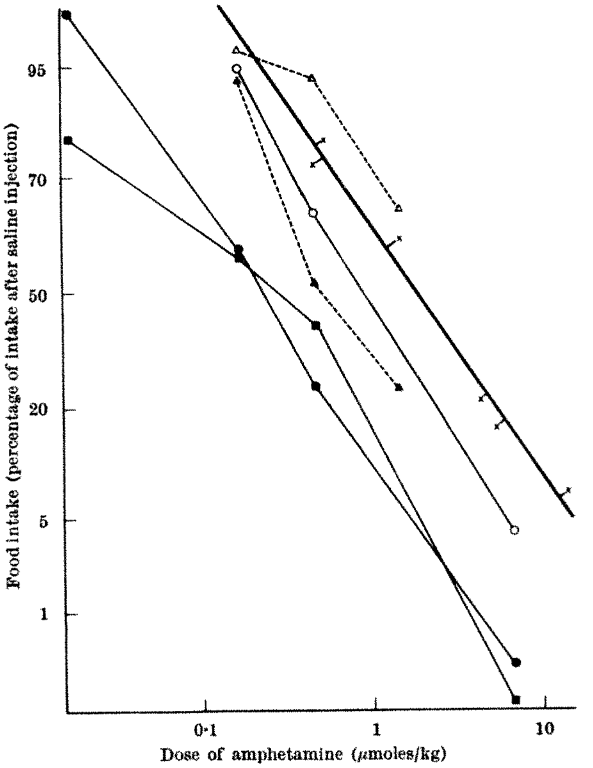

Fig. 1. Dose–response relationships for depression of food intake over 90 min after 6 h of food deprivation and injection of amphetamine by various routes. ×, Intraperitoneal injection ($N = 40$); solid symbols: "eaters" to noradrenaline; open symbols: "non-eaters"; ■, preoptic cannulae (2); ●, anterior hypothalamic cannulae (2); ○, posterior hypothalamus or diagonal band; ▲, △, anterior hypothalamic cannulae with surrounding lesion.

It has been stated that crystalline amphetamine placed in the lateral hypothalamus does not affect food intake[3]. Unilateral injection of 0·6 μl. of 65 mM *d*-amphetamine into the anterior lateral hypothalamic area does not elicit feeding in satiated rats[4]. Our data show, however, that both bilateral (Fig. 1) and unilateral (Table 1) injections of solutions of amphetamine into the lateral hypothalamic area depress food intake in deprived rats.

Various concentrations of *d*-amphetamine hydrochloride, made isotonic with sodium chloride, were injected in a volume of 1 μl. into the left side of the hypothalamus of 300–400 g Sprague–Dawley albino rats after 6 h of food deprivation. Each concentration was given twice. Injections followed a counterbalanced sequence and were at least 2 days apart. The injection needle was guided to the site by a stereotactically implanted cannula (outer diameter 0·4 mm)[5] the location of which was then histologically checked. At the time of implantation, an electrolytic lesion (1 mA, 30 s) had been placed in the classical hypothalamic feeding area (lateral part of the lateral hypothalamic area at the level of the dorsomedial nucleus) on the right-hand side of six of the rats. Access

Table 1. CHANGE IN FOOD INTAKE AFTER INTRAHYPOTHALAMIC INJECTION BEFORE ACCESS TO FOOD

Food deprivation (h)	Mean change from 1 h intake without injection					
	Saline		*dl*-Noradrenaline		*d*-Amphetamine	
	g	*P*	g	*P*	g	*P*
0	−0·02	n.s.	2·28	0·001	−0·31	n.s.
6	−0·10	n.s.	0·86	0·01	−1·24	0·05
22	−0·49	n.s.	−1·73	0·05	−2·32	0·01

to laboratory chow pellets was given immediately after injection. Food intake during the next 90 min is plotted in Fig. 1 as a percentage of the intake after intrahypothalamic saline injection.

Two pairs of rats showed very similar dose–response relationships: one pair had cannulae in the anterior preoptic area, above the olfactory tubercle (solid squares); the cannulae of the other pair ended at the root of the stria medullaris in the anterior lateral hypothalamic area. The dose–response lines are approximately parallel to that obtained for amphetamine hydrochloride or sulphate by the intraperitoneal route in forty rats from the same batch (crosses, Fig. 1), but are about ten times lower: the difference in depression of intake between the hypothalamic and the peritoneal routes was statistically reliable ($P < 0{\cdot}01$) at the dose of 0·47 μmoles/kg. Each of these four rats ate over 2 g more chow within 30 min after injection of 0·6 μl. of 65 mM noradrenaline than after the control injection. The other two of this group of six rats did not eat after noradrenaline: one cannula was in the posterior hypothalamus, the other was at the base of the brain in the diagonal band of Broca. The dose–response curve was reliably higher for this pair of "non-eaters" than for the two pairs of "eaters" after noradrenaline ($P < 0{\cdot}05$ at 0·47 μmoles/kg) and was not significantly different from the intraperitoneal dose–response.

Two more rats had a lesion placed in the left hand anterior lateral hypothalamic area and a cannula implanted with its tip at the same site. One rat ate more after noradrenaline than after saline and had more of the substantia innominata spared by the lesion than did the other, which did not eat more after noradrenaline. Each of three doses of amphetamine was injected through each cannula on three occasions after 6 h of food deprivation just before access to food. The dose–response curve for the "eater" (solid triangles, Fig. 1) was lower than that of the "non-eater" (open triangles; $P < 0{\cdot}05$).

The consistency of the correlation between the effectiveness of a cannula in guiding noradrenaline to elicit eating and as a route to the site through which amphetamine can elicit anorexia is direct evidence in support of a proposal that amphetamine depresses appetite by chemical interaction with the hypothalamic feeding system[1,6] and in particular the adrenergic portion of that system which is anterior to the classical feeding "centre"[5]. Amphetamine seems to have several different central actions[7], and although it has yet to be shown to have central adrenolytic effects such an action would explain disruption of the adrenergic feeding system. If it can be shown that amphetamine causes anorexia by postsynaptic blockade in the rat, there would then be reason to believe that the feeding system was adrenergic in all species showing amphetamine anorexia. This would be despite the failure of noradrenaline to elicit eating in species other than the rat[8], which may be attributable to interfering effects of intrahypothalamic noradrenaline such as those evident in the rat only after 24 h of starvation (Table 1).

Table 1 summarizes the effects of unilateral injections (1 μl.) of 155 mM sodium chloride with sodium replaced by amine to 65 mM in the case of noradrenaline and amphetamine. These fourteen rats had cannulae with tips in the anterior lateral hypothalamic area and no lesions. Because the effect of noradrenaline on feeding in satiated rats is diminished in rats deprived for 6 h and reversed in rats deprived for 24 h, it is conceivable that amphetamine causes anorexia by the release of endogenous noradrenaline it is known to cause centrally[7], the release being sufficiently great or rapid to disrupt the adrenergic feeding system or another connected system in the same brain region.

Obviously, proof that the anterior diencephalon contains the most sensitive site for amphetamine depression of feeding needs a survey of all the brain. Even then, the strictest criterion of localization of function requires double dissociation between the site of elicitation of anorexia and the site of some other action of amphetamine such as stereotypic motor movement by way of the caudate nucleus[9], temporal discrimination deficits[10] or centrally elicited autonomic or electroencephalogram reactions. So far the adrenergic feeding system in the rat has been localized only by single dissociation[5,11]. If the biochemical pharmacology of amphetamine anorexia differs from the release of dopamine in the caudate which mediates stereotypy, then even "triple dissociation" might be possible.

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[1] Carlisle, H. J., *J. Comp. Physiol. Psychol.*, **58**, 47 (1964).

[2] Miller, N. E., *Fed. Proc.*, **19**, 846 (1960).

[3] Stark, P., and Totty, C. W., *J. Pharm. Exp. Ther.*, **158**, 272 (1967).

[4] Booth, D. A., *J. Pharm. Exp. Ther.* (in the press).

[5] Booth, D. A., *Science*, **158**, 515 (1967).

[6] Reynolds, R. W., *J. Comp. Physiol. Psychol.*, **52**, 682 (1959); Brobeck, J. R., Larsson, S., and Reyes, E. A., *J. Physiol.*, **132**, 358 (1956).

[7] Glowinski, J., and Baldessarini, R. J., *Pharmacol. Rev.*, **18**, 1201 (1966).

[8] Myers, R. D., in *Thirst* (edit. by Wayner, M. J.), 533 (Pergamon, New York, 1964).

[9] Randrup, A., and Scheel-Krüger, J., *J. Pharm. Pharmacol.*, **18**, 752 (1966); Smelik, P. C., and Ernst, A. M., *Life Sci.*, **5**, 1485 (1966).

[10] Weiss, B., and Laties, V. G., *Fed. Proc.*, **26**, 1146 (1967).

[11] Grossman, S. P., *Amer. J. Physiol.*, **202**, 872 (1962); Wagner, J. W., and de Groot, J., *Amer. J. Physiol.*, **204**, 483 (1963).

BIOLOGY

Halogen Derivatives of 4-Hydroxybenzoic Acid as Root Growth Stimulants and the Importance of Light in this Response

The discovery by one of us[1] of the selective herbicidal activity of 3,5-diiodo-4-hydroxybenzonitrile (ioxynil) and its dibromo- analogue (bromoxynil) has led here to a number of other developments. Thus the high activity of these compounds in uncoupling oxidative phosphorylation[2] resulted in the finding that they are potent molluscicides[1]. Again, the demonstration that iodide ion is liberated when ioxynil is metabolized in soils and plants led to the discovery that inorganic iodides are useful and highly selective herbicides[3]. A further development from the ioxynil work was the examination of certain 2,6-disubstituted phenols as plant growth regulators[4]. Some of these were found to be highly active and equal to 2,4-dichlorophenoxyacetic acid (2,4-D) in certain tests[5]. We now report another development from our research on ioxynil.

In a study of related compounds, the product of hydrolysis of ioxynil, 3,5-diiodo-4-hydroxybenzoic acid (DIHB) was prepared. This acid, although not appreciably phytotoxic when applied to most plant species, was, however, very damaging to certain legumes. In particular, dwarf bean plants sprayed with a 500 p.p.m. solution of the sodium salt of the acid, or watered with this solution, were eventually killed. We have attributed this toxicity to the effect of the iodide ion which was found to be liberated during metabolism. The translocation of DIHB was then examined. In these experiments, in which seedlings were grown with their roots immersed in dilute solution of the compound, there was a marked stimulation

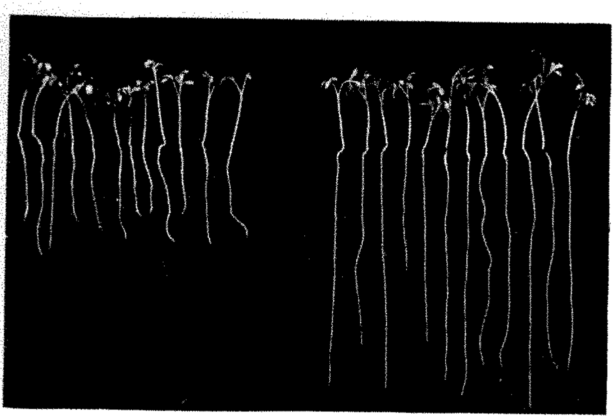

Fig. 1. Stimulation of root growth of cress seedlings (*Lepidium sativum* L.) by treatment with 10^{-5} M 3,5-diiodo-4-hydroxybenzoic acid. Control seedlings are on the left.

of root growth[6,7]. The effect was shown to be particularly striking with seedlings of cress (*Lepidium sativum* L.) where treated roots up to three times as long as control roots were obtained (Fig. 1). Similar stimulation of root growth was demonstrated with the seedlings of other plant species including rice (*Oryza sativa*) (variety 'Jao Leuang II'), although this compound has been reported to be inactive in tests with cucumber seedlings[8]. The nature of the stimulatory response was further investigated by estimating the number of cells in treated and control rice roots and also the mean length of samples of these cells. No evidence was obtained that treatment with DIHB produced an increase in cell number (Table 1); the mean length of treated cells, however, was about twice that of control cells.

Increases in root length resulting from chemical treatment have previously been reported. Thus it has been shown[8] that 3,5-dibromosalicylic acid and 3-nitro-4-fluorobenzoic acid are active in producing increased root elongation in cucumber seedlings. These compounds were therefore re-examined, together with the three 3,5-dihalogeno-4-hydroxybenzoic acids, in a test using cress seedlings carried out as follows.

Cress seeds were sown on 'Terylene' net floating on a solution of inorganic salts containing the test compound, and grown in controlled environmental conditions with 14 h of white light per day. After 64 h the mean length of treated roots was compared with that of roots grown on nutrients alone. The results are expressed as (length of 30 treated roots)/(length of 30 control roots) × 100 and are presented in Table 2.

It will be seen from Table 2 that all three dihalogen substituted 4-hydroxybenzoic acids are active in stimulating cress seedling root growth, the order of activity being iodine > bromine > chlorine. Furthermore, the salicylic and nitro-fluorobenzoic acids are both less active than the diiodo and the dibromo-hydroxybenzoic acid in this test. 3,5-Diiodotyrosine, which has been reported to stimulate root growth in rice seedlings[9], was also included in our experiments; this was found to be less effective than the three 4-hydroxybenzoic acids (Table 2).

Table 1. EFFECT ON RICE ROOTS OF TREATMENT WITH 5×10^{-6} M DIHB FOR VARIOUS TIMES

	Period of treatment (days)				
	3	4	5	6	7
Root length	158	214	200	189	202
Cell count	99·4	92·3	85·3	104·0	82·3*

Results are expressed as a percentage of control roots.
* Significant at the 5 per cent probability level.

Table 2. ROOT GROWTH STIMULATION INDUCED BY CHEMICAL TREATMENT

	Concentration (M)				
Compound	5×10^{-7}	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}
3,5-Diiodo-4-hydroxybenzoic acid (DIHB)	143*	175†	285‡	331‡	217‡
3,5-Dibromo-4-hydroxybenzoic acid	110	120	210‡	234‡	261‡
3,5-Dichloro-4-hydroxybenzoic acid	102	120	160*	176†	218‡
3,5-Dibromosalicylic acid	128	144*	131	108	71
3-Nitro-4-fluorobenzoic acid	93	121	129	185†	222‡
3,5-Diiodotyrosine	119	125	165*	152*	121

Results significant at: * 5 per cent probability level; † 1 per cent probability level; ‡ 0·1 per cent probability level.

Table 3. EFFECT OF DIHB TREATMENT (10^{-5} M THROUGH THE ROOTS) ON THE GROWTH OF THE ROOTS OF CRESS SEEDLINGS IN THE LIGHT AND IN THE DARK

	Mean root length (mm)					
	Light			Dark		
Experiment No.	Treated	Control	Per cent of control	Treated	Control	Per cent of control
I	32·8 34·7	13·6 13·8	247*	29·2 32·5	31·3 27·9	104
II	32·8 34·3	9·9 10·1	332*	31·3 31·8	28·9 28·4	110
III	31·1	8·8	353*	30·8 36·1	26·6 30·6	116

* Significantly different from control at 0·1 per cent probability level.

Further work with DIHB established that the promotion of root elongation did not occur when cress seedlings were grown in soil or sand. Because in this case the roots were in darkness it was decided to investigate the responses of cress roots to DIHB treatment in conditions where both roots and shoots were either in light or dark. The seeds were germinated in darkness for 16 h and then maintained either in total darkness or exposed to continuous white light (200–300 l/ft.^{2}) for the remainder of the growth period. The lengths of the roots were then recorded. The results of three experiments showing the growth of seedlings in nutrient solution alone and nutrient containing DIHB (10^{-5} M) are given in Table 3.

From these results it is clear that light inhibits the growth of control roots, and that this inhibition is removed by treatment with DIHB. The results also explain why the response is not shown with seedlings growing in sand or soil where the roots are not exposed to light.

The metabolic fate of DIHB in the roots of cress and rice seedlings has been investigated using the usual chromatographic techniques. It was established with cress that after growth for 64 h in a solution containing DIHB (10^{-5} M) none of the compound could be detected in the roots whether these had been exposed to light or not. A new substance (R_F value of 0·45 on paper chromatograms developed in 2 per cent acetic acid) was, however, shown to be present. This compound, which was also found in the experiments with rice seedlings, yielded DIHB and glucose on both acid and alkaline hydrolyses and showed other properties which indicated that it was a glucose ester.

It is clear from this work that 3,5-dichloro, dibromo and diiodo-4-hydroxybenzoic acids are all capable of modifying the normal responses of roots to environmental conditions. One of these has been shown to be light. We have evidence, however, that other factors operate in this system and these aspects of the work will be described later.

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[1] Wain, R. L., *Nature*, **200**, 28 (1963).

[2] Kerr, M. W., and Wain, R. L., *Ann. Appl. Biol.*, **54**, 441 (1964).

[3] Wain, R. L., Balayannis, P. G., Taylor, H. F., and Zaki, M. A., *Nature*, **209**, 98 (1966).

[4] Wain, R. L., and Taylor, H. F., *Nature*, **207**, 167 (1965).

[5] Wain, R. L., and Harper, D. B., *Nature*, **213**, 1115 (1967).

[6] Balayannis, P. G., thesis, Univ. London (1965).

[7] Zaki, M. A., thesis, Univ. London (1965).

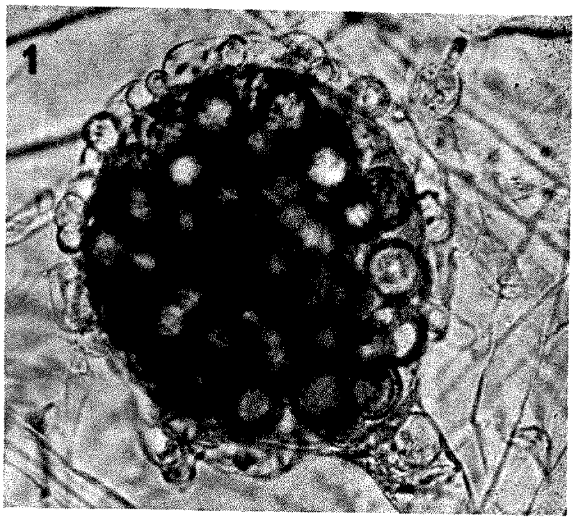
[8] Minarik, C. E., Ready, D., Norman, A. G., Thompson, H. E., and Owings, J. F., *Bot. Gaz.*, **133**, 135 (1951).

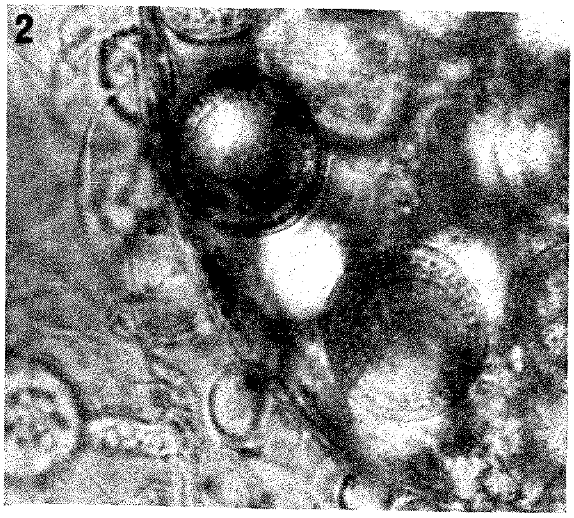
[9] Mukai, N., and Kinoshita, S., *Plant and Cell Physiol.*, **6**, 161 (1965).

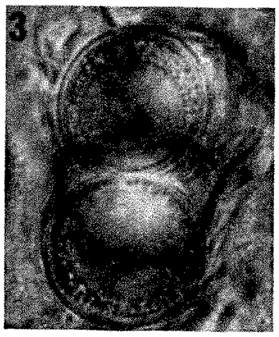
Atlantic Salmon Disease Fungus

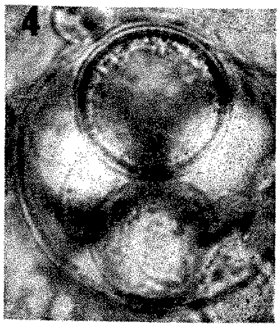
INITIAL reports of the salmon disease from Ireland suggested that the problem was purely bacteriological. This was despite the fact that the presence of a fungus, *Saprolegnia*, constituted the only clear outward symptom by which the disease was recognized. Stuart and Fuller have discussed the possible role of the fungus more realistically and have given details of their mycological researches[1]. With the advent of the disease to the English Lake District, especially in the autumn of 1967, I have also had the opportunity to make observations, and these to some extent parallel those of Stuart and Fuller. Further details of the fungus have, however, become known. *Saprolegnia* isolates were made from diseased salmon, trout and char (*Salvelinus willughbii*) netted in Windermere, and from further specimens of the latter which succumbed in hatchery ponds immediately adjacent to our laboratory. The water supply for the hatchery ponds derives directly from Windermere and is untreated. Using GYS-tellurite agar, a medium previously employed for ecological studies on the Saprolegniaceae[2], the isolates were soon free of bacteria and sterilized hemp seeds were added. These were removed after 8 h and transferred to dishes of sterile lake water where they grew abundant fringes of the fungus. These growth fringes produced sporangia releasing zoospores, but not oogonia. In the absence of these any specific epithet which may be applied to the purely asexual condition (for example, *Saprolegnia parasitica*) can have little or no meaning or usefulness. Accordingly attempts at oogonial induction were set in hand, using oatmeal and cholesterol[3] agars of different strengths with single or paired isolates. Isolates were also paired in simulated natural conditions in contact with sterile mud or soil, following the method of Sherwood[4] for *Dictyuchus*. All these attempts were unsuccessful.

An unexpected by-product of these otherwise abortive researches was the observation that the isolates produced sporangia directly on dry cholesterol agar (Fig. 5); thus this particular medium may prove to be of value for isolating and recognizing fish saprolegnias from mixed populations of fungi in nature. The hemp seed cultures in water were kept for several weeks, with repeated removal and replacement of the dish lids in order to make low power observations. Aerial fungal contaminants (especially of *Penicillium*), sustained by liquid nutrients in the dishes, became established on the surface meniscus in some cases. These contaminated dishes were not discarded because experience has shown that aquatic micro-organisms may give rare fruiting effects in such conditions. Recently, for example, an aquatic actinomycete of the family Actinoplanaceae formed sporangia in a liquid culture contaminated by a *Penicillium*, while uncontaminated cultures were quite devoid of them. This time the fruiting effect has involved the production of sexual structures. Of nine separate isolates under examination four formed oogonia, and these were all in contaminated cultures. For each of the four productive isolates there was at least one parallel uncontaminated culture and this material was all asexual only. The possibility of placing oogonial induction observations such as these on a more strictly scientific basis is encouraged by the work of Park[5]. He has shown that fungus interaction phenomena can be reproducible and even follow logical patterns of prediction. Despite its occurrence oogonial material has been very meagre, averaging only about five mature structures per dish, and a detailed description must be very tentative at this stage. Antheridia are probably diclinous, investing the oogonia so closely that their entire surface is often covered (Fig. 1). Difficulties of interpretation are increased by the frequent germination of zoospores on or near these structures (for example, Fig. 2). Oogonia usually terminate main hyphae and are spherical to clavate, the former 30–120μ in









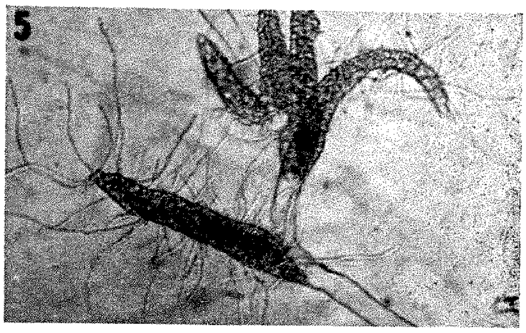


Fig. 1. Large oogonium with typically crowded antheridial attachment (× c. 475).

Fig. 2. Portion of same at higher magnification showing oospore structure. Germinated zoospore low left (× c. 1,135).

Figs. 3 and 4. Small complete oogonia (× c. 1,135).

Fig. 5. Growth on cholesterol agar showing fully formed sporangia. Zoospores encysted in them are sprouting (× c. 95).

diameter The wall is smooth and unpitted Oospores are subcentric 15–25μ in diameter, most being 18μ, and between four and a hundred or more per oogonium (Figs 2–4) Chlamydospores were rarely observed in these particular hemp seed cultures although they had been formed abundantly when paired isolates were grown on sterile mud An outstanding feature is the small size of the oospore These details suggest that the salmon disease *Saprolegnia* cannot be placed immediately in any existing species but may prove to be more closely related to the *S delica–S diclina* complex than to the *S ferax–S monoica* The fungus which Huxley[6] reported and figured from the previous British salmon disease outbreak in 1877 is believed by Coker[7] to have been *Saprolegnia monoica*, although by modern standards the description is quite inadequate The fact that he apparently obtained oogonia so readily, contrary to the experience of other contemporary investigators, casts some doubt on the whole account

Work by many investigators on the Saprolegniaceae has drawn attention to "non-fruiting" (that is, non-oogonial) forms in several genera While in the case of *Saprolegnia* itself these have traditionally been associated with isolations from fish, there is evidence that they constitute a definite proportion of the general saprobic microflora also On Fordwood Beck, a small afferent stream of Blelham Tarn (English Lake District), they were recovered in quantity from a sewage effluent derived from an aerobic percolating filter system[2] Until such forms can be adequately characterized and their distribution patterns examined more closely their full biological significance in the freshwater environment will not be known

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[1] Stuart, M R , and Fuller, H T , *Nature*, 217, 90 (1968).

[2] Willoughby, L G , and Collins, V G , *Nova Hedwigia*, 12, 149 (1966)

[3] Waterhouse, G M , *Mycol Papers*, 109 (1967)

[4] Sherwood, W A , *Mycologia*, 58, 215 (1966)

[5] Park, D , *Trans Brit Mycol Soc* , 46, 541 (1963)

[6] Huxley, T H , *Quart J Microsc Sci* , 22, 311 (1882)

[7] Coker, W C , *The Saprolegniaceae* (University of North Carolina Press, 1923)

Stimulation of Transpiration by Cytokinins

CYTOKININS[1] have received considerable attention from plant biologists because they play key parts in systems that regulate growth[2-4] These compounds influence physiological processes such as the induction of cell division, retardation of senescence, promotion of seed germination and promotion of bud developments[5,6] We have already observed that cytokinins initiate another physiological process—transpiration This observation is similar but not identical to a report[7] concerning the stimulation of transpiration of primary barley leaves

Our results indicate that cytokinin stimulates transpiration and induces cell division by a similar, if not the same, initiator system This communication describes similarities between stimulated transpiration and induced cell division and reports the reaction of a number of plant species to the former

The technique used to assess the activity of various compounds has already been described[8], but a brief description of the method is given here Basal ends of leaves from oat plants (*Avena sativa* L) were trimmed to 30 cm and severed ends were placed in an aqueous solution of the compound to be tested Leaves were maintained in the solution for 24 h under continuous fluorescent light (400 ft -candles), and transferred to 15 ml calibrated centrifuge tubes (two leaves in a tube) containing distilled water Treated leaves were placed under continuous fluorescent light for 24 h, and the amount of water lost from the tube was recorded The difference between the water loss from tubes containing treated and untreated leaves was a measure of the quantity of cytokinin in the test solution

Effects of cytokinins on transpiration (Table 1) show that zeatin (supplied by Dr C O Miller of Indiana University) is more active than kinetin and kinetin is more active than N^6-phenylaminopurine Furthermore, the order of sensitivity of the carrot phloem test[9] is similar to the transpiration test—both are more sensitive to zeatin than to kinetin In the cell division[6] and transpiration tests, N^6-hexylaminopurine is active but N^6-dimethylaminopurine is not Furthermore, our dosage response curves for N^6-(2-butoxyethyl) aminopurine in the transpiration test were similar to those obtained by Rothwell and Wright[10] in the tobacco pith test Results indicate a similarity between cytokinin-stimulated transpiration and induced cell division

Several compounds were tested to identify those that stimulate transpiration Certain substituted aminopurines (zeatin, kinetin, N^6-benzylaminopurine, N^6-phenylaminopurine, N^6-hexylaminopurine) increased transpiration, but others (N^6-methylaminopurine, N^6-dimethylaminopurine, 6-mercaptopurine) did not Recently we learned of some new synthetic compounds that possessed cytokinin activity (private communication from Professor R. L Wain, Wye College, who supplied compounds) These cytokinins were of interest because the N^6-substituted groups are straight chains (alkoxyalkyl) Two of these compounds (N^6-2-ethoxyethylaminopurine, N^6-2-butoxyethylaminopurine) were tested, and found to be active in the transpiration test Activity of the butoxyethyl derivative was twenty-fold greater than that of the ethoxyethyl derivative (Table 1)

Table 1 RANGE OF DETECTABLE CONCENTRATIONS OF CYTOKININS USING THE OAT LEAF TRANSPIRATION TEST

Cytokinin	Range (μg/ml)
Zeatin	0 001– 1 00
Kinetin	0 005– 8 00
N^6-Benzylaminopurine	0 005– 8 00
N^6-Phenylaminopurine	>0 01 –10 00
N^6-(2-Butoxyethyl) aminopurine	>0 05 – 4 00
N^6-Hexylaminopurine	0 10 –10 00
N^6-(2-Ethoxyethyl) aminopurine	1 00 – 2 00

Structural requirements for stimulated transpiration were similar to those required for induction of cell division In all cases, a substituted purine skeleton was essential The substituent group may be a saturated or unsaturated ring or a straight chain The length of the carbon chain seemed to influence activity This suggestion was made because N^6-dimethylaminopurine was not active, in the transpiration tests, but N^6-(2-ethoxyethyl) aminopurine was slightly active and N^6-(2-butoxyethyl) aminopurine was very active It appears therefore that straight chain substituents must have at least four carbon atoms.

Indoleacetic acid, 2 4-dichlorophenoxyacetic acid, sym-diphenyl urea and sym-diphenylthio urea as well as adenine, benzylamine and phenylamine did not increase transpiration Furthermore, gibberellic acid did not stimulate transpiration of excised oat leaves This was surprising because a previous report[7] indicated that gibberellic acid stimulated larger increases in transpiration of excised barley leaves than kinetin We observed no qualitative difference in stimulated transpiration within the family Gramineae If gibberellic acid does induce transpiration of primary barley leaves, *Avena* species possess a higher degree of specificity for cytokinins than *Hordeum* species

Although it is difficult to visualize a relation between cell division and transpiration, results suggest that this is the case This relation seems more feasible in view of a recent report by Meidner[11], who observed a rapid increase in the metabolisms of barley leaves treated with kinetins Although an exception (lack of kinetin–indole acetic acid synergism in the transpiration test) to the positive correlation between stimulated transpiration and cell division was observed, numerous similarities warrant attention to this phenomenon It is therefore suggested that increased transpiration, of species of Gramineae, be considered as one of the biological activities for cytokinins

Variation in cytokinin-stimulated transpiration was noted among various species of grasses[8], and so several dicotyledonous species were tested Varieties of *Vigna sinensis* (Torner) Savi (blackeyed peas), *Phaseolus lunatus* L (speckled butter beans), *P vulgaris* L. (pinto and kidney beans), *Glycine max* (L) Merr (soybean), *Helianthus annus* L (sunflower), and *Cucurbita pepo* L (sugar pumpkin) were grown in a growth room and treated as described, except that excised plants were used instead of excised leaves None of these exhibited increased transpiration when treated with various concentrations of kinetin

Excised leaves of *Acer saccharinum* L (silver maple), and *Liquidambar styraciflua* L (sweet gum), collected from field-grown plants, did not respond to kinetin treatment Excised leaves of field-grown *Lolium multiflorum* Lam (ryegrass), however, showed an increase in transpiration when treated with kinetin

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[1] Skoog, F , Strong, F M , and Miller, C O , *Science*, 148, 532 (1965)
[2] Galston, A W , *Amer Sci* , 55, 109 (1967)
[3] Letham, D S , *Ann Rev Plant Physiol* , 18, 354 (1967)
[4] Plant Growth Regulators, *N Y Acad Sci* , 144, Art 1, 1 (1967)
[5] Bruce, M I , Zwar, J A , and Keffard, N P *Life Sci* , 4, 461 (1965)
[6] Miller, C O , *Ann Rev Plant Physiol* , 12 395 (1961)
[7] Livne, A , and Vaadia, Y , *Physiol Plantarum*, 18, 658 (1965)
[8] Luke, H H , and Freeman, T E , *Nature*, 215, 874 (1967)
[9] Letham, D S , Shannon, J S , and McDonald, I R , *Proc London Chem Soc* , 230 (1964)
[10] Rothwell, K , and Wright, S T C , *Proc Roy Soc* , B, 167, 202 (1967).
[11] Meidner, H , *J Exp Bot* , 18, 556 (1967)

Historical Zoogeography of Antelopes

STUDIES of the Bovidae of the later Miocene of Fort Ternan, Kenya[1], and of the lower Pliocene of Samos are producing interesting zoogeographical findings In this communication, names above the species level are from Simpson[2]

Two groups among the Samos antelopes have special relevance, those of the *Palaeoryx* group, and various spirally horned antelopes which have been associated with *Prostrepsiceros* in the tribe Tragelaphini (Strepsicerotini of Simpson[2])

Antelope "species" in *Palaeoryx*, *Protoryx* and *Pachytragus*, and in the smaller *Microtragus*, *Leptotragus* and *Pseudotragus*, have been regarded as Hippotragini Some show clearly, others less clearly, however, resemblances to Caprini the short braincase (all except *Protoryx longiceps* Pilgrim and Hopwood), pronounced bending of the braincase on the facial axis in side view; an anterior surface (*Microtragus*) or an anterior keel on the horn cores (*Pachytragus crassicornis* Schlosser), rather abrupt bending back of the horn core tips (*Protoryx longiceps*); the lower edge of the auditory bulla passing downwards posteriorly to meet the paraoccipital process (*Palaeoryx pallasi* (Wagner) and *Protoryx longiceps*—the only two in which this character is visible), a long narrow ethmoidal fissure alongside the nasals (*Pachytragus crassicornis*, *Protoryx longiceps*), diminished basal pillars on the molar teeth (less in *Palaeoryx pallasi* than others), emphasized styles flanking weak ribs on the upper molars (*Pachytragus crassicornis*, *Protoryx longiceps*), short premolar rows (not *Palaeoryx pallasi*) and small anterior premolars Some characters of later Caprini are missing—for example, widely set anterior tuberosities of the basioccipital, moreover, no Bovid metapodials at Samos are as short as in modern goats, which seems to indicate occupation of different adaptive zones I would not assert that none of the *Palaeoryx* group could possibly be related to Hippotragini, but the evidence strongly favours their assignation to the Caprini, and they have no characters unequivocally resembling Hippotragini

Many names exist for Pliocene and Pleistocene spirally horned antelopes with anticlockwise torsion in the right horn core from the base upwards The three Samos species may be called *Protragelaphus skouzesi* Dames, *Prostrepsiceros woodwardi* Pilgrim and Hopwood, and *Palaeoreas lindermayeri* (Wagner) Pilgrim[3] and Pilgrim and Schaub[4] transferred *Protragelaphus* from Tragelaphini to Antilopini, and I now suggest the same course for *Prostrepsiceros* and *Palaeoreas* *Prostrepsiceros*, in which I include *Hemistrepsiceros*, differs from Tragelaphini by having its parieto-frontals suture not disappearing early in ontogeny, its braincase more bent on the facial axis in side view, the orbital surface of the lachrymal bone not at such a wide angle to the plane of its facial surface, and by having a preorbital fossa *Palaeoreas* shows the last three differences and a deep lateral postcornual groove not found in Tragelaphini It also much resembles *Protragelaphus*, which has such non-Tragelaphine characters as a short basioccipital, an occipital surface with a curved instead of a squared upper edge, and small foramina ovales

Thus the Tragelaphini and Hippotragini are absent from Samos, the Bovids there being wholly Boselaphini, Antilopini, Ovibovini and Caprini Wells[5] concluded 10 years ago that the Cephalophini, Neotragini and Alcelaphini were "essentially African" tribes The Tragelaphini can now be added to this group, but the Hippotragini, although now becoming almost totally African, occur in Arabia as *Oryx leucoryx* (Pallas) and also with Reduncini and an Alcelaphine, in the later Pliocene and early Pleistocene of the Indian and Pakistan Siwaliks[6]

Revision of the Samos Bovidae is thus accentuating the division of the family between Eurasiatic and African forms The family contains Tragelaphini, Cephalophini, Reduncini, Alcelaphini and Neotragini which, except for the Siwaliks record of two of them, are wholly African; and Boselaphini, Saigini, Rupicaprini and Ovibovini which are absent from sub-Saharan Africa The remaining Bovini, Hippotragini, Antilopini and Caprini occur both in Eurasia and sub-Saharan Africa, but in this group Gentry[7] could not trace phylogenetic connexions between African and Eurasiatic Bovini Also the Antilopini can be divided into a Eurasiatic spirally horned group, and into a group of African genera, an exception to this being the widely distributed *Gazella* (including *Procapra*) The Caprini are entirely Eurasiatic and North American except for *Capra walie* Ruppell in Ethiopia

Some fossils relevant to these conclusions remain for future consideration, for example, Bohlin's[8] Chinese "Hippotragini" related to *Protoryx*, Schlosser's[9] small Samos species *Tragoreas oryxoides* and Pilgrim's[10] Chinji "Tragelaphine" *Sivoreas* and his Dhok Pathan supposed Reduncini and Hippotragini, the affinities of Schlosser's and Pilgrim's forms are unclear

It becomes of great interest to see whether Fort Ternan shows us an earlier phase in the history of the African Bovidae At least four antelopes occur there A species of the Boselaphine *Protragocerus* is distinct from *P chantrei* Depéret of the European Tortonian and Sarmatian, and from another species in the Chinji and earliest Nagri of Pakistan to which Pilgrim[11] applied several names including *Strepsiportax gluten* (see also Thenius[12]) Second, there is a new species of Caprini generically inseparable from Pilgrim's[13] *Oioceros*(?) *grangeri* from the Tung Gur Miocene, Mongolia Pavlović and Thenius[14] mention a similar form from Yugoslavia *Oioceros*(?) limb bones at Fort Ternan show more cursorial adaptations than *Protragocerus* These two species constitute about 90 per cent of the recognizable Bovid remains at Fort Ternan Also present is a species agreeing closely with a frontlet from the Nagri gen indet (cf *Tragoreas*) *potwaricus*[15] The fourth species is a *Gazella* with mediolaterally compressed horn cores, rather brachyodont cheek teeth and long premolars The relative smallness of the Fort Ternan antelopes, vestigial alveoli for upper canines in *Protragocerus*, the resemblance of the *Oioceros*(?) to the Tung Gur species, and the brachyodonty of the gazelle give them a more archaic aspect than those of Samos What is most interesting is their relationship at the generic and higher levels to Eurasiatic forms, but not, apparently, to the later antelopes characteristic of Africa

The presence of Boselaphini and Caprini in the late Miocene of equatorial East Africa upsets the strictly geographical part of the concept of Eurasiatic and African divisions of the Bovidae We must evidently allow for past fluctuations of faunal boundaries, and, following Wells[5], can refer to such divisions as "essentially Eurasiatic" and "essentially African" Only in the Tatrot and Pinjor of the Siwaliks is any mixing of the divisions known, apart from that along the wide boundaries of the Palaearctic and Ethiopian realms Unanswered questions include Where did the essentially African Bovidae evolve ? Why are they absent from Fort Ternan but not from the later Pliocene and early Pleistocene of the Siwaliks ? Could more mixing of the divisions be found from further excavations or from further studies of puzzling fossils ?

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[1] Leakey, L S B , *Ann Mag Nat Hist* 4 (13), 689 (1961)
[2] Simpson, G G , *Bull Amer Mus Nat Hist* , 85, 157 (1945)
[3] Pilgrim, G E , *Palaeont Indica* (*n s*), 26, No 1, 44, 130 (1939)
[4] Pilgrim, G E , and Schaub, S , *Abh Schweiz Palaont Ges* , 62, No 3, 20 (1939)
[5] Wells, L H , *S Afr J Sci* , 53, 423 (1957)
[6] Pilgrim, G E , *Palaeont Indica* (*n s*), 26, No 1, 63, 71, 93 (1939)
[7] Gentry, A W , *Bull Brit Mus Nat Hist* (*Geol*), 14, 296 (1967)
[8] Bohlin, B , *Palaeont Sin C* , 9, No 4, 126, 130, 133 (1935)
[9] Schlosser, M , *Beitr Palaont Geol Öst -Ung* , 17, 34 (1904)
[10] Pilgrim, G E , *Palaeont Indica* (*n s*), 26, No 1, 90, 119, 122, 125, 130 (1939)
[11] Pilgrim, G E , *Bull Amer Mus Nat Hist* , 72, 758 (1937)
[12] Thenius E , *Geologie*, 5, 315 (1956)
[13] Pilgrim, G E , *Amer Mus Novit*, No 716, 4 (1934)
[14] Pavlović, M , and Thenius, E , *Anz Öst Akad Wiss*, No 11, 3 (1959)
[15] Pilgrim, G E , *Palaeont Indica* (*n s*), 26, No 1, 86 (1939)

Saprolegnia parasitica Coker in Estuaries

IN a recent article Stuart and Fuller[1] question whether *Saprolegnia parasitica* Coker should be regarded as a freshwater fungus or a fungus capable of growing and sporing in both freshwater and estuarine environments I find that their review of data concerning the tolerance of Saprolegniaceae to saline environments is misleading because (*a*) they fail to point out the discrepancy in the literature between the influence of temperature on spore production in the freshwater and saline environments, and (*b*) they do not distinguish between vegetative growth with asexual sporulation and the mere presence of viable but unidentified propagules

Some Saprolegniaceae such as *Leptolegnia baltica* Hohnk and Vallin[2] appear to be adapted to life in slightly saline (4–6$^{0}/_{00}$) conditions Te Strake[3] states that the Saprolegniaceae which she isolated from estuarine water of salinity up to 2 8$^{0}/_{00}$ would tolerate greater salinities in vegetative culture at higher temperatures, but that asexual sporulation occurred in the higher salinities only at temperatures above 20° C At 15° C sporulation did not occur above a salinity level of 0 3$^{0}/_{00}$ The temperature data for asexual sporulation quoted by Stuart and Fuller refer to freshwater environments and may not, therefore, be relevant

It is unfortunate that in their pure culture experiments Stuart and Fuller did not use fungi freshly isolated from their fish or the estuaries where they were working, and that they failed to give the temperature at which their experiments were carried out (What is the maximum water temperature of, for example, the Shannon estuary ?)

Te Strake's work shows that viable propagules, which are not identified, are present in estuarine sites The presence of propagules in moving water does not necessarily indicate their site of production This applies to spores of Oomycetes just as much as to spores of Fungi Imperfecti[1,4] Until the site of production is established comments on "habitat nomenclature" have no foundation[5]

To demonstrate that *Saprolegnia parasitica* is not confined to the freshwater environment for vegetative growth and the production of zoospores, it is essential to answer the following questions (1) Does the fungus sporulate equally well in freshwater and estuarine water at temperatures and salinity values comparable with those in the natural environments under investigation ? (2) Do the zoospores remain motile for the same length of time in both environments ? (3) How long do the motile or encysted zoospores remain viable in freshwater and estuarine water ? (4) Will oospores germinate equally well in both environments ? (5) Is the colonization of the substrate (living or dead) comparable in both environments ? (6) What is the computed propagule density required to cause disease in particular hosts in a given environment (river, estuary or the open sea) ?

If this fungus should prove to be a primary parasite rather than a facultative wound parasite, is it not more important to establish the origin and size of the potential spore inoculum and the survival rate of the inoculum in saline conditions, rather than the vegetative growth of the fungus in saline media at unspecified temperatures ?

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[1] Stuart, M R , and Fuller, H T , *Nature*, 217, 90 (1968)
[2] Höhnk, W , and Vallin, S , *Veroff Inst Meeresforsch Bremerh* , 2, 215 (1963)
[3] Te Strake, D , *Phyton, Intern J Exp Bot* , 12, 147 (1959)
[4] Willoughbv, L G , *J Ecol* , 50, 733 (1962)
[5] Dick, M W , *Veroff Inst Meeresforsch Bremerh* , Sonderband 3 (in the press)

Chlamydomonas: Adaptation Phenomena in Phototaxis

THE oriented movement of unicellular algae, such as *Chlamydomonas*, is usually regarded as being either positive or negative, when evoked by light of a fixed intensity Until recently it has been difficult to measure phototactic responses of algae quantitatively and rapidly, but methods have now been described for following the phototactic response of a population of algae[1,2] These methods are based on a photoelectric monitoring of the distribution of the algae in a suspension, during exposure to light which evokes oriented response We have adopted the method of Feinleib and Curry[2] essentially unmodified to follow the initial response of cultures of *Chlamydomonas reinhardii* ($WT+2$) The experiments were undertaken to study the variability of the photo-

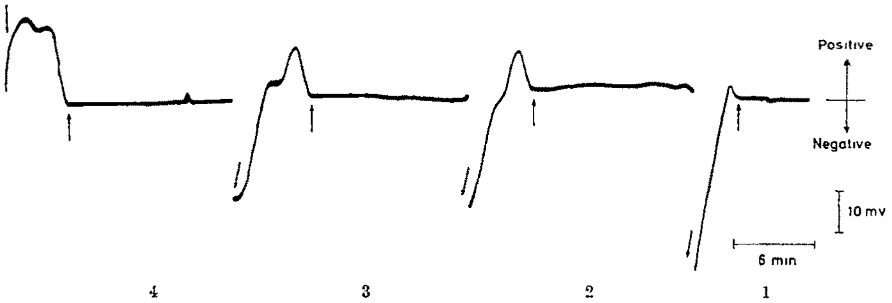

Fig 1 Phototactic response of a 2 day old culture of *Chlamydomonas reinhardii* (1) Initial response negative, (2) response after 3 min strong light followed by 12 min in the dark, (3) response after further 4 5 min in strong light followed by 12 min in dark, (4) response after additional 6 min in strong light and 12 min in dark Recorder speed was 10 in /h Orienting light was 540 ft -candles, white light, falling on blue green filter (Corning 9782) During the dark period the cells were exposed to weak red monitoring light ↑, Light on, ↓, light off, culture mixed

tactic response of cultures of *Chlamydomonas* which we previously observed[3,4] We will show that the same culture of *Chlamydomonas* can be negative or positive in its response to a given fixed intensity of orienting light The organism seems to adapt rapidly to conditions prevailing before measurement Cultures were grown as described before[3,4] in continuous light in an inorganic medium[2], with constant stirring by a magnetic stirrer The phototactic response of the cultures immediately after the orienting light was switched on was observed Cultures frequently did not respond in a clear cut manner, a slight positive response was often followed by a negative one. If cultures were exposed to light in the apparatus for increasing periods of the time and their phototactic responses were measured periodically, the initial positive response increased and the subsequent negative one decreased (Fig. 1) These results indicated that the cells were adapting to the light so that their phototactic response changed. The following experiment was therefore undertaken. A culture was selected the initial response of which was negative This culture was now illuminated for 2 h with 200 ft -candles light from an incandescent lamp and its response again measured. The culture which had shown a strong and immediate negative response now responded positively to the same light intensity The culture was now returned to darkness for 75 min and its response was measured again As Fig 2 shows, it now responded negatively

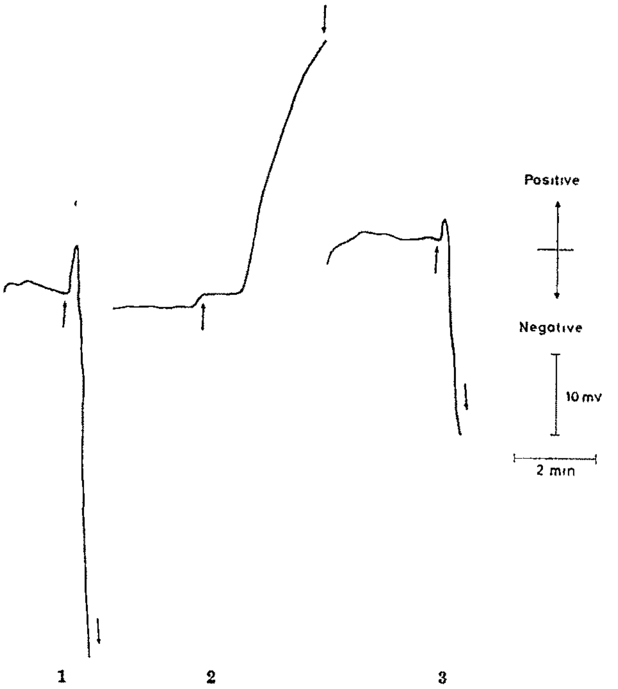

Fig 2 Phototactic response of 4 day old culture of *Chlamydomonas reinhardii* (1) Initial response, (2) response after exposure for 2 h to incandescent light (200 ft -candles), (3) response after additional period 75 min in dark The orienting light in all cases was monochromatic blue-green light (Corning filter No 9782) Intensity was 250 ft -candles, white light before filter Recorder speed was 30 in /h ↑, Light on, ↓, recorder off

These results clearly show that a culture of algae can rapidly change its phototactic response to a given fixed light intensity from negative to positive and vice versa It can be assumed that the algae adapt their phototactic behaviour in response to the light intensity to which they are exposed before measurement This adaptation is probably caused by metabolic changes which take place during the period of adaptation, and they are possibly connected with photosynthesis The cultures have a generation time of more than 12 h and were not synchronized, so division of the cells is not likely to be involved Microscopic observation gave no indication that the algae were dividing during the period of adaptation No changes in the *p*H of the medium could be observed during adaptation

These results indicate the extreme caution which must be exercised when referring to algal cultures as positive or negative in their response to light The nature of the response to a fixed intensity of orienting light can change rapidly, depending only on treatment immediately before measurement No absolute positive or negative responses seem to exist It seems to us that the diurnal upward and downward movement of algae in their natural environment, which has been ascribed to positive and negative phototaxis (ref 5), must also be re-examined

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[1] Lindes, D , Diehn, B , and Tollin, G , *Rev Sci Instrum* , **36**, 1721 (1963)
[2] Feinleib, M E H , and Curry, G M , *Physiol Plant* , **20**, 1683 (1967)
[3] Stahl, N , and Mayer, A M , *Science*, **141**, 1282 (1963)
[4] Chorin-Kirsch, I , and Mayer, A M , *Nature*, **203**, 1085 (1964)
[5] Soeder, C J , *Umschau in Wissenschaft und Technik*, **67**, 388 (1967)

Light Induced Uptake of Potassium and Chloride by *Chlorella pyrenoidosa*

RECENT studies of mechanisms of ion transport across the membranes of isolated mitochondria and chloroplasts have been designed to show whether the energy is derived directly from electron transport or from high energy chemical intermediates[1,2] A similar argument concerning the active transport of ions across the protoplasmic membranes of intact animal and plant cells[3] has also been considered for many years Perhaps the most convincing evidence that the active transport of an ionic

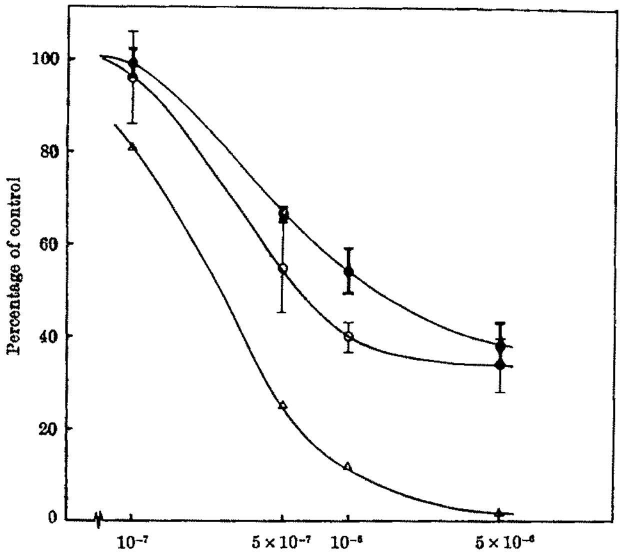

Fig 1 The effect of low concentrations of DCMU on the influx of potassium (●), chloride (○) and oxygen evolution (△) The results are presented as the percentage of the controls and the standard errors of the regression lines are given for the tracer experiments

species is intimately linked with electron transport has come from studies on the giant cells of *Nitella translucens*[4] and *Hydrodictyon africanum*[5] In these algae, it has been shown that the light stimulated influx of chloride, unlike the uptake of potassium, is independent of photophosphorylation and seems to be associated with the oxygen evolving system 2 of photosynthesis

This communication reports some work on *Chlorella pyrenoidosa* to test whether the light promoted uptake of potassium and chloride into these cells is driven by two different energy sources as suggested for the vacuolated coenocytes The unidirectional fluxes of these ions were measured using potassium-42 and chloride-36 and all the experiments were conducted in culture medium The internal radioactivity was assayed, after sedimenting the cells on 'Millipore' filters and washing, by means of an end-window Geiger tube

Table 1 LIGHT AND DARK INFLUXES* OF POTASSIUM AND CHLORIDE INTO *Chlorella pyrenoidosa*

Ion	Influxes from culture medium†			
	$\mu\mu$ moles/s 5 mm^3 packed cells		$\mu\mu$ moles/s cm^2	
	Dark	Light	Dark	Light
K	3 4 ± 0 1 (19)	19 1 ± 1 0 (31)	0 18 ± 0 01	1 03 ± 0 10
Cl	0 35 ± 0 01 (6)	2 32 ± 0 26 (9)	0 019 ± 0 006	0 12 ± 0 02

Results are quoted as ± standard error of the mean, number of experiments on which mean is based is given in parentheses The cells were illuminated by white light from an incandescent source at an intensity of about 500 ft -candles

* Unidirectional influxes in the absence of net ion movement

† The culture medium (*p*H 6 8) contained (mM) KNO_3 5 00, Na_2HPO_4 $2H_2O$ 0 50, KH_2PO_4 0 50, $MgSO_4$ $7H_2O$ 2 00, $Ca(NO_3)_2$ $4H_2O$ 0 25, KCl 1 00 and trace elements, 2 ml /l as given by Hutner *et al*[6]

Table 1 shows the mean values of the light and dark influxes and gives an estimate of each flux in terms of unit area of cell surface It can be seen that the action of light is to stimulate the influx of these ions by a factor of about six. Such a sensitivity to light does not necessarily suggest that an active pump is involved. It has been possible, however, to obtain a direct measurement of the potential difference between the interior and exterior of these cells when bathed in culture medium This procedure will be reported elsewhere, but the results strongly indicate that potassium and chloride are transported into *Chlorella* against the electrochemical potential gradient A mean potential difference (±*S E M*) of −40±2 mV was obtained from sixty-five cells while the Nernst equilibrium potentials, calculated from the distribution of these ions, were −71 mV for potassium and +8 mV for chloride Thus it would seem that, with respect to the direction of active transport of these two ions, *Chlorella* shows the same characteristics as *Nitella* and *Hydrodictyon*.

The sensitivity of these apparent active light stimulated influxes to 3 - (3,4 - dichlorophenyl) - 1,1 - dimethylurea (DCMU) can be seen in Fig. 1. Oxygen evolution, as measured with a platinum polarograph, was partially inhibited by concentrations of 10^{-7} to 10^{-6} M while complete inhibition occurred at 5×10^{-6} M. Over the same concentration range, the influxes of potassium and chloride, measured by double labelling, were reduced by equal amounts with about 40 per cent of the uptake of both ions persisting at concentrations of DCMU which totally inhibited oxygen evolution Such findings are quite different from those obtained with the two coenocytic algae, in which the influx of chloride was much more sensitive to this herbicide than was the potassium influx

The results suggest that both these photostimulated fluxes into *Chlorella* contain components which are independent of net electron flow and pseudocyclic flow. Such components could derive their energy from cyclic photophosphorylation in a manner similar to that suggested for glucose assimilation by this same alga[7]. Indeed, it was also found that when the cell suspensions were gassed with nitrogen free of carbon dioxide the influxes of the two ions were reduced to approximately 50 per cent of the aerobic controls To test whether these DCMU resistant and anaerobic components were partially or wholly dependent on cyclic electron flow, some experiments using the cytochrome *b* inhibitor, antimycin A, were carried out and the results shown in Fig 2 It can be seen that both these components were inhibited by this antibiotic, with the chloride influx showing the greater sensitivity This difference in the response of the two ions to increasing concentrations of the poison may simply reflect a larger passive component of the potassium influx as compared with the chloride uptake Nevertheless, it is clear that both ionic influxes show some requirement for the participation of cyclic electron flow

Further experiments using the uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) also showed the light stimulated aerobic uptake of potassium and chloride to be equally inhibited, with a maximum effect at concentrations of about 10^{-5} M. Moreover, when the relative quantal efficiencies of the influxes were measured for cells illuminated by 680 and 705 mμ light, the same

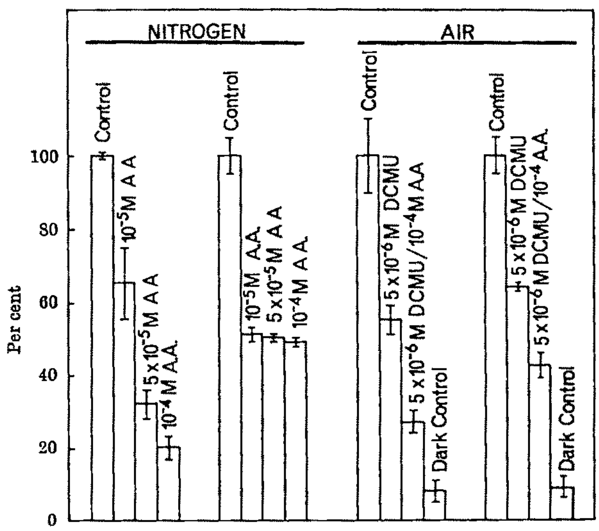

Fig 2 The effect of various concentrations of antimycin A (AA) on the anaerobic light stimulated uptake of potassium and chloride and also the effect of this antibiotic on the DCMU resistant influx components of these two ions The standard errors of the regression lines are given

values were obtained for both ions The measurement of oxygen evolution in the same lighting conditions, however, suggested that the two photosystems had not been separated This is in agreement with Hommersand[8], who encountered the same difficulty with this strain of *Chlorella* (stock number 252, Culture Collection of Algae, Indiana University) and who concluded that the overlap of the two pigment systems was better than usual

The only obvious difference between the influx of chloride and potassium into *Chlorella* found during this work was the light intensity at which the maximum rates were obtained The chloride uptake saturated at an intensity which was 25 per cent of that needed to saturate the rate of potassium uptake With an incandescent light source, the chloride uptake saturated at $1 5\times10^4$ ergs/cm^2 s, while both photosynthesis and the potassium influx saturated at $5 5\times10^4$ ergs/cm^2 s

In summary, it is most probable that the light stimulated uptake of potassium and uptake of chloride by *Chlorella* are active processes There is no clear evidence, however, that these two fluxes are dependent on different sources of energy It also seems unlikely that they obtain the necessary energy directly from electron transport but possibly utilize ATP derived from cyclic and non-cyclic photophosphorylation. The close association of active chloride influxes with oxygen evolution may be a feature only found with vacuolated cells, although some recent work on *Elodea densa*[9] does not support this

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[1] Chance, B , and Mela, L , *J Biol Chem* , **241**, 4588 (1966)
[2] Karlish, S J D , and Avron, M , *Nature*, **216**, 1107 (1967)
[3] Dainty, J , in *The Cellular Functions of Membrane Transport* (edit by Hoffman, J F) (Prentice-Hall, New Jersey, 1964)
[4] MacRobbie, E A C , *Biochim Biophys Acta*, **94**, 64 (1965)
[5] Raven, J A , *J Gen Physiol* , **50**, 1627 (1967)
[6] Hutner, S H , Provasdi, L , Schotg, A , and Haskin, C P , *Amer Phil Soc* , **94**, 152 (1950)
[7] Tanner, W , Dächsel, L , and Kandler, O , *Plant Physiol* , **40**, 1151 (1965)
[8] Hommersand, M H , in *Photosynthetic Mechanisms of Green Plants* (edit by Kok, B , and Jagendorf, A T) (Publication 1145 N A S –N R C , Washington, 1963)
[9] Jeschke, W D , *Planta*, **73**, 161 (1967)

Action of Potato Peel Extracts in modifying Tuber Dormancy

Differences in length of tuber dormancy which are related to mineral content have been found during work on the effect of the mineral nutrition of the potato plant on the performance of its progeny To relate these differences to tuber biochemistry, it was necessary to know the effect of tuber extracts on the dormancy of whole tubers Previous attempts to control dormancy by treating potato pieces with either tuber peel extracts containing the inhibitor-β complex[1] or with one of its components, abscisic acid, have failed[2], although the disappearance of this complex from the tuber has been correlated with the breaking of dormancy[3] It is also known that cutting a tuber causes a wound reaction which leads to the breaking of dormancy[4]. Furthermore, the practice of soaking tubers in concentrated solutions for up to 24 h produces anaerobic conditions which could have effects on dormancy as great as those of the extract being supplied

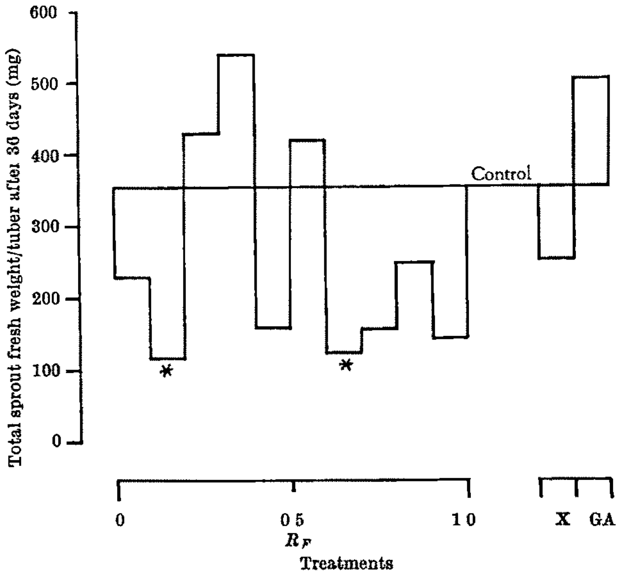

Fig 1 Sprout growth after 36 days, related to water control Treatments are R_F 0 1–1 0 of chromatograms of tuber peel extracts X, R_F 0 2, 0 4 and 0 7 combined, GA, 1 p p m gibberellic acid * Depression, significant at 5 per cent level, when compared with control

I felt that a more realistic technique would be to use whole tubers which were in contact with a very dilute solution for a long period but only partly immersed, so preventing anaeroby Accordingly, whole mature non-sprouting tubers each weighing 50–90 g were used as assay material in this experiment.

The peel from 25 kg of freshly collected mature tubers was extracted with 80 per cent methanol, filtered, and the filtrate was reduced under vacuum The resulting aqueous solution was acidified to *p*H 3 and partitioned with ethyl acetate The reduced ethyl acetate fraction was separated by descending paper chromatography using an isopropanol: ammonia water (10 1 1) solvent system Thirteen unreplicated treatments were used, comprising the 10 R_F strips from the chromatograms, a combination of strips of R_F 0 2, 0 4 and 0 7, gibberellic acid and a blank strip control The strips were placed in polythene bags and to each was added 100 ml of distilled water and nine potato tubers except for the treatment with gibberellic acid in which a 1 p p m solution of gibberellic acid was used instead This method of application is a modification of Goodwin's technique for breaking dormancy[5]

After 8 days in the dark at 13° C, many of the tubers had developed "soft-rot" (also noted by Goodwin), but the extent of this varied with the treatments (Table 1). There was no rotting of tubers treated with strip R_F 0 7 which is the R_F at which the inhibitor-β complex usually

Table 1 THE EFFECT OF TUBER EXTRACTS AND GIBBERELLIC ACID ON THE SPROUT GROWTH OF WHOLE TUBERS

	R_F												
Treatment	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9	1 0	Control water	X*	Gibberellic acid 1 p p m
No of tubers remaining healthy after 8 days	8	5	5	3	5	6	9	7	4	6	4	4	1
Total eye No on remaining tubers	63	58	36	30	46	49	68	75	29	58	33	33	10
Percentage eyes sprouted after 21 days	27	5	28	33	41	49	6	52	41	47	73	24	90
Total sprout length (mm) per tuber after 29 days	16	10	31	33	15	44	8	17	21	14	28	17	139
33 days	23	16	39	57	21	56	15	22	25	18	34	22	162
36 days	25	18	40	58	22	59	17	23	26	20	35	24	164
Total sprout dry weight (mg) per tuber after 36 days	30	16†	52	61	21	50	19†	23	32	22	43	34	64

* X, Mixture of R_F 0 2, 0 4 and 0 7 † Decrease significant at 5 per cent level, when compared with control

occurs in this system The rotten tubers were discarded and the healthy ones removed from the bags and placed in trays.

After 21 days sprouts had become visible and were recorded. Very few eyes were sprouting under treatments R_F 0·2 and 0 7, but almost half were sprouting under treatment R_F 0·6 and nearly three-quarters in the control R_F 0 2 and 0 6 represent the areas where a neutral inhibitor and gibberellic acid are usually found in this system

Records were made of total sprout length for each tuber after 29, 33 and 36 days, and total sprout weight for each tuber at 36 days All indicated a substantial reduction in sprout growth produced by treatments R_F 0 2, 0 5 and 0 7 when compared with the control Salicylic acid, a mild inhibitor, usually occurs at R_F 0 5 in this system There were increases in sprout lengths produced by R_F 0 3, 0 4 and 0 6 The mixture of R_F 0 2, 0 4 and 0 7 produced growth intermediate between the extremes given by treatments R_F 0 4 and 0 7, which suggests that the auxin usually found in R_F 0 4 (ref 6) will relieve the inhibition produced by the compounds at R_F 0 2 and 0 7.

These preliminary results suggest that the inhibitor-β complex does prolong dormancy, the evidence for which until now has been circumstantial A considerable effect was also produced by treatment R_F 0 2 This is the area of the chromatogram where neutral inhibitors noted by Varga and Ferenczy occur[6] Although these inhibitors do not disappear at dormancy break[7], this demonstration of what must be presumed to be a dormancy prolonging activity suggests an indirect role in the maintenance of dormancy which has still to be elucidated

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[1] Burton, W G , *The Growth of the Potato* (edit by Ivins, J D , and Milthorpe, F. L), 328 (Butterworths, London, 1963)

[2] El-Antably, H M M , Wareing, P F , and Hillman, J , *Planta*, 73, 74 (1967)

[3] [Hemberg, T , *Physiol Plant* ,2, 24 (1949)

[4] Burton, W G , *The Potato*, 242 (H Veenman and Zonen, N V , Netherlands, 1966)

[5] Goodwin, P B , *Europ Potato J* , 9, 53 (1966)

[6] Varga, M B , and Ferenczy, L , *Acta. Bot Acad Sci. Hung.*, III, 1-2, in (1957)

[7] Hemberg, T , *Physiol Plant* ,2, 615 (1958)

Vulval Bodies in Certain Species of *Heterodera*

DIFFERENCES in the vulval region of the Tylenchid nematodes of the genus *Heterodera* are of considerable systematic value[1-3] The "bullae", knob-like structures[4] not present in all species, are of particular importance They seem to be endocuticular, but their function is unknown This communication reports the presence in the four species so far examined of other endocuticular bodies closely associated with the vulva itself My attention was directed to them by Dr C. Ellenby, who first observed them in sectioned material of *H rostochiensis* and *H tabacum* (unpublished work)

The "vulval bodies", as it is proposed to call them, are clearly shown in Fig 1, a longitudinal section of a white cyst of *H rostochiensis* They have also been found in *H schachtii*, a bullate cyst with vulval cone, and in *H cacti*, an abullate cyst with vulval cone In both these species the vulval bodies are apparently less well developed than in *H rostochiensis* and *H tabacum* It is too early to say, however, whether the apparent differences in size between the vulval bodies of different species are of systematic value

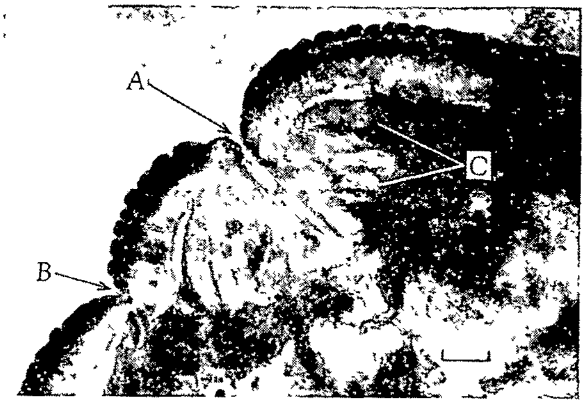

Fig 1 Longitudinal section of white cyst of *H rostochiensis* showing vulval bodies *A*, Vulva, *B*, anus, *C*, vulval bodies (Scale=10μ)

The vulval bodies are embedded in hypodermis, and in all the species examined they are closely associated with the fenestra, the thin peri-vulval region, it would be more correct to say that they are attached to the fenestra, because if this is detached from the cyst wall by manipulation, the vulval bodies are detached with it Their function is unknown If detachment of the fenestra of the fully formed cyst is vital for the emergence of larvae[1], then perhaps the vulval bodies may be involved in some way in this process. They do not seem to be involved in mating, for comparison of fertilized and non-fertilized cysts showed that the vulval bodies increased in size after fertilization in the former, but showed no such change in the latter

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[1] Cooper, B A , *Proc Second Easter Sch Agric Sci Univ Nott* , 269 (1955)

[2] Taylor, A L , *Proc S-19 Workshop in Phytonematology Univ Tennessee (H Taxon)*, 1 (1957)

[3] Mulvey, R H , *Canad J Zool* , 35, 421 (1957)

[4] Franklin, M T , *J Helminth*, 17, 127 (1939)

Dry Season Biology of *Anopheles gambiae* Giles in the Sudan

COLLECTIONS of mosquitoes have been made regularly throughout the dry season in the desert west of Omdurman, Sudan, by means of pyrethrum-spraying, well-traps and hand catching The results indicate that adult female *Anopheles gambiae* (sp B ?) Giles survive throughout the dry season, but with a change in their physiology and behaviour The insects were found to hide in dwelling huts (87 7 per cent), in cracks down dry wells (8 5 per cent), in disused or ruined houses (3 2 per cent) and in rabbit and rodent burrows (0 5 per cent) Dissection showed that 77 1 per cent of the nulliparous females captured were engorged with blood Of these, 90 6 per cent contained human blood The ovaries evidently undergo only one gonotrophic cycle during the dry season, developing extremely slowly so that, when the rains come, the gravid females are ready to oviposit These mosquitoes are apparently unable to lay eggs during the dry season

Laboratory studies have also been carried out on mosquitoes bred in an insectary About 10 per cent of nulliparous females, kept from the beginning of the cool dry season in conditions of temperature and humidity approximating to natural conditions, lived for periods up to 206 days Those maintained in similar conditions from the beginning of the hot dry season lived for a maximum of 51 days Individuals reared towards the end of the hot dry season did not live long. The ovaries of these experimental individuals did not mature, although the insects took frequent blood meals. Some of them survived for as long as 59 days' fasting. By weighing starved females before and after feeding, it was found that they took incomplete blood meals. Gonoactive females, reared in the insectary, however, took complete blood meals. Incomplete blood meals in gonoactive females evidently result in the failure of ovarian development.

Laboratory experiments also showed that low temperatures (20° ± 0.5° C) and a low threshold of relative humidity (10–20 per cent) induce a state of arrested development. Low humidities at normal temperature produce an inhibition of oviposition in gravid females for 4–14 days.

This work was supported by the World Health Organization, Division of Malaria Eradication, and the practical work was carried out by one of us (S. M. O.).

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ANIMAL BEHAVIOUR

Specific Habituation by Chicks

CERTAIN changes in the sensory input of an animal are immediately followed by a reaction from that animal which is the same for a variety of different changes. This orientation reaction was first described by Pavlov[1], who referred to observable changes in posture such as turning the head to look or listen in a particular direction. Many other changes within the animal have been found to coincide with such movements and are therefore considered to be part of the orientation reaction[2]. Lynn[3] has summarized the various components of the reaction which have been measured.

Repetition of a change in environment leads to reduction in the duration and extensiveness of the orientation reaction and, as far as present recording techniques can determine, to its final disappearance. After an environmental change has occurred at intervals until the disappearance of the orientation reaction, slight modification of that change may be followed by the reappearance of the reaction[2]. The degree of modification necessary for such a reappearance depends on various factors and is limited by the acuity of the sensory system concerned. Sokolov[2] has described experiments in which animals habituated to a tone of a particular pitch or duration showed a reaction, measured physiologically in various ways, when the pitch or duration was altered. To explain such results, Sokolov has proposed that with repeated presentation of a stimulus a "neuronal model", related to it, is gradually formed. Responses to subsequent stimuli depend on a comparison between the stimuli and the model. If that further input corresponds exactly with the model, no reaction occurs, but a mismatch of any sort is followed by an orientation reaction and, presumably, by a modification of the model.

This and other theories of the mechanisms of habituation are reviewed by Lynn[3]. The physiological mechanisms suggested by Sokolov[2] to account for his observations have recently been questioned by Horn[4], who points out that some aspects of habituation can be explained without postulating an active gating process after the matching of model and stimulus. Horn also quotes evidence, for some situations, against Sokolov's statement[2] that the model and matching system are located in the cortex, and that the afferent signal to the reticular formation, where the behavioural change is presumed to be initiated, is controlled by fibres originating in the cortex. Horn's proposals do, however, include a model forming system, which in the case of time dependent experiments may be two-stage, and thus do not conflict with Sokolov's behavioural hypothesis, only with some of the mechanisms he suggests.

The aim of this study was to test Sokolov's general hypothesis using behavioural measures and to see whether the theory applied also to birds. The reactions shown by young domestic chicks to a regularly repeated light flash and the effects of varying the duration of that flash are described.

Chicks were reared from hatching singly in grey-walled pens[5] with a 6.3 V torch-bulb present on the wall. This bulb could be silently illuminated by an automatic timing mechanism. The observer was never seen or heard by the chicks. The birds were watched individually and their behaviour was recorded continuously using a moving paper event recorder. Every initiation, termination or change of direction of a movement, and the bout length of the fifteen most frequent measures of behaviour, were recorded.

When the seventeen chicks used in the experiment were 2–3 days old, the torch-bulbs were illuminated on a regime of 10 s on, 20 s off. This was the first illumination change seen by the chicks since being placed in the pens. Each bird was watched for 2.5 min before and for 2.5 min after the initial illumination. The regime was maintained for 18–24 h, after which the chicks were watched for a further 2.5 min and then for 15 min while the duration of the bulb-on period was altered at intervals.

The changes in the behaviour of chicks which result from the illumination of a torch-bulb in their home pen have been reported elsewhere[5]. A major change in behaviour during the 3 s after the first bulb illumination was shown by 94 per cent of the chicks watched in this experiment. The exact nature of the change varied from one individual to another, but any call, jump, head-shake, head turn so as to fixate the bulb, or abrupt termination or change in direction of an activity, is referred to here as a major change. The beginning or end of bouts of frequently occurring activities are called minor changes in behaviour. A comparison of the 2.5 min before the initial bulb-on with the 2.5 min after, using two-tailed Wilcoxon matched pairs tests, showed increases in calling loudly and jumping ($P < 0.01$), fixating the torch-bulb ($P < 0.01$), and moving close to the torch-bulb ($P < 0.05$).

When each chick was watched for 2.5 min after the regime had been operating for 18–24 h, no bird showed an important change in behaviour during the first 3 s after any bulb-on, and minor changes occurred in only 2 per cent of these 3 s periods. Thus the reaction to the light flash, as assessed by measurement of overt behaviour, had disappeared.

The illumination regime was then altered in one of two ways (Fig. 1). The 30 s period which included 10 s bulb-on and 20 s bulb-off became (*a*) 5 s on, 25 s off ($n = 8$); or (*b*) 15 s on, 15 s off ($n = 9$). After one such alteration, the previous regime was reinstated for five 30 s periods before another change of the same type. This procedure was repeated so that each bird was subjected to the

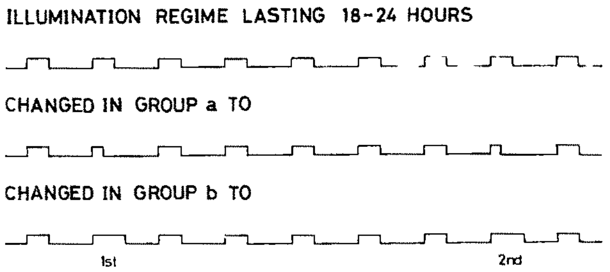

Fig. 1

changed regime five times at 3 min intervals The only change detectable by the chicks was in flash duration, for the timer controlling the regime was inaudible to them, being in a different room, and the use of a constant voltage supply prevented any change in bulb brightness

If a model of the regular input were formed and an overt reaction occurred when the subsequent input did not match that model, it would be expected that the behaviour of the chicks would show a change in group (*a*) at 5 s after bulb-on, that is to say, when the bulb went off too early, and in group (*b*) at 10 s after bulb-on, that is to say, when the bulb would normally have been extinguished but was not Such changes should be apparent within 3 s, so that data from the record of continuous observation which are quoted here are for 5–8 s after bulb-on in group (*a*) and for 10–13 s in group (*b*)

During the five 30 s periods before the alteration in flash duration, none of the chicks in group (*a*) showed a major change in behaviour between 5 and 8 s after bulb-on, and none of those in group (*b*) showed a major change between 10 and 13 s after bulb-on In the five periods during which the flash duration was altered, however, major behaviour changes were shown by 83 per cent of group (*a*) birds between 5 and 8 s after bulb-on and by 100 per cent of group (*b*) birds between 10 and 13 s after bulb-on

The most obvious changes in behaviour were the fixation of the bulb for at least 2 s and a sudden shake of the head or whole body accompanied by a brief cessation of whatever activity was being performed Other frequent changes included the initiation of a series of rapid pecks directed at the ground or the wall or sudden isolated pecks, scratches, calls or preening Some birds walked towards or away from the bulb A comparison of the frequency of major changes in behaviour before altering flash duration and in the five successive periods in which the duration was altered is shown in Table 1

Table 1 INCREASE IN BEHAVIOUR CHANGES AFTER ALTERING FLASH DURATION*

Group	*n*	Before altering flash duration			Total	After altering flash duration				
		0–3 s	5–8 s	10–13 s	%	1st	2nd	3rd	4th	5th
(*a*)	8	0	0	2	50	75	63	50	33	0
(*b*)	9	0	2	0	49	44	33	56	44	67

* Each figure is the percentage of major behaviour changes during 3 s observation

"Before altering flash duration" figures are for the 3 s starting at bulb on, and 5 and 10 s after "After altering flash duration" figures are for 5–8 s after in group (*a*) and 10–13 s in group (*b*) The total percentages for all five periods and the figures for successive periods are shown.

For group (*a*), a comparison of the behaviour of each bird in the 5–8 s after bulb-on during the five periods before altering flash duration, with the corresponding time during the five periods in which the duration was altered, gave a two-tailed value for P of 0 02 (Wilcoxon matched pairs test) For group (*b*), in a similar comparison but for the 10–13 s after bulb-on, $P < 0\ 01$ two-tailed

In group (*a*), 75 per cent of the chicks reacted to the early extinction of the bulb when this first occurred, but none reacted to the fifth duration change This change from a 10 to a 5 s flash is presumably large enough to be readily detected as a discrepancy between previous and present input but, as far as is apparent from behavioural observation, the "model" is modified rapidly Fewer birds reacted initially to a change from a 10 to a 15 s flash, but the total percentage of reactions over the five changes was almost the same This change may be less easily detected than that from 10 to 5 s and the "model" is not modified as rapidly

The recording was continuous, so it was possible to look for behaviour changes during periods other than those in which the flash duration was altered The chicks often showed changes in behaviour when the bulb was illuminated as usual in the 30 s period following that of altered duration For both group (*a*) and group (*b*), in 28 per cent of the bulb-illuminations following periods in which flash duration had been altered, major changes in behaviour occurred in the first 3 s Results of Wilcoxon tests comparing these 3 s periods with the corresponding periods before altering the duration for both major changes, and major and minor changes combined are as follows group (*a*), major change $P > 0\ 05$, major or minor change $P = 0\ 05$, group (*b*), major change $P = 0\ 05$, major or minor change $P = 0\ 01$ (all two-tailed) This effect of an environmental change on subsequent reactivity to a regular change is similar to some of those described for various mammals[2]

Using entirely behavioural measures, these results support the hypothesis that the mechanisms underlying such reactions include the formation of a model of the repeated sensory input and some system for comparing subsequent input with that model This comparison could be active, as required by Sokolov, or passive as suggested by Horn A mismatch may be followed by an overt reaction, a change in the likelihood that the animal will respond to further input, and modification of the model

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[1] Pavlov, I P, *Lectures on the Work of the Cerebral Hemispheres*, No 2 in *Selected Works* (edit by Koshtoyants, Kh S), 199 (Moscow, 1924)

[2] Sokolov, E N, in *Central Nervous System and Behaviour* (edit by Brazier, M A) (New York, 1960)

[3] Lynn, R, *Attention, Arousal, and the Orientation Reaction* (Pergamon, 1966)

[4] Horn, G, *Nature*, 215, 707 (1967)

[5] Broom, D M, Ph D thesis, Univ Cambridge (1967)

APPLIED SCIENCE

Argon Sorption at 77·4° K on a Microporous Glass

PHYSICAL adsorption should be a fast process, but several glasses have been observed to interact with gases—both inert[1] and otherwise[2,3]—slowly, the process taking a very long time During recent investigations into the structure of glass fibres using a selective leaching technique, we have prepared a porous glass, predominantly silica in composition, which exhibits slow sorption of gases over long periods of time These sorption properties can be very greatly modified by thermal pretreatment

A typical characterization of a porous product of a leaching process would involve the measurement of a pore size distribution and a surface area, and their variation as a function of thermal treatment This was found to be ineffective for the system studied because the adsorption isotherms of the usual probe gases showed a marked dependence on time A certain degree of characterization of the silica fibre properties was possible by observing the rates of inert gas sorption as a function of uptake and thermal treatment

The sorption kinetics of argon by the microporous glass were followed gravimetrically using a vacuum microbalance Before the sorption experiments, the samples were heated *in situ* at the required temperature for at least 16 h at pressures of less than 10^{-5} mm of mercury Further treatment at the same temperature did not normally result in any further loss of weight or change in the sorption kinetics

The rate of sorption of argon was found to be virtually independent of pressure in the relative pressure range 0 05 to 0 5 at 77 4° K but to be strongly dependent on

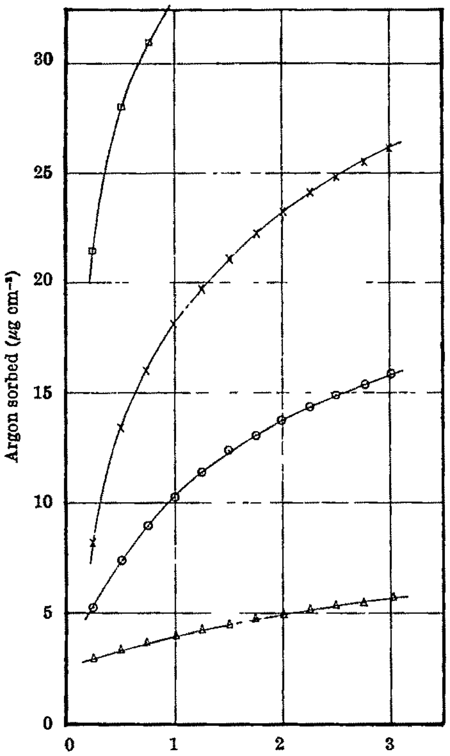

Fig 1 Argon sorption by a microporous glass produced by acid leaching Argon pressure ~0 8 cm of mercury Temperature 77 6° K □, 335° C (16 h), ×, 200° C (18 h), ○, 200° C (2 h), △, 120° C (16 h)

thermal treatment and history Adsorption experiments carried out with benzene at 26° C also showed some time dependence, but the rates were of the order of 10^4 times smaller and outside the limit of sensitivity of the apparatus

The krypton surface area was determined by using a volumetric technique and was found to agree to within 5 per cent with the value found for the original glass. The method was not of sufficient sensitivity to investigate slow processes The interaction of water with the thermally treated sample was quite complex and invalidated its use as a molecular probe, because the structure of the microporous glass was irreversibly altered during an absorption experiment

Figs 1 and 2 show the results of a series of sorption experiments on the microporous glass after a variety of pretreatments It is evident that water has had a sintering effect on the structure Fig 3 illustrates the sintering effect of temperature on the structure, in that the rate of argon sorption at constant uptake increases progressively as the temperature increases up to 450° C, and then sharply decreases, virtually ceasing below 600° C This behaviour is mirrored in the dimensional changes observed in the samples as a function of temperature, and is in contrast to the behaviour observed in porous 'Vycor' glass[4], where no appreciable consolidation occurred below 900° C

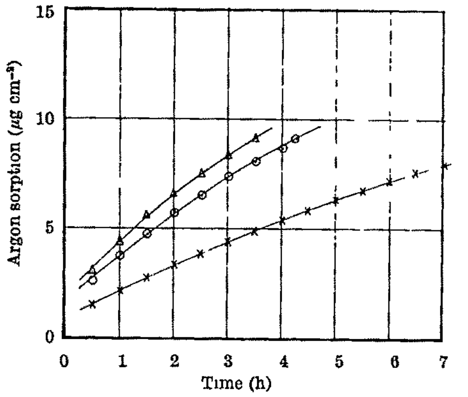

Fig 2 Argon sorption by a microporous glass after heat treatment to 450° C and rehydration at 20° C Argon pressure ~0 8 cm of mercury Temperature −195 6° C ×, 290° C (16 h), ○, 400° C (16 h), △, 490° C (16 h)

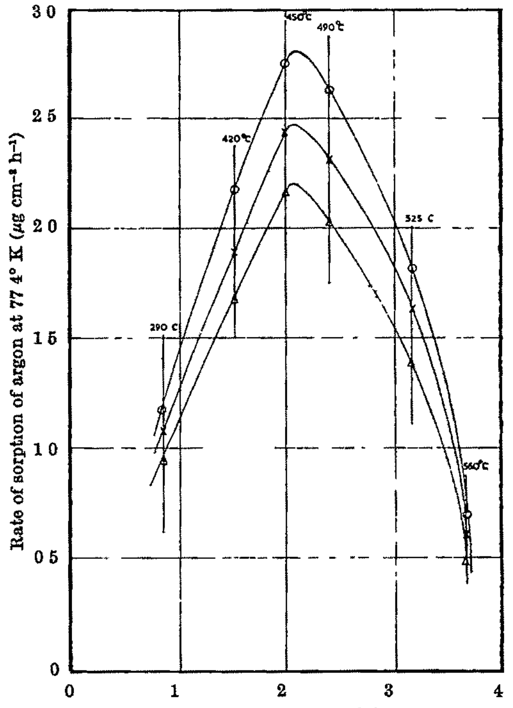

Fig 3 The influence of thermal treatment and associated weight loss on the rate of argon sorption at 77 4° K at constant uptake ○, 0 375 μg cm^{-2}, ×, 0 5 μg cm^{-2}, △, 0 625 μg cm^{-2}

The evidence points to the existence of an interior volume within the silica fibres, the entrance to which is controlled by fine pores of dimensions similar to the size of the argon molecule, for krypton and benzene are apparently excluded or have a very much slower sorption rate If its behaviour is compared with that of the molecular sieve chabazite, which shows a similar type of slow sorption process with hydrocarbon molecules[5], it would be necessary to assume a smaller controlling pore diameter than that present in chabazite because the latter shows no slow sorption with argon It would require a pore diameter in the region of 3 84 Å or less to produce the required time dependent sorption and, according to Kington and Laing[6], it is more likely that a diameter of 3 1 Å or less would in fact be necessary to cause the movement of argon to become an activated process in a porous system

The mechanism of sintering of the porous product of selective leaching of various glasses is to be studied in more detail with particular emphasis on the effects of water vapour and thermal pretreatment

B EVANS
T E WHITE

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Lancashire

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[1] Liang, S C , *J Phys Chem* , **57**, No 910 (1953)
[2] Bangham, D H , and Burt, F P , *Proc Roy Soc* , **A, 105**, 481 (1924)
[3] Whalen, J W , *J Phys Chem* , **67**, 2114 (1963)
[4] Elmer, T H , Chapman, I D , and Nordberg M E , *J Phys Chem* , **66**, 1517 (1962)
[5] Barrer, R M , and Ibbitson, D A , *Trans Faraday Soc* , **40**, 206 (1944)
[6] Kington, G L , and Laing, W , *Trans Faraday Soc* , **51**, 287 (1955)

BOOK REVIEWS

PROBLEMS OF THE QUASARS

Quasi-stellar Objects

(A Series of Books in Astronomy and Astrophysics.) By Geoffrey Burbidge and Margaret Burbidge. Pp. viii + 235. (San Francisco and London: W. H. Freeman and Company, 1967.) 70s.

Some five years ago several papers were published in one issue of *Nature*, and they were the first to introduce quasars to astronomers and to the scientific public. In his contribution Cyril Hazard showed how the position of the two parts of the radio source *3C* 273 fitted exactly on to the position of the corresponding optical object. In another paper Maarten Schmidt explained the emission spectrum of part B of the object by assuming that it had been red-shifted by an amount $z = 0.16$. Since then astronomers have been wrestling with the problem of the quasars.

The observers have made astonishing progress in their work. Identifications have been made for well over a hundred quasars, with red-shifts that range up to $z = 2.22$. The radio astronomers have measured angular diameters and found that many quasars have unresolved components smaller than 0.01 seconds of arc. Infrared observations have shown that *3C* 273, at any rate, emits most of its energy at sub-millimetre wavelengths. Most puzzling of all is the discovery that many quasars are variable, with relatively abrupt brightenings and darkenings that sometimes occur within one or a few days. Finally, some of the more highly red-shifted objects were found to have absorption as well as emission lines, and although z emission is usually close to z absorption the two values of z seem never quite to coincide.

Clearly the observers have done a vast amount of work, and Margaret and Geoffrey Burbidge, in particular, have contributed very largely to our knowledge of the observed properties of quasars. In this book they have collected together and assessed all the accessible observational results found by themselves and by other astronomers. All future investigations must begin with selected readings of Burbidge and Burbidge to find out what is already known—or rather what was known in early 1967.

But *Quasi-stellar Objects* goes further than this. In addition to describing the observations, the Burbidges give a full account of the attempts of theoreticians to explain the phenomena. If the reader comes away with a confused impression, then this only shows how well the authors have done their work. No one can even be sure how to interpret the red-shifts. Are they cosmological? If so, the energy output of the typical quasar must be exceedingly great and its life-time perhaps as long as a million years. One would expect such an object to be quite large; on the other hand the rapid fluctuations in brightness indicate that in an important sense it has an underlying structure with a much smaller linear scale.

Are the red-shifts gravitational? Schmidt and Greenstein long ago showed that the emission spectra cannot arise on the outside of a body that is massive enough. The resultant gravitational red-shift z would be too small even for *3C* 273. To make the gravitational theory work one would have to postulate a structure of almost Byzantine complexity and of rather doubtful stability.

Are the red-shifts due to the Doppler effect in a set of local objects rapidly receding from the vicinity of our Galaxy? If so, who can imagine the dynamics of the explosion that sent off individual masses with speeds up to 0.82 c, but did not completely disrupt them? Further, where are the groups of quasars surrounding other galaxies, many of which should look to us much brighter than our own quasars and should show considerable blue-shifts? These are only a few of the arguments that the theoreticians have been involved in. The Burbidges give a very good account of them, even if sometimes they plead a little too much on behalf of their own favourite view.

The theoretical situation is indeed fluid, even turbulent. By the discovery of quasars the observers have shown the astrophysicists how far they are from understanding the Universe, just when they were beginning to feel a little too satisfied with their recent successes. Long may such observers continue to provide us with such surprises.

F. D. Kahn

SPECIAL RELATIVITY

Elements and Formulae of Special Relativity

(The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Physics Division.) By E. A. Guggenheim. Pp. x + 63. (Oxford, London and New York: Pergamon Press, 1967.) 21s. net; $4.50.

This book gives an account of the essentials of special relativity theory. Aberration, mechanics, hydrodynamics, electromagnetism are all covered at a simple level, as deductions from the Lorentz transformation, which is, however, not proved. The book is compact and will suit those who want a compendium of formulae but who do not wish to enquire too deeply into conceptual problems such as: why do we need a relativity of simultaneity, anyway? I have one reservation concerning the author's five and a half page chapter on relativistic thermodynamics. He treats it as if everything were cut and dried in this field. This is, however, misleading, and there is a body of opinion which would not accept all the formulae derived in this chapter.

P. T. Landsberg

POLYMER RELAXATIONS

Anelastic and Dielectric Effects in Polymeric Solids

By N. G. McCrum, B. E. Read and G. Williams. Pp. xv + 617. (London and New York: John Wiley and Sons, 1967.) 160s.

Were it not for the development of polymer science, through exploratory polymer chemistry in particular, the subject of viscoelasticity might have remained little more than an interesting and mathematically speculative curiosity. With the tremendous output of new products in recent decades, albeit often within, rather than from, the research laboratory, there has been a corresponding availability of materials which exhibit this phenomenon to a substantially marked degree and thereby allow for a more extensive study. Perhaps not surprisingly, from the touchstone of the varied demands of characterization, there has been at least a dualistic experimental approach to the subject of viscoelasticity, with some workers on the one hand concentrating more on mechanical features, whereas others have found dielectric properties of greater interest. This book makes a serious attempt to unify the two approaches, and even the nomenclature, by an integration of the relevant mechanical and dielectric aspects of linear viscoelasticity in such a way that it will be of interest not only to chemists and physicists but also to researchers in the fields of chemical, electrical and mechanical engineering.

Following an introductory chapter and a general one on the chemical and physical structure of polymeric solids, there are three, mainly theoretical, chapters on theories of the limiting moduli and the static dielectric constant, phenomenological theories of mechanical and dielectric relaxation, and molecular theories of relaxation including an account of secondary relaxations in solids Two useful chapters on experimental methods, mechanical and dielectric, conclude the first two-fifths of the book The rest is devoted to chapters on results obtained on individual classes of polymers, including methacrylate and related polymers, polyvinyl esters and related polymers; hydrocarbon polymers; halogen polymers, polyamides and polyurethanes, polyesters and polycarbonates, and, finally, oxide polymers A reference section containing more than 800 references in alphabetical sequence, many of recent origin, an author index and subject index complete the publication

The overall impression is of an authoritative account, agreeably printed and adequately supplemented with tables and figures—and not one spring or dashpot in sight—presented with a textual precision which permits a high content of detailed information Perhaps because of this, readers from one discipline may wish for a little elaboration on some parts which will be sufficient for those from another, and yet there will be no difficulty for the serious reader in seeking appropriate information, whether it be theoretical or experimental Here it should be pointed out, as the authors admit, that the subject matter is such that it will be of more immediate interest to the researcher rather than to, say, the practising engineer Nevertheless, herein lie many of the explanations for the often maligned properties of polymers which have been used in the wrong context, and to this end I feel that the book, or its contents, may profitably be reported outside the research laboratory This is a valuable publication and, although there may be one or two books it will not supplant, except in date, there are many accounts it will, and it is therefore strongly recommended both as a textbook and reference book for the serious reader and researcher in polymer science

R P SHELDON

LAYERED ROCKS

Layered Igneous Rocks

By L R Wager and G M Brown Pp xv+588 (Edinburgh and London . Oliver and Boyd, Ltd , 1968) 168*s* net

THE phenomenon of layering in igneous rocks has fascinated petrologists for many years and the name of Professor Wager has been associated with the subject, in particular with the Skaergaard Intrusion of East Greenland, for some thirty of them. It is fortunate that he had completed his contribution to this book before his sudden death in 1965 and that Professor Brown was able to carry the book to publication

The first part, written by Professor Wager, gives a very detailed account of the Skaergaard Intrusion which serves as a basis for comparison with the other layered complexes The account is systematic and commences with a general introduction to the characteristic features of the complex. Having done this, the minerals and the rocks composing the layered series are described. The chemistry is given meticulous coverage and equal attention is paid to the mechanism of differentiation of the layering While putting forward a theory, the authors do not agree that an explanation for one locality may necessarily satisfy another, and point out (page 338) that "No single theory is capable of explaining, for all layered intrusions, the origin of a feature such as rhythmic layering, because, despite overall similarities, the details differ from one intrusion to another"

The second part of the book, written by Professor Brown, is devoted to the other layered complexes of the world and in particular to the Bushveld, Stillwater and Rhum Intrusions In each case the treatment is the same, which makes comparison easy The form of the intrusion is described together with an account of its minerals present, the principal subdivisions of the intrusions are discussed and finally the crystal history is given In the case of the Bushveld, this is probably the most up to date general account since Hall's memoir of 1924 In all cases reference is made back to Skaergaard and points of similarity and dissimilarity are noted

Other types of basic layered intrusions described include the Cuillins of Skye, Kap Edvard Holm, East Greenland, Duluth, Minnesota, and the Great Dyke of Southern Rhodesia Described in less detail are twenty-nine smaller layered and fractionated intrusions, which are grouped by continents They include Ardnamurchan, the Lizard and Dawros from the British Isles, several examples from North America and Africa and three from Russia A short chapter is included on Differentiated Sills and there is also one dealing with layering in syenites and granites

This is a very comprehensive work—a compendium of layered intrusions in fact—which has been brought together in one volume for the first time The detail is such that it will be of most use to the advanced student and postgraduate, although students of even first year level may derive benefit from reading the first and last chapters on general aspects of layering It will be, and deserves to be, a classic textbook and it is a fitting tribute to Professor Wager's work.

J R V BROOKS

CELLS IN PAPERBACK

The Cellular Role of Macromolecules

(Series in Undergraduate Biology, Vol 2) By P H Jellinck Pp 117 (Glenview, Ill Scott, Foresman and Company, 1967) $2 25

THERE have been considerable changes in the teaching of biology recently, both at school and in the university, and there is a demand for suitable books particularly at the elementary level Publishers have met this need with textbooks of wide coverage and uneven quality, which are often characterized by redundant illustration There has also been a tendency for the production of paperback series which, while certainly no cheaper in aggregate, allow individual selections A narrower coverage also provides the opportunity for two essential features of any elementary monograph which is to interest and educate a student· an informed selection from the vast amount of material available, and a coherent view of the subject by the author

These desirable but elusive features are not always found in every volume of a series "The cellular role of macromolecules" is a large subject and may be difficult to cover adequately, but this book makes the attempt It begins with the description of various cells, then covers dynamic aspects of biological systems, before proceeding to a discussion of intermediary metabolism, biosynthesis and regulatory mechanisms.

Unfortunately it is not always very accurate For example, Gurdon's work on nuclear transplantation indicates a position very different from that of Briggs's and King's Transcription of RNA from one strand only of the DNA double helix clearly was not first shown from the poly dAT experiment quoted, nor does the evidence quoted show that transcription occurs "within the minor groove" of the DNA helix.

The book is probably the better for not being lavishly illustrated, although the whole page diagram of the reactions involved in lipid biosynthesis would not be very clear to an elementary student and certainly fails to compensate for the two short paragraphs on the subject Holley's work on transfer-RNA sequence is mentioned, but no comment is made on the secondary structure, with which it is compatible, the hairpin configuration is still discussed and illustrated

There are liberal quotations from many research workers, which may, as the author hopes, illustrate their contribution and philosophy, unfortunately they often punctuate the narrative flow and sometimes illustrate a turgid style Students may like to read these extracts and it is certainly helpful to mention the authors of various experiments, although I am puzzled by the subtle gradations which have decreed that some have Christian names, others initials and a few neither

This book does not supersede other similar paperbacks in this well-publicized field, despite several useful features.

P M B. WALKER

BIOLOGICAL PROBLEMS

Problems in Quantitative Biology

(Biological Science Texts) By J F. Eggleston Pp ix+226 (London The English Universities Press, Ltd, 1968) 22*s* 6*d*

THIS collection of numerical examples is intended to promote the teaching of biology as an exact science by providing exercises which give sixth form and first year university students experience in handling quantitative biological information, and help them to apply mathematical skills and assess the error and degree of significance involved. It might in some ways be regarded as an English counterpart of Joseph Schwab's *Invitations to Enquiry*

The book is organized in two sections. The first part comprises eight groups of analytical and numerical problems concerning physiological processes in plants and animals These groups deal severally with respiration and gaseous exchange; photosynthesis, water relations of animals and plants; ion uptake and loss in animals and plants, muscle and skeletal physiology, kidney and excretion; and statistics and genetics The second part, printed on distinguishing green paper and coded to correlate easily with the first part, claims to give a detailed consideration of the problems posed in the first part, together with their solutions To a professional mathematician the explanations might appear somewhat imprecise and inadequate

It is a truism that the quality of learning is determined by the quality of the teacher, certainly the educational use of this book calls for knowledge, skill and discrimination of a high order on the teacher's part It would be his task to select the problems appropriate to his pupils' sophistication both in biological concepts, facts and principles and in mathematical expertise, unless the text were to provide merely a collection of arid exercises Such a concentration of examples might well daunt those who take a more broadly humanistic view of the biological sciences than is at present fashionable, though the insufficiently numerate biology teacher might well find some reassurance in the second part of the book

There is certainly wide variety in the problems posed and this gives a helpful idea of the many aspects of biology which lend themselves to numerical treatment, nevertheless, mathematical problems should come into biology only if they elucidate a particular aspect of the subject Too often these problems seem artificial, thought up to involve some mathematical skill or physico-chemical consideration

Far from "spreading alarm and despondency" as the author fears, the introduction of material from original papers and advanced texts is a welcome and redeeming feature of the book It is certainly in accord with the current demand for relevance at all educational levels Unfortunately, the text lacks documentation and, if the author's hope that pupils will be stimulated to refer to reference books is to be fulfilled, an adequate bibliography is essential

Mathematics in the forms suggested by this book can indeed be a most useful precision tool for occasional use by a knowledgeable and discriminating practitioner, but as a "weapon in his armoury" the average teacher might well find such a concentrated device as this rather dangerously two-edged

Problems in Quantitative Biology should be regarded with caution as a teacher's reference book rather than adopted unreservedly as a student's text

E J VINNICOMBE

GENETICS REVIEWED

Annual Review of Genetics

Vol. 1. Edited by Herschel L Roman in association with Laurence M. Sandler and Gunther S Stent Pp. 334 (Palo Alto, California Annual Reviews, Inc, 1967) n p

THIS volume inaugurates a new member of the Annual Reviews series The usual two questions arise is it required, and if so, is it good? For once the answers can both be in the affirmative. Though most first issues manage to be good, this is exceptionally so The quality and composition of the editorial committee—Crow, Luria, Owen, Rhoades, Roman and Tatum—promise that the standard will continue H L Roman's editorship, in association with L M Sandler and G S Stent, is further guarantee

Genetics has at last permeated the whole of biology. Often unaware, now every biologist "does" some genetics The result is that those more primarily interested in the genetic aspects of any one problem are faced with the difficulty of scattered literature An *Annual Review of Genetics* is required to make this task easier

The first issue covers both highly specialized and more general topics Picking and choosing the chapters on "Human Genetics" by H E Sutton and "Population Genetics" by R C. Lewontin stress the generality of balanced polymorphism from man to *Drosophila* A masterly article by C Yanofsky reviews the five principal sources of evidence—two from bacteriophage and three from *Escherichia coli*—which have demonstrated the co-linearity between the nucleotide sequence of a gene and the amino-acid sequence of the polypeptide chain encoded in that gene S Emerson, in "Fungal Genetics", stresses the unique contribution of tetrad analysis to the investigation of the mechanisms of recombination As to "Developmental Genetics", H Ursprung stresses the change from the analysis of developmental pathways disrupted in mutants to that of sequential activity of genes in terms of messenger production

Those mentioned are only five of the thirteen review articles of this volume, all of which are valuable

G PONTECORVO

TAXONOMY OF PLANTS

Plant Variation and Classification

By Ritchie C. Bell (Fundamentals of Botany Series) Pp vi+135 (London Macmillan and Co, Ltd, 1967) 12*s* 6*d*

THIS is a typical "curate's egg" book, the sections on variation are very well done, while those on classification are weak in a number of places It suffers all the way through, also, by leaning too heavily on theoretical examples where a little searching through the literature would have revealed actual ones of much greater interest and value to the student

After an introductory chapter on variation and classification the author goes on to discuss nomenclature and keys, this chapter is reasonably well done, but at least seven errors occur in Table 2–1, where the translations of certain specific epithets are given

The historical chapter on botanical classification is interesting and of considerable value until a discussion of phenetic and phylogenetic classifications is embarked on Here, the author is led into serious errors, for example (page 36) "In the phenetic system, the arrangement of the taxa of plants according to their relative evolutionary position is made on the basis of the apparent relationships observed among available plants or plant materials" This is far from the truth, for the whole purpose of a phenetic system is to classify on the basis of observed characters, without any reference to evolutionary position

On the other hand, the fourth and fifth chapters are well reasoned and clearly written The fourth chapter deals with variation patterns, isolating mechanisms, aneuploidy and polyploidy. The fifth chapter describes the sources of variation, clearly differentiating phenotypic from genotypic variation and dealing well with each

Taxonomic characters are listed in the sixth chapter, but the author has not really been allowed enough space to do justice to these, especially to the newer techniques of chemotaxonomy and serotaxonomy, while studies on fine structure are not mentioned at all The seventh chapter deals with the evaluation and presentation of data and mentions hybrid indices, polygonal graphs and scatter diagrams Brief notes on numerical taxonomy are also included.

Finally, the eighth chapter gives short descriptions, illustrated by drawings and diagrams of twenty-five flowering plant families, with an average of one family per page These are quite well presented, though of course not much can be said about each family in such a short space and only the plants thought of as "typical" can be mentioned at all Incidentally, the so-called angiosperm classification on page 96, modified from that of Bessey, is not really a classification but a phylogenetic tree, and as such might be rather confusing to the student, for whom this book is intended

The suggestions for further reading are far ranging in subject, but of the thirty-four references to books (excluding state floras) only six were published outside the United States, while only one of the eleven periodicals is a non-American one There is no bibliography as such, even though the text has numerous references to authors and dates of publication

No doubt the author was strictly limited by the size of this book, which forms part of a series, and for that reason could not hope to go into greater detail in all the aspects of taxonomy he has tried to cover It would surely have been better, however, to omit the descriptions of the twenty-five families in the eighth chapter altogether and to have used the space so provided to enlarge other sections The chemotaxonomy, serotaxonomy and taxometrics sections would well repay expansion and it would be valuable to provide more actual examples of the points described, and surely, in a book of this sort, species concepts ought to be discussed or at least receive passing mention

J G HAWKES

PRODUCTIVITY OF FORESTS

Methods for Estimating the Primary Production of Forests

By P J Newbould. (International Biological Programme Handbook No 2) Pp viii + 62 (Oxford and Edinburgh Blackwell Scientific Publications, 1967) 7*s* 6*d*

THIS booklet consists of forty-seven pages of text and ten pages of references, it is intended to serve as a guide to the methods that may be recommended "to scientists who themselves do not think that they have better methods" (as E B Worthington says in the foreword) for the "general investigations of primary production" in woodlands which form part of the International Biological Programme, thus it concerns the annual yield of timber, branches, leaves, roots, and so on The recommendations are mostly sound, as indeed they ought to be in view of the many specialists who contributed to the original draft, and should be useful to the planner of investigations into such matters, provided that he makes full use of the references, heeds the many important but often inconspicuous warnings that are issued by Professor Newbould, and has a sound grasp of the statistical principles of sampling

Perhaps a fuller discussion of the relative importance of the various sources of error would have been welcome, and also a clearer relation of method to purpose, for example, tables which it is both legitimate and desirable to use when ascertaining relative yields of a single plot during successive periods of time may be grossly misleading if used for comparing the yields of plots in different environments Again, it is suggested that a period of between three and five years is probably adequate for measuring the growth of all-aged forest, it all depends on the purpose In Europe a period of ten years is no more adequate for giving a reliable estimate of mean increment than it would be for giving a reliable mean rainfall This, however, may be asking for too much in so small a space, and the critic is disarmed by the admission that the work could undoubtedly be improved, together with a request for suggestions to this end

Here, then, are some suggestions The old and well-tried method of measuring the volume of irregular branches by immersing them in water is worth mentioning. There is a large amount of information available on the pattern of density of wood in tree boles, and this should be consulted before sampling them for density, partition into sapwood and heartwood is not necessarily the best procedure Foresters, when measuring sample plots, usually measure the volume of bole and, sometimes, of branches, greater than some conventional "timber size" (in Britain 9 5 in girth), it would be useful if biologists, when measuring the amount of wood in a forest, measured separately from the rest the same components as those measured by local forest research departments, this would facilitate the comparison of foresters' and biologists' work, greatly to the advantage of biologists

EUSTACE W JONES

VEGETATION MAPPING

Vegetation Mapping

By A W. Kuchler Pp vi + 472 (New York The Ronald Press Company, 1967) $15

WITH few exceptions, mainly in Europe, mapping of vegetation has proceeded largely in isolation from other ecological activities This book will probably bring to the notice of many ecologists for the first time the amount and interest of the work that has been going on in this field It is therefore welcome in a broader context than is indicated by its ostensible purpose of providing a handbook and guide to the procedures and technicalities of producing vegetation maps

The author of this book is a leading practitioner of vegetation mapping, who is particularly well qualified to bring together the diverse literature of the subject He has succeeded in producing what will be a definitive guide to this field, at least in English speaking countries, for some time

The book opens with an interesting "Historical Sketch" of the development of vegetation cartography The second section of the book is concerned with "Some Basic Considerations" This is somewhat disappointing Before units of vegetation can be mapped, a clear conception of the nature of these units is necessary and one expects some discussion of the thorny problems that surround concepts of the plant community. These problems are scarcely mentioned and one looks in vain in the extensive bibliography for many of the names of those who have figured in discussion of them. Tansley, Clements and Curtis receive incidental mention in this connexion but without indication of the fundamental differences between their points of view, others, for example Gleason and Ramensky, are not mentioned at all. Classification is likewise treated rather superficially. The author rightly emphasizes that only two types of features are available as criteria in the classification of vegetation (as opposed to classification of sites)—structure and floristic composition—but there is little discussion of what aspects of the floristic composition should receive most weight and there is no consideration at all of the development of numerical techniques that has been such a prominent feature of phytosociology in the past decade.

The third section of the book deals with "Technical Aspects", covering such questions as choice of scale, use of aerial photographs, choice of colour and symbol, the lay-out of maps and accompanying texts, and so on. This section raises few points of fundamental controversy (though many will consider the relegation of "Boundaries and Transitions" to a technical aspect somewhat surprising). These technical aspects are clearly and interestingly presented and treated in a well-balanced manner.

The fourth, and longest, section deals with the practice of mapping in various conditions and according to various schools. This account will be invaluable both to those considering possible methods to use in vegetation mapping and to those concerned to make use of vegetation maps emanating from different schools.

The final section is concerned with "Application of Vegetation Maps". In addition to the use of vegetation maps as tools in ecological and geographical research, examples, actual or hypothetical, are given of their use as climatic records, in pedology and geology, agriculture, forestry, land management and planning, education, for commercial engineering and fiscal interests, and in relation to military activities. This is a most impressive list. If only a small proportion of the suggested potential uses is realized, there is indeed a strong case for extensive allocation of resources to vegetation mapping.

A bibliography of some 600 references is particularly valuable for its coverage of literature in French and German. There are few references after 1963.

In spite of the reservations indicated here, this is a valuable and timely book, although few will go all the way with the author in assigning a central role in the understanding of vegetation to vegetation mapping. The author is an enthusiast for his subject and his enthusiasm makes his book a pleasure to read, even when we may disagree with what he has to say. It is written in an easy and informal style and well produced, though the price, by European standards, seems rather high.

P. GREIG-SMITH

PARENTS ARE DIFFERENT

Influences on Parent Behavior

By Lois Meek Stolz. (Stanford Studies in Psychology.) Pp. viii + 355. (London: Tavistock Publications, Ltd., 1967.) 65s. net.

IT is well accepted that parents, even when social variables such as religion or social class are controlled, will vary considerably in their child-rearing behaviour. The author of this lengthy book is concerned to isolate some of the factors—either conscious or easily recalled since unconscious ones are inaccessible by social research—which may influence them.

Repeated interviews were carried out with both parents in thirty-nine families. Most fathers were interviewed by men and most mothers by women. Four main groups of influences were described: (1) values, that is goals, (2) beliefs, that is (*a*) instrumental (children obey their fathers because they respect them), or (*b*) descriptive (girls seem to like horses), (3) others—(*a*) past and present experiences, (*b*) communications, mass and personal, and (*c*) behavioural settings.

It might have been better, in view of the original assumption that families vary in spite of social homogeneity, to have selected a group who were similar in all major respects. Instead the author took pains to keep the group as disparate as she could. There are some suggestive findings here, but the work is based on too slight information to prove ultimately reliable. There is a great overlap of influences described which can only be assigned weighted importance with a much more selective approach and with a larger sample. The book is too long for easy reading and would benefit by good cutting and editing.

ANNETTE LAWSON

HANDBOOK REVISED

Handbook of Chemistry and Physics

A Ready-reference Book of Chemical and Physical Data. Editor-in-chief, Robert C. Weast. Forty-eighth edition. (Cleveland, Ohio: The Chemical Rubber Co.; Oxford: Blackwell Scientific Publications, 1967.) 170s.

THE *Handbook of Chemistry and Physics* has progressed a long way since its first appearance as a pocket edition in 1913. Now in the 48th version, the handbook has gained so many tables over the years that it might be more appropriately labelled "satchel sized", but it nevertheless remains true to its description of a "ready reference book of chemical and physical data".

Information is classed into one of six sections, which cover mathematical tables, elements and inorganic compounds, organic compounds, general chemical constants, general physical constants and miscellaneous. Nearly 2,000 pages provide more than 550 tables, seventeen of them new, and more than eleven revised. Information that has been included for the first time covers absolute viscosity of liquid sodium and potassium, constants for satellite geodesy, factors for conversion of $\log_{10}$ to $RT\log_e X$, infrared correlation, far infrared vibrational frequency correlation and characteristic frequencies between 700–300 cm^{-1}. New charts also give characteristic nuclear magnetic resonance spectral positions for hydrogen in organic molecules, ionization constants for deuterium oxide from 10° C to 50° C, heats of combustion, density and composition of sulphuric acid, thermal conductivity of certain metals, liquids and gases and solubility products. There is a supplementary table of diamagnetic susceptibilities of organic compounds, and a table, of particular interest to bearded scientists perhaps, of mechanical and physical properties of whiskers.

The rate at which scientific knowledge increases these days must give the editors of a handbook like this rather a headache, for now it must be more a question of what to leave out than what to put in, if the book is to remain a manageable size. It has been thought impossible to produce one volume to cater for the biological as well as the physical sciences, but although concentrating on the physical sciences the editors have been conscious of the growing interdisciplinary attitude in science, and have included tables that extend beyond the physical field. The mathematical section has been growing steadily over the years to keep pace with the growing complexity of modern chemistry and physics, largely at the suggestion of users of the handbook. A large editorial board is involved in its production, with Robert C. Weast as editor-in-chief, so the handbook ends up full of information for the scientists, by the scientists, giving a vast quantity for the money.

ELISABETH LEWIS

University News : Edinburgh

Professor J I G Cadogan, at present Purdie professor of chemistry in the University of St Andrews, has been appointed to the Forbes chair of organic chemistry on the retirement of Professor E Hirst

Announcements

The Council of the Edinburgh Geological Society has awarded the Clough Medal for the period 1967–68 to Dr A G MacGregor in recognition of his contributions to Scottish geology, particularly in the fields of volcanology, tectonics and metamorphism

The Paul Instrument Fund Committee, which is composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, and was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science", has made the following grants

£2,000 to *Dr A P Anderson*, lecturer in the department of electronic and electrical engineering in the University of Sheffield, for the construction of an instrument for the measurement of energy in laser beams, £1,500 to *Dr W J Jones*, demonstrator in the department of physical chemistry, University of Cambridge, for the construction of a spectrometer employing frequency selective intensity modulation, £1,830 to *Mr A R Luxmoore*, division of civil engineering, University College of Swansea, for continuation of the construction of a 'Moiré-scope' for which he received a grant of £5,000 in November 1965 and £1,980 in June 1967, £6,250 to *Professor J D McGee*, professor of applied physics at the Imperial College of Science and Technology in the University of London, for continuation of his work on the development of a photoelectronic image device for time images for which in December 1964 he received a grant of £8,100 over three years, £4,000 to *Dr K I Mayne*, senior lecturer in the department of natural philosophy in the University of Edinburgh, for the construction of a polarized electron source.

Wolfson Foundation Grants for Technological Projects On October 14, 1967, the Wolfson Foundation made public its decision to set aside, in principle, up to £500,000 a year over the next five years to support large projects at universities in fields of applied science which, in the judgment of the trustees, would be most likely to improve the economic position of the United Kingdom and help the modernization of British industry Subsequently all universities and colleges of technology were informed of this decision The foundation now announces that applications in support of projects which conform to its intentions should be sent, in appropriate detail, to the foundation offices (Universal House, 251 Tottenham Court Road, London, W1) by July 1, 1968 It is hoped that it will be possible to decide by early October which schemes the foundation will support Applications will be judged in competition with each other on the basis of their technical merits Favourable consideration will be given to projects which are linked to industry Applicants who feel they require information about the relevance of possible projects should get in touch with the director of the foundation

An experimental Information Centre on Intestinal Absorption has been set up at the University of Sheffield with a grant from the Office for Scientific and Technical Information (OSTI) The project, initiated by Professor D H Smyth, is a joint venture of the Department of Physiology and the Postgraduate School of Librarianship and Information Science (director, Mr W L Saunders) At present the centre has a staff of three A monthly bulletin of literature references with brief abstracts is produced The emphasis is on mechanisms of transport across the absorptive epithelium, a field in which Professor Smyth's department is very active and has much experience References are collected both by conventional methods and by the computer services *ASCA*, *CBAC*, *Chemical Titles* and *MEDLARS* Author and subject indexes are produced using a computer in conjunction with offset lithography The aims of the project are 1, to investigate information needs of workers in this field and services already available, 2, to indicate ways in which a centre of this kind can provide improved information services, 3, to investigate the benefit, to users, of the association of the centre with an active group of research workers, 4, research and development in methods of information handling

During 1968 the service will be free, users will be asked to help to improve the service by providing details of their information requirements Further details may be obtained from Mr J K Barkla, Biomedical Information Project, Department of Physiology, University of Sheffield

Meetings

Biological Semiconductors, April 1–3, University of Nottingham (Dr J B Finean, Department of Medical Biochemistry and Pharmacology, The University, PO Box 363, Birmingham 15)

Heavy Particle Collisions, April 1–3, Queen's University of Belfast (The Conference Secretary, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1)

Business Information, April 8–9, University of Aston, Birmingham (The Education Officer, Aslib, 3 Belgrave Square, London SW1)

Some Aspects of Heterocyclic Sulphur Chemistry, April 9–10, University of Salford (Drs O Meth-Cohn and B Iddon, Department of Chemistry, University of Salford, Salford 5, Lancashire)

Spectroscopy, April 17–19, Brighton (The General Secretary, Institute of Petroleum, 61 New Cavendish Street, London W1)

Erratum In the introduction to the article "Multiphase Development of the Lunar Crater Tycho" by R G Strom and G Fielder (*Nature*, 217, 611, 1968) it was stated that Orbiter V landed on the Moon, which is, of course, wrong—the satellite orbited the Moon

CORRESPONDENCE

Inside Plants

Sir,—When I read a review such as that which you publish for *Plant Anatomy* (by Professor A Fahn) in *Nature* (216, 522, 1967), I realize how low the intellectual level is sinking and the disservice which is propagated It was a sad day when *Nature* forsook Wordsworth's advice*, for this meanness would never have been reached

Yours truly,

E. J H Corner

Botany School,
Downing Street,
Cambridge

**Nature* used to bear the legend "To the solid ground of Nature trusts the mind that builds for aye "

FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, March 4

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 10 a m —Colloquium on "Pulsed Code Modulation"

University of London (at the Royal Postgraduate Medical School, Du Cane Road, London, W12), at 4 p m —Professor R D Girdwood "Folic Acid Deficiency"

Institution of Civil Engineers (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "The Organization of the Port Planning Team and the Role of the Engineer", introduced by Mr J E G Palmer

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Need or Otherwise for Suppression of Harmonics Generated by Convertor Equipment" opened by Mr H J Sheppard

University of London (at the Royal Free Hospital School of Medicine, 8 Hunter Street, London, WC1), at 5 30 p m —Docent Annica Dahlstrom (Stockholm and Goteborg) "Monoamine-containing Neurones in Mammals I Monoamine-containing Neurones in Mammals as Studied by Fluorescence Microscopy" *

Institution of Mechanical Engineers, Applied Mechanics Group (at 1 Birdcage Walk Westminster, London, SW1), at 6 p m —Discussion Meeting on "Designing Components Subjected to High Surface Pressure"

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W1), at 6 p m —Mr F W Hyde "Progress in Radio Astronomy"

Society of Chemical Industry, London Section (joint meeting with the Chemical Engineering and Heavy Organic Chemicals Groups, at 14 Belgrave Square, London, SW1), at 6 30 p m —Sir Kenneth Hutchison, CBE "New Sources of Natural Gas for Britain" (Jubilee Memorial Lecture)

Tuesday, March 5

Institution of Electronic and Radio Engineers, Joint IEE/IERE Computer Groups (at the Institution of Electrical Engineers, Savoy Place, London WC2), at 10 a m —Colloquium on "Large Scale Integration"

Chemical Society, Chemical Crystallography Group (at the Geological Society, Burlington House Piccadilly, London, W1), at 2 p m —Discussion Meeting on "Penta-Co-Ordination"

Institution of Civil Engineers (at Great George Street, Westminster, London, SW1), at 5 30 p m —Mr P H Steel "The Bristol Regional Foul Water Drainage Scheme"

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I Mech E , and the Institute of Measurement and Control, at Savoy Place, London, WC2), at 5 30 p m —Mr I V Idelson "Towards Control Theory in Warehousing"

University of London (at the Royal Free Hospital School of Medicine, 8 Hunter Street, London, WC1), at 5 30 p m —Docent Annica Dahlstrom "The Monoamine-containing Neurones in Mammals II Distribution and Turnover of Noradrenaline and Storage Granules in Sympathetic Adrenergic Neurones" *

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion meeting on "How Shall We Use North Sea Gas ?"

Wednesday, March 6

Royal Institution, History of Science Discussion Group (at 21 Albemarle Street, London W1) at 1 p m —Dr N E Butcher "Geology as an Applied Science in the Nineteenth Century" *

Colour Group (Great Britain) (in the Physics Department, Imperial College, London, SW7) at 2 30 p m —Meeting on "Thin Films" Dr A Thetford "Interference Filters—Theory", Mr H A Macleod "Interference Filters—Manufacture"

Institute of Petroleum (at 61 New Cavendish Street, London, W1), at 5 30 p m —Mr D W N Dolbear "Vocational Training for Graduates in Exploration"

University of London (at the Institute of Child Health, Guilford Street, London, WC1), at 5 30 p m —Dr C L Cope "Steroids and Stress" (Last of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation)*

University of London (at the Institute of Archaeology, 31–34 Gordon Square, London, WC1) at 6 p m —Mr B Greenhill "Sources of Maritime Archaeology—The Building of Schooners" *

Society for Analytical Chemistry, Microchemical Methods Group (at "The Feathers", Tudor Street, London, EC4), at 6 30 p m —Discussion Meeting on "Automatic C H N Analysers", introduced by Mr T R F W Fennell

Society of Environmental Engineers, Packaging Group (at the Euston Tavern, 73 Euston Road, London, NW1), at 6 30 p m —Mr A Thompson "Tailoring Foams for Package Cushioning'

Pharmaceutical Society of Great Britain (at 17 Bloomsbury Square, London, WC1) at 7 p m —Professor Maurice Leclerc (Faculte de Pharmacie, Paris) ' Therapeutic Use of Anticoagulants"

Thursday, March 7

University of London (in the Anatomy Theatre, University College London, Gower Street, London, WC1), at 1 20 p m —Dr O C J Lippold "Art in Scientific Research" *

Royal Society (at 6 Carlton House Terrace, London, SW1) at 4 30 p m —Dr G D H Bell FRS "Plant Breeding for Crop Improvement in Britain —Methods, Achievements and Objectives" (Review Lecture)

Institution of Civil Engineers, Education and Training Group (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "The Higher National Diploma in Civil Engineering", introduced by Mr R J Ashby

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Discussion meeting on "Research in the Electricity Supply Industry" opened by Dr L Rotherham, FRS

University of London (in Mechanical Engineering Lecture Theatre A, Imperial College, London, SW7), at 5 30 p m —Professor A Gansser (Zurich) "Evolution of the Himalayas" (further lecture on March 8) *

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London SE1), at 6 p m —Mr J Templeton and Mr J H Merritt "Tests on Flexible Connections for Vertical Plate Freezers"

Institution of Electronic and Radio Engineers, Communication Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 6 p m —Mr W D Benson and Mr B Buckingham "Integrated Circuits for Radio Receivers"

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion meeting on "Steam Generation from Refuse"

Friday, March 8

Society for Water Treatment and Examination (at the College of Preceptors, 2 and 3 Bloomsbury Square, London, WC1), at 9 30 a m —Technical Sessions of the Sixteenth Annual General Meeting

Royal Institution, Photochemistry Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Dr A D Osborne "Transient Species in the Flash Photolysis of Some Dyes" *

Institution of Electrical Engineers, History of Technology Group (at Savoy Place, London, WC2), at 5 30 p m —Mr R J Halsey "British Contributions to Telecommunication"

Royal Institution (at 21 Albemarle Street, London, W1), at 9 p m —Mr C Maxwell Cade "Seeing by Heat-Waves"

Saturday, March 9

Inner London Education Authority (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3 30 p m —Mr Oliver S. Turner "The Canals of London" *

Monday, March 11

Society of Chemical Industry, Microbiology Group (joint meeting with the Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, SW1), at 5 30 p m —Dr D A Haydon "Some Recent Developments in the Study of Biomolecular Lipid Films"

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I Mech E , and the Institute of Marine Engineers, at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Meeting on "Roll Stabilisation and Auto Pilots in Marine Engineering Application"

Monday, March 11—Thursday, March 14

Institute of Metals (at Church House, Great Smith Street, London, SW1) —Spring Meeting

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Senior Lecturer (with research interests in some branch of solid mechanics) in Applied Mathematics—The Registrar, University of Strathclyde George Street, Glasgow, C1 (March 9)

Lecturer or Assistant Lecturer (graduate) in Botany—The Secretary, Bedford College (University of London), Regent's Park, London, NW1 (March 11)

Lecturer or Assistant Lecturer (preferably candidate working in the fields of helicopter dynamics or mathematical physics) in Applied Mathematics—The Registrar and Secretary, The University, Leeds, 2, quoting Ref No 68/21/3 (March 11)

Lecturer in Palaeontology and Stratigraphy at the University of Durban, Natal—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (South Africa and London, March 15)

Lecturers in Applied Mathematics in the fields of continuum mechanics, control theory or other branches of mathematics of practical importance—The Registrar, University of Warwick, Coventry, CV4 7AL, Warwickshire (March 15)

Lecturer or Assistant Lecturer (with a special interest in developmental problems) in Plant Physiology—The Registrar, University College of Wales, Aberystwyth (March 18)

Lecturer in Mechanical Engineering—The Secretary, The University, Dundee Scotland (March 21)

Assistant Experimental Officer (with a degree in chemistry or an equivalent qualification) to supervise chemical analyses and to take part in research projects related to the chemical aspects of quality in agricultural crops—The Secretary, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge (March 23)

Research Demonstrators (with qualifications in microbiology, bacteriology, biochemistry or related subjects) in the Department of Microbiology—The Assistant Bursar (Personnel), University of Reading, Reading, Berks (March 25)

Lecturer in Botany—The Secretary, University College, Cork, Republic of Ireland (March 29)

University Lecturer (with a good degree in an anthropological subject with sound training experience and teaching experience in material culture, including technology) in Ethnology in the Department of Ethnology

AND PREHISTORY—The Secretary of Faculties, University Registry, Broad Street, Oxford (March 30)

SENIOR LECTURER or LECTURER in the DEPARTMENT OF VETERINARY MEDICINE, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (April 1)

ASSISTANT PROFESSORS (3) in the DEPARTMENT OF GEOLOGY Haile Sellassie I University, Ethiopia—The Inter-University Council, 33 Bedford Place, London, WC1 (April 15)

CHAIR OF MEDICAL PROTOZOOLOGY at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, WC1 (April 30)

CHAIR OF TROPICAL HYGIENE and the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, WC1 (April 30)

SECOND CHAIR OF CHEMISTRY—The Secretary, University of Lancaster, University House, Bailrigg, Lancaster (April 30)

ASSISTANT PROFESSOR (biochemist or plant physiologist) in the Division of Plant Physiology and Biochemistry, Faculty of Agriculture—Head, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada

CURATORIAL ASSISTANT (with a BSc or MSc in earth sciences, and preferably some artistic talent) to act as Museum Guide for school groups, and to assist in organization, care and display of rock mineral and fossil collections—Chairman, Museum Committee, Department of Geology, University of Alberta, Edmonton, Alberta, Canada

READERS, SENIOR LECTURERS, LECTURERS IN PHYSICS, CHEMISTRY, BIOLOGY or MATHEMATICS, with opportunity for Professorship—The President, Hacettepe University, Ankara, Turkey

RESEARCH ASSISTANT (preferably with experience in one of the following fields gas dynamics, electrical discharges, combustion) to study electrogasdynamic power generation—Dr J Lawton, Department of Clerical Engineering and Chemical Technology, Imperial College, London, SW7

SENIOR LECTURER, LECTURER or ASSISTANT LECTURER IN ASTRONOMY—The Academic Registrar, Hatfield College of Technology, Hatfield, Herts

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The Schools Council Working Paper No 14 Mathematics for the Majority a Programme in Mathematics for the Young School Leaver Pp v+45 (London H M Stationery Office, 1967) 5*s* net [1812

BBC Engineering Monograph No 71 The Programme Effects Generator By H Davies Pp 19 (London British Broadcasting Corporation, 1967) 5*s* [1812

Atomic Energy Research Establishment, Health Physics and Medical Division AERE–R 5575 Radioactive Fallout in Air and Rain—Results to the Middle of 1967 By R S Cambray, Miss E M R Fisher, W L Brooks and D H Peirson Pp 40 (Harwell UKAEA, 1967 Available from H M Stationery Office) 7*s* net [1912

The Science Undergraduate a Study of Science Students at Five English Universities By Donald Hutchings Pp 80 (Oxford University of Oxford Department of Education, 1967) 14*s* 6*d* [2012

Water Resources Act, 1963 Fourth Annual Report of the Water Resources Board for the year ending 30th September, 1967 Pp vii+60 (London H M Stationery Office, 1967) 7*s* 9*d* net [2112

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Geological Society of America Memoir 98 Caribbean Geological Investigations By H H Hess, Carl O Bowin, Thomas W Donnelly, John T Whetten and E R Oxburgh Pp 310 $18 Memoir 100 Texas Mojsisovicziinae (Ammonoidea) and the Zonation of the Fredericksburg By Keith Young Pp viii+225 (38 plates) $6 75 Memoir 101 Ostracoda from the Gulf of California By Frederick M Swain Pp ix+139 (9 plates) $6 75 Memoir 102 Brachiopoda of the Keyser Limestone (Silurian-Devonian) of Maryland and Adjacent Areas By Paul Bowen Pp vii +103 (8 plates) $6 75 Special Paper 86 Scapolite in the Belt Series in the St Joe Clearwater Region, Idaho By Anna Hietanen Pp 56+8 plates $5 Special Paper 91 Petrology of Cornucopia Tonalite Unit, Cornucopia Stock, Wallowa Mountains, Northeastern Oregon By William H Taubeneck Pp 56+2 plates $3 Special Paper 87 Abstracts for 1965 Abstracts of papers submitted for six meetings with which the Society was associated Pp 366 $7 Special Paper 92 The Damaran Episode in the Upper Proterozoic—Lower Paleozoic Structural History of Southern Africa By Tom N Clifford Pp 100 $4 75 Special Paper 93 Hydrology of Limestone Terrains in the Coastal Plain of the Southeastern United States By V T Stringfield and H E Le Grand Pp 46 $2 Special Paper 94 Loess Deposits of Mississippi By E L Krinitsky and W J N Turnbull 64+6 plates $3 50 Special Paper 97 Time and Space Relationships of the Taconic Allochthon and Autochthon By E-an Zen Pp 107+3 plates $5 50 (New York The Geological Society of America, 1966 and 1967) [2112

United States Department of the Interior Geological Survey Professional Paper 581-A Seismic Reflection Profiles along the Drill Holes on the Continental Margin Off Florida By K O Emery and E F K Zarudzki Pp vi+8 (Washington, D C Government Printing Office, 1967) $0 20 [2112

East African Common Services Organization Annual Report of the East African Freshwater Research Organization 1966 Pp 46 (Jinja, Uganda East African Freshwater Fisheries Research Organization, 1967) 6*s* [2212

Bulletin of the Museum of Comparative Zoology, Harvard University Vol 136, No 6 (November 14, 1967) Delimitation of the Genus *Ceracis* (Coleoptera Ciidae) with a Revision of North American Species By John F Lawrence Pp 91–144 (Cambridge, Mass Museum of Comparative Zoology, Harvard University, 1967) [2212

Institutt for Atomenergi Kjeller Research Establishment Kjeller Report 121 A Model for the Dynamics of Nuclear Reactors with Boiling Coolant with a New Approach to the Vapour Generating Process By P Bakstad and K O Solberg Pp iv+68 (Kjeller, Norway Institutt for Atomenergi, Kjeller Research Establishment, 1967) [2212

United States Department of the Interior Geological Survey Professional Paper 557 Geology of the Connors Pass Quadrangle, Schell Creek Range, East-Central Nevada By H Drewes Pp iv+93+plates 1–3 (Washington, D C Government Printing Office, 1967) [2212

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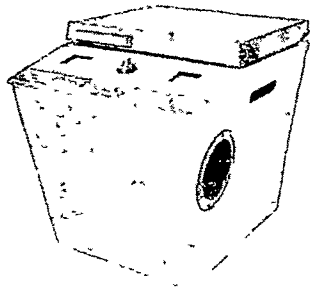

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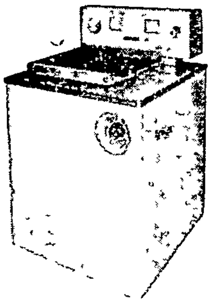

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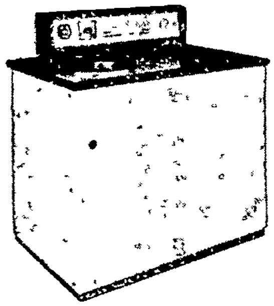

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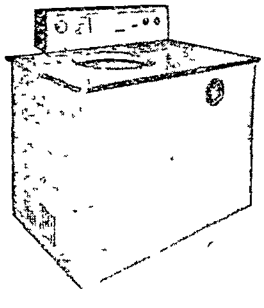

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Amino acid content of hyaluronic acid - D A Swann (Boston, USA)

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(b) Lectureship in ARCHITECTURE Appointee required to organize courses in fundamental draughtsmanship

Appointees to (a) and (b) required to take charge of one year of the course and set and supervise studio programmes

(c) Lectureship in INORGANIC or ANALYTICAL CHEMISTRY

(d) Senior Lectureship in GEOGRAPHY

(e) Lectureship in GEOLOGY Appointee will teach branches of Geophysics

(f) Lectureship in MECHANICAL ENGINEERING Candidates should possess good academic qualifications with industrial, research or teaching experience Special interest and experience should be in the fields of Mechanical Design, Production Engineering or Dynamics

Salary scales Senior Lecturer, £EA1 950 to £EA2 615 per annum, Lecturer, £EA1 350 to £EA2 230 per annum (one EA shilling=1s 2d sterling) Salary supplemented in appropriate cases under British Expatriates Supplementation Scheme in range £345 to £505 per annum (sterling) Expatriates are usually appointed on two-year tours F.S S U Subsidised accommodation Passages for appointee and family (up to five adult passages) on appointment and termination

Detailed applications (six copies) naming three referees by March 31, 1968, to Inter-University Council, 33 Bedford Place London, W C 1, from whom particulars are available (910)

UNITED BRISTOL HOSPITALS
BRISTOL ROYAL INFIRMARY
RESEARCH ASSISTANT IN HOSPITAL BIOCHEMISTRY

Applications are invited from science graduates (or those expecting to graduate in 1968) for the post of Research Assistant in the chemical pathology section of the clinical pathological laboratory of the United Bristol Hospitals The work will involve development of methods of saturation analysis and in particular the radioimmunoassay of hormones in the blood The salary will be according to qualifications and experience within the scale for basic grade hospital biochemists (from £963 for first- or second-class honours graduates or £855 for others up to £1 210) Opportunity for taking higher degree

Applications with the names of two referees to Dr K G McGowan, Department of Clinical Pathology, Royal Infirmary, Bristol, 2 (882)

SOUTHAMPTON GENERAL HOSPITAL
(586 beds)
TREMONA ROAD SHIRLEY,
SOUTHAMPTON S09 4XY

Science Graduate or **Medical Laboratory Technician** required for new Endocrine Laboratory within a Group Pathology organization Work in the radioimmunoassay of peptide hormones particularly parathyroid hormone and in associated research Previous practical experience desirable Envisaged grading is that of Basic Biochemist or Senior II Medical Laboratory Technician

Further information obtainable from Dr R M Buckle Department of Endocrinology Applications to the Hospital Secretary at the above address (879)

CSIRO BIOCHEMIST
AUSTRALIA
DIVISION OF PLANT INDUSTRY
MICROBIOLOGY SECTION

GENERAL The Division of Plant Industry of the Commonwealth Scientific and Industrial Research Organization which has regional laboratories and experiment stations in many parts of Australia and a large staff of experimental and research scientists, carries out research in a wide range of plant sciences on problems fundamental to agricultural production The research staff of the Microbiology Section of the Division is working in a variety of fields including physiology and biochemistry of disease resistance, biochemistry of nitrogen fixation, serology and immunochemistry of *Rhizobium* species and biochemistry of spore germination in fungi The Section has a wide range of equipment available and has access to CERES, the Division's Phytotron

DUTIES The appointee will join a research group investigating the physiological and biochemical basis of disease resistance in plants using model host-pathogen systems He will be expected to undertake a research programme investigating the underlying biochemical and enzymological reactions in plants which, in response to fungal infection and to certain fungal metabolites, lead to the production in the plant of antifungal defence compounds (phytoalexins) Opportunities exist for independent and team research both with this group and with other biochemists and molecular biologists in the Division

QUALIFICATIONS Applicants should have a Ph D degree, or relevant postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability Prior experience in the metabolism and regulatory mechanisms of plants would be an advantage

SALARY Depending upon qualifications and experience, the appointment will be made within the salary ranges of Research Scientist, $A5,250–$A6,622 p a , or Senior Research Scientist, $A6,892–$A7,974 p a Salary rates for women are $A428 p a less than the corresponding rates for men

CONDITIONS The duration of the appointment will be determined in consultation with the successful candidate, some applicants may prefer a fixed term appointment of three years

Fares paid for the appointee and his dependent family If the appointee is at present domiciled outside Australia, return fares will be paid at the conclusion of a fixed term appointment Applications (quoting Appointment No 130/896), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach —

Mr R F Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
Africa House Kingsway,
LONDON, W C 2 by the 29th March, 1968

(906)

UNIVERSITY OF GUELPH

The University of Guelph wishes to appoint a

DIRECTOR
of the Institute of Biomedical Research

The Institute is now in the planning stage, and the early appointment of the Director is desirable so that he can participate in all aspects of planning The Institute, under the administration of the University of Guelph is intended to serve the biological and medical sciences at Guelph and at the other Universities in Ontario

The Institute will provide facilities for the production of laboratory animals and for an active educational programme in laboratory animal medicine and technology The director will be expected to initiate and stimulate research and educational programmes in comparative medicine

The Director will be a member of the faculty of the University of Guelph with an academic appointment in an appropriate department

Applicants must have advanced training in biological and medical science and, preferably, considerable experience in comparative medicine

Applications and enquiries should be addressed to the **Associate Dean (Research), Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.**

(760)

AGRICULTURE

General agriculturists and agricultural specialists of many kinds are frequently required in the effort to match the large demand from the developing countries The vacancies advertised below are a selection from the many openings which exist now While they are being filled, other vacancies are arising Salaries within the ranges quoted are assessed on qualifications and experience, and the terms of most appointments include free or subsidised accommodation, education grants, family passages, good leave on full pay, etc Most appointments are limited to nationals of the United Kingdom or the Republic of Ireland who are normally resident in those countries

AFGHANISTAN

Cotton Adviser RC 213/2/02

Duties To advise on the development and expansion of cotton production, mainly in the Helmand Valley, to design, carry out and analyse experiments, to train technical assistants and give advice to Government officials and farmers

Qualifications A good degree in agriculture or related science, with experience of the experimental agronomy of tropical field crops, especially cotton

Salary £3,000–4,000 a year, according to experience subject to British income tax, plus a variable tax-free foreign service allowance ranging at present from £345–£880 a year, depending on marital status Contract 2 years

BOLIVIA

Animal Husbandry Expert (British Tropical Agricultural Mission) RC 213/22/05

Duties To advise and work in collaboration with Bolivian technicians on all aspects of the improvement of ranch management

Qualifications A degree in agriculture, preferably with a post graduate qualification or specialisation in the animal husbandry field, and ten years' experience in cattle ranch management

Salary £2,500–£3,100 a year, depending on qualifications and experience, subject to British income tax, plus a variable non-taxable overseas allowance currently payable at rates from £540–£1,310 a year depending on marital status Contract 2 tours of 18 months

BOTSWANA

Insecticide Formulations Expert RC 213/19/07

Duties To work on toxicity of insecticides on various pests on formulation deposits and residues, to undertake work for the protection of stored grain and to train a local officer

Qualifications and Terms An Honours Degree in either Zoology or Chemistry A practical knowledge of both subjects and an interest in the practical application of pesticides for crop protection is desirable

Basic salary Rands 4,200 (£ sterling 2,450) plus an inducement allowance at present £ Sterling 312 Terminal gratuity 25% of basic salary and inducement allowance Contract 2–3 years

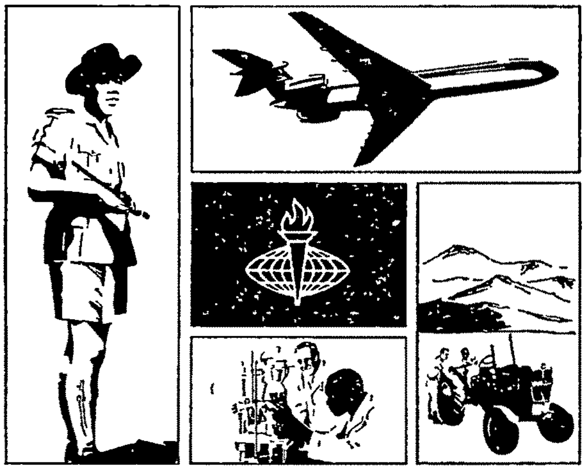
ETHIOPIA

Lecturers RC 213/58/04

Lecturers in Agricultural Science, Agricultural Mechanics, Agronomy or Farm Management are required to teach at the Jimma and Ambo Agricultural Schools

Qualifications and Terms A university degree with practical and teaching experience

Salaries £1,760–£2,700 a year, subject to British income tax, plus a variable non taxable overseas allowance ranging from £750–£1,580 a year, depending on marital status Contracts 3 years



MALAWI

Agricultural Research Officer RC 213/134/016

Duties To draw up and execute a programme investigating all aspects of rice agronomy under rain fed and irrigated conditions

Qualifications and Terms A degree in Agriculture and 5 years' experience in rice agronomy under tropical conditions

Salary £1,485–£2,600 a year, in addition a supplement of £100 a year is payable direct to the Officer's bank account outside Malawi and Rhodesia Tax free terminal gratuity of 15% to 25% Contract 24–36 months

MALAYSIA

Oil Palm Development Adviser RC 213/106/01

Duties To formulate an oil palm planting programme for the Federal Land Development Authority

Qualifications and Terms At least 15 years' practical and commercial experience in oil palm production A degree in agriculture is desirable

Salary £2,900–£3,000 a year subject to British income tax, plus a variable non-taxable overseas allowance ranging from £425–£1,275 a year depending on marital status Contract 2 years

SABAH

Agricultural Research Officers (Plant Breeders) RC 213/150/03

Duties To deal with the selection and breeding of oil palm and cocoa throughout the State of Sabah

Qualification and Terms An Honours degree in Natural Science with 2 years' post graduate training A good general knowledge of the subject is essential Field experience desirable

Salary $9,000–$17,040 a year (£ Sterling 1,226–2,321 a year) plus an inducement allowance at present in the range of £ Sterling 739–1,206 a year Terminal gratuity approximately 20% of basic salary and inducement allowance Contract 24–36 months

TRISTAN DA CUNHA

Agricultural Officer RC 213/179/01

Duties To stimulate agriculture and encourage young farmers

Qualifications A degree or diploma in agriculture with a good farming background and preferably some experience in dealing with small island communities

Salary £1,420–£1,966 a year 12½% gratuity Contract 2 years

UGANDA

Principal/Senior Research Officer RC 213/183/05

Duties To take charge of a small section of scientists investigating the breeding and selection of food crops

Qualifications and Terms At least a second class honours degree in Botany or Agricultural Botany with post-graduate training in plant breeding, and overseas experience

Basic Salary £ Ugandan 1,839–2,175 a year (£ Sterling 2,145–2,537) liable to Uganda income tax In addition an allowance normally tax free at present in the range of £ Sterling 888–936 a year will be paid by the British Government on behalf of the Uganda Government, direct to an Officer's bank account in Uganda 25% gratuity Contract 21–27 months

For further particulars of these vacancies and an application form please write giving your full name, age and brief details of professional qualifications and experience, and quoting appropriate reference number, to the Appointments Officer, Room 324/N/14 Ministry of Overseas Development, Eland House, Stag Place, London, S W 1.

MINISTRY OF OVERSEAS DEVELOPMENT

(922)

THE MEDICAL RESEARCH COUNCIL

invite applications for a newly established

SENIOR POST

in a group working on the

aetiology of tumours with special reference to man.

Applicants may be either scientifically or medically qualified and research experience in immunology, virology or genetics would be an advantage The unit has good access to clinical material and also has facilities for experimental work in virology, cell biology and biochemistry In addition to working in collaboration with other members of the group the successful candidate will be expected to develop original lines of research in this field

The salary will be in the range £1,905 to £2,630 per annum, depending on qualifications and experience

Applications giving a full curriculum vitae should be sent to the Administrative Officer, Medical Research Council, Clinical and Population Cytogenetics Research Unit, Western General Hospital, Edinburgh 4

(862)

MEDISCHE FACULTEIT ROTTERDAM

The Laboratory of Pathology of the Medical Faculty Rotterdam has available the following vacancies for senior staff-members

1. **Chief morbid anatomy service**
2. **Chief surgical pathology service and senior consultants in charge of**
3. **Cardiopathology**
4. **Pathology of the lung**
5. **Tumour pathology**

Qualified pathologists seriously interested in taking the responsibility for one of these functions are invited to send full information concerning their training and qualifications including a list of publications to Prof Dr H E Schornagel and Prof Dr M J de Vries, Academisch Ziekenhuis Dijkzigt, Dr Molewaterplein 40, Rotterdam, the Netherlands

(867)

UNIVERSITY STAFF

CALGARY, ALBERTA

The Geology Department expects to make appointments of one of the following specialists

GEOPHYSICIST Person expected to develop research and courses with emphasis on exploration for metallic mineral deposits

ENGINEERING GEOLOGIST Person expected to develop research and courses in engineering geology Experience in ground water hydrology and surficial geology an asset

Level of appointment for above positions would be commensurate with ability and experience Applicants with Ph D degrees will receive preference Presently the Department has 11 staff members and modern facilities Calgary is 40 miles east of the Rocky Mountains, has a population of 360 000 and is the centre of the oil industry in Canada

Submit applications, with résume and names of three referees to Dr P S Simony, Geology Department, The University of Calgary, Calgary, Alberta, Canada (603)

CANADA

THE UNIVERSITY OF MANITOBA

Invites applications from

LOW ENERGY NUCLEAR PHYSICS THEORISTS

DUTIES Teaching and Research

SALARIES Probably $12,000 to $18,000 per annum depending on qualifications All fringe benefits apply

FOR FURTHER INFORMATION WRITE

Dr A H Morrish,
Head
Department of Physics,
University of Manitoba
Winnipeg 19 Manitoba (498)

UNIVERSITY OF BRISTOL

DEPARTMENT OF ANATOMY

Applications are invited for a LECTURESHIP (Grade II) in the above Department, available from April 1, 1968 The duties of the post will be mainly concerned with the teaching of general and dental anatomy to dental students A good dental, medical or science degree is essential, teaching experience and ability in independent research would be an advantage Good facilities for research are available in the Department

Initial salary within the range £1,470 to £2,630 per annum, according to qualifications and experience

Applications (eight copies), together with the names of not more than two referees, should reach the Registrar, Senate House, Bristol, 2, from whom further particulars may be obtained not later than Monday, March 18 (Quote reference AL1) (919)

WOLVERHAMPTON HOSPITAL MANAGEMENT COMMITTEE

BIOCHEMIST

Applications are invited for the post of Biochemist (Basic Grade) Candidates should possess an Honours Degree in Chemistry or Biochemistry or equivalent qualifications in Chemistry In addition to routine work opportunities exist for instructon in background pathology and for research Candidates may visit the Laboratory by arrangement with Dr G Walters at New Cross Hospital, Wolverhampton

Applications, with names of two referees to the Group Secretary, Royal Hospital, Wolverhampton (918)

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

MARINE SCIENCE LABORATORIES

DEPARTMENT OF MARINE BIOLOGY

Applications are invited for three teaching posts in zooplankton, phytoplankton and marine microbiology The posts will be offered on one of the following scales

Demonstrator (two posts) £865 per annum

Assistant Lecturer £1,105 by £75 to £1,180 by £80 to £1,340

Lecturer £1,470 by £90 to £2,010 by £85 to £2,180 by £90 to £2 630

In addition there will be F S S U benefits

Applicants for the Demonstratorships should have a good Honours degree in an appropriate subject The successful candidates may register for a higher degree of the University of Wales

Applicants for the post of Lecturer or Assistant Lecturer must have relevant research experience

Two copies of the application, with the names of three referees, should be sent, not later than March 11, 1968, to the Registrar, University College of North Wales, Bangor, from whom further particulars may be obtained (843)

UNIVERSITY OF LAGOS

NIGERIA

FACULTY OF ENGINEERING

Applications are invited for the following posts tenable from September, 1968, or soonest thereafter

(a) Civil Engineering (I) Chair (II) Senior Lectureships or Lectureships in the following fields (i) Hydraulics and Hydrology and (ii) Transportation

(b) Electrical Engineering (I) Chair (II) Senior Lectureships or Lectureships in the following fields (i) Control, (ii) Power, and (iii) Telecommunications

(c) Mechanical Engineering (I) Chair (II) Senior Lectureships or Lectureships in the following fields (i) Thermodynamics and (ii) Fluid Mechanics

Salary scales Professor £N3,000 per annum Senior Lecturer, £N2,275 to £N2,575 per annum Lecturer, £N1,200 to £N2,175 per annum (£N1 equals £1 3s 4d sterling) Salary supperannuation in range £240 to £600 per annum (sterling) in appropriate cases under British Expatriates Supplementation Scheme Family passages; various allowances, superannuation scheme

Detailed applications (6 copies) naming three referees, by April 22 1968 to Inter-University Council, 33 Bedford Place London W C 1, from whom particulars are available (886)

UNIVERSITY OF THE WEST INDIES

TRINIDAD

Applications invited for (a) Senior Lectureship or (b) Lectureship in Agricultural Economics in Department of Agricultural Economics and Farm Management Competence in production economics advantageous but extreme specialisation is not sought Salary (a) £2 250 to £3,405 per annum, (b) £1,550 to £2 630 per annum Child allowance F S S U Family passages

Detailed applications (6 copies) naming three referees by April 1, 1968 by persons living in the Americas and the Caribbean area, to Secretary, University of the West Indies, St Augustine Trinidad, and by all others to Inter-University Council, 33 Bedford Place, London W C 1 Further particulars may be obtained similarly (887)

UNIVERSITY OF ST. ANDREWS

LECTURESHIP OR ASSISTANT LECTURESHIP IN PHYSICS

Applications are invited for the post of LECTURER OR ASSISTANT LECTURER IN EXPERIMENTAL PHYSICS Candidates should have special qualifications or interests in one of the following fields Low Temperature, including solid state, Laser Physics, X-ray Crystallography Further information from Professor J F Allen, Department of Physics Salary scales Lecturers £1 470 to £2,630, Assistant Lecturers, £1 105 to £1,340, starting salary in each case according to qualifications and experience F S S U, grants towards expenses of furniture removal

Five copies of applications, with the names of three referees, should be lodged with the Secretary of the University, College Gate, St Andrews, by March 23, 1968 (856)

BEECHAM RESEARCH LABORATORIES

have a vacancy for a

TECHNICIAN FOR THE TOXICOLOGY UNIT

The successful applicant will be required to work primarily on serum biochemical determination, including enzyme techniques, in a variety of animal species He or she should have had some experience of routine hospital or other pathology laboratory techniques and should have reached the intermediate I M L T. standard Experience of automated or work simplification methods would be an advantage, but is not essential

Applications are invited from young men or women, who would be given the facilities of day release if required These laboratories are recognized by the I M L T for the purpose of study for further qualifications

An attractive starting salary is offered and reviewed regularly, along with generous fringe benefits including sick pay, profit sharing, and non-contributory pension with life assurance schemes

Please write, stating briefly age, qualifications and experience, to **The Personnel Officer, Beecham Research Laboratories, Brockham Park, Betchworth, Surrey.**

(841)

UNIVERSITY OF EXETER

CHAIRS IN BIOLOGICAL SCIENCES

Applications are invited from suitably qualified persons for two Chairs from October, 1969, when, following the retirement of Professor Caldwell and Professor Harvey, the existing Departments of Botany and Zoology will be merged into a Department of Biological Sciences The Professors-Designate will in 1968/69 be expected to act in a consultative capacity in relation to planning courses and accommodation

The salary will be not less than £3,570 per annum

Further particulars may be obtained from the Secretary of the University, Northcote House, The Queen's Drive, Exeter to whom applications should be submitted (fourteen copies one copy from candidates overseas) by April 1, 1968 (846)

UNIVERSITY OF NOTTINGHAM

Applications are invited from Honours graduates in Zoology, Entomology or Agricultural Zoology (including 1968 graduands) for DEMONSTRATORSHIP IN AGRICULTURAL ZOOLOGY in the Department of Physiology and Environmental Studies, School of Agriculture Sutton Bonington Candidates should preferably be interested in Soil Zoology, Acarology or Nematology Salary scale £800 by £25 to £850 per annum

Conditions of appointment and forms of application, to be returned by March 4, 1968, from the Registrar, University of Nottingham University Park, Nottingham, NG7 2RD (842)

Organic Chemists

Vacancies exist in our Process Development Department for Organic Chemists (preferably 24 to 27 years of age) with Ph D degrees or equivalent research experience, and a lively interest in synthetic organic chemistry

The work is largely concerned with the development of processes for new medicinal products, and covers a wide field of organic chemistry The Department is closely associated with both Research Department and the Plant, and is housed in modern, well equipped laboratories in pleasant surroundings

The attractive conditions of service would be discussed at interview The successful candidates, if married, may receive a reasonable refund of removal and travel expenses and, if necessary, assistance in house purchase

Applications giving brief details of age, qualifications and experience should be addressed to

G W Peters,
Senior Personnel Officer,
IMPERIAL CHEMICAL INDUSTRIES LIMITED,
PHARMACEUTICALS DIVISION,
Alderley House, Alderley Park,
Macclesfield, Cheshire

(861)

A large industrial Company in the North of England has a vacancy for an experienced

APPLIED MATHEMATICIAN

of high ability to work on selected projects of great mathematical interest. It is expected that the first project will be the construction of a highly sophisticated mathematical model of a tidal river. The successful applicant would find himself a member of a substantial mathematical group inside a keen technical management team, and would have easy access to first class computer facilities. The starting salary would be negotiable , a salary above £2,500 p a could be offered to a highly qualified and experienced applicant Please apply to Box No 951, *Nature,* T G Scott & Son Ltd , 1 Clements Inn, Strand, W.C 2

(854)

ELECTRON MICROSCOPIST

for Industrial Research

Gillette Research Laboratories have a vacancy for an electron microscopist to work on hair structure and polymers

The work entails the use of ultra-thin sectioning techniques, as well as general electron microscopy Experience in one or both of these fields will be required, and candidates should be qualified to HNC, BSc, or equivalent standard

Please write with details in confidence to,

Dr. N C. Welsh (Ref N.4401), Head of Laboratory,
Gillette Research Laboratory,
454 Basingstoke Road, Reading, Berks.

(938)

UNIVERSITY OF MANCHESTER
MANCHESTER, 13

LECTURERS AND ASSISTANT LECTURERS IN CHEMISTRY

Applications invited for a number of posts vacant now or from October 1968 It is hoped to make at least one appointment in each of the fields inorganic, organic theoretical No field of interest excluded from consideration Salary ranges per annum Assistant Lecturer, £1 105 to £1 340 , Lecturer, £1,470 to £2,630 F S S U

Particulars and application forms (returnable by March 22) from the Registrar Quote ref 20/68/Na (914)

NEW ZEALAND

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Applications are invited for the undermentioned vacancy

SCIENTIST

Vacancy B 13/18/66/533

Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand

Salary Up to $6,830 per annum is available according to qualifications and experience

The position would suit either an established Scientist or a recent graduate with training in microbiology with interests in one of the following fields—ruminant digestion, microbial ecology or ruminants, pregastric fermentation, biochemistry of pure species of rumen bacteria and protozoa or rhizobium research These are established lines of work within the Division in which an intensified effort is required

Passages Fares for appointee and his wife and family, if married, will be paid

Incidental expenses Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S W 1 with whom applications will close on March 27, 1968

Please quote reference B 13/18/66/533 when inquiring (915)

UNIVERSITY OF BRISTOL

DEPARTMENT OF ANATOMY

It is hoped to appoint two LECTURERS (Grade II) in the above Department after May 1, 1968 A good medical, dental or science degree is essential together with teaching experience and ability in independent research (e g , neurobiology cytogenetics, electron microscopy, histochemistry)

Initial salary within the range £1 470 to £2,630 per annum, according to qualifications and experience

Applications (eight copies), together with the names of not more than two referees, should reach the Registrar, Senate House, Bristol 2, from whom further particulars may be obtained, not later than Monday, March 18, 1968 (Quote reference AL23)

(920)

SIMON FRASER UNIVERSITY

(Located in the Vancouver area of British Columbia, Canada)

Department of Chemistry currently 24 faculty members has senior-level vacancy for a BIOCHEMIST or PHYSICAL ORGANIC CHEMIST

Enquiries to Brian D Pate, Head, Department of Chemistry Simon Fraser University, Burnaby 2, B C , Canada

(875)

UNIVERSITY OF SURREY

DEPARTMENT OF BIOCHEMISTRY

SENIOR LECTURER

Applications are invited for the post of Senior Lecturer in the Department of Biochemistry Candidates should have a wide experience in the teaching of biochemistry and have a research interest in pharmacological biochemistry and/or biochemical methodology

The commencing salary will be at an appropriate point on the Senior Lecturer Scale £2 520 to £3 150 (bar) to £3 415 per annum with a deduction of 5 per cent for superannuation (F S S U)

Applications together with the names and addresses of not more than 3 referees should be received not later than March 15 1968 by the Academic Registrar (LFG), University of Surrey Battersea Park Road London S W 11 from whom further particulars may be obtained (901)

UNIVERSITY OF OXFORD

LECTURESHIPS IN EXPERIMENTAL PHYSICS

Applications are invited for University Lectureships in Experimental Physics (pure or applied) in the Clarendon Laboratory and in the Department of Nuclear Physics which may be held in conjunction with Fellowships at Magdalen College or St Edmund Hall or (for a woman) at St Anne's College

Applications (four copies) with a statement of career and qualifications and the names of three referees must be received by April 15 1968 For the University Lectureships further details are available from the Secretary of Faculties University Registry Broad Street Oxford , for the College Fellowships from the President Magdalen College , The Principal St Edmund Hall , The Principal, St Anne's College, Oxford (881)

MAGDALEN COLLEGE OXFORD

OFFICIAL FELLOWSHIP IN PHYSICS

The Governing Body proposes to appoint an OFFICIAL FELLOW IN EXPERIMENTAL PHYSICS from October 1 1968 The post may be held in conjunction with one of the University Lectureships currently advertised by the Clarendon Laboratory and the Department of Nuclear Physics from the same date

Applications, with a brief statement of academic record research interests and names of three referees should be sent, not later than April 15, 1968, to the President Magdalen College Oxford from whom further details may be obtained (880)

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

Commonwealth Bureau of Soils,
Harpenden Herts

Duties include preparing abstracts for the abstracting journal SOILS AND FERTILIZERS, which covers the world scientific literature on soils and soil/plant relationships dealing with scientific inquiries within these fields Essential qualifications a degree in agricultural science or in sciences basic to it ability to write good English Reading knowledge of foreign languages and experience or training in information work would be advantages Salary in scale £926 to £2 431 with provision for superannuation Starting salary according to qualifications experience and age Two extra increments after two years' satisfactory service for applicant aged 32 or under on date of appointment

Application forms and full particulars from Director Commonwealth Bureau of Soils Rothamsted Experimental Station, Harpenden, Herts (878)

POSTGRADUATE STUDIES IN PHYSICS

UNIVERSITY OF VICTORIA

VICTORIA BRITISH COLUMBIA CANADA

The Department of Physics offers assistantships for students working towards M Sc and Ph D degrees in Physics Fields of research include theoretical physics astrophysics physics of fluid geophysics and nuclear physics Financial assistance is available in the range from $2 300 to $4 600 per annum

Details of this assistance and of the fields of research may be obtained from Professor J L Climenhaga, Head, Department of Physics University of Victoria, Victoria, British Columbia, Canada (873)

Scientific Officer, Senior Scientific Officer

Metallurgy Division • A.E.R.E. Harwell

Many challenging scientific problems remain to be solved in the development of advanced reactor systems In particular, a better understanding is needed of irradiation effects in high temperature alloys and ceramic fuels Also there is need to apply the latest ideas to develop materials with improved mechanical properties and corrosion resistance for use in nuclear technology A vigorous research programme on these topics is in progress in the Metallurgy Division at Harwell and we have a vacancy for a scientist, with energy and imagination, in each of the two research teams engaged on these studies The work is closely linked both to more applied studies in the Authority's Reactor Group and to more fundamental studies at Harwell and in the universities

The facilities at Harwell for carrying out this work are among the best in the world and include three research reactors, post-irradiation examination cells, particle accelerators, computers, electron microscopes, (a 1 MeV microscope will be installed in 1969), electron microprobes, a scanning electron microscope, low-energy electron diffraction sets, field ion microscopes, and a wide range of mechanical testing equipment

Candidates should have a first or good second class honours degree, preferably in physics, metallurgy or engineering Previous experience in these fields is not essential but some experience of the mechanical and physical properties of solids is desirable There is ample opportunity to keep abreast with modern developments by attending Colloquia and courses run by the Harwell Post-Graduate Education Centre.

Starting salary will be at a point on the scales £995 to £1,685 or £1,870 to £2,310 depending upon age and experience Married officers living beyond daily travelling distance will be eligible for Authority Housing or Assisted House Purchase

Application forms and further details may be obtained from

Appointments Section 'A', A 523034
A E R.E , Harwell, DIDCOT, Berks.

(839)

SIMON FRASER UNIVERSITY

Research Opportunities in Chemistry

The Department of Chemistry of this rapidly growing university, which is situated on the outskirts of the City of Vancouver, has openings for suitably qualified students in the graduate programme leading to the degrees of M Sc and Ph D Qualified honours degree in Chemistry, or A R I C Financial assistance in the form of teaching assistantships and research assistantships, to the value of $3,100 per annum in the first year, will be available to suitable candidates The trimester system in operation at Simon Fraser University allows students to enter the graduate programme in September or January

Interested persons should write in the first place to

The Chairman, Graduate Admissions Committee,
Department of Chemistry,
Simon Fraser University,
Burnaby 2, B.C. CANADA

(874)

SCIENTIFIC INFORMATION OFFICER

(Female)

The Industrial Hygiene Research Laboratories of Imperial Chemical Industries Limited, situated at Alderley Park in Cheshire, are responsible for determining the toxicological hazards of the chemicals used by the manufacturing divisions of the Company As part of this service there is an Information Bureau staffed by female graduates who index items of toxicological interest in the literature and answer enquiries on toxicological subjects

Applications are invited for a vacancy in this department Candidates should be up to 30 years of age and graduates in chemistry (with a biological subsidiary subject), biochemistry, pharmacy or zoology (with chemistry as the subsidiary) Previous experience in information work is not essential

Progressive salary plan profit sharing scheme pleasant rural surroundings

Applications and enquiries should be addressed to **O. B. Sayer, Assistant Staff Manager, I C.I. Limited, Fulshaw Hall, Wilmslow, Cheshire.**

(840)

ANIMAL HEALTH TRUST

Applications are invited from registered members of the veterinary profession for the post of DIRECTOR of the Trust's Small Animals Centre at Lanwades Park Kennett, near Newmarket, Suffolk The applicant should have had research experience relating to the improvement of health and the control of disease in dogs cats and other small animals He should be capable of directing the work of a small but busy centre dealing with these species

Salary according to qualifications, commencing in the range of £3 600 to £4 100 per annum Superannuation under F S S U

Further particulars and form of application may be obtained from the Secretary, Animal Health Trust, 14 Ashley Place, London, S W 1 (799)

UNIVERSITY OF EXETER

ASSISTANT LECTURER IN PSYCHOLOGY

Applications are invited for a post of Assistant Lecturer in Psychology tenable from October 1, 1968

Postgraduate training or research in experimental or clinical psychology is required

Salary scale £1,105 by £75 to £1,180 by £80 to £1,340 per annum Initial salary will depend upon qualifications and experience

Further particulars may be obtained from the Secretary of the University, Northcote House, The Queen's Drive Exeter Closing date for applications (eight copies one copy from overseas candidates) April 16, 1968 (898)

UNIVERSITY OF EDINBURGH

Chair of Geophysics

Applications are invited for the Chair of Geophysics, tenable from October 1, 1968, or from a date to be arranged The Professor will be Head of a newly established department Salary within the professorial range with membership of F S S U

Further particulars may be obtained from The Secretary to the University, Old College, South Bridge, Edinburgh, with whom applications (twelve copies), giving the names of three referees, should be lodged not later than April 1, 1968 Overseas candidates need submit only one copy of their application (853)

UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF AGRICULTURAL BOTANY

Applications are invited for the post of ASSISTANT LECTURER in the above Department with a special interest in Genetics and Biological Statistics Postgraduate experience desirable

Application forms are obtainable from the Registrar, to whom all applications should be sent by April 1, 1968 (746)

UNIVERSITY OF EAST AFRICA

MAKERERE UNIVERSITY COLLEGE, UGANDA

Applications are invited for a Readership in the Department of Zoology Appointment may be on permanent terms (East African citizens only) or on contract terms Appointee must have interests in fresh water ecology hydrobiology and/or ichthyology Salary scale £EA2 540 to £EA2 825 per annum (one E A shilling equals 1s 2d sterling) Salary supplemented in range £495 to £485 (sic) per annum (sterling) in appropriate cases under British Expatriate Supplementation Scheme Part-furnished quarters at rent not exceeding £EA114 per annum Expatriate appointees receive up to five adult economy class air passages on appointment and leave, up to five adult sea passages on termination additional children's air passages for children educated overseas, leave of 3 months after 21 months

Detailed applications (6 copies) naming three referees, by April 11, 1968 to Inter-University Council 33 Bedford Place, London W C 1, from whom particulars may be obtained (888)

UNIVERSITY OF EAST AFRICA

MAKERERE UNIVERSITY COLLEGE— UGANDA

Applications are invited for (a) Lectureship or (b) Assistant Lectureship in Geography available from July, 1968 Specialists in any fields considered existing knowledge of East Africa advantageous Salary (a) £EA1,350 to £EA2,230 per annum, (b) £EA1,125 to £EA1 245 per annum (one EA shilling=1s 2d sterling) Salary supplemented in appropriate cases under British Expatriates Supplementation Scheme in range £265 to £410 per annum (sterling) F S S U Family passages, biennial overseas leave

Detailed applications (six copies), naming three referees by April 5 1968, to Inter-University Council, 33 Bedford Place, London, W C 1, from whom particulars are obtainable (925)

UNIVERSITY OF EAST AFRICA

UNIVERSITY COLLEGE, NAIROBI

Applications are invited for the Chair of Physics Salary £EA3,150 per annum (one EA Shilling=1s 2d Sterling) Salary supplemented in appropriate cases by £505 per annum (Sterling) under British Expatriates Supplementation Scheme Expatriates are usually appointed on two-year tours F S S U Subsidised accommodation Passages for appointee and family (up to five adult passages) on appointment and termination

Detailed applications (six copies), naming three referees by March 31, 1968, to Inter-University Council, 33 Bedford Place, London, W C 1 from whom particulars are available (909)

UNIVERSITY OF THE WEST INDIES

TRINIDAD

Applications are invited for (a) **Lectureship** or (b) **Assistant Lectureship** in Agriculture, Department of Crop Science Applicants should possess a degree in agriculture (or its equivalent) and preferably some experience in teaching and research Tropical experience very desirable but not essential Salary scales (a) £1 550 to £2,630 per annum (b) £1,250 to £1,410 per annum Child allowance F S S U Family passages Triennial study leave

Detailed applications (6 copies), naming 3 referees by April 5, 1968, by persons living in the Americas and Caribbean area to Secretary, University of the West Indies, St Augustine Trinidad, and by all others to Inter-University Council, 33 Bedford Place, London, W C 1 Further particulars obtainable similarly (924)

CAMBRIDGE UNIVERSITY SCHOOL OF Agriculture Applications invited for posts of University Demonstrator and Farm Demonstrator Qualifications an honours degree in Agriculture or Agricultural Science or an honours degree in pure science followed by an adequate postgraduate qualification in Agriculture For University Demonstrator, candidates should also have had postgraduate experience in research and/or teaching For Farm Demonstrator, preference will be given to candidates with previous training in field experimentation Salary scales University Demonstrator, £1,260 by £80 to £1,420 by £50 to £1 470 by £90 to £1,650, Farm Demonstrator, £1,050, £1 105, £1,180 by £80 to £1,420 Starting point according to age qualifications and experience Both posts pensionable under F S S U Contribution may be made towards removal expenses Further details on request —Apply in writing giving full personal particulars and names and addresses of not more than three referees, to the Secretary, School of Agriculture, Cambridge, not later than March 28, 1968 (905)

NATURE Volume 217
MARCH 9, 1968

Can Europe Stay the Same?

The third meeting of science ministers which the OECD is promoting in Paris next week will return to a theme which has not been very far beneath the surface in the past decade—the supposed technology gap between Europe and the United States. This is perhaps inevitable, if only because the ministers who will be meeting in Paris are almost all of them conscious of the need somehow to justify their expenditure in economic terms, which is often taken to imply that the object of European policies in science and technology should be somehow to make Europe as prosperous as the United States. On this occasion, it is also possible that discussion of the technology gap will provide a convenient means by which the Minister of Technology, Mr Wedgwood Benn, can explain just what is to be understood by the recently canvassed concept of a European Institute of Technology. This institute, still nebulous, seems to be most of what is left of the brave talk a year ago of how the United Kingdom would with the mainland of Europe forge what was fondly called a European Technological Community. Other governments than the British will no doubt be fascinated to know whether the institute which the Ministry of Technology has in mind will be a kind of academic body or whether, as has sometimes been suggested, the institute would emerge as Mintech writ large, and presumably diffuse. At the same time, it will not necessarily be a pointless exercise if they are induced by circumstances to consider yet again the contrast between the two sides of the Atlantic, for there is no question that Europe has much to learn.

But what? The greatest danger is that, in making the necessarily somewhat hackneyed comparisons with the United States which have almost become second nature, Europeans may be tempted to consider, as harbingers of industrial change and economic prosperity, attributes of the industrialized American society which are, in reality, no more than consequences. For one thing, it is not a trivial question to ask which comes first, the technology gap or the prosperity gap. There may even be something in the view that research and development are not only preconditions for prosperity but also luxuries which only the well-to-do can properly afford. Certainly it is no accident that both Japan and Italy, when bent on really rapid economic progress, chose to rely much more heavily on licences to exploit other people's inventions than on research and development of their own. And which but the most prosperous nation in the world would have been able to afford the exploits of the National Aeronautics and Space Administration this past decade? The trouble is that this plain truth and others like it have not been nearly as widely appreciated as they should have been. In Britain and elsewhere, there has been quite a clamour in favour, for example, of emulating the United States in unrestrained expenditure on space research and development in the fond belief that this will somehow create prosperity. To be sure, this is a trap which almost everybody should be able to avoid, but many other traps are much more carefully concealed.

The chances are that Europe has in any case already done a great deal to learn one of the most important lessons to be learned—the need to make Europe commercially competitive with the United States. In part, this objective raises problems of scale. On the mainland there is the EEC, which will in time become a powerful device for increasing industrial production and thus, in the end, for supporting a buoyant and stimulating effort in research and development. Positive feedback is what the electrical engineers would call it. In Britain, mergers between previously competing companies have become the order of the day, legislation on monopolies notwithstanding, in part at least because people seem to recognize that it has become difficult for small companies to be at once progressive and comprehensive. To the extent that the Ministry of Technology has deliberately created the climate in which mergers have become fashionable, Mr Wedgwood Benn will be able to preen himself a little in Paris. But everyone will recognize that problems of scale are not the only ones to be resolved before the technology gap will disappear. Management—its quality and skill—should come high on the list of lessons appreciated but not yet learned. Financial institutions will also have to change, and this is likely to be painfully slow. So, too, will governments and, in the long run, it is more than probable that Europe will not be able to close what seems to be the technology gap until the whole fabric of society has been in some way touched. Science and technology have an important part to play, and without them nothing would be possible, but it is folly to expect that they can be the mainsprings of the changes which the ministers wish to see.

The general truth of this proposition is splendidly illustrated by the perceptive study of the relationship between *Fundamental Research and the Universities*

INSTRUMENTATION

This is the time of the year when British universities make plans for spending money on equipment. The Labex exhibition was held in Manchester last month and next week there will be the annual exhibition of the Institute of Physics and the Physical Society at Alexandra Palace. To mark these events, this issue of *Nature* includes two articles on recent developments in laboratory technique (page 913).

which has been prepared by Professor Ben-David of the University of Jerusalem as one of the documents for the meeting of ministers. To summarize his persuasive argument is almost unjust, but it goes something like this. The disparity between the United States and Europe which Europeans are willing enough to acknowledge where the application of science is concerned is also now apparent in basic and fundamental science, however those terms are defined. Professor Ben-David splits a few hairs in adding up Nobel Prizes and published papers in the learned journals, but the essence of his case is qualitative and not arithmetical. American science is vigorous and creative because of the way in which it is organized, with plenty of opportunities for teams of people to compete against each other and to seek out new problems to solve. Not merely are there competing sources of funds for the support of research, but a variety of institutions in which people can pursue their scientific ends. Professor Ben-David calls this entrepreneurship, and in his view Europe falls behind the United States because it lacks the kind of institutional framework within which this kind of activity can be carried forward.

The most distinctive part of Professor Ben-David's argument consists of a plea for the removal of the hierarchical structure of European universities. In his view, Britain is a kind of half-way house between the rest of Europe and the United States, and what he calls the Cambridge–Oxford–London triangle is in every way comparable with the Cambridge complex in Massachusetts. The concentration around Paris of universities and other institutions for research is a similar hotbed of innovation, particularly because of the way the Collège de France provides an analogue for the reputable institutes for advanced study which have sprung up in the United States. But exceptions to the general rule that European science is organized too rigidly, and that it is as a consequence too constrained and even too inhibited, are at once fewer and less effective than they might be.

In the past few months in Europe, this has become a familiar cry. With luck, it may become a clamour in the years ahead, and there is every chance that Professor Ben-David's tract will have an important influence. His statement of the need for a structural upheaval of the administration of academic institutions is persuasive and subversive (which is, no doubt, why the OECD's introduction emphasizes that Professor Ben-David's views are not official policy). The truth is, of course, that even in Britain, which is only comparatively enlightened in these things, there is a tendency to assume that existing institutions must continue to exist substantially unchanged.

In this climate, for example, new kinds of university departments can be created only with the funds which are not required for established disciplinary structures. It is hard to shut down laboratories which already exist but which have outlived their immediate usefulness, but extremely difficult to embark on laboratories which the European Molecular Biology Organization would like to establish. Brooding over all of this is a sophisticated system of committees which has become increasingly devoted to the belief that it is possible to make supposedly judicial decisions about the spending of money in circumstances when it may well be that the only meaningful decisions are those which enable vigorous people to keep on being vigorous. And if this complaint is valid in Britain, Professor Ben-David is right in saying that Britain and France are luckier than most other European nations. He is also right to imply that it would be possible for Europe as a whole to attend to these structural problems without compromising its attitude towards other aspects of the comparison between Europe and the United States. The point is that a more flexible, and a more European, framework for the administration of fundamental research in Europe is desirable in its own right. The fact that it would help to close whatever gap there may be across the Atlantic is an extra benefit.

Science Policy Meeting

On March 11 and 12, ministers of science and education from 22 different countries will be meeting in Paris at the headquarters of the Organization for Economic Co-operation and Development. Representing Britain will be Mr Patrick Gordon Walker and Mr Anthony Wedgwood Benn. Among other subjects scheduled for discussion is one of Europe's recurrent preoccupations, the technological gap between Europe and the United States. The ministers will be discussing a report produced by OECD which sets out to establish the factual basis for the technological gap. The report, which will not be published in full until after the Paris meeting, studies nine sectors of industry—computers, scientific instruments, pharmaceuticals, plastics, non-ferrous metals, man-made fibres, iron and steel and machine tools. The report assesses the performance of the various industries both as performers of research and development and as industrial innovators, and attempts to relate these to industrial prosperity.

The ministers will also be discussing fundamental research policy, and scientific and technical information systems and policies. The discussion on fundamental research will hinge on several reports published by OECD in recent years; the most recent is an independent survey carried out for the organization by Professor Joseph Ben-David, professor of sociology at the Hebrew University of Jerusalem. Professor Ben-David concludes that European universities are inefficient; this, he says, is because chairs are established on the assumption that one man is capable of representing, more or less on his own, an entire discipline for about the lifetime of a generation. This, Professor Ben-David says, is palpably out of date, and he suggests instead the formation of large multidisciplinary research institutes able to award at least research degrees. Professor Ben-David recognizes the danger that institutes of this kind, far from being centres of innovation, often become bulwarks of traditionalism, and he suggests that they should be experiments in the true sense of the word. "Whatever schemes are adopted or considered, the expected objects should be defined and the results tested and published" The discussion next week is intended to make these ideas more precise, and the OECD is certainly hoping that a pilot scheme for a project of this sort may be approved

The final discussion will be on the problems of information retrieval As well as the technical difficulties of retrieval, there are distinct dangers that the various types of retrieval system now being developed will be incompatible If the United States devises a retrieval system which Europe is unable to plug into, many of the advantages of the system will be lost

Not Enough Uranium

THE rapid expansion of nuclear power has meant that electrical utilities, particularly in the United States, have had to take a hard look at the supplies of uranium Like most mineral resources, the stocks seem to be limited Some argue that this will enforce a much greater reliance on coal fired stations—or, at the other extreme, on the successful development of fusion reactors More optimistic observers point out that this fear of shortage is a familiar part of mining operations, without which no mining company would ever feel like prospecting Between 1935 and 1965, for example, petroleum reserves ranged from ten to fourteen times annual requirements Despite or, some might argue, because of the potential shortage, exploration kept ahead of demand

The European Nuclear Energy Agency and the International Atomic Energy Agency have now got together to produce revised estimates of uranium resources Briefly, the conclusion is that not enough has yet been found Substantial new reserves are needed if estimated demand is to be met The report says that "there is no reason to suppose that such deposits will not be found if a massive, well-financed and well-conceived prospecting effort is undertaken However, the magnitude and urgency of the effort that will be required cannot be too strongly emphasized"

Uranium is widely distributed throughout the world, although Canada, the United States and South Africa have the lion's share The report estimates that the amount of uranium available at a sensible price—less than $10 per lb of U_3O_8—is about 7,000 short tons, with a further 126,000 short tons available as by-products of other industrial processes For a higher price, of course, much more is available In the range between $10 and $30 per lb, there are probably a further 1,400,000 short tons For comparison, the US Atomic Energy Commission estimated in 1966 that the probable world demand for U_3O_8 would be 65,000 short tons by 1980 Assured reserves at a fair price probably therefore represent ten to fifteen years supply, and the estimate means that annual production will have to be more than trebled by 1980

All this is going to make uranium mining a growth industry over the next ten years. On the face of it, there is no great cause for alarm—large areas of the world have yet to be thoroughly prospected, and little is known in the West about reserves in Communist countries The sea may also provide an alternative source of uranium, and the United Kingdom Atomic Energy Authority has estimated that the sea contains some 4,000 million tons of the mineral. This could be extracted at a price, although it seems to be too great a price to make the process immediately attractive Technological development will also help, and the most important element in this is likely to be fast breeder reactors, which will make much better use of uranium-238, converting it to plutonium and burning that as fuel While modern water reactors reflect a dollar increase in the price per lb of U_3O_8 by an increase in generation cost of 0 08 mill per kWh, fast breeders will be less sensitive to raw material costs.

Science School

THE Science Foundation for Physics within the University of Sydney is to hold its second International Science School for high school students from August 26 to September 6 this year The school, directed by Professor H Messel, who first conceived the idea, will be on the general theme, "Man in Inner and Outer Space" Twenty students from the United States, Japan and Britain will join one hundred and four students from Australia and New Zealand for the course of twenty lectures given by Australian and overseas scientists—seven of the lectures, for some reason, will be on America's projected manned Moon landing

Overseas scholarships were first offered last year to ten American students This year is the first time that the scholarships have been extended to Britain and Japan The ten American students will once again receive their awards personally from President Johnson Similarly, the Japanese Prime Minister, Mr Eisaku Sato, will sponsor the five Japanese representatives The British students, on the other hand, will have to be content with the Royal Institution, under the patronage of the Duke of Edinburgh The five British recipients of the scholarships will be known as the "Royal Institution Australian Science Scholars" The five lucky students were chosen from schools all over the country with the assistance of the Association for Science Education. All the winners will get a round-the-world air ticket with a two-week stay in Sydney for the Science School

More Chemists Needed

How is industry to attract its fair share of science graduates? The chemical industry is tackling this problem by organizing a conference with the theme "Modern Chemistry in Industry", to be held at Eastbourne between March 11 and 14 An attendance of about 1,000 is expected at the conference, including representatives from industry, government and the trade unions, but the majority will be science teachers and lecturers from the schools and universities.

The problem to be faced is two-fold—the flow of school leavers into the sciences needs to be increased, and more graduates must be encouraged to work in industry The organizers of the conference point to a lack of balance in the educational system. The drift away from science in the schools continues, leaving unfilled places in university science departments, while the arts faculties are having to turn away highly qualified candidates Even if the school leaver can be persuaded to enter a university science department, he is likely to fall prey to the notion widespread among graduates that research by industrial organizations is in some way inferior to that done in the universities

The conference will try to improve industry's scientific reputation among science teachers, in the hope that some of their enthusiasm will filter through to their pupils. To this end the conference will mainly consist of a series of lectures on various aspects of contemporary industrial chemistry, designed to illustrate the volume and quality of industrial research.

The Royal Society is sponsoring the meeting through the British National Committee for Chemistry. The majority of the speakers come from the chemical and allied industries, which are also paying for the conference. The speakers include Lord Beeching, a deputy chairman of ICI, Lord Sherfield, chairman of the Industrial and Commercial Finance Corporation, and Dr M. E. Spaght, a managing director of the Royal Dutch Shell Group. The president of the conference is Lord Todd, professor of organic chemistry at the University of Cambridge.

This is not the first conference to be held along these lines. The Science Research Council, in association with the Careers Research and Advisory Centre, staged an experimental summer school in Oxford last year to persuade postgraduate students carrying out research in university science departments to consider management as a career. The students and the managers from industry who attended must have benefited from the free exchange of ideas encouraged by a summer school.

It is difficult to see how a similar exchange of views, essential if managers are to become more aware of the academic scientist's impression of industry, will be possible within the formal framework of the Eastbourne meeting. The chemical industry is nevertheless probably correct in realizing the important part played by the schools in influencing choice of careers. If it can demonstrate to the teachers that industrial research is as challenging and as intellectually demanding as academic work, it may go some way towards helping its own recruitment of scientists.

Whence Foot and Mouth?

Lamb from Argentina was the probable source of the British foot-and-mouth epidemic that first broke out on a Shropshire farm last October and has since led to the slaughter of some 443,000 animals. This conclusion—hotly disputed by the Argentinian Embassy in London—is made in the report compiled by the Ministry of Agriculture and published this week.

The report bases its case on the circumstantial evidence that Argentine lamb was supplied to the farm where the epidemic first began, and on the failure to discover any other possible source of infection. In an admirably thorough piece of detection, Mr John Reid, the Ministry's chief veterinary officer, has established that the disease could not have been introduced to the Shropshire farm by the movement of humans or animals, and he considers that, although there were 3,000 cases of the disease last year in Western Germany, it could not have been transmitted either by the winds or by birds. "The distance is too great for the disease to have been brought from Germany to Shropshire by birds", he says, and presumably—though this is not stated in the report—the route does not correspond to any migratory pattern.

Ruling out imported hay and a previous outbreak of the disease in Warwickshire, Mr Reid narrows down the possible source of infection to imported meat. The butcher who supplied meat to the Shropshire farm received New Zealand lamb and Argentine lamb from his distributor. It is not known which kind of lamb the farm received, but New Zealand is free from foot-and-mouth whereas in Argentina the disease is endemic. The owner of the farm fed bones to his dogs and although he boiled them first he "did not claim that this was always done effectively". The disease first broke out in two gilt pigs and the bones fed to the dogs "could have been carried into the yard or neighbouring fields where the gilts had been". From this reconstruction of the crime, the report says, "it was not possible to establish conclusively that imported frozen lamb carried foot-and-mouth disease virus to the farm, but, as all other generally recognized sources of infection had been eliminated, it remained the most probable vector". The majority of subsequent outbreaks were probably the result of carriage by birds and the wind, but the large number of cases which occurred simultaneously over a wide area suggests a common primary cause. The Argentine lamb supplied to the Shropshire farm came from a consignment of 770 carcasses, all from a particular establishment in the Argentine, which was distributed over a wide area of the country. This was the area of the greatest concentration of the infection in the first month of the epidemic.

The only type of virus isolated in the epidemic was O_1. Although this type is prevalent in South America, it is not yet known whether it is prevalent in the area which supplies the establishment from which the suspected consignment was supplied.

How did the virus bypass the measures for its detection? The Argentinian Embassy in London has pointed out that there is a permanent veterinary staff attached to the British Embassy in the Argentine which collaborates with the local authorities in inspecting hygienic conditions in canning factories and slaughter houses. The staff was established on the recommendation of the Gowers Report, presented in 1954 after the last serious outbreak of the disease in Britain, but it has no power to sample carcasses intended for export to Britain for the disease. This, the British Ministry of Agriculture has stated, is the responsibility of the Argentinian Veterinary Service. It is impracticable to sample the carcasses coming into Britain because the heads and feet, which bear the most obvious signs of the disease, have been removed.

The most recent cases of the present epidemic occurred on February 21. The Ministry of Agriculture hopes that these will be the last, but is keeping its fingers crossed until the three-week incubation period of the virus has passed.

Carnegie Research

A review of the research being done at the six research departments of the Carnegie Institution of Washington is given in the institution's latest annual report for 1966–67. The departments concerned are the Geophysical Laboratory and the Department of Terrestrial Magnetism, both in Washington, DC, the Department of Plant Biology at Stanford, California, the Department of Embryology at Baltimore, Maryland, the Genetics Research Unit, Cold Spring Harbor, New York, and the Mount Wilson and Palomar Observatories, Pasadena, California, operated jointly with the California Institute of Technology

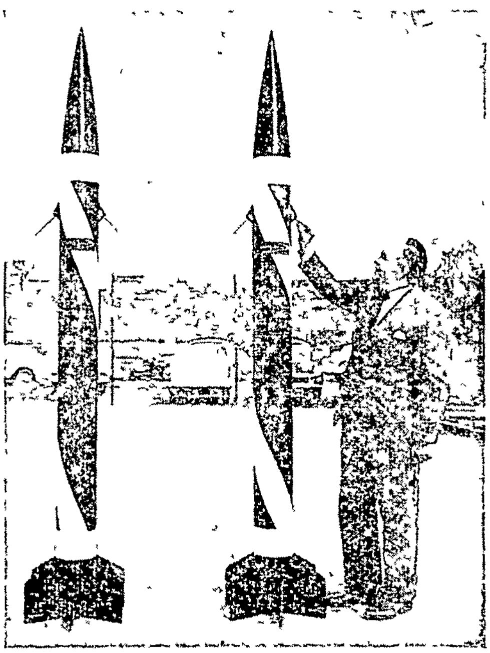
Research in the astronomical field during the year included the discovery of more information about quasars, and the discovery of a probable physical inter-galactic connexion between our galaxy and the Magellanic Clouds—scientists from the Instituto Argentino de Radioastronomía working at the radio telescope constructed by the institution at La Plata, Argentina, investigated what seems to be a bridge of neutral hydrogen between the Milky Way and the small Magellanic Cloud

Workers at the Department of Terrestrial Magnetism have found that the Earth's crust is very thin around Washington, DC They have constructed a map showing the thickness of the crust from North Carolina to central Pennsylvania The map shows that the crust varies in thickness from 30 to 60 kilometres, with the thinnest area underlying Washington and adjacent Virginia, and the thickest areas beneath the Appalachian Mountains. The Biophysics Section of this same department is interested in the biochemistry of evolution It has reported work on the search for repeated DNA sequences in different species representing all the main forms of life, from viruses to humans The investigators found that fifty-five different DNAs from plants and animals showed DNA sequence repetition In other words, in each case, a large fraction of the genetic material consists of many copies of short DNA sequences or packets of genetic information—only bacterial and viral DNA failed to show sequence repetition It is believed that the repeated DNA sequences are slowly mutated or changed during the course of evolution Thus the copies (members of families of repeated sequences) do not remain identical The degree of divergence may be a measure of the age of the family of nucleotide sequences—this divergence may be very relevant to evolutionary history From time to time certain segments of DNA are copied and new families of many identical members are produced

Among other research reported is the discovery at the Department of Plant Pathology of the way to make some common plants double their growth by reducing the amount of oxygen in the air surrounding them

New Research Rocket

THE proving trials of a new British research rocket have been completed and a series of test flights have started in which scientific experiments are carried The rocket is the Petrel, a small solid fuel vehicle designed to provide high altitude research facilities, mainly for universities, at low cost It was developed for the Science Research Council by the Ministry of Technology's Rocket Propulsion Establishment, the Atomic Weapons Research Establishment and Bristol Aerojet Ltd Petrel can reach altitudes of up to 140 km and can carry a payload of 13 kilograms, which has to be reduced if telemetry facilities are required



The first of the series of test flights was launched successfully on February 3 and a second firing took place on February 29, both from the South Uist range in the Hebrides The experiments carried on these flights were designed by the Radio and Space Research Station at Slough to investigate high speed electrons from space bombarding the atmosphere The flights were tests in the sense that their purpose was primarily to evaluate the behaviour of the rocket rather than to gather scientific information

Several more Petrel rockets are planned to be launched from South Uist this spring, carrying experiments for university research groups These include payloads from Birmingham University to investigate ions and electrons in the atmosphere, from Southampton to study sporadic layers of ionization and from Sheffield to study fine structure effects in the ionosphere

Rules for Heart Transplants

THE Board on Medicine of the US National Academy of Sciences has issued a set of guidelines for heart transplants In a statement published on February 28 it says that because cardiac transplantation in man cannot yet be regarded as an accepted form of therapy, but only as a scientific exploration of the unknown, the operation should only be carried out in institutions where a special set of criteria can be met The surgical team, the statement says, should have extensive laboratory experience of heart transplantation and a thorough understanding of the biological processes involved in rejection and its control—immunologists should be readily available to collaborate in the operation

The statement goes on to say that the primary justification for transplanting hearts, with respect to donor and recipient, is that the knowledge so gained will be of benefit to other members of society The ethical issues involved are part of the whole problem of the ethics of human experimentation, which are at present being studied intensely, as the statement says, by a number of well-qualified groups in the United States and other countries Until some ethical guidelines are available the Board on Medicine can only advise every institution to assure itself that it has protected the interests of all parties involved to the fullest possible extent. The donor, the board says, should be unanimously selected by doctors who are not directly involved in the transplantation effort Their opinion as to the acceptability of the donor should be based on crucial and irreversible bodily damage and imminent death The prospective recipient should also be examined by an independent group of doctors, including a cardiologist and an immunologist Their decision should be based on all the evidence, including the presence of advanced, irreversible cardiac damage and the likelihood of benefit from the procedure

School Leavers

VOLUME 2 of *Statistics of Education*—one of at least five volumes covering different educational subjects—has been published this week by HMSO (price 20*s*) This particular volume deals with General Certificate of Education examinations, Certificate of Secondary Education examinations, and school leavers

From the numerous lists of figures, a rising trend in the number of students qualified in GCE can be seen In fact, during the ten years ending in 1966, the number of passes at O-level almost doubled The biggest increases were in economics (and associated subjects)—490 per cent—and in the other social science and vocational subjects group—200 per cent Naturally enough, the greatest number of entrants were in English and mathematics, with geography and French not far behind Physics also seems to have been popular, with an average of 54 7 per cent passes Successes in the A-level examinations showed an increase of nearly 150 per cent during the last decade and the only decrease was in botany which fell by 9 per cent English literature and physics were the two most popular subjects The total of 140,000 CSE candidates for 1966 was more than twice the number for 1965, with entries for boys averaging 4·3 subjects each and for girls 3 2 subjects each

In 1965–66, 38 5 per cent of all leavers attempted O-level, and of these 21 per cent obtained five or more passes Of over 15 per cent who attempted A-level, 11 per cent obtained two or more passes The data also show that 16·5 per cent of leavers went on to further education 7 5 per cent each to universities and to other full-time further education, and 1 5 per cent to colleges of further education

Regional statistics show that the proportion of all leavers going to full-time further education was above the national average in Wales and in the south of England except Greater London, and that the number leaving with two or more A-levels was highest in south-east and south-west England A similar trend was shown for those leaving with five or more A-levels, with a percentage of 24 7 per cent for Wales closely following the figures of 26 8 and 26 4 for the south-east and south-west respectively

Committee on Education

THE British Parliament has now proceeded with the plan to set up a specialist committee of the House of Commons to deal with educational topics The membership of the committee, announced last week, includes ten Labour members, seven Conservatives and no others, a source of some frustration to the dozen Liberals in the House of Commons As well as being large, the select committee is also eminent. It includes a former Conservative Minister of Education, Sir Edward Boyle, and a former Labour Minister, Mr Fred Willey, who was Minister of Land and Natural Resources until that ministry disappeared Before that he was for many years a Labour spokesman on education The Labour Party, with almost as many teachers in the House of Commons as the Conservatives have lawyers, ran the risk of turning the committee into little more than a teachers' lobby. This seems to have been resisted, although several of the members are former teachers—Mr Christopher Price, Mr Trevor Park and Mr Richard Mitchell have all taught at schools, and Mr Stanley Henig is a former university lecturer Among the Conservatives there are no former teachers, except Mr Richard Hornby, who taught for two years at Eton There is, however, Mr Esmond Wright, who until his election as member for Glasgow Pollock was professor of modern history at the University of Glasgow

The final terms of reference of the committee are a compromise In its original form, the motion establishing the committee put down by Mr Richard Crossman, Leader of the House of Commons, did not mention Scotland, and the committee had no Scottish members This was later changed, and the committee is now empowered to examine the activities of the Scottish Education Department as well as the Department of Education and Science It now has two Scottish members But this has not satisfied members from Scotland, who are hard to satisfy They say, quite truthfully, that Scotland has an entirely separate system of education, and deserves a committee to itself—a committee empowered to examine the work of the Scottish Office as a whole

Mr Crossman has made it quite clear that the committee is not a committee on education. Rather, it is a committee charged to examine the activities of the Department of Education and Science and the Scottish Education Department—it is a departmental rather than a subject committee. Mr Crossman already seems halfway to admitting that it was a mistake to establish the committee on this basis "It is arguable that subject committees are, on the whole, better and easier to work It may be in the final resort that we come only to subject committees, but we were pledged to try out a departmental committee " There is now a select committee on agriculture and one on education, both organized on departmental lines This has been done, it is thought, because Mr Anthony Crosland and Mr Fred Peart were the two ministers initially most enthusiastic about specialist committees Mr Crosland has now moved on to the Board of Trade, leaving Mr Patrick Gordon Walker, still having an unhappy time as a minister, to handle the select committee as best he can Mr Peart, still Minister of Agriculture, is probably finding the committee on agriculture more of a problem than he bargained for.

The committee will be meeting in public, and its chairman will be Mr Fred Willey As its first subject, it has chosen to examine the Inspectorate of Schools This is the body which certifies the standards of all schools in Britain, and is likely to prove a tepid subject politically The committee has clearly decided to play itself in gently, and is likely to start the examination by summoning civil servants rather than ministers from the Department of Education and Science

New Degrees

RIPPLES from the permissive society are beginning in Britain to be felt in the strict world of the PhD degree, where ordinances are being revised Bristol is the latest university to relax its requirements so that graduates of Bristol of eight years standing can now submit "substantial published work pursued in the university or elsewhere" for a PhD These conditions supplement and do not replace the conventional supervised postgraduate work in the university

Other universities have already introduced similar schemes That at Cambridge has been in action for more than a year and a considerable number of applications and enquiries has been received The idea is to provide some kind of recognition for the work that individuals, such as teachers or industrial research workers, have published, often as a result of studies in their spare time

Mathematics for All

ONE of the principal recommendations of the Dainton Report, published on February 29, is that all pupils should study mathematics until they leave school It seems from the report that a high proportion of pupils with O-level passes in this subject abandon it in the sixth form, but the Dainton Committee suggests that the overwhelming majority are capable of benefiting from its continued study It considers that mathematics is a "key factor" in restoring flexibility to the present situation, and stresses that England and Wales are exceptional in western Europe in abandoning science and mathematics for a significant proportion of those in school over the age of 15

One difficulty with this proposal may be the shortage of science teachers paragraph 86 of the report itself states that there is a shortage—especially in mathematics How then is mathematical teaching to be increased when teachers are themselves in short supply ? The latest available figure for the number of mathematicians whose qualifications can be regarded as equivalent to a degree who are teaching mathematics in schools is 11,033 for 1965—an increase of only 252 over the figure of 10,781 for 1962 Moreover, in a sample of secondary schools, 36 per cent of mathematics teaching was given by teachers with no qualifications in mathematics But the report stresses that the way in which these teachers are used and the support they are given is probably as important as their actual supply It also emphasizes the encouraging fact that recent policies for the recruitment of young scientists into teaching have been quite successful, a survey in 1965 showed that 40 per cent of science teachers (including mathematics) with second class honours were under 30 compared with 33 per cent for languages

Dr Bryan Thwaites of Westfield College, this year's president of the Institute of Mathematics, feels sure that the various professional institutes will back up the proposal, and maintains that it is right both educationally and from the point of view of manpower supply He is surprised, however, at the committee's assurance that the quality of science teachers is being maintained, instead of declining In his experience, at least one headmaster has suggested that he could not obtain science teachers of sufficiently high quality

EMPLOYMENT OF MATHEMATICS GRADUATES IN MAINTAINED AND DIRECT GRANT SCHOOLS—UNIVERSITY GRADUATES ONLY

	1962	1963	1964
1st class honours	512	504	507
2nd class honours	1,523	1,581	1,663
3rd class honours	3,445	3,434	3,432
Total	5,480	5,519	5,602

The headmaster of the City of Bath Boys' Grammar School, Mr L H Scott, thinks that "everyone ought to know the language of mathematics", but thinks that a great many pupils will not be willing to be pushed into it He emphasizes the necessity for a new type of course, particularly in the sixth form Like Dr Thwaites, he believes that there is a definite fall in the quality of science teachers and expressed concern that the shortage is likely to become more acute within the next ten years—a study of the age distribution of teachers has shown that a high proportion of those with first class honours are approaching retirement He thinks that teachers in this age bracket are still to a large extent holding the fort

Professor Sir Nevill Mott of the University of Cambridge is also an advocate of a new mathematics course What the report fails to bring out, he suggests, is that pupils are unlikely to study mathematics beyond O-level unless they are good at it, for the simple reason that they do not think it will help them in securing a university place He suggests that there should be a new, less advanced mathematics course which makes use of mechanical aids and films, and that universities should be encouraged to accept reasonable performance in such a subject offered by pupils hoping to read biological subjects at university

Parks and Countryside

THE eighteenth report of the National Parks Commission for the year ended September 30, 1967, has recently been published (HMSO, 8*s*. 6*d*) This is probably the last time that the report will appear in its present form, for the commission's functions will be altered when the Government's Countryside Commission comes into being—though when this will be is uncertain, for a parliamentary standing committee is still considering the Countryside Bill which had its second reading in November 1967 The National Parks Commission, which reports to the Minister of Housing and Local Government and the Secretary of State for Wales, gives the impression in its report that it does not welcome the proposed Countryside Commission with open arms—"it seems certain that the Bill will not give us all that we asked for in the way of financial and executive independence " The commission, however, "looks forward" to the wider responsibilities implicit in the new Bill

At present, the National Parks Commission is continuing its job of preserving the ten National Parks and other "areas of outstanding natural beauty" in England and Wales Last year, orders designating two new areas—Anglesey and the South Hampshire coast—were made by the commission, and plans were advanced for further areas including the Kent Downs, the Norfolk coast and Dedham Vale Some of these orders have since been confirmed Early in 1967, when a large estate in the heart of the Snowdonia National Park came on to the market, the commission urged the Government to purchase an area to which public access could be secured Though the estate was sold privately, the Secretary of State for Wales announced in November 1967 that the Government had purchased from the new owners a critical area of some 13,000 acres including the summit of Snowdon and Llanberis Pass. The Countryside Commission will replace and extend this work of the National Parks Commission in the context of the promotion of the countryside generally The National Parks Commission seems to think, however, that the National Parks will continue to occupy a central position in the new organization The progress made in each park during the year is recorded in the report

During the year under review, links with the Council of Europe were strengthened by the appointment of the National Parks Commission as the United Kingdom correspondent to a new European Information Centre on Nature Conservation As a result of the work of the Standing Committee set up by the second "Countryside in 1970" conference, the commission has also been invited to act as the United Kingdom "focal point" for all information activities relating to the countryside

One of the functions of the commission is to make proposals for the creation of long-distance footpaths and bridleways In this connexion the commission submitted information and recommendations to the Committee on Footpaths appointed by the Government in 1967. An important job of the commission is the consideration of proposals for development affecting the landscape in designated and other areas of coast and countryside During the year, 302 such proposals were considered, of which one-third were within areas of outstanding natural beauty One of the most important of the proposals was one for a North Sea gas terminal at Bacton on the Norfolk coast (see *Nature*, **217**, 10, 1968) At the public inquiry, at which the commission was represented, the Minister of Housing and Local Government agreed that the works proposed would involve "a massive and alien intrusion into the coastal landscape". He allowed the development, however, to the "great distress" of the commission The commission points out that the inquiry did at least establish the technical possibility of taking sea gas treatment plant away from the coast, previously considered impractical

Science in Parliament

The Parliamentary and Scientific Committee, an organization which sets out to keep both Houses of the British Parliament informed on scientific and technological subjects, has elected a new chairman He is Mr David Ginsburg, Labour Member of Parliament for Dewsbury since 1959, and he takes over from Mr Arthur Palmer Mr Ginsburg, who is 47 this year, was educated at University College School and Balliol College, Oxford, where he got a degree in politics, philosophy and economics For some years he was secretary of the research department of the Labour Party. Mr Ginsburg, whose political views are in the mainstream of the Labour Party, has been a member of the Select Committee on Science and Technology since its formation His contributions to the sessions of the committee are always pleasant and well informed, which suggests that he is admirably equipped to be chairman of the inter-party Parliamentary and Scientific Committee

Parliament in Britain

from our Parliamentary Correspondent

Mr Robert Woof, a mining Member of Parliament, won a tiny victory on February 23, when he asked the Minister of Power which nuclear power station he expected to be producing electricity at 0·54*d* per unit by 1970. Mr Marsh had to admit that none would, and was generous enough to point out that the best coal-fired stations, Ratcliffe and Cottam, coming on stream in 1968, would generate at 0 54*d* and 0·56*d* per unit respectively Dungeness B, the advanced gas cooled station due to start in 1971, would produce power at 0 57*d* per unit (Written answer, February 23)

Concorde

Mr Wedgwood Benn, Minister of Technology, announced approval for the production financing of the Concorde supersonic airliner The amount of money would be large, he warned The net working capital needed over the next five years would be of the order of £100 to £125 million, and the companies were arranging for their banks to advance up to £25 million, which the Government would guarantee Mr Benn said that the rate of production would be three a month, although it depended to some extent on firm orders Only Mr Hugh Jenkins struck a dissident note Did the minister not agree that the proposal was a gross misuse of public funds ? That depended, Mr Benn said, on whether it was a success If it was a great success, he said, it would be a profitable investment (Statement, February 27)

Bristol Siddeley

Later in the week, Mr Benn made a statement about the report of the committee of inquiry into the pricing of contracts made with Bristol Siddeley Engines The committee, under Sir Roy Wilson, said that the company had budgeted for, and achieved, exorbitant profits on overhaul contracts "The approximate extent of the profitability on these contracts was, at the time, known to the company at all levels of management We do not accept that the overcharging has been justified by any of the arguments put forward by the company " The negotiations amounted to intentional misrepresentation, despite this, Mr Benn said that the Attorney General, after consultation with the Director of Public Prosecutions, had decided that the evidence did not justify proceedings The company had already paid back some £3 96 million (Oral answer, February 27)

Chapelcross Breakdown

Mr Tom Swain asked the Minister of Technology how long it would be before the nuclear reactor at Chapelcross would be in operation again It was shut down after an accident in May 1967. Mr Benn said that remedial work was making good progress, but the Atomic Energy Authority could not say how soon the station would be back on stream The loss of sales of electricity up to the end of February had cost £0·8 million Mr Benn also told Mr Swain that the growing volume of exploration work should ensure enough uranium for the United Kingdom nuclear power programme at reasonable prices A price variation of 10 per cent meant a change of 1 25 per cent in generating costs, he said (Written answer, February 29)

NEWS AND VIEWS

Biosatellites are a Waste of Money

THE National Aeronautics and Space Administration (NASA) is currently asking the United States Congress for $32 5 million to finance bioscience programmes during the fiscal year 1969 It is perhaps no coincidence that NASA also convened a symposium at the National Academy of Sciences in Washington on February 23 and 24 to publicize the preliminary results of the Biosatellite II mission, even though the data from most of the experiments were still only incompletely analysed and essential post-flight controls had not even begun That might not have mattered, of course, if there had been particularly exciting results But there were not, and the proceedings of the symposium are unlikely to win the biosatellite programme many new friends among the scientific community Indeed, they give more ammunition to the many and eminent biologists who believe Biosatellites I and II were a waste of money and time Congress, however, even though it is cutting the budgets of many government agencies, may not think the same way

Biosatellite II was essentially a repetition of Biosatellite I, which was successfully launched in December 1966 but which, because of a failure in retro-rocket ignition, was not recovered And as the NASA engineers and administration explained in the first session of the symposium, Biosatellite II has also had its technical problems First, during the final count-down on September 7, 1967, there was a delay of about three hours because of minor faults in the spacecraft and launching vehicle which unfortunately meant that the biological experiments were kept on the Earth three hours longer than expected This greatly reduced the value of an experiment with fertilized frogs' eggs Second, although the satellite was successfully launched into a nearly circular orbit at 315 km, the planned three-day flight had to be curtailed to 45 hours because of a storm threatening the recovery area in the Pacific so that the experiments were not run to completion. Third, and most important, the vibrations to which the satellite was subjected during the launch were apparently greater than anticipated, so that the pre-launch controls of the effects of vibration and acceleration on the experiments have to be repeated at the Ames Research Center beginning on March 12

The thirteen experiments which were flown in Biosatellite II were apparently selected from some two hundred proposals submitted to NASA, and they fall into two main classes First, those designed to study the effect of weightlessness (or, more accurately—at least until the effects of vibration have been cleared up—space flight conditions) on the growth and development of the plants and frogs' eggs The rationale behind these experiments is clear, because both plant growth and frog embryogenesis are known to be sensitive to gravity The second class of experiments was designed to tell whether organisms are more deleteriously affected by radiation under space flight conditions than on Earth Presumably the experimenters argued that if weightlessness has any effect on cellular metabolism, this might show up as an increased mutational rate which is indicative of an upset in the cellular repair mechanisms

Without going into great detail—the full symposium proceedings are to be published in *Bioscience* later this year—the results of the first class of experiments involving plants can be summarized briefly All the responses during the flight, the epinasty of pepper plants, the growth and orientation of wheat root and coleoptiles, the behaviour of the so-called statolith starch granules in wheat seedlings as revealed by histochemistry and the distribution of a few enzymes in wheat seedlings, also occurred in controls on klinostats on the Earth's surface As Dr K. V Thimann, the general chairman of the symposium, said in his summary, there is no real basis for believing that zero gravity causes any responses different from those due to balanced gravity Equally, space flight has no effect on an experiment to measure the growth, feeding and cell division of the multinucleate amoeba *Pelomyxa carolinensis*

Perhaps the most interesting experiment flown on Biosatellite II was the one intended to find out whether frogs' eggs (sp *Rana pipiens*) develop normally under zero gravity Since Schultze's classic experiment in the late nineteenth century, evidence has accumulated that gravity plays a part in determining bilateral symmetry and normal development of the embryo The stage of development most sensitive to disorientation with respect to gravity is immediately after fertilization, and sensitivity decays steadily during the onset of the first cleavage The best experiment for Biosatellite II would clearly have been to fertilize the eggs in space This apparently was too difficult, so eggs were fertilized before launch, held at 6° C and then heated in space Unfortunately, the three-hour delay in the launch was critical to the experiment It meant that by the time the eggs were in orbit, they had reached the middle of the first cleavage stage, although they had not completed cleavage The result of the experiment was that the eggs developed quite normally from this stage during the flight and subsequently after recovery Weightlessness and the vibrations during launching do not therefore affect development beyond the first mid-cleavage stage That is not a trivial statement, but because of the three-hour delay the most gravity sensitive period in development was not tested

The six experiments designed to test for an enhancement-effect between radiation and space flight conditions involved a fairly wide range of organisms two plants, *Tradescantia* and *Neurospora*, and three insect species, *Drosophila*, the flour beetle *Tribolium*, and a parasite wasp *Habrobracon* As expected, the response of cells to radiation in space flight varies greatly from one type of cell to another depending on the metabolic state of the cells, just as on Earth. But what was not expected was that there does seem to be a slight but statistically significant increase in the sensitivity of some cells to radiation damage under space flight conditions The biggest effect detected was with *Drosophila* scored for ' dumpy wing" mutation The flight group had 14 mutants out of the total 20,562, whereas the ground control had 4 mutants out of a total of 24,838 Strontium-85 in the satellite was the source radiation in all these experiments

These radiation experiments at first sight do suggest that weightlessness has some enhancing effect on radiation damage, but the interpretation is complicated because vibration or some other factor in the space flight environment may be responsible Clearly there can be no definite conclusion until thorough post-flight vibration tests have been performed at the NASA Ames Laboratory And NASA might be more highly respected if, in its public statements on Biosatellite II, it had not tried to gloss over this and other problems NASA blandly asserts that "the only condition flight organisms experienced was weightlessness", yet Dr O E Reynolds, Director of Bioscience Programs, in his summary of the symposium in Washington, said the immediate problem was to clear up the vibration radiation interaction problem which he described as the "muddiest issue" Similarly, the NASA public statement asserts that "all 13 experiments carried on the satellite were successful", but does not mention the critical three-hour delay which greatly reduced the significance of the experiment with frogs' eggs

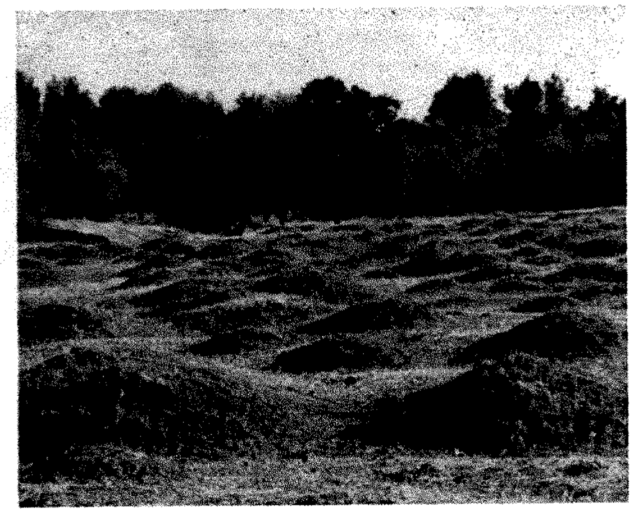
Have the results of the Biosatellite II justified the cost of the mission ? And what implications do they have for future biosatellite programmes ? Dr C A. Wilson of the Ames Research Center, who was in charge of the launchings of Biosatellites I and II, estimated that about three-quarters of the NASA bioscience programme budget was being spent on biosatellites and that the combined cost of Biosatellites I and II was $50 million But according to at least one of the experimenters, this is a conservative estimate Obviously it is impossible to get a unanimous decision about how much money any particular scientific fact or piece of research is worth, but the $50 million NASA has spent has really shown only two things First, zero gravity and balanced gravity have the same effect on plant growth and development, and, second, that some factor in the space flight environment appears to enhance the deleterious effect of radiation on some cell types There is always the argument that it is impossible to predict the effects of space flight, so the experiments had to be done once But how many biologists honestly believed that $50 million would not have produced far more significant information if it had been spent by the National Science Foundation, the National Institutes of Health or even on the exobiology sponsored by NASA ? Dr Thimann must surely have been voicing the thoughts of most American biologists, including many of the most eminent, when he said forthrightly at the end of the symposium that there was no real basis for requesting further flights carrying experiments similar to those on Biosatellite II Unfortunately, that is unlikely to deter NASA

What implications do the results of the Biosatellite II have on the four biosatellites that are scheduled for flight in the next three years ? Dr Reynolds said there was nothing in the results of Biosatellite II which suggested to him that the experiments already scheduled for the next four flights should be changed That may well be true for the two thirty-day missions to study the physiology of Macaque monkeys during space flight Those are legitimate experiments in which the bioscience programme could contribute significantly to the manned space flight programme, space medicine and basic physiology What other justification can there be for the biosatellite programme, especially at a time when the budgets for NSF and NIH are being held back ? The two other flights already planned, however, are for 21 days, and two of the proposed experiments must surely be critically re-examined The first is to observe the morphogenesis of the plant *Arabidopsis thaliana* under weightless conditions, and the second is to study the effect of weightlessness on human cells in tissue culture In the light of the Biosatellite II results, can either of these experiments be justified ? If NASA really wants its biosatellite programmes to be taken seriously by the scientific community, and that is certainly not the case so far, it would do well to abandon both these experiments, possibly replacing them by a proper experiment on frog embryogenesis with fertilization during flight If NASA perseveres with its biosatellite programme unchanged, it can only expect that most biologists will remain determined not to be associated, no matter how big a financial carrot is dangled in front of them

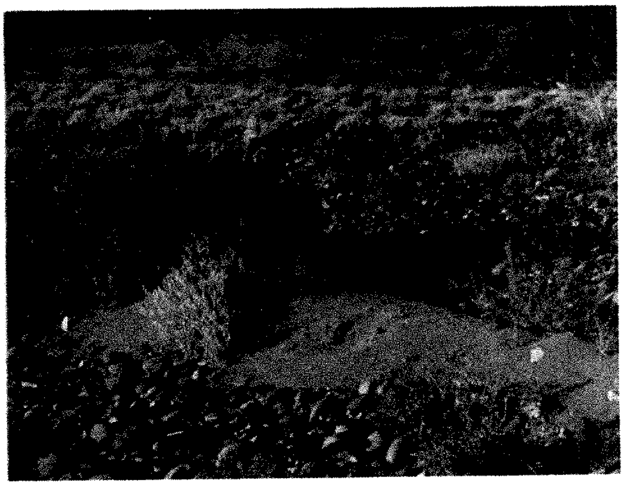
Earth Mounds

PICTURES of sand dunes formed in the lee of a hedge during a Yorkshire dust storm and reproduced recently in *Nature* (**216**, 20, 1967) have stimulated Harold A Gangmark and F Bruce Sanford of the United States Bureau of Commercial Fisheries, Seattle, to write in support of their theory for the formation of earth mounds (*Fishery Bulletin* Vol 6, No 1) These earth mounds can be found in the flood zones of rivers in many parts of America and probably other parts of the world, but none of the many theories of how they are formed has yet been universally accepted. Mr Gangmark and Mr Sanford, as fishery biologists, are interested in flooding and soil erosion because of the effect on fish and incubating spawn, and believe that an explanation of the way in which mounds are formed will indicate how streams behave and which one will be most suitable for fish rearing.

Plants such as brickellbush growing in the flood path of a stream are believed by Mr Gangmark and



Mounds near Red Bluff, California.



Silt being deposited downstream of a plant.

Mr Sanford to be the primary cause of the mounds. During flooding, the plants slow down the stream to such an extent that the mud being carried along is deposited and a pile of silt builds up in an elongated shape downstream from the plants. The stream flow produces ellipsoidal mounds with their axes parallel in the direction of flow. Any plant in a flood plain is thought to produce the beginnings of a mound, but only brickellbush and other plants with strong roots and long lifespan will stand up to continuous pressure to form large mounds, some of them more than three feet high and twenty feet long. Mounds have been found in places where streams no longer run, even when in flood. In many cases this change of environment kills the plant which caused the mound, leaving no visible remains, but Mr Gangmark and Mr Sanford have found old roots at the upstream end of all the mounds investigated.

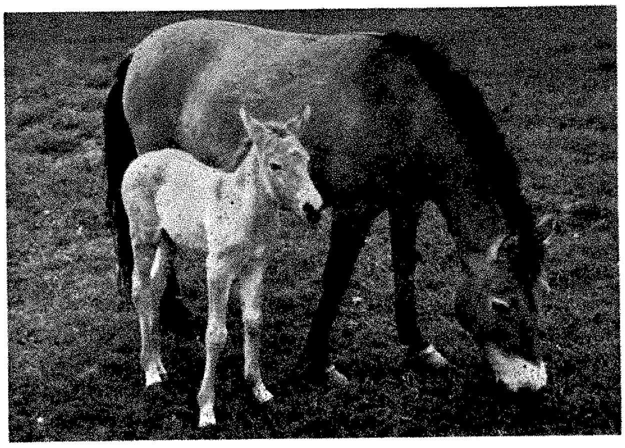
This theory for the method of formation of the mounds is reinforced by comparison with the dunes formed in dust storms. The transport medium is different: air for dunes and water for mounds, but the dunes build up in ellipsoidal shapes behind clumps of hedge like large-scale mounds, suggesting that the methods of formation are similar.

Rare Horses

THE Przewalski horse, one of the rarest mammals in the world, has excited interest for several reasons. It is believed to be the horse pictured in the Caves of Lascaux by Stone Age man ten to fifteen thousand years ago and it is considered by most authorities to be the ancestor of modern breeds. At one time herds roamed wild over Europe and Central Asia, but the numbers gradually declined as civilization spread. The modern form was found less than a century ago, in 1879, by Colonel N. Przewalski in a restricted area in the region of the Gobi Desert on the borders of China and Outer Mongolia.

It was named *Equus przewalskii* by Poliakof in 1881 to distinguish it from the modern domestic horse, *E. caballus*. Unfortunately, once discovered, the numbers of Przewalski horses in the wild declined further. Many were captured and sent to zoos. Quite recently it was thought that the truly wild horse had become completely extinct and the species was only to be found in captivity—even those in zoos are not all true Przewalski horses, because many of those originally captured were thought to have been hybrids between Przewalski horses and Mongolian ponies run wild. There may still be some horses left, however, for in 1966 a herd of eight (a stallion and seven mares) was sighted in Mongolia near the Chinese border. Unfortunately, the herd galloped off into China and so little may be known about it for some time. Several skeletons of course remain, and zoos have bred from the original captured horses. The latest *International Zoo Yearbook* gives figures of 64 male and 83 female Przewalski horses living in 35 zoos between February and August last year. The mare and foal pictured here are in the Zoological Society of London's garden. The foal was born in 1966, but it is not certain whether it is a true Przewalski horse as it has a lighter mane and tail than usual.

There have been several papers and books written on the Przewalski horse and its phylogenetic relationships. The Prague Zoo has been outstanding in keeping records of the horse, and a pedigree book is compiled there every year. Dr R. M. Stecher of the Western Reserve Medical School, Cleveland, Ohio, has been particularly concerned with the anatomical characteristics of *E. przewalskii* which distinguish it from other Equidae. In a recent paper (*Acta Zoologica et Pathologica*, No. 43, 45 ; 1967) he gives results of vertebrae



Przewalski mare and foal (The Zoological Society).

counts from spines of 61 skeletons of the Przewalski horse and he correlates these with counts from four other horses—the domestic horse (*E. caballus*), mule (*E. caballus* × *E. asinus*), donkey (*E. asinus*), and hemione (*E. hemionus*). He has found that Przewalski horses are differentiated from other Equidae by having the longest thoracic segment of the spine, the next-to-the-longest lumbar segment, the shortest sacral segment and the next-to-the-smallest number of lateral joints in the lumbar region. Stecher attempts to correlate the spinal characteristics of the five horses with their chromosomal complements. The chromosomal counts of the horses are found in the following descending order: Przewalski horse $2n=66$, *E. przewalskii* × *E. caballus* $2n=65$, *E. caballus* $2n=64$, *E. caballus* × *E. asinus* $2n=63$, *E. asinus* $2n=62$, and *E. hemionus* $2n=54$. Stecher has found that there is a complete correlation between the decreases in chromosome counts and the decrease in presacral vertebrae. There is also a partial correlation with the increase in the anatomical as well as the functional length of the sacrum, additions to the functional length of the sacrum by ankylosis of caudal vertebrae, and the increase of lateral joints in the lumbar region. It may be, therefore, that in the course of equine evolution the chromosome number and the number of presacral vertebrae have decreased, and the numbers of sacral vertebrae and lateral joints have increased.

More Plant Taxonomy

Some of the latest developments in plant taxonomy were discussed on March 1 when the Experimental Plant Taxonomy Group of the Linnean Society held its tenth annual symposium.

The section Eu-Cochlearia of the genus *Cochlearia*, scurvy-grass, has always given trouble; Linnaeus described four species and since then there has been considerable taxonomic confusion. Mr J. J. B. Gill of the department of genetics in Liverpool has been investigating cytologically the relationships between thirteen species of *Cochlearia*. Within these species chromosome numbers of 12, 14, 25, 26, 36, 42 and 48 occur, and Mr Gill has found that hybridization between chromosome levels is easy, except when crosses are made between diploids ($2n=12$ or $2n=14$) and octoploids ($2n=48$). Analysis of chromosome pairing in hybrids showed Mr Gill that all the diploid species which he examined are essentially the same; the species with two sets of seven chromosomes have arisen from those species with two sets of six, by aneuploidy—one chromosome has been added to the basic number of six. Evidence for this comes from intercrossing these species, when at meiosis the chromosomes show three pairs and one trivalent or six pairs and one univalent.

Mr Gill found that all thirteen species of *Cochlearia* are related, and that autoploidy has been very important in their evolution. For example, *C. officinalis* ($2n=24$) when crossed with a $2n=12$ species shows between one and six trivalents, and in a cross between two populations of *C. officinalis*, one from Sweden and the other from Italy, there was strong secondary association of bivalents. This clearly suggests that *C. officinalis* developed by autoploidy from a $2n=12$ species.

Miss Brenda Matfield of the department of botany at Westfield College, London, has been hybridizing species of the sub-series Tormentillae of the genus *Potentilla*. The species concerned are *P. reptans* ($2n=28$), *P. erecta* ($2n=28$) and *P. anglica* ($2n=56$). The last is thought to be an alltetraploid of the other two—at meiosis it shows only trivalents—but Miss Matfield has so far been unable to synthesize it. Hexaploid natural hybrids are common and three have been given binomials: *P.X. suberecta* (*P. anglica* × *P. erecta*), *P.X. italica* (*P. erecta* × *P. reptans*) and *P.X. mixta* (*P. anglica* × *P. reptans*). Experimental hybridizations have indicated that *P.X. mixta* may arise in two ways, by hybridization between *P. anglica* and *P. reptans*, or by the fusion of an unreduced gamete of *P. reptans* with a reduced gamete of *P. erecta*. Miss Matfield has shown, by backcrossing *P.X. suberecta* with the parent species, that introgression is theoretically possible from *P. erecta* to *P. anglica* through the hybrid, although this is not known in the field.

Attempts to hybridize *P. erecta* and *P. reptans* were unsuccessful, and Miss Matfield suggests that *P.X. italica* is not a separate species and that the name is a synonym for *P.X. mixta*—the descriptions for the two are very similar.

Dr M. J. Lawrence, Dr J. S. Gale and Mr A. E. Arthur at the department of genetics in Birmingham have been investigating the causes of genetic diversity in *Papaver dubium*, the long-headed poppy. The character which they have studied is time of flowering, and for the purpose they set up a synthetic natural population in 1964. In this, natural conditions were simulated as nearly as possible—hand raking and light weeding were carried out in the autumn, but no fertilizer was used. Outbreeding was dismissed as a possible cause of genetic diversity when no overall difference between the mean expression of flowering time was found between crossed and selfed progeny of seven plants sampled from this population. The possibility that genetic variation is a result of environmental heterogeneity of habitat was also discounted when poppies were sampled from a fairly wide climatic range and showed no correlation of climate with flowering time.

The third possible cause of the genetic diversity is selection, and Dr Lawrence and his colleagues have found that this is the operative factor. They found that early flowering poppies seem to be the fittest in that they produce the most viable seed, but populations do not become uniformly early flowering because dormancy is also involved.

Some of these poppies germinate in the autumn and some in the spring, and many of the former are the early flowering types. Thus when the winter is mild and many autumn germinators survive, these will be the fittest, but when the winter is severe there will be more spring germinators, and these, which will in this case produce the most viable seed, will be the fittest. Because the selective advantage fluctuates from one season to another, progress towards the fixation of one genotype with respect to flowering time is retarded and genetic diversity is maintained.

Voltes Face

from our Molecular Biology Correspondent

Last week (*Nature*, **217**, 803; 1968) I described three papers dealing with the sub-unit structure of aspartate transcarbamylase and its relation to the function of the enzyme The central finding (Gerhart and Schachman, *Biochemistry*, **7**, 538, 1968) was that there are six subunits, two bearing catalytic and four bearing regulatory sites This unique situation was considered in terms of an allosteric scheme for the enzyme Hard on the heels of these papers comes a study of the same enzyme by Weber (*J Biol Chem*, **243**, 543, 1968) from which it appears that these results are in error, inasmuch as aspartate transcarbamylase has in fact a rather more commonplace constitution, being made up of four regulatory and *four* catalytic sub-units

Whereas Gerhart and Schachman worked with the fragments obtained when the enzyme is dissociated by treatment with an aromatic mercurial, and assumed these products to represent monomeric chains, Weber performed molecular weight determinations in 8 M urea Sedimentation equilibrium led to values of some 42,000 and 23,000, respectively, for the catalytic and regulatory chains These results were supported by N-terminal residue determinations and amino-acid analysis one N-terminal alanine and one threonine were found for an equivalent weight of 73,000, and the amino-acid compositions for the two sub-units together reproduced closely the composition of the intact enzyme It therefore now seems safe to conclude that the protein is an octamer and that the catalytic and regulatory chains each possess one active site The number of sites (four of each kind in the native enzyme) was reported by Changeux, Gerhart and Schachman, who have discarded an earlier estimate of eight regulatory sites

The moral yet again would seem to be that it is hazardous and unnecessary to assume that monomeric chains are present under any but extreme denaturing conditions Numerous molecular weight determinations of proteins in guanidine hydrochloride solutions—the use of which has been urged notably by Tanford and by Harrington—are now known to have given correct monomer molecular weights, and it is, one must suppose, an indication of the success of this approach that its re-discovery on this side of the Atlantic should already have been described (Ullmann, Goldberg, Perrin and Monod, *Biochemistry*, **7**, 261, 1968)

It may be mentioned that Sia and Horecker (*Arch Biochem*, **123**, 186, 1968) are now in agreement with the four-chain structure of the enzyme aldolase, long a subject of contention They elaborate, moreover, on the remarkable degradation of the polypeptide chain of this enzyme in quite mildly acid solution The possibility that such phenomena may seriously vitiate molecular weight determinations in special cases must be recognized

T_4 Regulation

from our Cell Biology Correspondent

DURING the replication of T-even phages, gene functions appear in a time sequence, which is why most viral functions have been classified as either early or late Refinements in methods of analysis have, however, shown that this is an oversimplification Levinthal, Hosoda and Shub, for example, have found that early proteins are synthesized asynchronously, they are not a single co-ordinately controlled set There are also sequential changes in the population of phage *m*RNA molecules (see *Nature*, **216**, 117, 1967) But only recently, with the improvements in the sensitivity of DNA/RNA hybridization techniques, has it been possible to measure accurately gene control at the level of *m*RNA transcription In *J Mol Biol* (**31**, 325, 1968), Bolle, Epstein, Salser and Geiduschek describe DNA/RNA hybridization experiments which begin to show the complexity of the regulation of T_4 transcription

Twenty minutes after infection, a third to a half of the phage *m*RNA molecules are late *m*RNA species The remainder of the population of *m*RNA at 20 minutes consists of early-type molecules which are also present 5 minutes after infection, when the late species are between a hundred and several thousand times less abundant There is no difference in the average stability of early and late *m*RNA It is also clear that the whole T_4 genome is not translated for a brief period immediately after infection, some late species seem not to be made at all in the first 5 minutes after infection Bolle *et al* also mention briefly other results to be published in detail later Apparently there are three classes of early *m*RNA molecules which are turned on rapidly in succession during the first 5 minutes of infection Synthesis of the majority of these RNA species is then reduced several-fold after between 5 minutes and 20 minutes, during the period in which the late *m*RNAs are switched on Even so, a few of the RNA species present after 5 minutes increase during this period

It is clear that regulation of T_4 gene expression cannot be solely at the translational level, and the most reasonable explanation of the 200 to 4,000-fold increase in late *m*RNA between 5 and 20 minutes is that late *m*RNA transcription is specifically switched on in this period What the switch mechanism is and whether all late genes are activated at the same time remains to be seen

In the same issue of *J Mol Biol* (**31**, 463, 1968), Chrispeels, Boyd, Williams and Neidhardt report that during T_4 infection of *E coli*, a new valyl *t*RNA-synthetase activity appears with an accompanying decrease in the amount of the original *E coli* valyl *t*RNA-synthetase The T_4 induced enzyme differs in sedimentation properties and thermal stability from the *E coli* enzyme, but heavy labelling experiments prove that it is formed from polypeptide chain synthesized before infection Chrispeels *et al* believe that the phage-induced enzyme is a modified form of the host enzyme, possibly a dimer This conversion is specific (arginine, aspartic, leucine, phenylalanine, and serine-*t*-RNA synthetases of *E coli* are not modified by T_4 infection) and it is sensitive to chloramphenicol For these reasons, Chrispeels *et al* suggest as a working hypothesis that a new or altered valine *t*RNA produced by T_4 promotes the conversion of the host enzyme Whether the new enzyme plays an important part in the physiology of the phage is undecided, but since conversion of all the host enzyme occurs by about 15 to 20 minutes after infection, it is just possible that the conversion is involved in the regulation of early and late protein synthesis

Globin Metabolism in Thalassaemia

from our Medical Biochemistry Correspondent

THALASSAEMIA is a chronic haemolytic anaemia which is quite common in some parts of the world, particularly around the Mediterranean. The condition is due to an abnormal recessive gene which affects haemoglobin synthesis. Heterozygotes (with only one thalassaemia gene) usually show a mild chronic anaemia with a low haemoglobin concentration in the red cell. This condition, known as thalassaemia minor, can be more serious if the patient also has some abnormal haemoglobins. The homozygous condition known as thalassaemia major or Cooley's anaemia is a very serious disease which is usually fatal before the patient is adult.

The obvious explanation is that this is a serious defect of haemoglobin synthesis in thalassaemics, which have a substantial amount of foetal haemoglobin in their blood. It seemed probable that there was also a defect in the structure of the red cell stroma (the insoluble part of the red cell), for the cells have an abnormally short life-span. Recent work has shown that homozygous thalassaemics cannot synthesize the β-chain of haemoglobin, and that they also produce much more of the α-chain than is necessary for combination with the γ- and δ-chains synthesized. Labelling experiments have indicated that, in erythroid cells, the ratio of α-globin to γ-globin plus δ-globin was 3·0, but this ratio was never found in mature cells. The ratio there was 1·0, corresponding to the presence of normal haemoglobin A_2 and haemoglobin F.

It has now been shown (Bergollesi, Pontremoli, Manini and Conconi, *European J. Biochem.*, **3**, 364; 1968) that the excess of α-globin formed in reticulocytes from patients with thalassaemia major becomes associated with the stroma. Blood samples from nine patients with thalassaemia major and two normal controls (and bone marrow samples from two patients) were incubated in an amino-acid mixture containing tritium-labelled valine, leucine and lysine. In the control preparations, the activity of both cytoplasmic and stromal proteins increased for two to five hours and then remained constant, with very little label in the stromal proteins. In the patients' samples, the total cell label remained the same after five hours but the label was redistributed. The label in the cytoplasmic proteins increased rapidly for the first five hours and then decreased slowly, while the label in the stromal proteins increased slowly throughout the twenty-five hour incubation period.

Chromatographic separation of the cytoplasmic proteins showed that initially the ratio $\alpha/\gamma+\delta$ globins was 3·0, but as the tritium concentration in the cytoplasm decreased the ratio dropped to 1·0. Nearly all the labelled protein in the stroma was α-globin. The authors could find no α-globin in the stroma of mature red cells from thalassaemics, indicating that the α-globin must be further metabolized or firmly associated with the membrane in some way. It is tempting to assume that this reaction is the reason for the fragility of thalassaemic erythrocytes. Certainly further work on this interaction may indicate some of the factors affecting red cell stability.

Regulation of Lambda Genes

from a Temperate Bacteriophage Correspondent

It looks as though the temperate coliphage lambda is becoming another gold mine for molecular biologists. The phage has recently been attracting increasing attention, largely because it provides an experimental system for tackling problems of gene regulation at a higher level of complexity than those presented by the venerable lactose operon of *E. coli*. In the lactose operon, a single repressor protein prevents the expression of the three structural genes of the operon; similarly a lambda repressor prevents the expression of the entire phage genome, which has some thirty to fifty genes (see *Nature*, **217**, 509; 1968). Unlike the lactose genes, however, the genes of lambda are not all expressed co-ordinately after the repressor is removed, but instead they divide into several groups which differ in both the time and the level of expression during phage growth. Thus current interest in lambda centres on the mechanisms which control this ordered expression. Which lambda genes are controlled directly by the lambda repressor and which are controlled by other means during phage growth? A possible alternative to direct repression could involve regions of the phage genome which can be transcribed only by a phage specific RNA polymerase which might appear after the initial stages of infection.

Pereira da Silva and Jacob (*Virology*, **33**, 618; 1967) have just reported an investigation of the control of the expression of two genes, $C_{\rm II}$ and O, which function early in the growth cycle. $C_{\rm II}$ is involved in the establishment of the lysogenic (totally repressed) state and O is required for the vegetative replication of the phage DNA. Pereira da Silva and Jacob asked whether $C_{\rm II}$ and O are under direct repressor control or whether they are controlled by some other gene which is in turn under repressor control. Thomas (*J. Mol. Biol.*, **22**, 79; 1966) has proposed that the N gene, which maps adjacent to the lambda repressor gene and is itself controlled by the repressor, may control the expression of other lambda genes; he suggested that the expression of O requires the presence of the N gene product so that O is only controlled indirectly by the repressor through the mediation of the N protein. Pereira da Silva and Jacob's experiments, however, indicate that both O and $C_{\rm II}$ are directly controlled by the repressor and that they can be expressed in the absence of the N protein.

In their first experiment, they used lysogenic *E. coli* in which the prophage (the totally repressed phage chromosome) had a defective N gene; they irradiated these cells with ultraviolet light to inactivate the repressor and then showed that the $C_{\rm II}$ and O functions appeared in the cells. They detected the presence of $C_{\rm II}$ and O gene products by showing that they were supplied to superinfecting phages which lacked functional $C_{\rm II}$ and O genes. For both $C_{\rm II}$ and O the magnitude of the "rescue" effect increased if they delayed superinfection after irradiation, which implies that the $C_{\rm II}$ and O gene products accumulate after inactivation of the repressor.

In a second and similar experiment they used a cell lysogenic for the defective prophage *t*11 which, after induction, fails to produce a functional O gene product. These rescue experiments showed that the *t*11 genome also does not produce the $C_{\rm II}$ function; this finding lends further support to previous suggestions that $C_{\rm II}$ and O belong to a single operon.

Pereira da Silva and Jacob have thus shown that the $C_{\rm II}$ and O genes can be expressed in the absence of the N gene product, although their experiments do not eliminate the possibility that the N gene product enhances the expression of $C_{\rm II}$ and O during normal infection.

Memory and the Increase of Knowledge

by

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In his Henry Tizard Memorial Lecture, delivered at Westminster School on February 29, 1968, Professor Young discussed the increase of knowledge and its storage in the brain.

THIS is an absurdly wide canvas I like to think that Henry Tizard would have approved of its objectives, though he might have been suspicious of its breadth I got to know him fairly well when he became president of Magdalen and I was his vice-president It was not altogether an easy task. His aims and ideas were on such a scale that I think he found it hard to be patient with our donnish concern with detailed studies, especially those not apparently directly relevant to current problems It is indeed difficult for the technologist, anxious to make immediate practical improvements, to know how to be patient with those who claim that theirs are the fundamental investigations, although they are not at present of any use. This is one of the problems that lies at the root of our worries about how to organize our search for knowledge and how to assess the results of research

I propose that we should handle it by considering that this is a problem about how the brain works This, of course, involves a whole host of questions about the nature of knowledge—whether it is a proper use of words to say that the brain acquires knowledge, if so, how does it do it, and many more questions In view of all that philosophers have said on these topics it is obvious that the scientist cannot really have the information to handle them But this knowledge about the brain, though it is so rudimentary, is growing very fast indeed. We have got to begin to get used to living with it.

A great deal is known by psychologists about the conditions under which memory operates, especially in man and the rat Almost nothing is known about its physical basis in the brain We do not even know what sort of thing or system the record or engram may be Before we can hope to find it, we must answer the very awkward question "What is the status of the concept of 'a record' ?". We are so familiar with the use of books, pictures, computer memories and other forms of physical records that we may find it hard to realize how complex they are They are all physical systems that in some sense have been made to represent or stand for other systems. What is meant by saying that one system represents another ? The words only have meaning of systems that have been devised by certain operations involving some conventional system of coding and addressing The process by which words of, say, the poet Dylan Thomas written on a page, represent for the reader a village in Wales is really most complex Similarly, a given point in the memory of a computer only represents part of, say, a bank overdraft if the numbers that pinpoint its position in a set of registers are activated at the right position in a sequence essentially of questions asked according to the programme with which the record in the memory was written The point in the computer memory in no other sense represents the bank balance, any more than the blot of ink of the L in Llaregyb represents the village of Laugharne in West Wales

Is the memory of the brain, like written or computer records, something highly addressed, with each point only of significance within the programme ? We do not know the answer to this and that is why I say we have almost no information about the physical nature of memory There is a strong presumption from what we do know about the organization of the nervous system that each unit for receiving and storing is specific for some particular feature that is relevant to the animal's life The method of encoding used is, in general, to put each item of information into a distinct channel. Thus in the cat the visual field is dissected in the sense that each cell of the primary visual cortex responds to an illuminated bar or contour, set at a particular angle. Other cells respond to more complex figures, but we do not know how the response to a whole scene, say, a saucer of milk or a dog, is computed by this multiplicity of channels There can be little doubt that within a few years we shall find out, and so perhaps gain great help with one of the most important technological problems of today—the design of machines for pattern recognition. Only when we have got far enough to know what the record is like shall we really be able to study how it becomes changed by learning But we can begin by dealing with units such as we know are present for the detection of particular features How could they be changed as a result of experience ?

The value of a memory to any system depends on the presence of more than one possibility of action in any given situation In the Pavlovian conditioned reflex the sound of a given note can produce either more saliva or less, or no change We have investigated the means by which such changes occur in octopuses, which, when a figure such as a horizontal rectangle is shown to them, can be taught either to come out to attack it or to retreat We can localize the part of the brain in which the record is written Moreover, octopuses can also learn to pull in a rough plastic ball and reject a smooth one, and the record ensuring this has been shown to be in a different part of the brain from the visual record

Remembering how the nervous system encodes, we postulate that for, say, horizontal rectangles there are special detector cells in the visual memory, and similarly there are detectors of roughness in the tactile memory

Each detector (or "classifying cell") must then be able initially to produce either the positive or negative action, whereas after training it produces only one or the other We have some evidence about how this limitation of possibilities occurs—perhaps through the secretion by certain small cells of substances that inhibit the unwanted channel Each classifying cell and its attendant cells thus constitute a unit of memory or mnemon, storing one bit of information Each mnemon is switched in one direction or the other by the action of nerve fibres signalling that a good taste or a trauma has occurred There is thus built up what may be called a model in the brain (following Kenneth Craik) This model indicates which of the possible actions that the animal can take is likely to be appropriate in the light of what has happened to it in the past

This concept of a model is quite helpful in a general way But the problems remain of finding out how it is used, how items of information are retrieved from it If it is made of units such as I have mentioned, how are they addressed, how is the action of millions of them combined to produce the behaviour of the whole organism ? We simply do not know the answers to these questions and it may be that it is wrong to try to isolate units of any sort Evidence is conflicting as to how far the records of particular events is localized Since Lashley it has been traditional to regard engrams as dispersed, because local lesions do not remove the record for individual items An attractive suggestion is that the record is like a hologram, in that it is dispersed and that activation of a part (in the appropriate way) evokes the whole There is, however, some evidence that there is more localization than Lashley supposed Thus electrical stimulation of one particular point on what Penfield calls the experiential cortex of the hemisphere of a patient under local anaesthesia may repeatedly elicit reports of a single scene Of course, these sites may not contain the actual record but be functionally connected with the neuronal record The nature of what is reported suggests that the report is itself part of the classifying system Reports are often of watching or hearing, seldom of acting, speaking or feeling

Perhaps what we have to think of is not so much how items are looked up in a great brain book as how the brain operates to solve each problem. We can even see a little of how the model grows in the developing brain, all the time serving the child's needs every day Children do not learn to speak by rote Finding the right words at 10–15,000 neuromuscular events/minute is not a matter of some demon darting about the brain looking them up in an addressed dictionary It is the result of the use of a speech-producing system, developed partly by maturation, partly by learning[1] First, the child's babblings acquire tonal contours, then single words are used as whole sentences, such as "daddy", with various intonations Then, strictly in sequence, come the use of two and three words, often one strong word, a so-called grammatical functor or "pivot", such as "it", "here" or "more", paired with a word from the growing vocabulary At each stage the speaker is making the response to input that is most likely to produce a satisfactory result in view of past experience The input finds the address by operating the system in the way that has been successful in the past

Such a system might be developed by the switching of mnemons There are said to be twelve places in the airway where antagonistic muscles produce speech Any classifying cell, say, one recording red light, may influence (indirectly) each of these points If it gives the "correct" response when red light occurs, the child will make a noise a bit more like "red" and will be rewarded, if only by approval, and the probability of this channel will be increased Once again we must be careful not to assume that what is being learned is a simple set of conditioned responses as Skinner once tried to demonstrate This position was subjected to a devastating criticism by Chomsky, but we need not be so frightened by his polemic as to give up the search for the changes that take place during the acquisition of speech It is not impossible that "at its origin grammar may well be acquired by mechanisms as naive as those of object-learning"[2] It is well to remember that object naming itself remains to some extent a flexible and creative process The category "a house" does not really refer simply to the class of all houses It is a word used in a certain way in speech—including, for example, house of God or House of Lords As Lenneberg[1] puts it, "Words are not the labels of concepts completed earlier and stored away, they are the labels of a categorization process or family of such processes"

Probably, however, the machinery of the brain contains systems the very nature of which we have not yet glimpsed. The timing devices and temporal sequences that are involved in nearly all behaviour patterns have been completely left out here, but may be essential features of the memory record Whatever the systems may be, we shall not find them unless we look And in order to look we must have words and hypotheses with which to describe and discuss our findings

This is the state of the study of one aspect of cerebral function today I have used it because, of course, it is the only subject that I know even a little about But perhaps it may serve as an example of what one field of research means today and of its significance for all of us Such studies of the brain have their immediate practical usefulness in education and medicine and surgery, by no means negligible But it would be grotesque to say that we only investigate them with these uses in mind The methods by which we think, remember and know are the background of all our activities A sound system with which to discuss them is likely to be productive of useful and satisfactory ways of proceeding in almost any other field of human action, certainly in all those that involve the brain

Of course, the achievement of such a system has been the aim of philosophers and scientists at least since Plato It seems to me that improvement of our system has been proceeding at an accelerating rate in recent years We may, of course, be deceived in this and certainly not all parts of our society show even a relatively secure confidence about the bases on which life can be lived I think this is a great pity and that we should actively proclaim that such insecurity need not continue.

The bases on which a useful system can be built become more secure every year, from the discoveries—for they are truly discoveries—of philosophy, linguistics and aesthetics, as well as those of science and technology How are we to ensure at the same time the further progress of discovery and the making widely available of its fruits both of detailed practice and of general theory ?

It has been in British academic life that many of the most important intellectual and scientific developments have begun I will not try to make a list for either philosophy or science, but I venture to think that in both fields our system has carried us far ahead of most countries, and many in those countries think so too. The combination of teaching and research that is made possible in our universities through the UGC system is in many ways the envy of the world We want to open it for large numbers of people and to give them a broader training, but let us be very careful that in doing so we do not give up its special virtues and relapse into mediocrity As some students put it to me the other day "We fear they want to turn the universities into battery farms for broiler technicians". Most people will agree that we need technologists, business administrators and many sorts of specialist, and that it is a large part of the function of universities as well as technical colleges to supply them Students themselves are anxious that their studies shall enable them to find their role (as they put it) in society Those who take degree courses that do not satisfy this role-seeking are the focus of unhappiness in universities all over the world[3] They complain that their courses do not prepare them to operate in the real world Interestingly enough, one group of complaints is against the breadth of education (as many as twelve subjects in one term in one course) Such complaints are common not only in North as well as South American universities but in those of Europe, which we are often asked to emulate by reducing our specialization This question should not be one of "taking sides" for or against specialization We should find out what the student needs in order to develop best at each stage At a given age, say, sixteen, he may begin to want to get down to details about some things and also to continue to widen his knowledge. Can this not be arranged ? In the university, can specialist teachers not arrange their subject matter so as to open wider horizons ? Certainly medical science provides a marvellous opportunity for a general education as well as a special one, if only we really insist that the student's growth requires both equally, and act accordingly They do not want to be part of battery farms What is the answer ? Do we want more different types and grades of education with segregation of the best ? The élite solution is appealing to many and is used in some countries, but I believe we should go the other way I have the greatest admiration for what our élite system has done for us in the past, and we can build on it Our best has been very good

But there is no reason why we should not make many more who are like them, provided we take real trouble to make sure that development is encouraged and not thwarted Let us get rid of the whole concept of a pool of ability, first and second class and so on Heredity has, of course, an influence on the brain, some studies would say "up to a half"—but half of what? No one really knows how to train the different sorts of brain or to estimate their productivity anyhow Let us find out before we condemn anyone as second-rate Let us say every day in every school and university that we are determined that our output shall be the very best Many "practical" people will say that this is unrealistic nonsense, that the less able will dilute and thwart the able I say in all seriousness and from thirty years of teaching that I do not believe it is necessarily so

Of course there are dangers in expansion Mediocrity is ready to creep in with the excuse that more must mean worse No one is likely to minimize the danger. But this is not, as some people seem to think, a law of nature. More can mean better, indeed, most concerns welcome more customers. We should be proud and glad that people want to go to universities Very sensible of them, I think But we must make sure that what we offer continues to be up to standard. This should be the main aim of all of us who want to improve university productivity I hope that the vice-chancellor of Lancaster and his colleagues who are investigating aspects of academic productivity for the Vice-Chancellors Committee will put the problem of standards at the very front of their studies and statements. We know how hard it is to measure amounts of knowledge—absurd to try, most people will say But yet we all admire some academics more than others, so we must be applying some sort of measure. At any rate we must give special attention to finding ways to ensure the maintenance of standards, however difficult it may be We should make this our first priority. Unless we do so, education becomes a fraud Counting heads is a poor method of measurement if you neglect to consider what you are putting into them

The majority of people will reach astonishingly high standards of intellectual interest and understanding, provided they are challenged with the highest by meeting not complex dogma but problems to be faced in a manner they can understand We have hardly begun the detailed study of how to help the development of the brain as a problem-solving device. The child learns to speak by experimenting with additions to his grammar and vocabulary as his needs expand The six year old nowadays learns set theory by doing experiments with numerods The university student will learn to understand and operate with complex concepts if he is taught by someone who is himself wrestling to improve those concepts He is much less likely to do so if he is presented with them as material to be learned dogmatically

How does all this affect the problems with which we started as to how knowledge increases and how the increase may be assessed? We have seen that studies of the brain suggest that it is not a classified record like a book but a calculating machine that gives useful answers when it is operated by the demands of experience. The specification is terribly vague and could be greatly improved even now, but it is interesting to compare it with a definition of a modern view of epistemology "Belief, like knowledge, is not an act . . . it is a disposition or tendency to act (including to speak) in certain ways under certain conditions"[4] Looked at from our point of view, the knowledge of an individual is the organization incorporated in the model in his brain. The details of this model, of course, are built largely by the influence of other people on him, but what then is new knowledge? It can only be the acquisition of new tendencies or dispositions to act and these can only be acquired by experience and trial and error and practice. When any student acquires "new dispositions" there is an increase of knowledge, provided, of course, that these are "correct", in the sense of enabling him to operate more usefully (though we might have further trouble defining that word). I believe that all learning proceeds best when it is a form of research and that all research is a form of learning. The activities cannot and should not be separated at any stage, from the work of the youngest child to that of the oldest don. No method has been devised by which the increase in knowledge from teaching and research can be measured. Yet schools and especially universities are places whose function is the increase of knowledge, the improvement of "dispositions to act" so that more correct and useful decisions shall be reached And in case anyone should condemn this as utilitarian, I will agree with them that increase of knowledge is good for its own sake There is perhaps nothing more beautiful or satisfying than to learn, to know and above all to find out new facts and pass on new ways of thinking to others. Our university system will continue to be healthy in spite of expansion so long as those who are responsible for sponsoring academic productivity and assessing its output continue to emphasize loudly and repeatedly that the aim of the system is the increase of knowledge, jointly by teaching and research.

[1] Lenneberg, E , *The Biological Foundations of Language* (Wiley, 1967)

[2] Geschwind, Norman, *The Development of the Brain and the Evolution of Language*, Monog Ser on Languages and Linguistics, No 17 Rep Fifteenth Annual R T M on Linguistic and Language Studies (1964)

[3] *Students and Politics*, in *Daedalus* (American Academy of Arts and Sciences, 1968)

[4] *Encyclopaedia Britannica*, 1967.

Optical Identification of the First Neutron Star?

by
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A more precise position for one of the rapidly pulsating radio sources has been found. This has suggested that the source is associated with an eighteenth magnitude blue star

In a recent communication from this observatory, Hewish *et al*[1] have described the discovery of a new class of celestial radio source of very small physical size which emits short pulses of radiation with an extremely constant repetition frequency It was suggested that the radio signals might be associated with the gravitational vibration of a white dwarf or neutron star which could excite repetitive shock disturbances in the stellar atmosphere.

The discovery and detailed study of a neutron star would prove of the greatest importance, both in relation to the properties of super-dense matter and in the mechanism occurring in the gravitational collapse of a star. The possibility that the object discovered by Hewish and his collaborators is indeed a neutron star is thus of great interest, and further investigations by all available methods and over the widest possible range of wavelengths are now required.

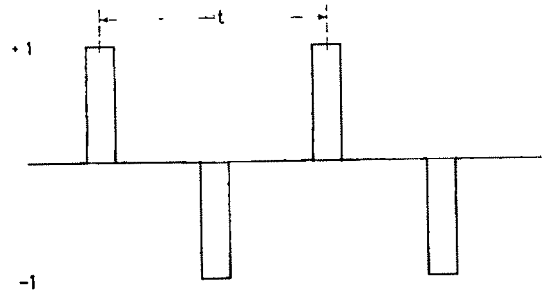

Fig. 1 The time sequence of the commutator

The original observations at 81.5 MHz allowed the position to be determined with an accuracy $\Delta\alpha = \pm 3^s$ $\Delta\delta = \pm 30'$. The measurement of the variation of the received repetition frequency caused by the Earth's orbital motion over a period of a month gave an independent value of the declination which was in good agreement with that obtained from the directivity of the aerial system, but the errors were still too large to allow the recognition of any faint optical object which might be associated with the source.

Observations have recently been made at higher frequencies of 408 and 1,407 MHz using the one-mile radio telescope in order (*a*) to establish a more precise position and (*b*) to obtain information on the radio spectrum of the pulses. The 81.5 MHz observations have shown that the source is very weak with a flux density in the pulse which varies both from minute to minute and over the months of observation. During most periods the power received from each pulse is $\sim 0.5\times10^{-26}$ J m^{-2} Hz^{-1}, so that the mean flux density $S_{81.5} \simeq 0.35\times10^{-26}$ W m^{-2} Hz^{-1}. Special recording methods have therefore been used with the one-mile telescope, both to allow the measurement of the small mean flux density and to distinguish the source from the more powerful normal sources which are likely to be found in its neighbourhood. The method makes use of a filtering process based on the characteristic recurrence frequency of the signal both to reject the response due to constant sources and to permit the use of a long effective integration time. In order to avoid the necessity for recording at a high sampling rate, which would be necessary if the filtering were carried out entirely in the computer, the reduction was performed in two stages.

(*a*) The output of the receiver was switched, using commutating relays, in the time sequence shown in Fig. 1, where the widths of the positive and negative gates are each one-eighth of the total period t, which was made close to the known period of the pulses observed at 81.5 MHz. The commutator was driven from the sidereal crystal clock.

If the commutator is synchronized so that the incoming pulses fall in one or other of the gates, the output from the relays contains a d.c. component proportional to the mean flux density, and integration can be used to increase the signal to noise ratio; the noise level is also reduced relative to that of an unswitched receiver output, while all constant sources produce alternating outputs at the switching frequency which will be rejected by the low-pass filter.

By choosing the period t to differ slightly from that of the incoming pulses (t_0), the output from the filter will alternate with a waveform similar to that shown in Fig. 1, but with a greatly increased period T given by

$$\frac{1}{T} = \frac{1}{t} - \frac{1}{t_0}$$

In practice a value of $t = 1.344328$ sidereal seconds (equal to 1.340654 solar seconds) was used, giving a value of $T \sim 520$ s and the signal from the commutator was smoothed with a time constant of 10 s and sampled at intervals of 20 s. This signal was digitized and recorded on punched paper tape in the manner normally used with the one-mile telescope[2].

(*b*) The reduction in the computer followed that normally employed in the determination of the position of a source[3] except that a convolution operation was first carried out using a function similar to that shown in Fig. 1 but having a periodicity close to T. This operation was necessary because the phase of the incoming pulses and hence of the signal of period T is unknown. The convolution, which corresponded to integration over a period of 30^m, was carried out with displacements of $T/16$. The results were presented as a series of 12^h plots giving the amplitude and radio frequency phase relative to that of a source at an assumed position, the residual variation of the phase then allowed the difference between the true and assumed positions of the source to be determined.

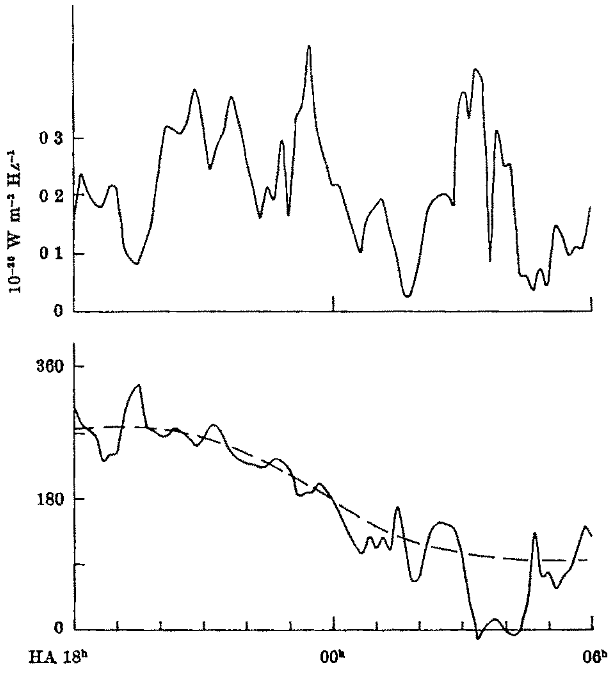

Fig. 2 Record obtained at 408 MHz using an aerial spacing of 900 λ on January 23, 1968. The top trace shows the variation of flux density during the 12^h observing period. The lower trace shows the variation of the radio-frequency phase relative to that of a source at an assumed position close to the actual position. The dashed curve shows the variation of phase computed for a source at the final adopted position.

If the values adopted for t and T are sufficiently accurate, the signals will continue, with particular displacements of the convolving function, for the whole 12^h period. If the adopted value of T is incorrect, the signal on any one output will disappear during the run and reappear with another displacement.

One of the output records for the observations of January 23, 1968, with a value of $T = 532.773$ sidereal seconds, which is very close to the optimum value, is shown in Fig. 2, the assumed position was about 4′ arc south of the true position, and the dashed line shows the variation of radio-frequency phase appropriate to the final adopted

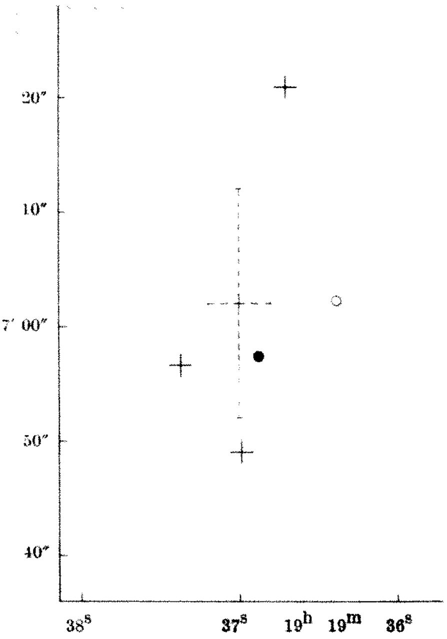

Fig. 3. The positions of the source obtained from successive observations at 408 MHz are indicated by the crosses; the dashed cross represents the mean position and error. The 18^m blue star is indicated by the disk and the faint red object by the open circle.

position. From this record and from others reduced with a slightly different value of T (525·0 sidereal seconds) the periodicity of the incoming pulses was derived; when corrected for the orbital velocity of the Earth, a value of $P_0 = 1{\cdot}337283$ solar seconds was obtained in good agreement with the more accurate value of 1·3372795 derived by Hewish *et al.*

The small flux density and the variability of the source make it difficult to obtain an accurate measurement of its position, but the results from three 12^h observations at 408 MHz are presented in Fig. 3. The mean position is

$$\alpha(1950{\cdot}0) = 19^h\ 19^m\ 37^{s}{\cdot}0 \pm 0^{s}{\cdot}2$$

$$\delta(1950{\cdot}0) = 21^\circ\ \ 47'\ \ 02'' \pm 10''$$

A search has been made using an overlay technique[4] to locate the source on the prints of the Palomar Sky Survey, and enlargements of both red and blue prints are shown in Fig. 4. These photographs show that close to the position of the radio source there is an 18^m blue star, with a fainter red object some 8″ arc north-west. More precise positions of these objects were therefore obtained by measuring the distances from a number of *AGK*2 stars. The results support the association of the radio source with the blue star the co-ordinates of which are

$$\alpha(950{\cdot}0) = 19^h\ 19^m\ 36^{s}{\cdot}88 \pm 0^{s}{\cdot}1$$

$$\delta(1950{\cdot}0) = 21^\circ\ \ 46'\ \ 57''.4\ \pm 1''{\cdot}5$$

Although this position corresponds to the epoch of the Sky Survey (1950·5) the interpretation of the observed dispersion of the 81·5 MHz signals in terms of the effect of the interstellar medium suggests a distance of 50–100 pc so that any proper motion is unlikely to exceed 3″ arc.

The discrepancy in the measured position of only 5″ arc suggests strongly that this blue star should be associated with the radio source and further optical observations are now needed. It is possible that measurements of its spectrum and proper motion will provide further evidence to relate it to the radio source. Conclusive evidence would, however, be provided if it were found to exhibit fluctuations of light intensity having the same period as that of the radio emission; the radial oscillations of the star may excite shock fronts in the atmosphere which are responsible for both optical and radio emission. It is possible that electromagnetically accelerated electrons might account for the very high optical surface brightness required without the intense X-ray emission which would accompany thermal excitation; in this case the optical emission might well exhibit the same pulsed characteristics.

Observations at 1,407 MHz, while not having sufficient signal to noise to provide an improved position, have given some evidence on the flux density, and the results have been combined with those at 81·5 and 408 MHz to provide information on the radio spectrum. The spectrum of a given pulse would be of particular interest, and could be determined in principle by correcting for the different times of travel (amounting to some 8 s) derived from the observed dispersion. Such a measurement is not, how-

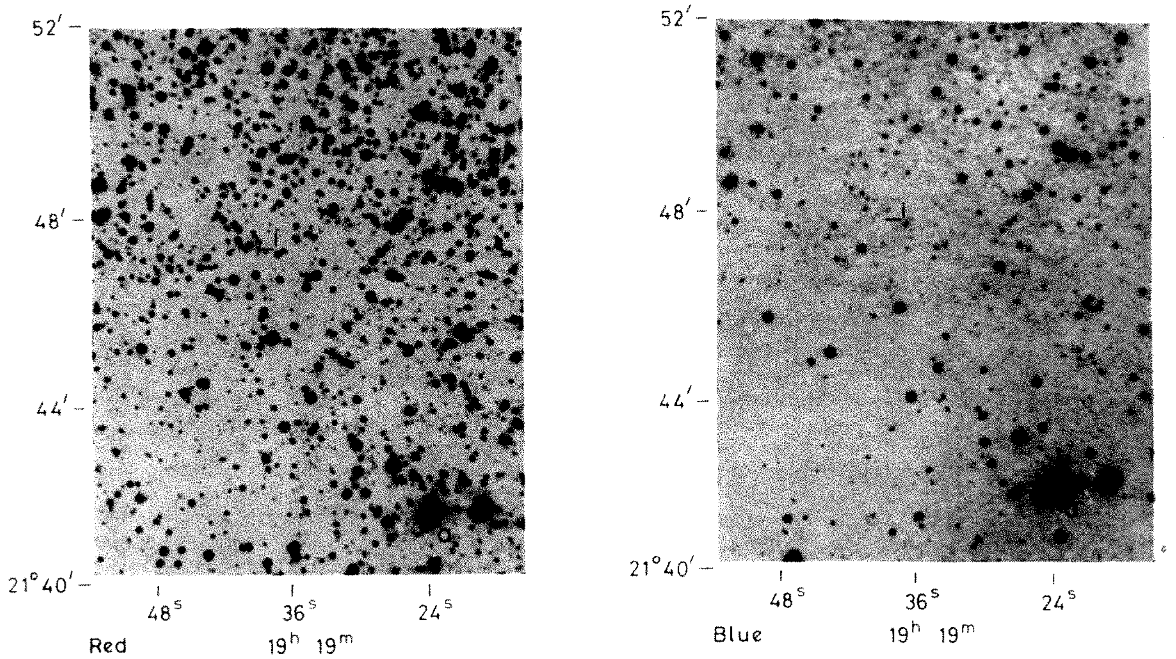

Fig. 4. Enlargements of the red and blue prints of the Palomar Sky Survey showing the radio position. The bright star (*a*) in the south-west of the area is *AGK*2 + 21°. 1963. (© 1957 National Geographic Society—Palomar Observatory Sky Survey.)

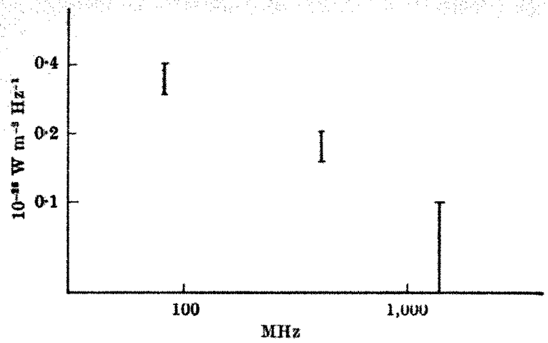

Fig. 5. The mean flux density of the source at the three observing frequencies.

ever, practicable both because of the poor signal to noise ratio in the present observations, and because of the effect of interplanetary scintillation[5] at 81·5 MHz. Instead a mean spectrum has been derived by comparing the average flux densities over the 12^h observing periods with the average flux density observed at 81·5 MHz over a comparable total time during the period December 1967 to February 1968.

The results are shown in Fig. 5. The very high surface brightness (which is probably some 10^5 times greater even than those occurring in QSS) and the absence of a cut-off at low frequencies indicate that the emission cannot be attributed to the synchrotron mechanism[6]; as with the emission from sunspots and from the compact low frequency source in the Crab Nebula[7], it is more likely that it is due to coherent electron motion in plasma oscillations.

Although this new class of object probably only represents about 1 in 5,000 of the total source population at a given flux density, the remarkable character of their emission makes it possible to distinguish them for a more detailed examination of their characteristics.

We thank Drs P. F. Scott and D. M. A. Wilson for helping to assemble the special recording system, and Mr C. D. Mackay and Dr D. Wills for locating the position of the source on the Sky Survey prints, and for making accurate measurements of the position of the blue star.

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[1] Hewish, A., Bell, S. J., Pilkington, J. D. H., Scott, P. F., and Collins, R. A., *Nature*, **217**, 709 (1968).

[2] Elsmore, B., Kenderdine, S., and Ryle, M., *Mon. Not. Roy. Astro. Soc.*, **134**, 87 (1966).

[3] Parker, E. A., Elsmore, B., and Shakeshaft, J. R., *Nature*, **210**, 22 (1966).

[4] Longair, M. S., *Mon. Not. Roy. Astro. Soc.*, **129**, 419 (1965).

[5] Hewish, A., Scott, P. F., and Wills, D., *Nature*, **203**, 1214 (1964).

[6] Hornby, J. M., and Williams, P. J. S., *Mon. Not. Roy. Astro. Soc.*, **131**, 237 (1966).

[7] Hewish, A., and Okoye, S. E., *Nature*, **207**, 59 (1965).

Pulsating Radio Source at $\alpha = 19^h 19^m$, $\delta = +22^\circ$

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Signals from one of the rapidly pulsating radio sources have been recorded on three frequencies using the mark I radio telescope at the Nuffield Radio Astronomy Laboratories. The size of the source, deduced from the form of the signals, again seems consistent with an origin in a white dwarf or neutron star.

At the time of publication of the remarkable discovery by a group of Cambridge radio astronomers[1] of the pulsating radio source at $\alpha = 19^h 19^m 39^s \pm 3^s$, $\delta = +22^\circ \pm 30'$ (1950), the mark I radio telescope was equipped for reception at frequencies of 151, 240 and 408 MHz. The receivers were intended for studies of flare stars and interplanetary scintillations, and an Argus 400 digital computer was in use for the on-line analysis of the receiver outputs. It was therefore decided to search for radio pulses from this source at these receiver frequencies, which were above that of the original observation at 81 MHz. Radio pulses were found at all three frequencies, the first observation being on February 24, 1968. The position of the source agreed well with the original observations; the declination was measured as 21° 40′ ± 7′ (1950).

The method of analysis was to obtain an average pulse shape at each frequency by superposing receiver outputs over a number of cycles of the pulse repetition period of 1.33724 s. Each cycle was divided into shorter intervals, originally of 20 ms duration. When the position of the pulse within the period became clear, the interval was reduced progressively and the time scale was expanded, until for some experiments only 64 ms of each period were examined, with a time resolution of 1 ms. Most of the observations were made using three receiver channels simultaneously, recording the average in 32 intervals of 10 ms on each channel. Integration of pulses by this technique usually lasted for periods of 2 min.

Observations were then directed toward measurements of pulse shape at the three frequencies, and to the measurement of the relative times of arrival. The source was also followed for some hours on several days to record the variations of pulse amplitude which occurred on all three frequencies.

Shape of the Pulses

Fig. 1 shows mean pulse shapes at 408 MHz. Individual pulses at 408 MHz have also been recorded directly on a fast ultraviolet recorder, using a receiver time constant of 1 ms. These are shown in Fig. 2.

The average pulse shows a very sharp rise on all frequencies. This steep rise may have been appreciably slowed in these measurements by the differential time of arrival across the receiver bandwidth, which was 1 MHz. At 151 MHz this effect would stretch the pulse by 22 ms, at 240 MHz by 7 ms, and at 408 MHz by 1·5 ms. It appears that the initial rise may in fact occur within 2 ms at 408 MHz, and there is no evidence that the rise is slower at the lower frequencies.

The pulse lasts for about 35 ms on all three frequencies. This duration is consistent with the measurements on

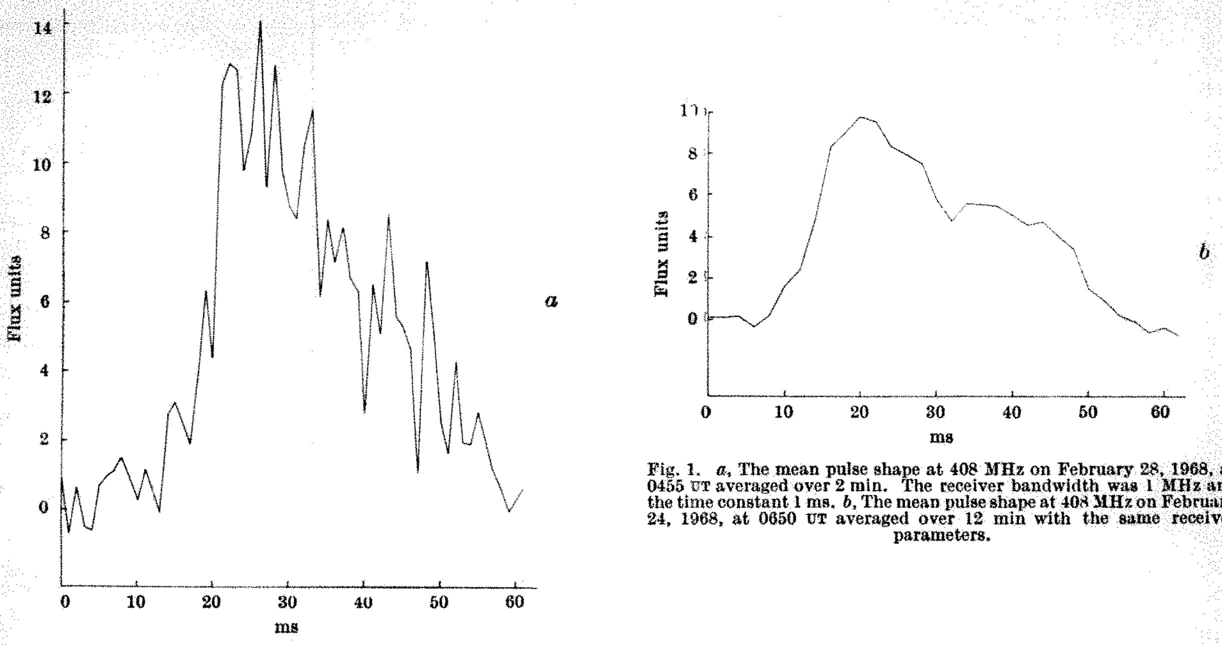

Fig. 1. *a*, The mean pulse shape at 408 MHz on February 28, 1968, at 0455 UT averaged over 2 min. The receiver bandwidth was 1 MHz and the time constant 1 ms. *b*, The mean pulse shape at 408 MHz on February 24, 1968, at 0650 UT averaged over 12 min with the same receiver parameters.

81 MHz, where a delay technique was used to measure the autocorrelation function of the pulse envelope. The autocorrelation function was originally interpreted in terms of a gaussian shaped pulse with width 17 ms. The roughly triangular pulse shape now observed gives a similar width of autocorrelation function, and we conclude that the duration of the pulse is in fact the same at all frequencies so far observed. There is no sign of any impulsive emission outside the normal duration of the pulse.

The measured pulse shape is variable in detail, both between successive pulses, as seen in Fig. 2, and also on averaged pulse shapes. Fluctuations are observed on

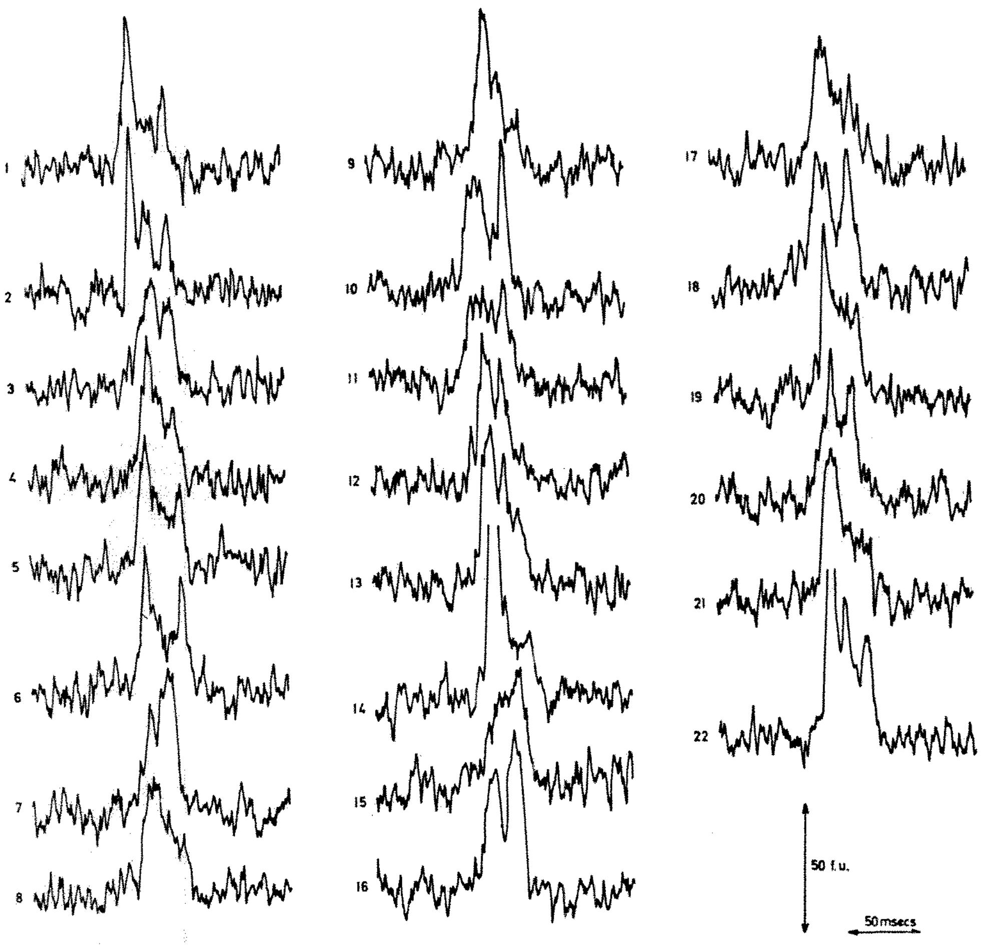

Fig. 2. A series of pulses recorded directly at 408 MHz (bandwidth 4 MHz, time constant 1 ms) on February 29, 1968.

time scales down to 1 ms, the limit of time resolution. The time of onset within the period of 1,337 ms is, however, stable within about 5 ms, a remarkably small limit considering the wide variability of pulse amplitude which is observed on all frequencies.

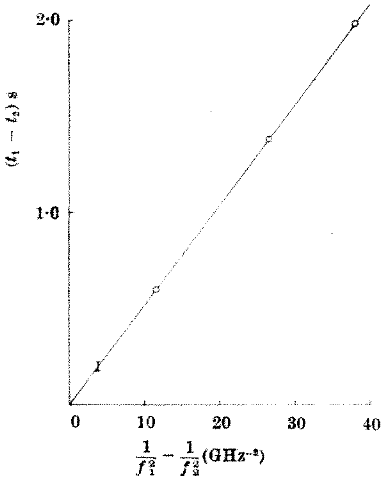

Fig. 3. Dispersion in arrival time of pulses at 151, 240 and 408 MHz. The errors are too small to show. An additional point at 0·2 s delay is obtained from the Cambridge observation at 80·5 and 81·5 MHz.

Pulse Delay

Differences in arrival times of the sharp leading edge could be determined with an accuracy of about 5 ms. We therefore tested the suggestion that the dispersion in arrival time on the three frequencies was due to group delay in the propagation path through ionized interstellar gas. If the pulse at frequency f_1 was emitted at time T_1 and observed at time t_1, then

$$t_1 - T_1 = A + \frac{B_2}{f_1^2}$$

where A is the free-space travel time and B is the dispersion constant. Fig. 3 shows the difference $t_1 - t_2$ plotted against $\left(\frac{1}{f_1^2} - \frac{1}{f_2^2}\right)$. The points lie on a straight line whose intercept with the vertical axis is 0 ± 5 ms, showing that the differences in emission times over the frequency band are either very small or that they themselves follow a similar dispersion law. We suggest that the emission in fact takes place nearly simultaneously on all frequencies.

The observed dispersion constant B yields a measure of the integrated electron density in the line of sight equal to $12{\cdot}55 \pm 0{\cdot}06$ pc cm^{-3}.

Pulse Amplitude

It is difficult to determine the spectrum of the radio emission, because the amplitudes on all three frequencies are variable, and the variations are not obviously related between the three frequencies. On all three frequencies the flux density during the pulse was usually between 2 and 20×10^{-26} W m^{-2} Hz^{-1}, measured as the peak of the mean profile averaged over 2 min. The direct recordings of single pulses on 408 MHz show short peaks of over 100×10^{-26} W m^{-2} Hz^{-1}.

Fig. 4 shows the variation of pulse amplitude on 408 MHz for a period of 6 h on February 28, 1968. Variations occur on a wide range of time scales. The most prominent feature is a rapid rise by a factor of more than 10, which may persist for 10 min or more and recur after 1–2 h. Variations over a period of 10 s seem to be less than those reported at 81 MHz. No truly periodic variability has been observed.

A receiver at 2,695 MHz on the mark II telescope was also used, and it soon appeared that the flux density of the pulses was much lower at this high frequency. Observations for a period of 3·25 h (1316–1631 UT on February 29, 1968) would have detected a flux density during the pulse, averaged over any one minute, of $0{\cdot}1 \times 10^{-26}$ W m^{-2} Hz^{-1}, but nothing was detected on this frequency.

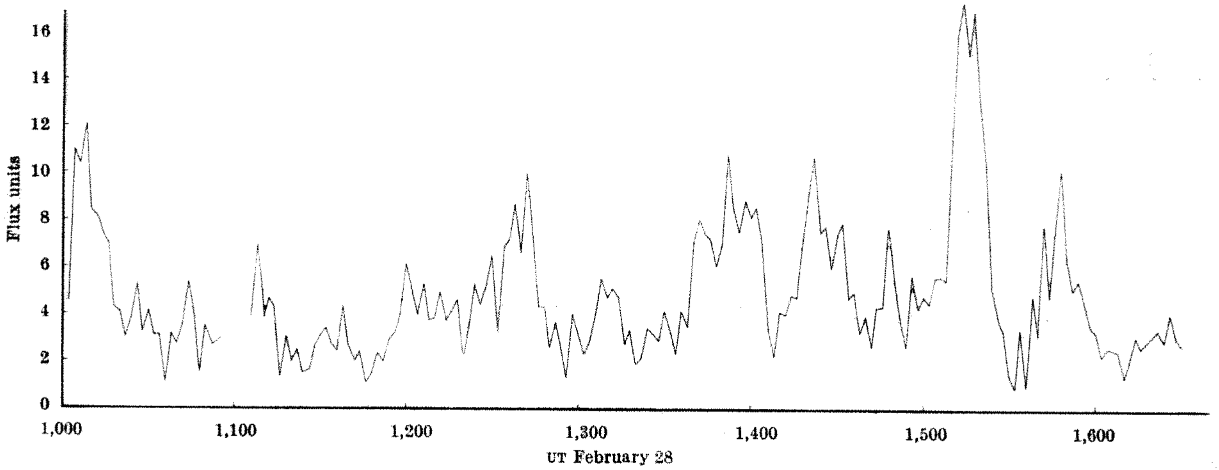

Fig. 4. The variation of pulse amplitude at 408 MHz, averaged over 2 min, for an observing period of 6 h on February 28, 1968.

Nature of the Source

The observation of the nearly simultaneous onset of emission of the pulses on three widely spaced frequencies suggests that the pulses on the various frequencies originate in regions which are separated by less than 1,000 km. Again, the total duration of the pulse, which is the same at all four frequencies, indicates that all sources of the emission lie within a distance of 10,000 km. These distances are consistent with an origin in the very condensed corona of a collapsed star, such as a white dwarf or neutron star, as suggested in ref. 1.

If the origin of the radiation is at a distance of 60 pc, as was suggested to explain the dispersion of arrival time, the simultaneous generation of pulses over a wide bandwidth implies a radiated pulse power of about 10^{21} W and a pulse energy of 2×10^{19} J. If the source is a star with radius 10,000 km, the power flux at the surface is about 3×10^{5} W m^{-2}, corresponding to a field strength of 10^{4} V m^{-1}. These values would, of course, be even larger for a smaller star.

Received March 2, 1968.

[1] Hewish, A., Bell, S. J., Pilkington, J. D. H., Scott, P. F., and Collins, R. A., *Nature*, **217**, 709 (1968).

INSTRUMENTATION

Development of High Energy Neutrino Experiments at CERN

by

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Equipment at present under construction and the new developments foreseen will increase neutrino event rates by large factors. Neutrino experiments will become a major field of high energy physics, leading to a further understanding of the interactions of these important particles.

RECENTLY the interactions of high energy neutrinos with free protons were photographed for the first time. This is the latest of a series of experiments which started some 40 yr ago in the Cavendish group when Ellis and Wooster were so perplexed by an apparent violation of the Law of Conservation of Energy that they devised an apparatus to determine whether a new form of neutral radiation accompanied the electrons from β-emitters. Their experiment was at least 10^{20} times too insensitive; it was not until a quarter of a century later, after the invention of the atomic reactor and much more perceptive detectors, that the existence of free antineutrinos was confirmed experimentally by Cowan and Reines and their co-workers. Still another decade elapsed before the first interaction of high energy neutrinos was observed.

There are probably more neutrinos than there are atoms in the universe; nevertheless, Pauli, who first postulated the existence of the neutrino to conserve momentum, energy and angular momentum in β-decay, wagered they would never be detected. The cross-sections for neutrino interactions, which are governed entirely by the weak force, must have seemed to him impossibly small for laboratory experiments. Bethe and Peierls first estimated that the capture of low energy antineutrinos by protons had a cross-section of about 10^{-43} cm². Lee and Yang showed that the cross-section for such a process of inverse beta decay increases with energy and reaches a maximum of about 10^{-38} cm², at about 1 GeV for neutrinos ($\nu_e + n \rightarrow e^- + p$), and at a somewhat higher energy for antineutrinos ($\bar{\nu}_e + p \rightarrow e^+ + n$). The weak interaction rates are so low that it is very difficult to invent experiments to observe them for particles which also exhibit strong and electromagnetic interactions. Before 1961 all experiments with the weak force had been concerned with the spontaneous decay of nuclei or particles, or the capture of reactor antineutrinos by protons. Although significant discoveries and deductions were made, including the violation of parity in weak decays of nuclei, and the two-neutrino hypothesis, the understanding of the weak phenomena was faced with a handicap analogous to that which would exist if the strong force could only be studied in low energy nuclear interactions.

The simplest theoretical approach to the weak process—and still the most successful—is the concept of the interaction of four leptons at a single point in space and time. To resolve certain impossible consequences of the theory, such as the ever increasing interaction cross-sections with energy, the concept of the intermediate boson was developed as a means of giving such an interaction a spatial distribution. Experimentalists saw this situation as an additional compulsion to study directly weak interactions at high energies to try to understand the fundamental phenomena; the only evident means was by the direct observation of high energy neutrino interactions.

Many unstable nuclei and particles have some mode of decay which produces neutrinos. The most energetic nuclear processes produce $\bar{\nu}_e$ of less than 15 MeV; pions, kaons and muons at rest yield higher energy neutrinos: $\pi^+ \rightarrow \mu^+ + \nu_\mu + 34$ MeV, $K^+ \rightarrow \mu^+ + \nu_\mu + 388$ MeV, $\mu^+ \rightarrow e^+ + \nu_e + \bar{\nu}_\mu + 105$ MeV. Both pions and kaons also produce ν_e, but only kaons ($K^+ \rightarrow e^+ + \pi^0 + \nu_e$, $K^0 \rightarrow e^+ + \pi^- + \nu_e$) have useful branching ratios. The cosmic neutrino flux comes from such origins, and others too, particularly ν_e from the formation of the elements in the Sun and other stars. Although these fluxes have a large total intensity—more than 10^{14} m^{-2} s^{-1} from the Sun—they are diffuse and the interaction rates are small so that no interaction of low energy neutrinos and only a few tens of interactions of high energy cosmic neutrinos have so far been detected. Nevertheless, as always, cosmic ray experiments have access to the highest possible energies.

Laboratory experiments have become feasible with ν_μ and $\bar{\nu}_\mu$ derived from the secondary beams available from the most powerful proton accelerators, the 31 GeV AGS at Brookhaven, the 25 GeV PS at CERN and the 12·5 GeV ZGS at Argonne. In the first high energy experiment[1] in 1962, the Brookhaven–Columbia group, with an allocation of about 1,000 h of the AGS, succeeded in recording 51 muons generated by muon neutrinos in 10 tons of spark chambers. This mixed beam of ν_μ and

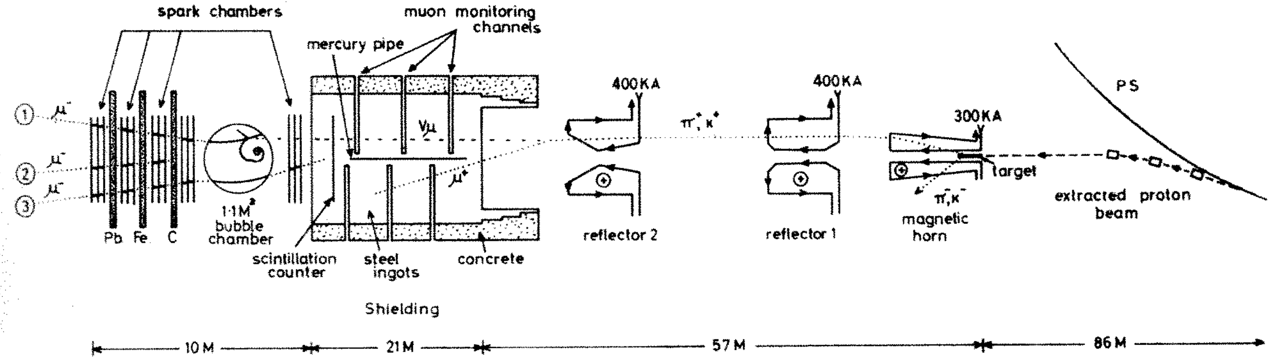


Fig. 1. CERN—new neutrino facility. The disposition of the various elements is shown schematically, including the 6,000 tons of steel shielding. Three types of ν_μ events are shown; (1) is an event produced in the bubble chamber, (2) is an event in a carbon plate in the spark chamber installation and (3) is an event in which the muon produced in a part of the shielding traverses the bubble chamber, where its sign and momentum can be distinguished.

$\bar{\nu}_\mu$ was derived at 7·5° from a fraction of the pions and kaons produced in an internal target. Due to the construction of the accelerator, most of the neutrino parents, which are concentrated near the forward direction, were lost. After a free flight of 21 m all particles except neutrinos were absorbed in a massive steel shielding in front of the detectors, otherwise the very rare neutrino interactions could never have been detected against the overwhelming background of accompanying particles. Almost no evidence of interactions producing an electron was seen and so it was concluded that interactions of ν_μ and ν_μ produce muons and not electrons. Thus the two-neutrino hypothesis that ν_μ and $\bar{\nu}_\mu$ are different particles from ν_e and $\bar{\nu}_e$ was confirmed. It was evident also that much higher neutrino interaction rates were essential for further work in this field.

In the subsequent experiments at CERN[2,3] and later at Brookhaven[4] and Argonne[5], two fundamental technical advances were made; the proton beam was extracted[6] from the accelerators, so that forward going neutrino parents could also be used, and focusing devices were developed to direct the parents towards the detectors so that the neutrinos had the highest probability of traversing the detectors. Neutrino parents from various parts of the target in which they are produced by the protons have a broad distribution in energy and direction. By means of computer explorations, various toroidal magnetic lenses have been invented for guiding such parents; the first such device, called a "magnetic horn", was developed at CERN[7] and also used at Argonne; it is shown in Fig. 2a and b. While the proton beam bombards the target, the two conical coaxial conductors carry a current

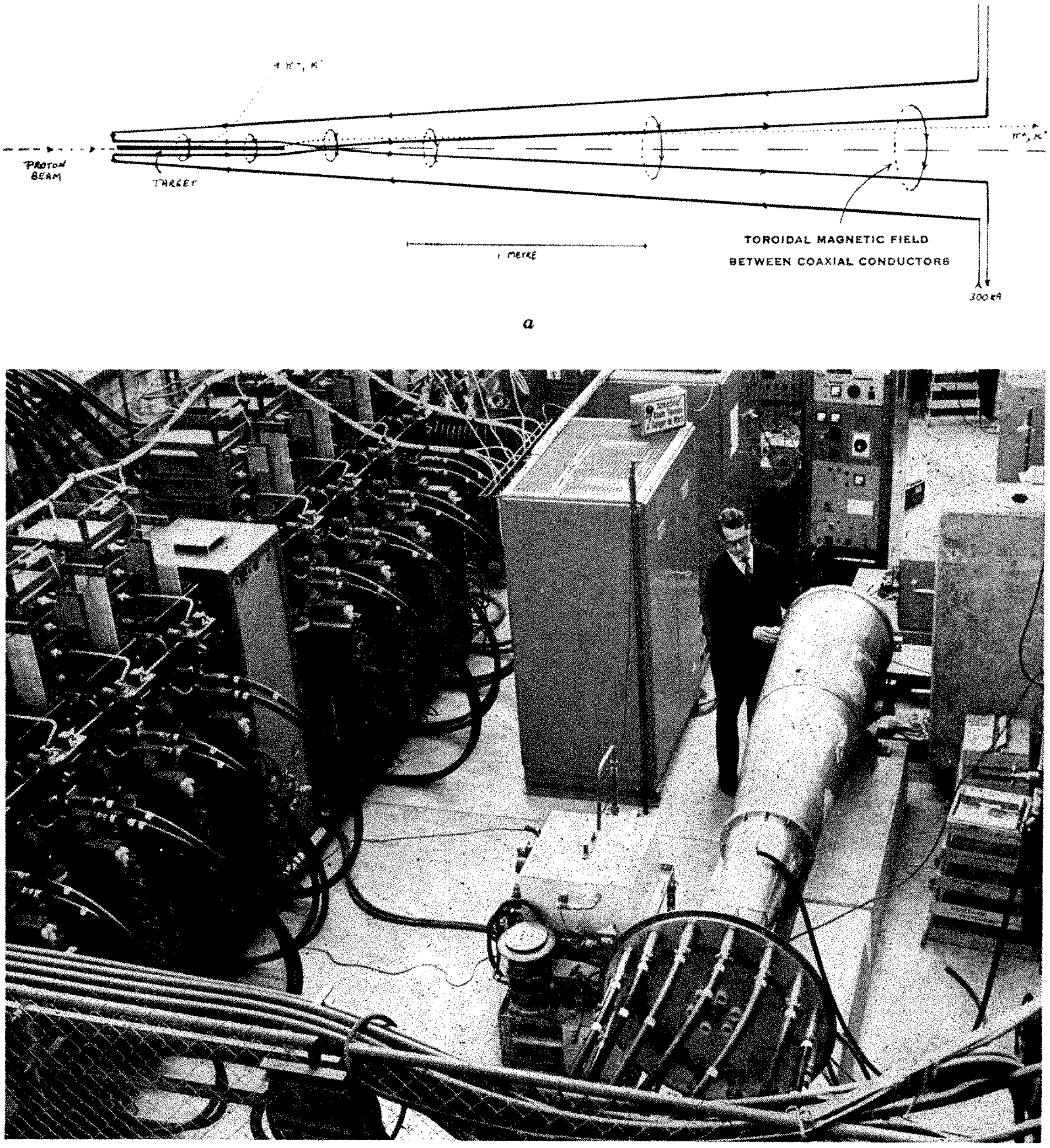


Fig. 2. *a*, Principle of the magnetic horn. The current pulse in the two axial conical conductors produces an azimuthal magnetic field between them which deflects charged particles towards or away from the axis according to their sign. The outline is the latest version of the magnetic horn. *b*, Testing the first magnetic horn. The condenser banks and ignition switches are on the left, the multiple coaxial cables which carry a total current of 300 kA are in the foreground. (CERN Photo 317-1-63.)

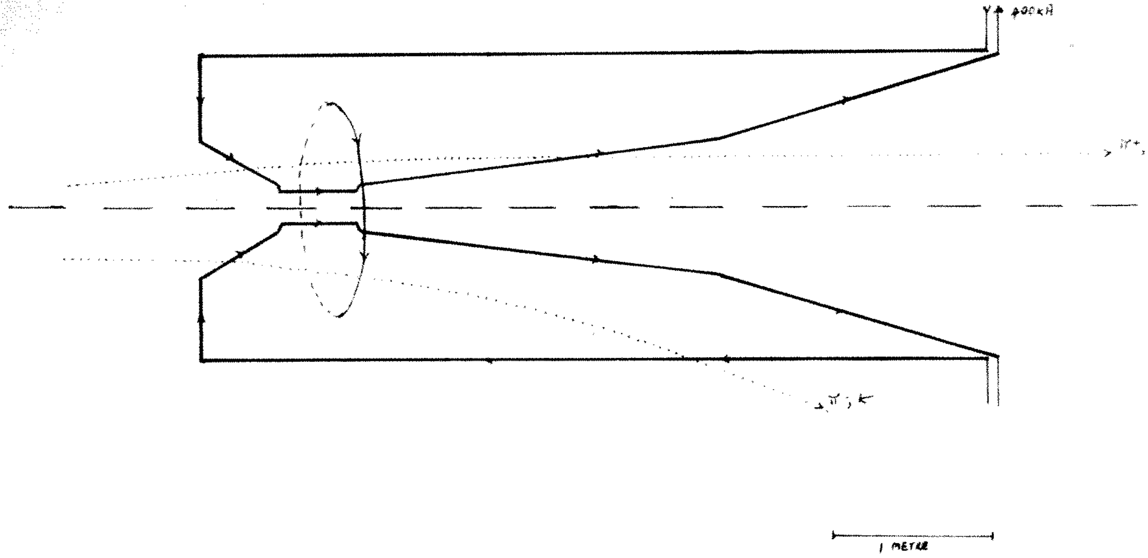


Fig. 3. Principle of the magnetic reflector. The major difference between this device and the magnetic horn shown in Fig. 1*a* is that the source of neutrino parents is some distance away.

of 300,000 A. With the current in the inner conductor in the direction of the neutrino parents, the magnetic field deviates the neutrino parents nearer to the axis, and the antineutrino parents further away, so that the neutrino flux at the detector is enhanced, and the antineutrino flux reduced to a very low value. At its narrowest place the inner cone is about 15 mm in diameter; such a conductor can carry a current of 300,000 A only momentarily. The mechanical shock, from the magnetic field and from the sudden heating by the passage of the current, produces such high stresses that a long development was needed, both for the magnetic horn and its energy storage system, to obtain the operating life of more than a million pulses necessary in an experiment.

The neutrino flux derived from the pions and kaons, produced from the extracted PS beam and focused by the magnetic horn, was almost two orders of magnitude higher in the experiments of 1963–64 than in the first neutrino experiment at Brookhaven. Thus it was possible to use a bubble chamber as a detector of neutrino interaction and a total of about 1,000 events in freon (CF_3Br) were photographed; a spark chamber of 30 tons operating at the same time recorded about 10,000 interactions. Many results were obtained, including a confirmation of the Brookhaven observation that ν_μ and ν_e carry independent quantum numbers. The ν_μ beam was contaminated by 0·5 per cent of ν_e, principally from kaons. The number of ν_e events was consistent with similar interaction cross-sections for ν_e and ν_μ, which was a new test of "μ–e" universality, not as precise as in decay processes, but in a quite different range of momentum transfer. From the statistics of the possible production of μ^+ by the ν_μ, it was concluded that any such interactions violating lepton conservation are less than 0·5 per cent of the total. There were several other conclusions, including that neutrinos from kaon parents seem to have the same properties as neutrinos from pion parents.

Of particular interest was the possibility of searching for the intermediate boson. Its expected signature was a muon pair in the spark chamber from the process $\nu_\mu + Z \rightarrow \mu^- + W^+ + Z$, followed in about 10^{-18} s by $W^+ \rightarrow \mu^+ + \nu_\mu$; or a μ^-, e^+ pair in the HLBC from $W^+ \rightarrow e^+ + \nu_e$, or perhaps pions from decay modes of the type $W^+ \rightarrow \pi^+ + \pi^0$ or $W^+ \rightarrow \pi^+ + \pi^+ + \pi^-$. No such evidence was found and it was concluded that, if the W as predicted exists at all, M_W must be greater than 2 GeV. To raise this mass limit appreciably requires neutrino beams of much higher energy.

From the bubble chamber data a preliminary determination was made of the nucleon form factors involved in the elastic interaction: $\nu_\mu + n \rightarrow \mu^- + p$. Although the neutron was bound in a nucleus of the CF_3Br, the analysis was valid because the muon escapes from the nucleus where it is produced without interaction and the proton has only a low probability of a disturbing interaction in the same nucleus. For pionic events the possibilities of analysis were much less favourable, since a pion is likely to be absorbed in the nucleus where it is produced. This aspect, together with clear evidence of a substantial inelastic event rate, made it obvious that the next experiment at CERN should investigate the neutrino interactions on free protons in propane in the bubble chamber.

To make such an experiment feasible, a new and substantial increase in the neutrino event rate was again imperative. It was achieved, partly by rebuilding the bubble chamber body to give a visible volume of 1·1 m³, and partly by constructing the new neutrino facility, depicted in Fig. 1. In this facility, the PS beam was extracted by the same apparatus as previously, but an entirely new system of elements for neutrino parent focusing was developed. The shielding was also rebuilt with provisions for monitoring the muon absorption. A new magnetic horn was designed with an efficiency about 1·3 times greater than the first one (unpublished results of D. H. Perkins and W. Venus). The essential modification, determined from more elaborate computer studies, was that parents were accepted from a longer, lighter target, actually of boron carbide instead of copper, as previously used. Particular attention was given to reducing the losses from interactions of the neutrino parents in the horn conductors, which they actually traverse. To focus the protons for the PS to a beam only 2 mm diameter at the horn target 90 m away was another essential requirement which has required much technical development. Some ten magnetic elements are pulsed simultaneously so that during the passage of the protons their guiding fields are precise to about 0·1 per cent.

The principal increase in the neutrino flux in the new facility has come from the invention of two toroidal lenses[8], called reflectors, which correct the trajectories of neutrino parents which are not focused adequately by the horn. The beam optical principles of the reflectors are similar to those of the magnetic horn. The essential developments have been to discover from computer studies that elements with such shapes, currents and locations can contribute so effectively to the neutrino flux, and then to excite them with the enormous currents required. Fig. 3 shows the principles of the first reflector.

The flux on the axis at the detector is inversely proportional to the shielding length. The best shielding therefore is the shortest consistent with sufficient stopping power, which for high energy experiments is determined by the maximum energy of the unwanted muons from the ν_μ parents. The disposition of the 6,000 tons of steel ingots, lent by the Swiss Government, and the many thousands of tons of concrete is shown in Fig. 1. For testing the synchronization and sensitivity of the neutrino detectors, mercury can be let out of an axial iron pipe to provide a controllable muon leakage through the shielding. The muon flux distribution can be scanned by detectors which can be moved in radial channels in the shielding. When the data obtained from these are finally reduced it will be possible to estimate a neutrino spectrum, which can be compared with that deduced from a knowledge of the parent production spectra in the target in the horn, the computed trajectories and the effects of absorption in the focusing devices. During the actual experiment, the muon monitoring equipment also enabled a rapid study of the optimum target material and best operation of the neutrino parent focusing elements. This CERN installation is at present the most intense high energy neutrino facility. In pulses 2 s apart about 10^{10} neutrinos traverse the detectors, to produce about five interactions per ton of material per hour.

For the first experiments in the new facility, in 1967, the 1·1 m^3 bubble chamber was used with propane, C_3H_8. Such a chamber installed in the experiment in which the existence of the two neutrinos was first demonstrated would have recorded only two events. In an allocation of accelerator time similar to that experiment about 1,000 events have been obtained in $1 \cdot 1 \times 10^6$ pictures. Simultaneously the PS beam was shared with several independent experiments, and the neutrino beam with two spark chamber experiments, the success of which depended on requirements which reduced markedly the maximum neutrino flux. About 80 per cent of the neutrino interactions in C_3H_8 are in carbon nuclei, but of those on free protons, about 100 will be good candidates for analysis as events of the type: $\nu_\mu + p \rightarrow \mu^- + \pi^+ + p$, shown in Fig. 4. By a kinematical study of the momentum unbalance, these events can be distinguished from interactions on bound protons in the carbon nucleus, which have appreciable Fermi momentum. For the final analysis a more refined fitting procedure will be used and a determination of the rate of production of N^*_{33} on free protons will be possible, together with a preliminary estimation of the appropriate form factors. Because of the more precise momentum determination possible in C_3H_8 compared with CF_3Br, the new bubble chamber data will permit a thorough re-examination of many of the conclusions of the 1963–64 experiments. The advantages of the lighter nuclei are, however, also accompanied by many disadvantages. The total event rate in C_3H_8 is only about 0·3 of that in CF_3Br and the manifestation of γ-rays is much rarer. Because the surroundings of the chamber and the shielding were of the same density in both series of experiments, the ratio of interactions due to neutral particles in equilibrium with the neutrino flux, to true neutrino interactions, is higher in C_3H_8 than in CF_3Br. This reduces the certitude of identification of events in propane. It is evident that in future experiments, according to the particular features of the interactions which are being studied, both heavy and light working fluids in bubble chambers will each have their useful roles.

Spark chambers operated in association with the bubble chamber will elucidate two further questions. The first question is—does the ν_μ ever produce a μ^+ instead of a μ^-? From the previous experiments this happens less than once in a hundred times. The present data should explore a much lower limit. For this aspect of the operation part of the neutrino shielding was defined as a target for ν_μ interactions by means of a scintillation counter. Emerging particles were located by a spark chamber, the charge of those which traversed the bubble chamber was identified and their range determined in the subsequent spark chambers; thus muons could be distinguished from pions, and μ^- from μ^+. During most of the operation an absorber was placed in front of the target in the magnetic horn to eliminate antineutrino parents which otherwise would not have been rejected by the focusing fields. The spark chamber group is at present analysing the relative significance of the residual muon leakage through the shielding, $\bar{\nu}_\mu$ interactions and any possible production of μ^+ by ν_μ. The other question it is investigating with the same installation is how the total neutrino interaction cross-section depends on the atomic weight of the interacting nucleus. This is done by measuring the interaction rate per unit mass in plates of carbon, iron and lead in the spark chamber array.

The equipment already under construction and the developments foreseen will increase event rates by such large factors that neutrino experiments will become a major

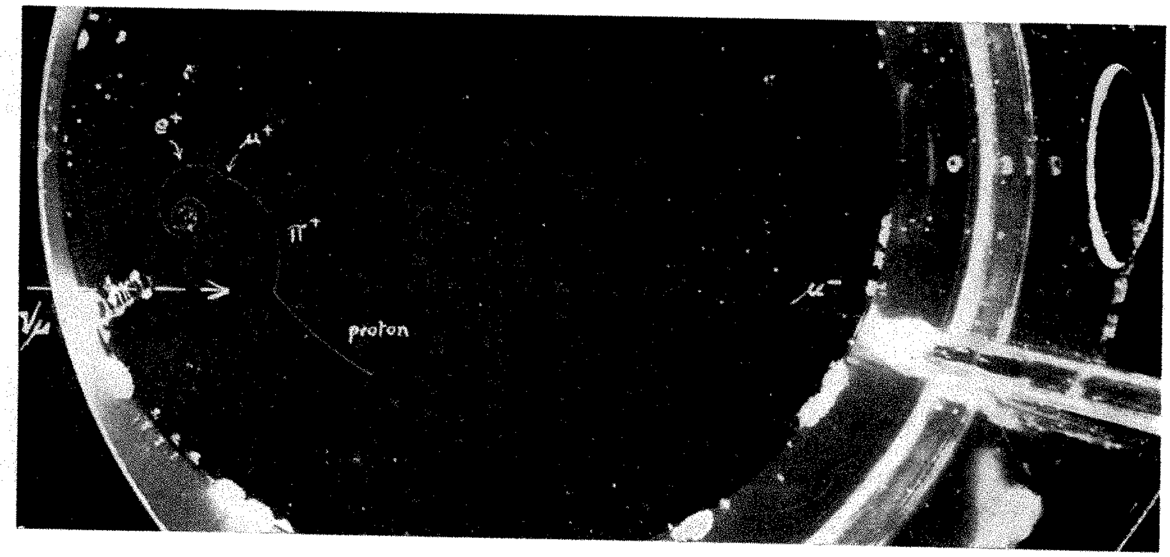


Fig. 4. Candidate for an interaction of a ν_μ of energy 2·62 GeV with a free proton $\nu_\mu + p \rightarrow \mu^- + \pi^+ + p$. The μ^- leaves the chamber, the proton is the heaviest track and the decay of the pion at rest ($\pi^+ \rightarrow \mu^+ + \nu_\mu$, $\mu^+ \rightarrow e^+ + \nu_e + \nu_\mu$) can be seen as the middle track.

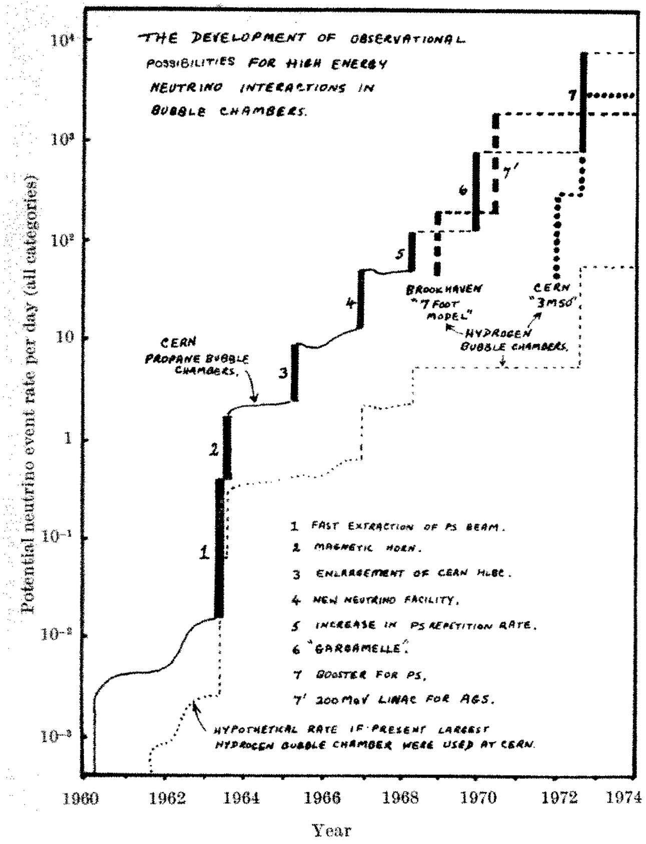


Fig. 5. Potential neutrino event rates at the PS and AGS: The diagram refers to the propane and hydrogen bubble chambers. The steps represent the various contributions to the event rate and already planned future developments. No account is taken of possible improvements in the accelerated proton beam except for the new injectors, or for possible improvements in neutrino parent focusing devices.

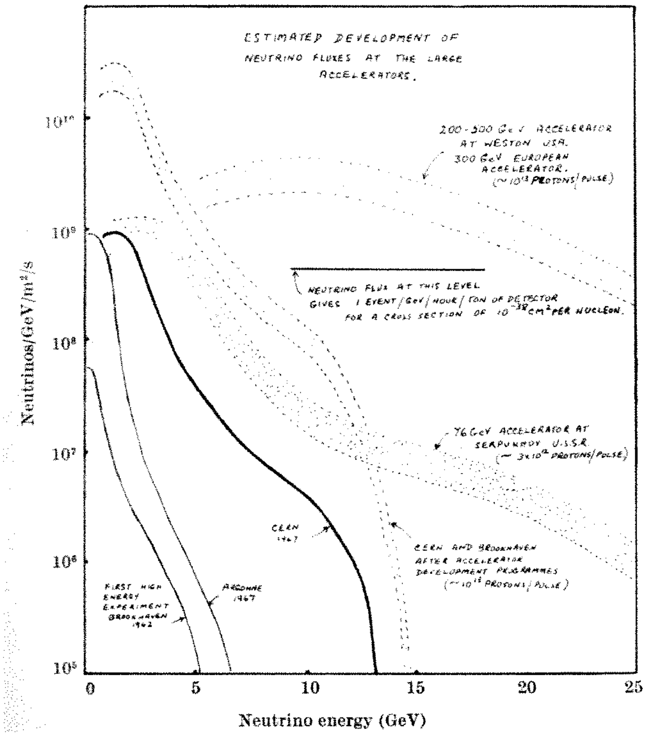
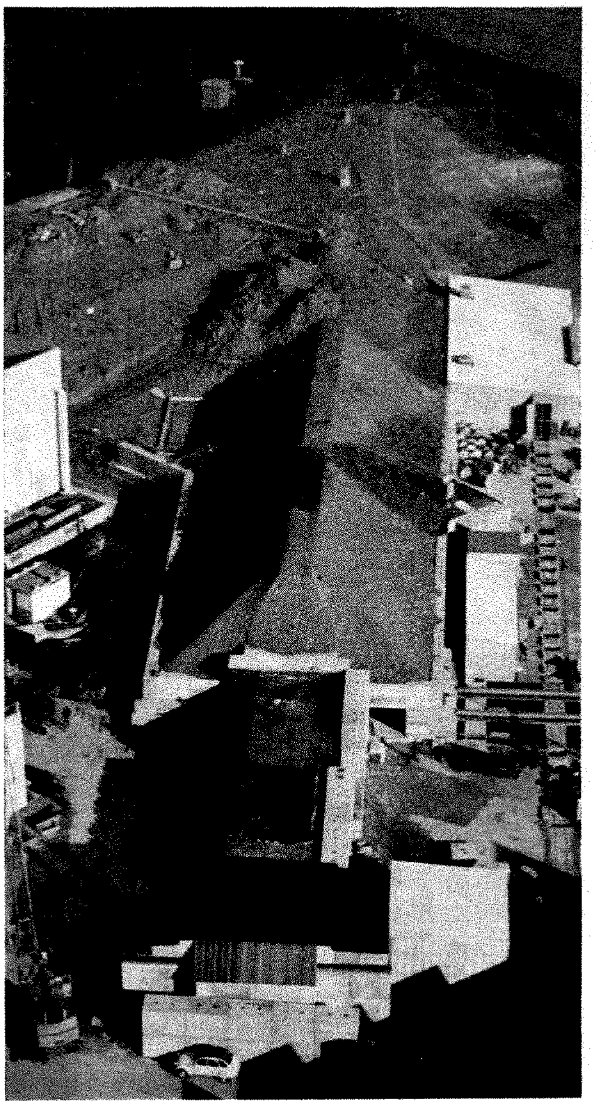


Fig. 6. Estimated development of neutrino fluxes at the large accelerators: The fluxes already used in three experiments are shown together with tentative estimates of the future fluxes for the accelerator intensities shown. A spectrum at the level indicated produces one event per GeV, per hour, per ton of detector for an interaction cross-section of 10^{-38} cm² per nucleon.

field of high energy physics. The AGS and the PS have development programmes to raise their intensities. Both accelerators will have new power supplies to increase the cycling rate of their magnet systems and both will have new injectors. A factor of twenty in the average extracted proton current will be gained within five years, with a corresponding gain in neutrino fluxes. A neutrino experiment is first priority on the experimental programme of each of the very large bubble chambers being built for the AGS, PS and ZGS. Gargamelle, the 10 m³ heavy liquid bubble chamber being constructed for CERN by the French Atomic Energy Commission, will increase the neutrino detection efficiency by a factor of about 7 compared with the present 1·1 m³ chamber. The half scale model of the projected "14 ft." hydrogen bubble chamber for the AGS will probably be operational near the end of this year. After testing, it will be used immediately for a neutrino experiment, probably with a deuterium filling, which will permit a refined study of form factors both in the process $\nu_\mu + n \rightarrow \mu^- + p$ and $\nu_\mu + p \rightarrow \mu^- + \pi^+ + p$. A great advantage of these larger chambers will be the increased efficiency of identification of the high energy reaction products. The previous results, which cannot be regarded as other than preliminary, will be improved by



Aerial view of the CERN neutrino facility: The annular earth covered tunnel of the 25 GeV PS can be seen at the top left. The neutrino beam tunnel is also earth covered, for radiation protection, and leads to the 6,000 ton shielding of steel ingots and finally the bubble chamber block-house in the foreground. (CERN Photo 115-10-66.)

the better statistics. A hundred-fold increase in the present event rates will probably be sufficient to study the polarization of some of the reaction products. With a thousand-fold increase it may be possible to study the neutral interactions of neutrinos: $\nu + e \rightarrow \nu + e$ and $\nu + p \rightarrow \nu + p$, which have never yet been seen but which are predicted to take place. Success will depend crucially on a detailed and accurate understanding of the background due to the neutral particles which are in statistical equilibrium with every neutrino beam passing through shielding material. The development of bubble chamber observations of neutrino events is shown in Fig. 5.

In parallel with the increases in neutrino event rate which the new developments will bring, neutrino beams with a smaller spread in energy[9] will be feasible. Until now the parent focusing devices have been designed to produce broad spectra, but given sufficient event rates neutrino beams with a narrow energy spectrum are also possible. The usefulness of such beams will depend greatly on what will be learned about neutrino interactions. It is of the greatest interest to experiment with higher energy neutrinos because at this stage even a knowledge of the variation of the total interaction cross-section with energy is of fundamental theoretical importance. The next major extension of laboratory neutrino spectra will be made at Serpukhov[10] which now has the highest energy proton accelerator in the world. If the mass of the W is less than 3 GeV, and its properties are as predicted, it can be found at Serpukhov. The interaction $\nu_\mu + e^- \rightarrow \mu^- + \nu_e$, which has a threshold of about 10·7 GeV, should be seen for the first time at Serpukhov. A less spectacular but extremely important aspect of the Serpukhov neutrino spectra will be the high flux in the region above 5 GeV which is also accessible at CERN and Brookhaven. At Serpukhov, given adequate facilities, it will be possible to obtain data equivalent to that of all preceding experiments in this region in a very short time of operation. Somewhat further into the future will be the neutrino facilities of the American 200–500 GeV proton synchrotron already being planned at Weston near Argonne, and the possible European 300 GeV accelerator. From such accelerators, very high neutrino fluxes[11] can be derived as shown in Fig. 6. With the detectors foreseen, total event rates 10^3 to 10^4 times greater than those possible today will be feasible. In this field, where even Pauli misjudged the future, it is improbable that we can foresee the problems which will be studied in the future. We know instinctively, however, that the new observational possibilities will lead to profound contributions to the understanding of the interactions of these particles, which seem to be the most abundant material constituent of nature.

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[1] Danby, A., *et al.*, *Phys. Rev. Lett.*, **9**, 36 (1962).

[2] Block, M. M., *et al.*, *Phys. Lett.*, **12**, 281 (1964).

[3] Bernardini, G., *et al.*, *Phys. Lett.*, **13**, 86 (1964).

[4] Burns, R., *et al.*, *Informal Conf. on Exp. Neutrino Phys.*, *CERN Report* 65–32, 1 (1966).

[5] Novey, T. B., *Proc. Roy. Soc.*, **A**, **301**, 113 (1967).

[6] Bertolotto, R., *et al.*, *Proc. Sienna Int. Conf. on Elementary Particles*, **1**, 523 (1963).

[7] Giesch, M., Van der Meer, S., Pluym, G., and Vahlbruch, K. M., *Proc. Sienna Intern. Conf. on Elementary Particles*, **1**, 536 (1963).

[8] Asner, A., and Iselin, C., *CERN Report* 65–17 (1965).

[9] Peterson, V. Z., and Keefe, D., *Informal Conf. on Exp. Neutrino Phys.*, *CERN Report* 32–65, 241 (1965).

[10] Alekseev, N. V., Gerstein, S. S., Markova, S. M., Folomeshkin, V. N., and Shukeilo, I. A., *Serpukhov Rep.* No. *IFVE* 6733*K* (1968).

[11] Perkins, D. H., *CERN/EFCA* 67/16, **2**, 1 (1967).

Applications of Magnetic Circular Dichroism and Optical Rotatory Dispersion Measurements

by

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Recent progress in the fields of magnetic circular dichroism (MCD) and magneto-optical rotatory dispersion (MORD) is reviewed and these techniques are contrasted with those of natural circular dichroism and optical rotatory dispersion. Apart from the fact that MCD and MORD do not require optically active compounds (optical activity being induced by the magnetic field), these physical tools offer spectroscopic information which is different or impossible to obtain by other means.

OPTICAL rotatory dispersion (ORD)[1] and circular dichroism (CD)[2] have become important tools in organic, inorganic and biological chemistry for the solution of many structural and stereochemical problems. This, in turn, has stimulated renewed interest in the phenomenon of magnetically induced optical activity[3,4] (the Faraday effect), which can be produced in all substances by exposure to a properly oriented magnetic field, whereas ORD and CD are applicable only to substances possessing natural optical activity. It will be shown that the utility in chemistry of magneto-optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD) is quite different from that of ORD and CD.

The principle of the Faraday effect is illustrated in Fig. 1. Left (L) and right (R) circularly polarized light (for the sake of convenience plane polarized light is employed because it is equivalent to the sum of the R and L circularly polarized components) is passed through a medium, and a magnetic field (H) is applied along the direction of propagation of the light beam. Because the magnetic field possesses helical symmetry, circular dissymmetry will be produced in the medium with the result that a different response will be elicited by optical stimulation with right and left circularly polarized light. Consequently, the refractive indices (n_L and n_R) as well as the molar extinction coefficients (ε_R and ε_L) will be different for the two (L and R) circularly polarized components. The angle α, which is proportional to $n_L - n_R$, is called the magneto-optical rotation, while $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ refers to the magnetic circular dichroism.

The magnitude of the Faraday effect phenomenon is proportional to the intensity of the magnetic field. In

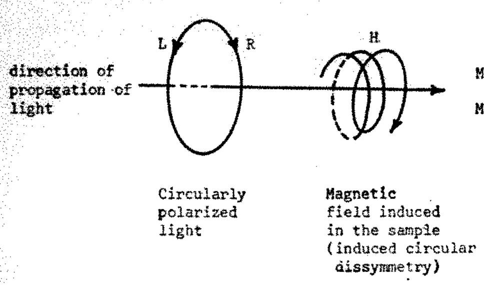
L
R
H
direction of propagation of light
MORD: $\alpha \sim n_L - n_R$
MCD: $\Delta\varepsilon \sim \varepsilon_L - \varepsilon_R$
Circularly polarized light
Magnetic field induced in the sample (induced circular dissymmetry)

Fig. 1. Principle of a Faraday effect experiment.

order to obtain maximal signals with a minimum of material the most recent experiments[5-9] have been conducted with superconducting magnets capable of field strengths in the neighbourhood of 50 kgauss. Experimentally the same instruments are employed for the measurement of MORD and MCD as for ORD and CD, the only requirement being that the sample compartment is of sufficient size to accept the appropriate magnet. The spectacular success of ORD with naturally optically active compounds has been associated with measurements performed through the region of the relevant absorption bands of the appropriate compounds, thus leading to anomalous dispersion (the well known "Cotton effects"[1]). Similarly, the most interesting results in MORD work[10,14] have been encountered in the region of maximum absorption. In the case of CD or MCD, this is an absolute requirement, because no circular dichroism is encountered outside the region of the absorption bands. Because most measurements are performed in solution utilizing optically transparent solvents (water, methanol and iso-octane, for instance), the MCD signals will only be associated with the absorption bands of the solute. On the other hand, magneto-optical rotation (related to refractive index, rather than just absorption—see Fig. 1) is also observable outside an absorption band, so the solvent also becomes optically active and its contribution must be subtracted from that of the solute in order to obtain the latter's MORD curve. This is one of the reasons why MCD is generally preferred over MORD.

Characteristics of MCD and MORD Spectra

In order to illustrate the areas of chemical applications, it is first necessary to point out the main features of the spectra encountered in measurements through absorption bands, MCD rather than MORD being chosen for the sake of convenience. The phenomenon is associated with an electronic transition, that is, with the promotion of an electron from the ground state to an excited one. An account of the dispersion curves can be given by considering the perturbation by the magnetic field of the various states and electronic transitions. In the absence of a magnetic field, a given transition at frequency ν_0 (Fig. 2*a*) gives rise to a corresponding absorption band (Fig. 2*b*). When the magnetic field is turned on, however, three perturbations are produced.

(1) The ground state may be split (in paramagnetic materials) and lead to two sub-levels; theory then predicts that the R and L circular absorption components occur at frequencies $\nu_0 - m_G H$ and $\nu_0 + m_G H$, respectively, where m_G is the so-called magnetic moment of the ground state, the magnitude of the splitting being $2H$ times m_G.

(2) R and L components have different probabilities of transitions, one being $\varepsilon_m(1 - aH)$ and the other $\varepsilon_m(1 + aH)$, because the perturbation is linear with the field.

(3) The sub-levels in the ground state have a different population, for example, 1 for the lower and $1 - 2m_G H/kT$ for the upper (according to the Boltzmann law). ε_L and ε_R can now be expressed as shown in Fig. 2*c* and the resulting expression for $\Delta\varepsilon$ becomes

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R = 2\varepsilon_m H \left\{ -m_G \frac{\mathrm{d}f}{\mathrm{d}\nu} + \left(\frac{m_G}{kT} + a\right) f(\nu) \right\}$$

In general, however, the excited state can also be split by the field, with a corresponding m_E value. It can then be easily established that $\Delta\varepsilon$ becomes

$$\Delta\varepsilon = 2\varepsilon_m H \left\{ -(m_G + m_E) \frac{\mathrm{d}f}{\mathrm{d}\nu} + \left(\frac{m_G}{kT} + a\right) f(\nu) \right\} \qquad (1)$$

This formula is similar to that obtained by more refined treatments[4].

Three important results follow. First, it shows that $\Delta\varepsilon$ is proportional to H. Second, an MCD experimental dispersion curve can be mathematically analysed as a linear combination of the absorption curve (this term often being referred to as $B + C/kT$) and its first derivative (A term). In other words, an MCD curve resembles a bell-shaped or an S-shaped curve, depending on the relative magnitude of the two terms (see, for example, Fig. 3). Third, B and C/kT can only be separated by measurements at various temperatures because they have the same frequency dependence. As in the case of natural optical activity[1,2], there is a Kronig–Kramers (K.K.) relation between MCD and MORD. The angle of rotation, α, is therefore expressed in a manner similar to $\Delta\varepsilon$, the K.K. transforms of $f(\nu)$ and $\mathrm{d}f/\mathrm{d}\nu$ being substituted in equation (1) and every other term being left unchanged. It follows that the MCD and MORD curves (for relationship, see Fig. 3) provide the same information, namely, the magnitude and sign of the three parameters A, B and C. It should be stated, however, that they are more easily extracted from MCD curves[6].

Comparison of Natural and Magneto-optical Activity

Equation (1) is appropriate for describing natural as well as magneto-optical activity. In the former case,

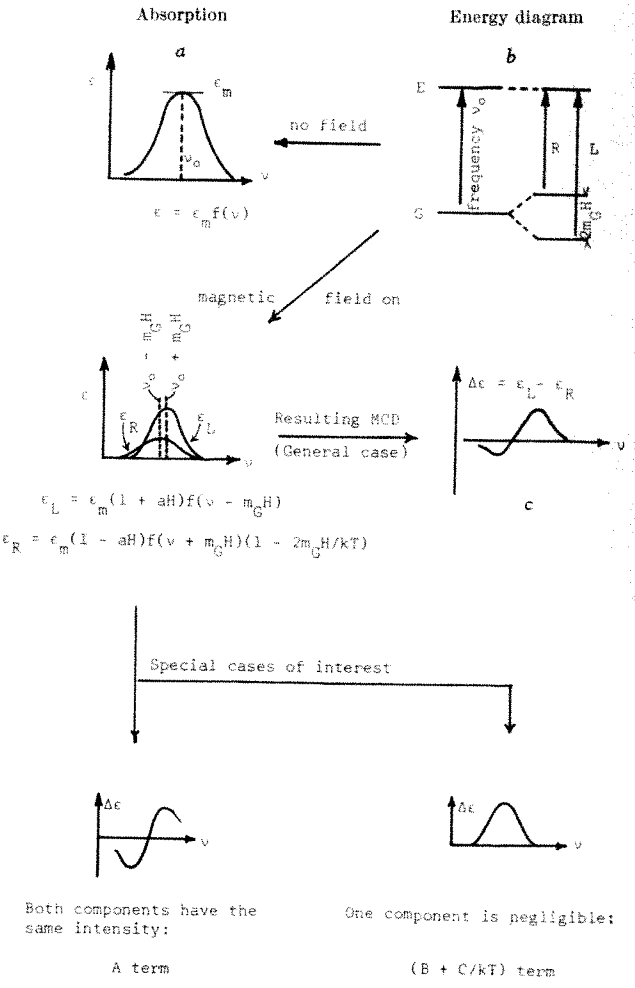
Absorption
Energy diagram
a
b
ε
ε_m
ν_0
ν
$\varepsilon = \varepsilon_m f(\nu)$
no field
E
G
Frequency ν_0
R
L
$2m_G H$
magnetic field on
$\nu_0 - m_G H$
$\nu_0 + m_G H$
ε
ε_R
ε_L
ν
Resulting MCD
(General case)
$\Delta\varepsilon = \varepsilon_L - \varepsilon_R$
ν
c
$\varepsilon_L = \varepsilon_m(1 + aH)f(\nu - m_G H)$
$\varepsilon_R = \varepsilon_m(1 - aH)f(\nu + m_G H)(1 - 2m_G H/kT)$
Special cases of interest
$\Delta\varepsilon$
ν
$\Delta\varepsilon$
ν
Both components have the same intensity:
A term
One component is negligible:
(B + C/kT) term

Fig. 2. Main features of magnetic circular dichroism (MCD) spectra.

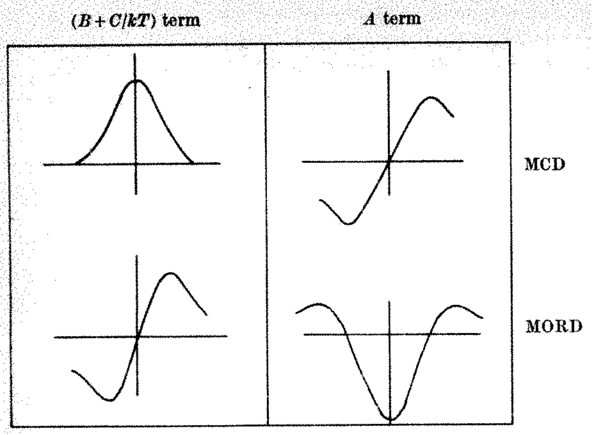

Fig. 3. Relationship between magneto-optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD) curves.

however, the B term only is non-zero—that is, a CD curve has the typical bell-shape. It is then clear that MCD provides new information through the A and C parameters. Moreover, it should be kept in mind that CD and MCD are of a different physical origin[11] and that the quantum mechanical expression of B is different in the two cases. This aspect can be illustrated by considering an inherently symmetric chromophore, in which optical activity is induced by disturbance from a dissymmetric substituent which does not form part of the chromophore itself (a typical example being the saturated carbonyl group[1,2]). Such a chromophore is very sensitive to the relative position of the substituent and the extrachromophoric part of the skeleton. This is why the sign and magnitude of the ORD and CD Cotton effects are so dependent on the conformation of the molecule and why ORD and CD have found such utility in stereochemistry. This does not apply to magneto-optical activity because the circularly dissymmetric perturbing field (leading to B terms) is now external and not inherent to the molecule. One can therefore predict that MORD and MCD will not usually be useful in the solution of conformational problems. This does not mean that Faraday effects are useless for solving chemical problems. Two examples bearing on this question will be considered briefly, one referring to an optically active and the other to an optically inactive substance.

In Fig. 4 the circular dichroism[6] of a chlorophyll-like molecule is reproduced without and with a magnetic field. It is clear that MCD signals are here far more intense than CD ones. Moreover, it has been established that the magnitude of the MCD peak around 539 nm depends considerably on the nature of the substituents attached to the skeleton. A similar conclusion can be reached from the MCD spectra (Fig. 5) of two optically inactive corrins[12] related to vitamin B_{12} in which the MCD peak at

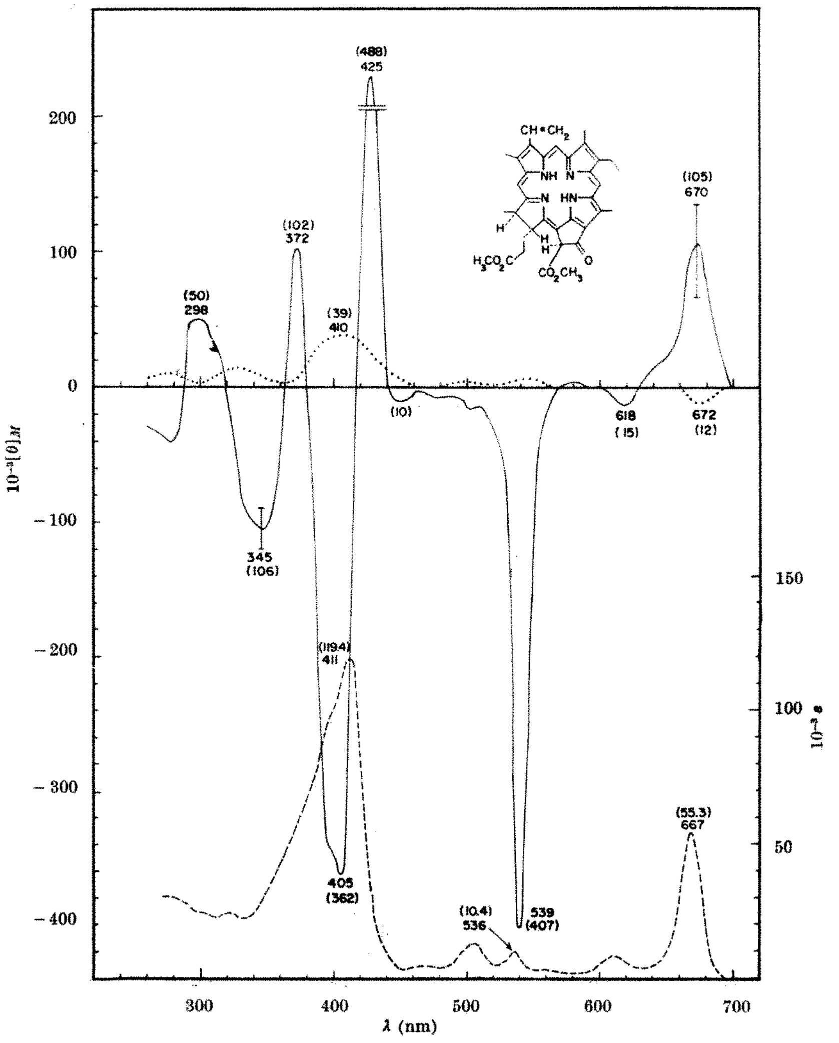

Fig. 4. Absorption (– – –), circular dichroism (. . . .) and magnetic circular dichroism (———) of methylpheophorbide a. MCD $[\theta]_M$ is reported for a 41·7 kG magnetic field. (Taken from ref. 6.)

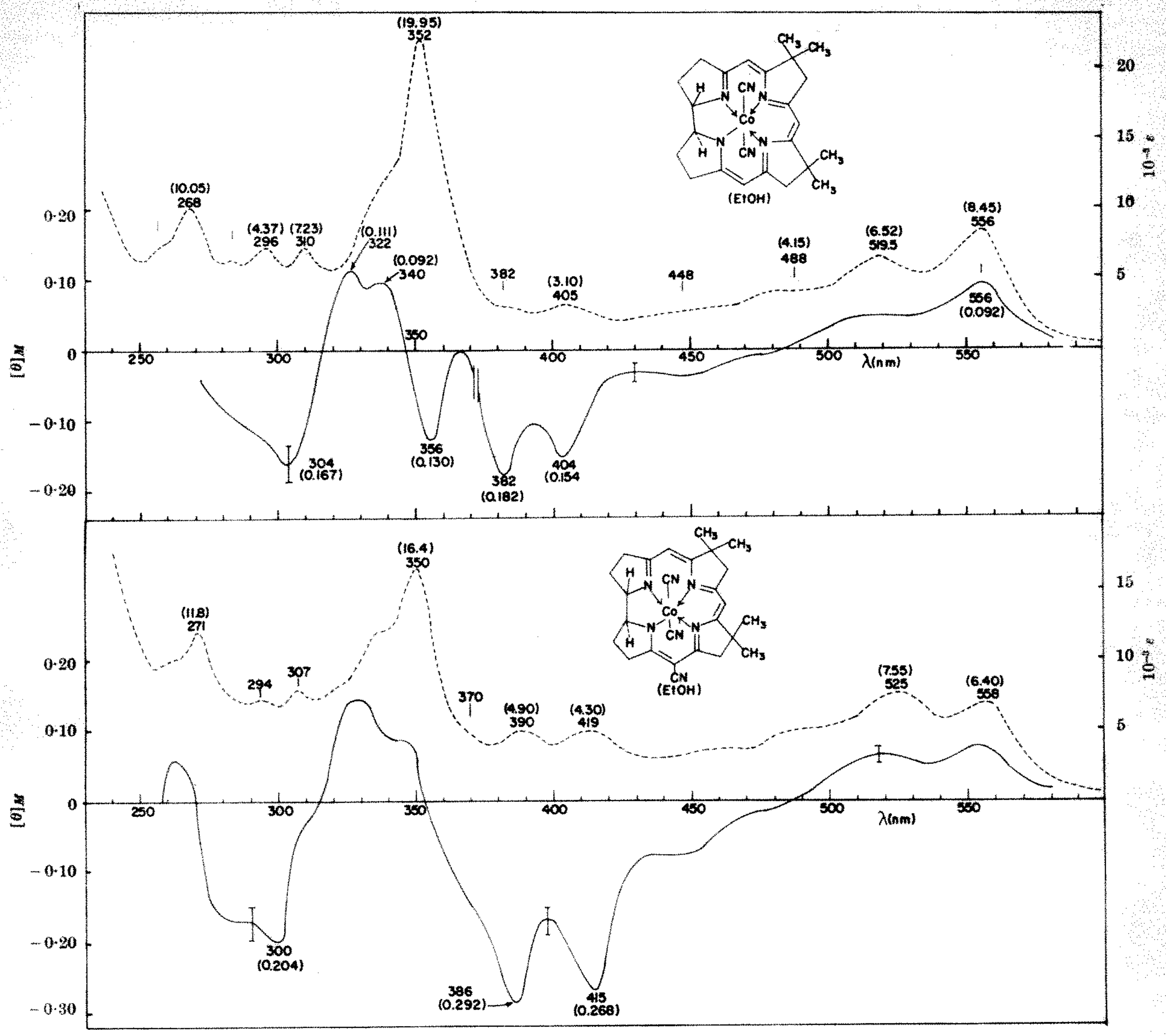

Fig. 5. Magnetic circular dichroism (———) and absorption spectra (– – –) of two optically inactive corrins.

356 nm disappears when one of the nuclear hydrogen atoms is replaced by a cyano group.

Unpublished studies in the nucleotide field by Voelter, Bunnenberg and Djerassi have also demonstrated the utility of MCD measurements with optically active as well as inactive molecules. The CD signals of optically active nucleosides and nucleotides are greatly magnified in the presence of a magnetic field (similar to the example of Fig. 4), so that one can obtain good MCD curves with quantities of material on which no CD signals can be discerned. The analytical and structural implications of such results are obvious. Furthermore, the MCD curves of the optically inactive purine and pyrimidine bases corresponding to these nucleotides and nucleosides can also be employed for purposes of structural differentiation, whereas ordinary CD or ORD cannot, of course, be employed because of the lack of natural optical activity.

Some Spectroscopic Applications of MCD and MORD

The utility of Faraday effect measurements can be demonstrated by considering again equation (1), and by comparing them with a few other techniques. The *C* and *A* terms allow the magnetic moment of the ground or excited states to be determined experimentally, *C* referring to the ground state only. The absolute value of *C* is already known from electron paramagnetic resonance (EPR) measurements, but its sign depends greatly on the nature and symmetry of the excited state corresponding to a given electronic transition. MCD and MORD therefore constitute a very powerful tool for aiding in assigning excited states. As shown recently in the case of the ferricyanide ion[8], this offers a new challenge to the study of charge transfer transitions. The *A* term is especially useful when it demonstrates the presence of a magnetic moment in the excited states. This is of great value because EPR—except for a few occasions—does not provide information on excited states. Zeeman effect measurements, which would solve such a problem, are limited to very sharp lines (gas or crystals at very low temperature) while Faraday effect measurements are possible on wide absorption bands, even at room temperature. Already a number of practical applications of these observations have been published pertaining to the assignment of excited states[8,9] and to the experimental determination of their magnetic moments[7,9]. These data allow in turn a quantitative verification of various molecular orbital calculations and constitute significant support for the theoretician. It is worth emphasizing that the substances studied so far range from inorganic complex ions to biological systems or complex conjugated organic molecules. Several general conclusions have been drawn from recent experimental work. It has been shown that the *A* and/or *C* terms are predicted to be non-zero in the case of molecules or complex ions possessing at least a three-fold axis of symmetry. Their sign and absolute value can be determined for allowed electronic transitions from group theory and quantum mechanical calculations respectively. On the other hand, the *B* term is the only one expected when the symmetry is lower, which is actually the case for most molecules. Theory then predicts[11,13] the *B* term to be large when two energy levels exist strongly coupled by the magnetic field and close together, while each of them is coupled to the ground state via an electric dipole moment. Group theory again shows that a large number of molecules, relevant to both chemistry and biology, exhibit such properties. Recent examples include various (ten) annulene systems[13] and vitamin B_{12} derivatives[12]. Although *B* is very difficult to estimate theoretically, its existence (and its sign) or non-existence is very significant by itself. For example, absorption bands which are hidden in the absorption spectrum can sometimes be revealed in the MCD spectrum[13]. Furthermore, the sign of the *B* terms permits the relative polarization of electronic or vibrational absorption components to be predicted or verified.

No attempt has been made to review all the recent MCD and MORD studies[14–16] of organic and inorganic compounds. Judging from the ever increasing tempo of activity in this field since 1965, one may anticipate rapid advances, and because there is practically no overlap between ORD and CD on the one hand, and MORD and MCD on the other, it is likely that the latter two techniques will soon be added to the instrumental repertoire of many chemists. Fortunately, this requires only the addition of a suitable magnet to existing instruments rather than the purchase or construction of completely new ones. In view of the wide distribution of spectropolarimeters and circular dichroism instruments, publications on many different types of compounds from numerous laboratories will surely be forthcoming with concomitant broadening of the scope of these two techniques.

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[1] Djerassi, C., *Optical Rotatory Dispersion* (McGraw-Hill, New York, 1960).

[2] Crabbé, P., *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry* (Holden-Day, San Francisco, 1965). Velluz, L., Legrand, M., and Grosjean, M., *Optical Circular Dichroism* (Academic Press, New York, 1965).

[3] Lowry, T. M., *Optical Rotatory Power* (Longmans Green, London, 1935, reprinted by Dover, New York, 1964).

[4] Buckingham, A. D., and Stephens, P. J., *Ann. Rev. Phys. Chem.*, **17**, 399 (1966).

[5] Schooley, D. A., Bunnenberg, E., and Djerassi, C., *Proc. US Nat. Acad. Sci.*, **56**, 1377 (1966).

[6] Briat, B., Schooley, D. A., Records, R., Bunnenberg, E., and Djerassi, C., *J. Amer. Chem. Soc.*, **89**, 6170 (1967).

[7] Briat, B., Schooley, D. A., Records, R., Bunnenberg, E., and Djerassi, C., *J. Amer. Chem. Soc.*, **89**, 7062 (1967).

[8] Schatz, P. N., McCaffery, A. J., Suetaka, W., Henning, G. N., Ritchie, A. B., and Stephens, P. J., *J. Chem. Phys.*, **45**, 722 (1966).

[9] McCaffery, A. J., Stephens, P. J., and Schatz, P. N., *Inorg. Chem.*, **6**, 1614 (1967).

[10] Shashoua, V. E., *J. Amer. Chem. Soc.*, **87**, 4044 (1965); *Arch. Biochem. Biophys.*, **111**, 550 (1965).

[11] Moscowitz, A., *Proc. Roy. Soc.*, A, **297**, 16 (1967).

[12] Briat, B., and Djerassi. C., *Bull. Soc. Chim. France* (in the press).

[13] Briat, B., Schooley, D. A., Records, R., Bunnenberg, B., Djerassi, C., and Vogel, E., *J. Amer. Chem. Soc.* (in the press).

[14] *Inter al.*, Briat, B., Billardon, M., Badoz, J., and Loriers, J., *Anal. Chim. Acta*, **34**, 465 (1966).

[15] Foss, J. G., and McCarville, M. E., *J. Amer. Chem. Soc.*, **89**, 30 (1967).

[16] Clerc, J. T., Wipf, H. K., and Simon, W., *Helv. Chim. Acta*, **50**, 1794 (1967).

Physiological, Morphological and Behavioural Adaptation to a Sodium Deficient Environment by Wild Native Australian and Introduced Species of Animals

by

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Native Australian and introduced species of animals are adapted to severe sodium deficiency. Effects of such a deficiency are aggravated by pregnancy and lactation, and it has been shown that mechanisms controlling sodium homeostasis in wild rabbits are subject to profound stress.

THERE are many reports of wild animals exhibiting an appetite for salt in nature, but there are few data concerning the metabolism of these animals which would establish beyond doubt that they are, in fact, salt depleted.

Stags, deer, chamois and other plant eaters are reported to have licked out caves in the salty slates of the Altai Mountains[1], and hunters lie in wait for them near salty pools, as they do with kudu and other buck near salt licks in Africa[2]. Rooseveldt elk and deer, bighorn sheep, goat, moose, caribou, mule deer and white tailed deer all seek out natural dry or wet licks in the Rocky Mountains in spring or summer or are attracted to salt blocks[3,4].

Game expose themselves to predation by black and grizzly bears and mountain lions when they leave open country and traverse the trails leading to licks. As many as 200 elk collect in a day at licks in Idaho. Cows and yearlings are most numerous at licks which are usually not shared, and the dominant bull elk exercises choice. Fighting occurs, and elk drive deer away and are themselves displaced by cattle[3]. Analyses of Canadian licks showed a high sodium content but other trace metals were present. In Montana only three of twenty-five potential licks were used by wild ruminants, and sodium bicarbonate and sodium sulphate were the only compounds significantly more abundant in these licks[5]. In Alaska, snow shoe hares will gnaw wood which has been immersed in salt water, and other animals will damage buildings where wood has been impregnated with salt from evaporated urine (personal communication). Reindeer are reported to drink sea water, and sheep made sodium deficient in the field by a unilateral parotid fistula will selectively graze plants with a high salt content[6]. Schaller observed that, on occasions, mountain gorillas ate soil which, on analysis, showed a high salt content[7].

Cattle and kangaroos in the mountains of south-eastern Australia exhibit an appetite for salt, and Bott *et al.*[8] showed that cattle which were eating pasture with only 1–2 mequiv. of sodium/kg wet weight excreted urine that was virtually free of sodium, and had a reduced salivary ratio of sodium to potassium indicative of increased aldosterone secretion. Myers[9] has found that a large increase of the zona glomerulosa occurs in the adrenals of wild rabbits in sub-alpine and sub-tropical regions, and that this can be associated also with the formation of cortical nodules. In the alps, these striking adrenal changes occurred in spring and early summer, and were associated with an avid appetite for wooden pegs impregnated with various salts. Sodium chloride and $NaHCO_3$ were particularly attacked, and some gnawing of $MgCl_2$ and KCl occurred if the pegs were exposed over a long period.

This article describes an attempt to analyse comprehensively the spectrum of adaptations by both native Australian and introduced species of animals to ecological conditions involving severe sodium deficiency. The studies of behaviour, physiological and biochemical processes, and morphology were made in the Snowy Mountains of southern Australia—an area larger than Switzerland—where analyses of soil and plants showed that the environment was severely sodium deficient. Control observations on similar parameters were made on an ocean coast region of Victoria, where the high sodium content of rain water provided vegetation with a high sodium content, and in a desert region near Broken Hill, central Australia, where the salt content of vegetation was high. A temperate grassland region with a moderately low salt content near the national capital, Canberra, was also studied. A principal aim was to evaluate salt appetite in relation to the verified sodium status of the animals as reflected by sodium excretion, and also the hormone secretory processes which are concerned with sodium balance. We have found some striking morphological changes concurrent with the functional observations. Some interesting seasonal, as well as regional, differences have emerged in relation to the alpine areas.

In examining the physiological effects of, and adaptive

Table 1. ANALYSES OF SAMPLES OF MIXED GRASSES AND SPECIES DESIGNATED AND SOIL SAMPLES FROM THE REGIONS OF CONTINENTAL AUSTRALIA IN WHICH THE ANIMALS WERE STUDIED

Location	Season	Sample type	Grass analyses mequiv./kg dry weight Na	K	No. of samples	Soil samples mequiv./kg dry weight Na	K	Mg	Ca	P	Cl
Snowy Mountains	Summer	Mixed	1·7	648							
		Mixed	1·3	170							
		Mixed	0·9	160							
		Mixed	1·9	117							
		Mixed	1·9	446							
	Autumn	Mixed	1·6	170							
		Mixed	2·0	160							
		Rumex	1·1	226	7	0·5	11	28	108	1·7	0·56
		Poa	3·6	141							
		Restio	2·2	163							
		Stylidium									
		Seeds	20	103							
		Stalks	83	90							
		Seed husks	175	110							
	Winter	Mixed	3·8	50							
		Mixed	3·6	106							
		Mixed	0·6	52							
	Spring	Mixed	< 0·2	428							
		Mixed	< 0·1	485							
		Mixed	8·5	165							
Grasslands (Canberra)		Mixed	5	320							
		Mixed	10	280							
		Mixed	10	105	3	0·8	4·3	27	45	0·3	0·60
		Mixed	5	450							
Central desert		Atriplex	2,200	310							
		Mixed	200	110	3	3·6	11	25	52	1·2	0·51
		Mixed (young shoots)	96	65							
Victorian sea coast		Mixed	309	349							
		Mixed	68	135	7	3·3	6·0	29	59	0·5	1·19
		Mixed	90	173							
		Mixed	208	395							

Table 2. MEASURES (MEAN ± *S.D.*) OF PHYSIOLOGICAL PARAMETERS IN WILD RABBITS IN THE REGIONS DESIGNATED. THE NUMBER OF ANIMALS IN EACH GROUP (*n*) IS INCLUDED

Region	Urine mequiv./l.		Peripheral blood aldosterone (ng/100 ml.)	Kidney renin (U/g cortex)	Faeces Hard* mequiv./kg/ dry weight		Faeces Soft* mequiv./kg/ dry weight	
Snowy Mountains	Na	K			Na	K	Na	K
Spring	0·59 ± 0·36 (19)	208 (19)	130 ± 104 (15)	162 ± 184 (10)	2	155	5	518
Summer	0·53 ± 0·12 (18)	269 (18)	69 ± 66 (12)	103 ± 80 (10)				
Autumn	2·6 ± 2·9 (12)	466 (6)		18 ± 7 (6)				
Winter	6·4 ± 1·2 (5)	390 (5)	74 ± 58 (11)					
Grasslands (Canberra)	18·0 ± 14·6 (12)	219 (12)	21 ± 10 (14)	39 ± 17 (8)				
Desert	139 ± 97 (15)	319 (15)	9 ± 6 (11)	30 ± 8 (7)	28	90	42	343

* Hard pellets are normal faeces voided directly onto the ground. Soft pellets, resulting from caecal fermentation, are eaten from the anus.

reactions to, a sodium deficient environment, it may be noted that, if the sodium content of the diet is virtually nil, conservatory processes may not be adequate to avoid a progressive depletion of body sodium. There may be species differences in this respect. Cattle are more prone to suffer severely from sodium deficiency because of the high sodium content of their semi-liquid faeces[10] than sheep which are able to reduce faecal sodium content to very low levels when sodium depletion supervenes[11].

The effect of a sodium deficient environment is aggravated by pregnancy and lactation, because of sodium accumulated in the growing products of conception and the augmented blood volume, and sodium lost in milk[12]. The stress can also be increased by any intestinal disturbance of microbial or nematodal origin, which jeopardizes the efficiency of sodium absorption from the gut[13], or results in sodium sequestration in the nematodal parasites.

Seasonal variation in the characteristics of vegetation, such as the onset of lush spring growth, can result in a rapid reduction in the sodium content/unit weight of vegetation, a decreased sodium to potassium ratio and sodium content/calorie of intake, and increased fluid turnover. In this context, it has been suggested that a high rate of potassium excretion causes some sodium loss[14]. If the sodium deficient areas are in the tropics, and temperature regulating mechanisms are stressed, according to the species, there is some sodium loss from skin in sweating or from saliva as a result of drooling associated with panting[12].

Christian (personal communication) has proposed that a high population density increases the size and activity of the zona fasciculata and reticularis, and that the high glucocorticoid secretion causes natriuresis and kaliuresis. Glucocorticoids may antagonize the sodium-retaining effect of aldosterone in adrenalectomized rats[15]. This renal effect would further aggravate the effects of a low sodium environment, and the trophic effects on the fasciculata–reticularis may possibly limit the functional capacity of glomerulosa to hypertrophy in response to sodium deficiency. Aumann and Emlen[16] have reported that the sodium level of soil and the density of populations of microtine rodents are directly related, and concurrent laboratory experiments showed increased salt intake of microtine groups in response to crowding.

The studies on the various biological parameters indicative of overall adaptation by the organism to differing ecological conditions were made on introduced species—wild rabbits *Oryctolagus cuniculus* (L.) and sheep—and on native animals—wombats *Vombatus hirsutus* (Perry) and kangaroos *Macropus giganteus* (Shaw). One principal aim was to measure the concentration of steroids in peripheral blood by using recently developed highly sensitive methods of double isotope dilution derivative assay[17]. Laboratory experiments[18] on the time course of action of ACTH following direct adrenal arterial infusion have shown that 2–3 min elapse before a significant rise of corticosteroid secretion in adrenal venous effluent. In order to obtain a measurement in conditions where the concentrations were not modified by probable pituitary stimulation associated with pursuit and capture of wild animals, it was considered that the most practical approximation to the normal would be achieved by locating the nocturnal grazing animals by spotlight from a travelling car, instant killing by shooting through the brain, and immediate withdrawal and frozen storage of cardiac blood. In these conditions, the peripheral blood level should reflect that of the grazing animal before disturbance.

Table 1 shows the analyses of soil and mixed grasses from the regions which were studied. With the Snowy Mountains, seasonal analyses were made. The higher sodium content of soil and grasses in the desert and on the sea coast is evident. Very low sodium content of grass was found in the Snowy Mountains in spring and summer. The exceptional observation in the spring was on an area where a bush fire had burnt 2 years previously. The sodium content of grasses was greater in the mountains in autumn and winter. One species of plant, *Stylidium graminiforum*, the seeds and other parts of which were eaten by rabbits in autumn, had a high sodium content. In relation to the alpine figures (*c.* 1 mequiv./kg wet weight) approximately a week's intake of food by a sheep or rabbit is required to provide up to 5 per cent of the sodium content of the circulating plasma whereas, on normal pasture, sodium equivalent to 10–25 per cent of the plasma content is ingested each day. At birth, a rabbit weighs about 40 g and total body sodium is about 5 mequiv. Subsequent to birth a litter of four rabbits each grows about 9 g/day. If the doe provides 100 g milk of normal composition/day, of which the ash content is 2·5 per cent and sodium is 5 per cent of the ash[19], it may be calculated that 20 per cent of the sodium content of circulating plasma would be required each day if lactation were normal. The profound stress on mechanisms controlling sodium homeostasis is evident from the following data.

Table 2 shows that in spring and summer the mountain rabbits excreted urine which was virtually free of sodium whereas, in autumn and winter, urine contained 2–6 mequiv./l. Correspondingly, the sodium content of hard and soft faeces was much lower than in the desert region. Fig. 1 shows that the sodium content of urine of kangaroos and wombats in the alps was virtually nil compared with that of sea coast animals—kangaroos a mean of 215 mequiv./l. and wombats 23 mequiv./l. The lower sodium content of the urine of the sea coast wombat possibly reflects different habits of the latter as root eaters. A large unexplained variation was found in urinary sodium content of sheep grazing in the mountain areas in early summer (from 0·3 to 130 mequiv./l., $n = 14$); this requires further investigation in relation to the possibility of selective grazing of plants or access to an undetected source of salt.

Only limited observations of blood electrolytes were made. The mean sodium content of plasma of the alpine kangaroos—139 mequiv./l.—was reduced relative to the sea coast animals—148 mequiv./l.

Table 2 shows that the concentration of peripheral blood aldosterone was greatly increased in the alpine rabbits relative to that of the desert and Canberra grassland animals. The highest aldosterone levels were found in pregnant animals during the spring in the alps.

As reported in detail elsewhere[20], the four mountain kangaroos which were examined had a mean aldosterone concentration (40 ng/100 ml.), four times that found with nine plain and sea coast animals—9 ng/100 ml. (Fig. 1). The mean aldosterone in the five mountain wombats (12·5 ng/100 ml.) was double that of the sea coast animals (6 ng/100 ml.)—both small groups included observations with very low levels. Cortisol and corticosterone were present in the blood of both species, cortisol being present in the larger amount. There was little difference in the levels of glucocorticoids in the sodium deficient and sodium replete animals.

Sheep in the alpine areas were anaesthetized, the parotid ducts were cannulated and saliva was collected. Adrenal vein blood was collected from the cannulated adrenal vein. Consistent with the unexplained variation of the concentration of urinary sodium referred to here, the secretion rates of aldosterone varied from the usual range of sodium replete anaesthetized sheep to levels seen in severe depletion (that is, from 1·7 to 15·6 μg/h), the high value being associated with virtual absence of sodium from urine and a large reduction of salivary sodium to potassium ratio.

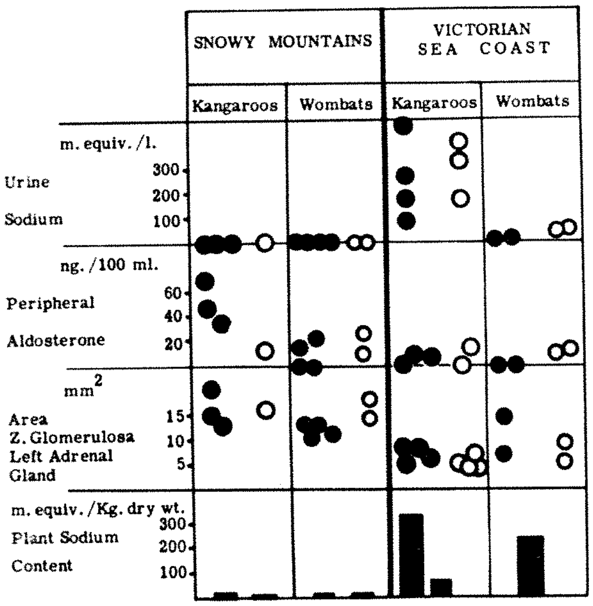

Fig. 1. The urinary sodium concentration, peripheral blood aldosterone and percentage of adrenal area represented by zona glomerulosa in two marsupial species—the grey kangaroo and the wombat studied in an alpine and a sea coast area.

The concentration of renin was measured in the kidney cortex[21]. In rabbits (Table 2) the mean concentration (U/g) was greatly elevated in the alps in spring and summer. Highest values were recorded in females—possibly suggesting, as with aldosterone values, the additional stress on sodium balance of the reproductive process. The mean value for animals from the Canberra grasslands area was slightly higher than for desert animals.

As with rabbits, kangaroo and wombat kidneys were incubated with prepared sheep substrate[21]. The concentrations were less than 1 U/g, which may be caused by species specificity of the renin/renin substrate reaction. The kidneys of sheep in the alps had renin levels in the high normal range but no values were found near to those found in laboratory animals which were sodium depleted by a parotid fistula.

Table 3 shows that the adrenal glands of the alpine rabbits in spring were heavier than in other regions, and

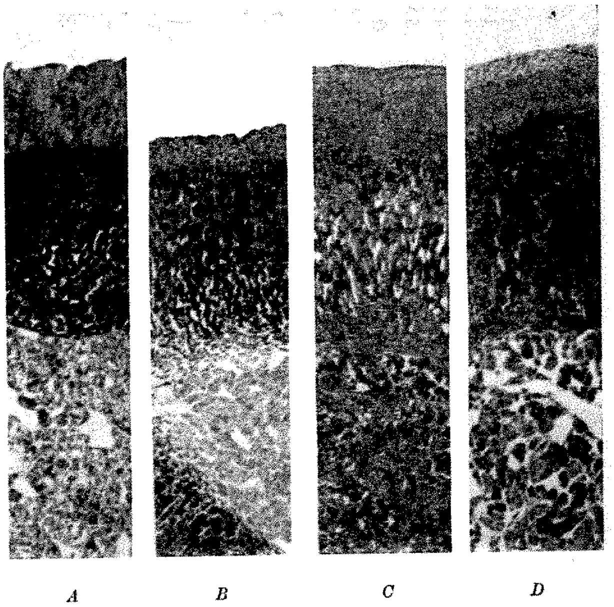

Fig. 2. Section of the adrenal glands of the kangaroo (*Macropus giganteus* (Shaw)), (*A*) sodium deficient, (*B*) sodium replete, and the wombat (*Vombatus hirsutus* (Perry)), (*C*) sodium deficient, (*D*) sodium replete. The zona glomerulosa is wider in deficient animals. The capsule is more prominent in the wombat adrenal. (× 27.)

that seasonal variation occurred in the alpine animals. The area of zona glomerulosa represented a much greater percentage of the total area of the adrenal in the alps in spring and summer than in the desert and grassland animals. The adrenal glands of *Macropus* (1·1 g) and wombats (0·7 g) examined in the mountains were approximately double the weight of the control animals from the sea coast (0·6 and 0·4 g, respectively), and the zona glomerulosa represented twice the percentage of total area in kangaroos, but was only 20 per cent greater in mountain wombats (Figs. 1 and 2).

There were striking structural changes in the salivary glands of the alpine animals when compared with the same species living in the sodium rich sea coast areas. Table 4 records the much more extensive duct system of the parotid gland of sodium depleted specimens of *Macropus giganteus* (Shaw) (the grey kangaroo). A similar finding was made in the submandibular glands of the same species where the percentage of total gland represented by the striated ducts was four times that of the

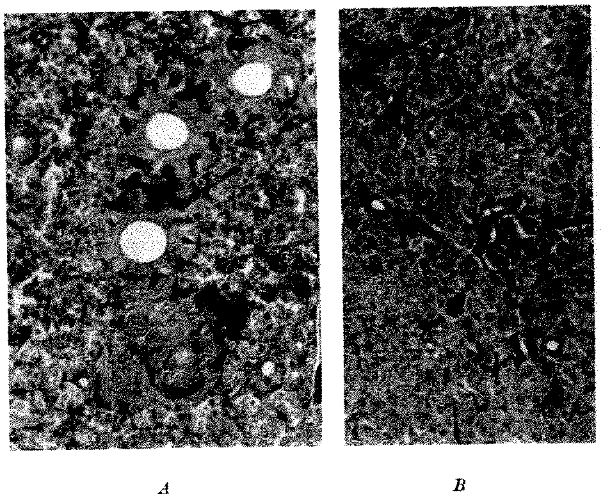

Fig. 3. Section of the parotid gland of sodium deficient alpine kangaroo (*A*) compared with that of a sodium replete sea coast animal of the same species (*B*). (× 60.)

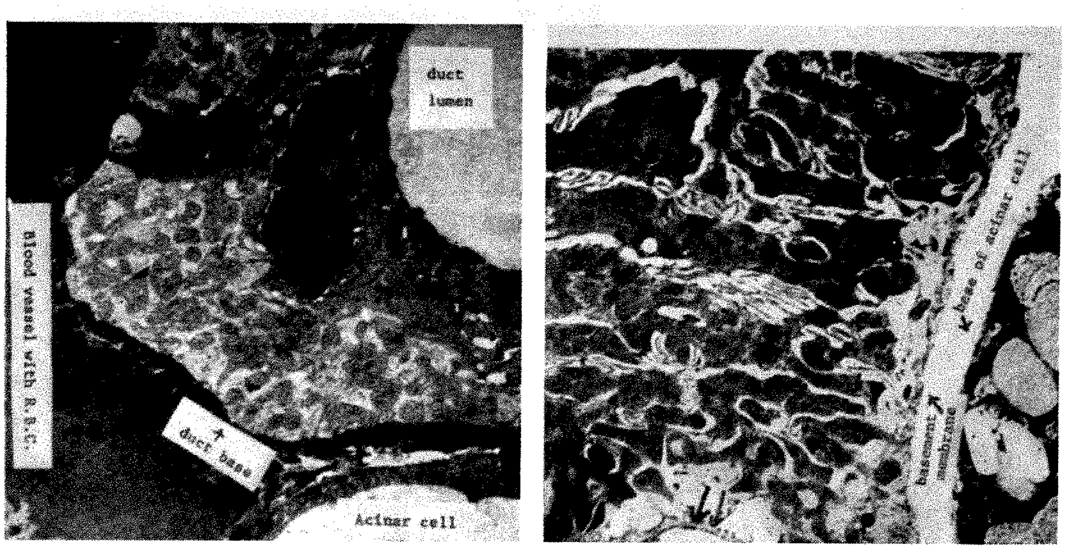

Fig. 4. Electron micrograph of the parotid gland of (left) a sodium replete sea coast wild rabbit (*Oryctolagus cuniculus* (L.)) compared with that of (right) a sodium deficient alpine wild rabbit. (× 10,000.)

Table 3. THE WEIGHT AND AREA OF ADRENAL AND THE PERCENTAGE OF TOTAL AREA OF ADRENAL REPRESENTED BY GLOMERULOSA IN WILD RABBITS IN DIFFERENT REGIONS OF AUSTRALIA

Region	Adrenal weight (g) (*n*)	Total area of adrenal (mm²)	Area of zona glomerulosa (percentage total)
Snowy Mountains			
Spring	0·18 (24)	22·6	34·4
Summer	0·12 (21)	18·2	22·6
Autumn	0·14 (12)	21·2	17·3
Winter	0·14 (13)	22·2	17·8
Central desert			
Summer	0·11 (14)	18·4	15·6
Canberra grasslands			
Spring	0·13 (12)	20·6	15·3

replete animals. Measurement of the height of parotid duct cells ($n = 100$) with a micrometer ocular showed that the value for sodium deficient animals was 19·51 ± 3·20μ, and for replete animals, 14·04 ± 3·05μ—($P < 0·01$). The vascular pattern of the sodium deficient animals was considerably different from that usually observed. Blood vessels were extraordinarily abundant around striated ducts. Fig. 3 shows that loops of capillaries penetrate the outer wall of the striated ducts and run in the epithelial zone. The wombat material showed similar changes in the epithelium though not to the same degree.

Table 4. CONSTITUTION OF *Macropus giganteus* (GREY KANGAROO) PAROTID GLAND EXPRESSED IN PERCENTAGE OF TOTAL VOLUME

Condition	Serous cell	Striated duct	Excretory duct
Sodium deficient	86·3	5·3	3·1
Sodium deficient	79·6	12·4	3·6
Sodium deficient	83·2	9·5	4·8
Sodium replete	95·4	0·0	1·5
Sodium replete	94·3	0·3	0·6
Sodium replete	92·7	0·2	1·3

In the parotids of rabbits deficient in sodium the volume of striated ducts was 5·9 per cent of the total volume compared with 1·5 per cent in the replete. The number of mitochondria in these cells was considerably less in the sodium replete animals. The epithelium was approximately twice as high (18·3μ) as in the sodium replete (10·9μ). Striated ducts were present in the submandibular glands whereas they were virtually absent in the sodium replete rabbits. EMG study of the rabbit parotids showed increased density of acinar granules in sodium deficiency. The striated ducts in sodium replete animals (Fig. 4) had low prismatic or cubic cells with very few or no infoldings of the basal cell membrane. Mitochondria were sparsely distributed and separated by cytoplasm. Their cristae were not very closely packed and no intracellular channels were visible. In the sodium deficient animals (Fig. 4), not only were the cells higher but there were profuse infoldings of the basal cell membranes. These infoldings are limiting portions of cell cytoplasm that are packed with mitochondria with very little cytoplasm between them. Furthermore, the cristae of the mitochondria were also very closely arranged suggesting a higher content of cristae per unit volume. The conspicuous intercellular clear channels are suggestive of intercellular channels involved in ion and fluid transport.

The kidneys of the sodium deficient wombats showed hypertrophy of the juxtaglomerular region and much greater height of proximal tubular cells. Changes in the kangaroo kidneys were less marked.

Experiments were undertaken in which groups of softwood sticks impregnated with different inorganic salts were firmly driven into the ground in the region of rabbit warrens. Analysis showed that the impregnation process caused the outer wood to contain up to 1,800 mequiv./kg of sodium whereas the core contained 20 mequiv. The content varied with different salts. Fig. 5 shows that in the spring and early summer in the Snowy Mountains (top three panels) the wild rabbits avidly devoured the pegs impregnated with NaCl and $NaHCO_3$, but largely ignored $MgCl_2$ and distilled water. Some gnawing of sticks impregnated with KCl occurred. In some instances the pegs impregnated with sodium were dug out of the ground and completely eaten. In the late autumn the rabbits did not show significant salt appetite. This corresponded with the urine analyses (mean sodium content, 3 mequiv./l.) zona glomerulosa width (Table 3) and grass analyses (Table 1) including *Stylidium*. Fig. 6 records behavioural observations on peg groups and shows the predominant feeding on pegs with sodium salts. Pegs put out near warrens at Canberra (mean urinary sodium, 18 mequiv./l.) were untouched and so were pegs in the desert area. A fascinating field of future behavioural investigation will centre on the process of initial recognition of the salt sticks by the naive wild depleted rabbits and the specific selection of the sodium containing pegs (Fig. 6). Some instances of intake of magnesium have been seen in pegs left out in the alps for a long time in late summer[9]. This requires further investigation but might be related to the influence of a sustained very high blood

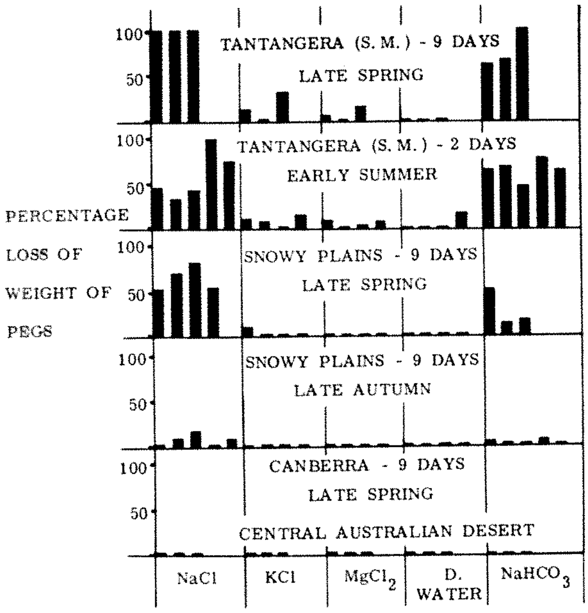

Fig. 5. The percentage loss of weight of soft wooden pegs heavily impregnated with the salts designated when they were placed in the vicinity of rabbit warrens for 9 days. The upper four panels record observations in the alpine region, the bottom panel in the desert and plateau grassland area.

aldosterone in spring and early summer in causing magnesium depletion in the tissue[22].

These studies show that the southern alps of Australia is a region severely deficient in sodium. Rainfall low in sodium together with annual leaching of soil by melting snow contributes to this ecological circumstance[12]. Possibly the most important finding emerging is that severe sodium deficiency does occur in wild animals in nature. In view of the additional stress imposed by the reproductive process, it is evident that a strong selection pressure would operate favouring animals with effective conservatory mechanisms. Similarly, selection would favour animals with central nervous mechanisms such that, when depleted, appetitive drive would cause more extensive exploration of the environment and detection and ingestion of sodium from licks, and plants which accumulate this ion[12]. This statement, as indicated[4], requires qualification in so far as such behaviour under stress of depletion may involve more extensive exposure to predation by carnivores.

The conservatory mechanisms correspond to the physiological responses to sodium deficiency defined in laboratory and clinical conditions[12,14,18]. The function of the renin–angiotensin system in the response to sodium deficiency in the marsupials will be a field for future comparative interest in the light of the preliminary difficulty in obtaining pressor material when incubating kidneys from sodium deficient wombats or kangaroos with sheep substrate.

The morphological changes in the duct system of salivary glands were an important new finding presumably indicative of chronic hyperactivity in active sodium reabsorption. Such structural changes might be attributable to either chronic sodium deficiency and/or the action of aldosterone. Previous observations on the influence of DOCA in producing a great increase in the height of duct epithelium in sheep suggest a hormonal trophic influence. On the question of detrimental effects of sodium deficiency, experiments with wild rabbits (Myers, unpublished) studied in enclosures with thirty-five, fifty or two hundred animals to the acre showed a highly significant reduction of glomerulosa and an increase in fasciculata areas in animals at high density (Table 5). This is consistent with the proposal that density is an additional aggravating stress in sodium deficient zones. In the course of preliminary experiments examining the nodule producing effects of ACTH (Myers and Bults, unpublished) it was found that, of itself, the stress of simple handling of the sodium deficient mountain rabbits resulted in weight loss and morbidity which were not seen in animals from sodium replete regions.

The presence or absence of an appetite for salt in naive wild rabbits was clearly related to sodium balance and physiological reactions of the animals. No controlled observations have been made to date on kangaroos, but

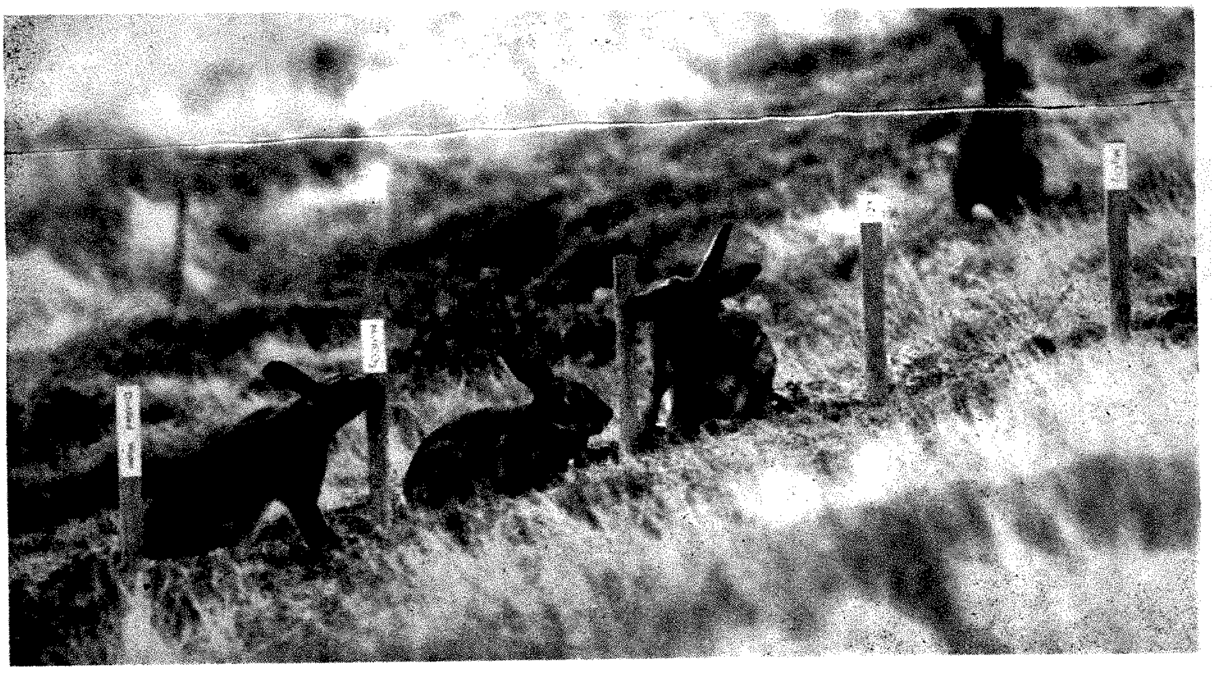

Fig. 6. A telescopic lens photograph of sodium deficient alpine wild rabbits attacking pegs impregnated with $NaHCO_3$ and NaCl (label detached).

Table 5. EFFECTS OF DENSITY ON ZONATION OF ADRENAL GLANDS OF WILD RABBITS IN EXPERIMENTAL POPULATIONS

	Population density (rabbits/acre)	N	Total adrenal (g)	Total area (mm²)	Median cross-section of right adrenal: Area Z. glomerulosa (mm²)	Area Z. glomerulosa (percentage total)	Area Z. fasciculata–reticularis (percentage total)	Area medulla (percentage total)
Males	200	20	0·38	26·27	† 2·50	10·29	82·49	7·23
	50	10	0·40	28·53	3·18 †	11·43 †	80·84 †	7·62
	35	5	0·30	25·59	3·78	16·07	76·71	7·27
Females	200	19	0·29	21·94	† 2·47	11·92	80·54	7·54
	50	10	0·25	19·10	3·35 *	17·83 *	73·25 *	8·93
	35	10	0·24	20·65	† 3·56	17·92	73·94	8·12

* $P < 0·001$. † $P < 0·01$.

these animals are known to be attracted to salt blocks put out for cattle in the mountain regions. The findings on wild rabbits highlight the problem of the explanation of the biological relevance of the need-free salt appetite exhibited by caged laboratory animals[23]. The latter observation, firmly established in the case of rats, is consistent with Richter's proposition[24] of a genetic ability in mammals to taste salt and that the ability to taste nutritious substances confers survival advantage. Salt taste fibres have been identified in a number of mammalian and more primitive vertebrate species. Bare[25] has suggested that the normal animal is protected against specific food deprivation by this unlearned stimulus response connexion. In this regard it is noteworthy that sodium chloride is a substance likely to be encountered in nature, either solid or in solution. The observations recorded here on various species of wild herbivores in the Rocky Mountains[3,4] indicate, however, a definite seasonal basis in intake, and this is clearly the case with wild rabbits, and is related to the sodium balance. It seems likely that this will also be the case with animals in the Rocky Mountains and Idaho. In the Florey Laboratories, self-selection of solutions of NaCl, $MgCl_2$, $CaCl_2$, KCl has been studied in rabbits captured in the wild when young. Most of these caged animals exhibit only a small need-free intake (sodium in the diet, 60 mequiv./kg) of isotonic or 500 mequiv./l. NaCl solution (mean intake of 0·16 and 0·4 mequiv./day, respectively). It therefore becomes of considerable importance to examine the salt appetite of the many species of the genus *Rattus* which live wild on several continents. Behaviour may depend considerably on the mode of presentation of the substance, and the animal without any metabolic deficiency may accept some isotonic solution but not gnaw wood impregnated with salt. The possibility has to be considered, however, that in natural conditions with a much more diverse range of stimuli engaging the attention of active animals the behaviour does not parallel that seen under the relatively stimulus deprived condition of the laboratory cage[23].

Obviously it is of general biological importance that herbivores are subject to ecological stress in relation to sodium variation, as shown here with the plants of regions varying from below 0·1 to 2,000 mequiv. of sodium/kg of dry weight. The carnivores probably largely avoid sodium deficiency because they feed on herbivores which, though stressed, have nonetheless a high tissue content of sodium. Thus it is unlikely that selection pressures, similar to those with herbivores, operate in carnivores in relation to evolutionary emergence of neural mechanisms evoking salt appetite. In this general context, the situation with regard to omnivores such as man or the rat is particularly interesting. With feral man, as represented by the primitive mountain tribes of New Guinea, dietetic studies have shown that with their essentially vegetarian diet the Highland Papuan consumes 200–400 times more potassium than sodium[26] with sodium intake as low as about 3 mequiv./day. The diet of Papuan communities is often largely dependent on a single staple food—sago, taro, the introduced sweet potato, or more rarely yam or banana. Peripheral whole blood aldosterone levels of Papuans in primitive highland areas have been compared with those in other areas where European food and salt is available. These studies have been done by the Howard Florey Laboratories as part of Macfarlane's extensive metabolic investigations of the peoples of the region. In the former instance, the level of aldosterone was consistent with other evidence of sodium deficiency—that is, mean 25·8 ng/100 ml. ($n = 7$), compared with 9·9 ng/100 ml. ($n = 14$) in the European areas, which is comparable with levels in hospitalized sodium replete patients on a standard diet[17]. The preparation of salt cake and the ancient salt trade in New Guinea Highlands have been described by anthropological investigators[27,28].

A further general consideration emerges from the finding of severe sodium deficiency in herbivores including ruminants in natural conditions. Digestion in the ruminant involves circulation of much larger volumes of alimentary fluid of high sodium content than in other species. The capacity to replace sodium by potassium in this cycle commensurately with sodium deficiency reduces the haemodynamic stress of sodium deficiency and thus is pivotal to adaptation. The data support the hypothesis previously advanced[11,12] that the laboratory demonstration of a large reduction in the ratio of sodium to potassium in the saliva of sodium deficient ruminants mediated by aldosterone reflects a principal mechanism in evolutionary emergence of the capacity of these animals to adapt to stringent environmental conditions.

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[1] Bunge, G., *Zeits. Biol.*, **9**, 104 (1873).

[2] Hemingway, E., *Green Hills of Africa* (Penguin, London, 1966).

[3] Dalke, P. D., Beeman, R. D., Kindel, F. J., Robel, R. J., and Williams, T. R., *J. Wildlife Management*, **29**, 319 (1965).

[4] Cowan, I. McT., and Brink, V. C., *J. Mamm.*, **30**, 379 (1949).

[5] Knight, R. R., and Mudge, M. R., *J. Wildlife Management*, **31**, 293 (1967).

[6] Arnold, G. W., *Proc. Austral. Soc. Anim. Prod.*, **5**, 258 (1964).

[7] Schaller, G., *The Year of the Gorilla* (Collins, London, 1965).

[8] Bott, E. A., Denton, D. A., Goding, J. R., and Sabine, J. R., *Nature*, **202**, 461 (1964).

[9] Myers, K., *Nature*, **213**, 147 (1967).

[10] Renkema, J. A., Senshu, T., Gaillard, B. D. E., and Brouwer, E., *Nature*, **195**, 389 (1962).

[11] Denton, D. A., *Quart. J. Exp. Physiol.*, **42**, 72 (1957).

[12] Denton, D. A., *Physiol. Rev.*, **45**, 245 (1965).

[13] Symons, L. E. A., *Austral. J. Biol. Sci.*, **13**, 171 (1960).

[14] Eisenstein, A. B., in *The Adrenal Cortex* (edit. by Eisenstein, A. B.), 335 (Little, Brown and Co., Boston, 1967).

[15] Uete, T., and Venning, E. H., *Endocrinology*, **71**, 768 (1962).

[16] Aumann, G. D., and Emlen, J. T., *Nature*, **208**, 198 (1965).

[17] Coghlan, J. P., and Scoggins, B. A., *J. Clin. Endocrinol. Metab.* (in the press).

[18] Blair-West, J. R., Coghlan, J. P., Denton, D. A., Goding, J. R., Orchard, E., Scoggins, B. A., Wintour, M., and Wright, R. D., in *Symposium: Angiotensin and Aldosterone. Proc. Third Intern. Cong. Nephrol.*, Abstr. 1 (1966).

[19] Blaxter, K. L., in *Milk: The Mammary Gland and its Secretion* (edit. by Kon, S. K., and Cowie, A. T.), **2**, 305 (Academic Press, New York and London, 1961).

[20] Coghlan, J. P., and Scoggins, B. A., *J. Endocrinol.* (in the press).

[21] Blair-West, J. R., Coghlan, J. P., Denton, D. A., Scoggins, B. A., Wintour, M., and Wright, R. D., *Med. J. Austral.*, **2**, 290 (1967).

[22] Care, A. D., and Ross, D. B., *Res. Vet. Sci.*, **4**, 24 (1963).

[23] Denton, D. A., in *Handbook of Physiology* (American Physiological Society) (in the press).

[24] Richter, C. P., in *L'Instinct dans le Comportement des Animaux et de l'Homme*, 577 (Masson et Cie., Paris, 1956).

[25] Bare, J. K., *J. Comp. Physiol. Psychol.*, **42**, 242 (1949).

[26] Oomen, H. A. P. C., Spoon, W., Heesterman, J. E., Ruinard, J., Luyken, R., and Slump, P., *Trop. Geog. Med.*, **13**, 55 (1961).

[27] Wills, P. A., *Philippine J. Sci.*, **87**, 169 (1958).

[28] Meggitt, M. J., *Austral. Museum Mag.*, **12**, 309 (1958).

Discrimination Learning in Animals: an Analysis through Side-effects

by

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Different techniques used in discrimination training of pigeons have different side-effects on the birds. These side-effects of training are important in analysing discrimination learning in animals.

PIGEONS have recently been the subjects of an increasing number of experiments on discrimination learning. The apparatus used in most of these experiments consists of a 1 ft ^{3} sound-attenuated chamber in which a hungry pigeon is placed. Pecks at a 0.75 in diameter disk (response key) on one wall of the chamber can be rewarded by brief access to a grain mixture. If unpredictable rewards (averaging, for example, one per min) follow pecks at the key, the pigeon comes to peck steadily at a rate of between 30 and 90 pecks per min[1]. In experiments on discrimination learning with the pigeon, the desired outcome is that the pigeon should peck to a signal for reward, and not peck to a signal for absence of reward. Three techniques of training have been developed which have this result, but which have different side-effects on the pigeon's behaviour. These by-products of discrimination training are important in analysing what is involved in discriminative behaviour, and they point to the need for revision of the concepts traditionally used in explaining animal discrimination learning[2].

Methods of Discrimination Training

Two training techniques date from earlier studies with rats[3], and can be termed "choice" and "go/no go" methods. In the choice situation, the pigeon is confronted with two illuminated keys in the experimental box. Pecks at one of these (the positive signal, or $S+$) bring occasional, unpredictable rewards, while pecks at the other (the negative signal, or $S-$) are not rewarded. Ultimately the pigeon comes to choose $S+$. In the go/no go method, only one key is present, illuminated with either $S+$ or $S-$. During the positive signal, pecks are rewarded, but during the negative signal, no rewards are forthcoming. A pigeon trained by this procedure pecks to the positive signal, and does not peck to the negative one. In the usual method of training a pigeon on a go/no go discrimination, many pecks are made to the signal for no reward ($S-$) in the first few sessions. Usually, $S+$ and $S-$ are similar, for example, red and green colours, or lines of different angles to the vertical. Recently, however, Terrace[4] has evolved a method of "errorless" training, by which almost all pecks to $S-$ are avoided. The technique consists of presenting the negative signal at first for short durations and at a low intensity of illumination. In these conditions, no pecks are made to it. The duration and intensity of $S-$ are gradually increased to equal those of $S+$. In this way, the discrimination is learned without errors.

Although the end results of the training methods are similar to the extent that the pigeon's behaviour matches the signal, there are differences in other behaviour in the discrimination situation. As the pigeon begins to differentiate the two signals in the go/no go situation, the rate of pecking to $S-$ declines. At the same time, the rate of pecking to $S+$ increases[5]. Pavlov[6] termed a similar effect in salivary conditioning "positive induction", while Reynolds[5] adopted the term "behavioural contrast". When the go/no go discrimination has been acquired with two colours as $S+$ and $S-$, and with many errors, subsequent testing reveals that a new colour is preferred to the original $S+$ colour[7]. This colour is located at a point on the wavelength dimension shifted from $S+$ in a direction away from $S-$. This effect is termed "peak-shift". These phenomena, shown in Fig. 1, occur only after go/no go discrimination learning with errors. Training on a choice discrimination, or on a go/no go discrimination where no errors are made, produces no shift in preference along the continuum, nor an increase in pecking rate to $S+$.

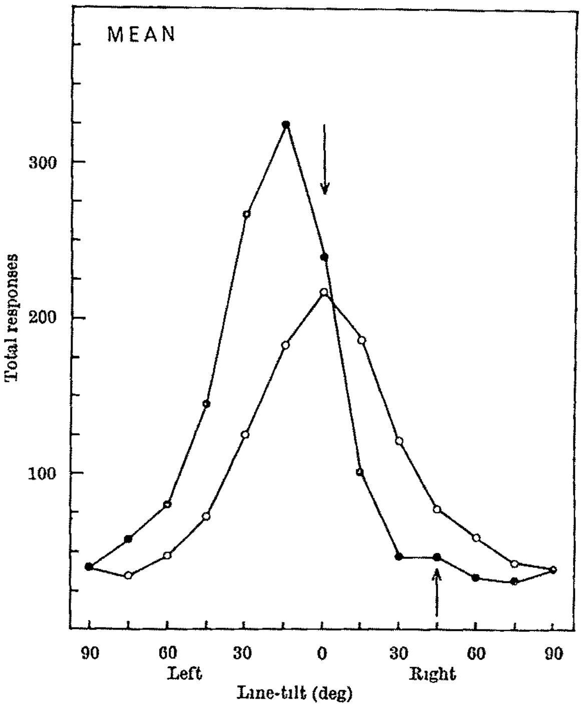

Fig. 1. Total responses during generalization testing of two groups of five pigeons. ●, Results from pigeons trained to discriminate vertical (0°) from 45° in a go/no go situation; ○, results from pigeons trained to discriminate 0° from absence of a line. This training is necessary to ensure attention to the dimension of line-orientation. Pigeons on the 0°/45° discrimination show the peak-shift effect: the maximum point on the gradient is not 0° ($S+$) but 15° left

Two attempts have been made to explain these effects, neither of which is compatible with all the evidence. First, Terrace[8] has suggested that induction or contrast results from a lowered pecking rate to $S-$. There are data, however, to show that a reduced pecking rate is neither necessary[9] nor sufficient[10] for contrast and peak-shift effects. In most studies, frequency of reward is confounded with pecking rate, but where conditions during $S-$ become worse without a lowering of pecking rate (that is, in a schedule where the pigeon must peck slowly to obtain rewards), it is change in reward frequency, not in pecking rate, that correlates with contrast effects. A recent experiment (T. M. Bloomfield, unpublished) shows that contrast is linked to an increased evaluation of reward by the animal, a pigeon will choose the reward given for pecks to $S+$ in a discrimination, in preference to one given outside the discrimination situation. Once again, this result is restricted to the go/no go situation where errors occur in training, and throws doubt on the second line taken to explain contrast and peak-shift.

This is the "adaptation-level" theory of Helson[11], which suggests that rewards (among other events) are assessed against the context of rewards the animal has experienced. A moderate reward, according to this theory, will seem large in a context of small rewards and small in a context of large rewards. As it stands, however, this account is of little use in differentiating between discrimination learning with and without errors. In both cases, the pigeon has been exposed to a certain frequency of reward during the initial training with $S+$. Later, no rewards are available for pecks to the $S-$, and the theory of adaptation-level predicts the reward available in $S+$ will increase in value. Clearly, the effects of absence of reward in $S-$ could depend on prior response to $S-$, which is absent in errorless learning. To answer this point it would be necessary, however, to take the discussion away from adaptation-levels into questions of similarity of, and thus into classification of, the positive and negative signals. These questions are raised later.

Rules governing Discrimination Performance

In attempts to explain the side-effects of discrimination acquisition, it was assumed that they were awkward phenomena to be explained away. It has been too often taken for granted[2] that discrimination learning is simply a matter of a response being attached to one "stimulus" and eliminated in the presence of another. It might be more profitable, however, to use these effects in the analysis of discrimination learning itself, by asking whether such behaviour reveals anything about what the animal has learned as a result of discrimination training. One rule that could obviously govern behaviour following go/no go discrimination acquisition would be: peck at $S+$, don't peck at $S-$. A rule with a "don't" in it is equivalent to inhibition of behaviour—the pigeon suppresses pecking during $S-$. Pavlov[6] has listed some methods of checking for inhibition involved in a negative rule, one of which is relevant here. If pecking during $S-$ is inhibited or suppressed, it should be possible to recover it. In fact, inducing extra stress in rats by electric shock[12], and administering "tranquillizing" drugs to pigeons[13], both increase responding during $S-$ in a go/no go discrimination learned with errors. The latter effect does not appear after errorless discrimination learning.

Other evidence[7] shows that a choice discrimination does not transfer to a go/no go situation with the same signals. The fact that a pigeon has learned to choose one signal in preference to another does not mean that it has learned to inhibit pecking when $S-$ alone is present. Choice discriminations thus seem explicable in terms of the single rule: peck to $S+$. Go/no go discriminations, on the other hand, involve the use of two rules, as explained. The fact that errorless go/no go discriminations are not susceptible to disinhibitory procedures suggests that these, too, come under the single rule: peck to $S+$. Such an analysis is supported by the restriction of contrast and peak-shift effects to go/no go discriminations learned with errors—the one discrimination situation that seems to require a negative rule to govern the pigeon's behaviour. The reward for pecks to $S+$ is overvalued only where the pigeon is inhibiting pecks to $S-$.

Classification of Discriminative Signals

This analysis, however, does not make clear the processes involved in errorless learning. Why is there no inhibition of pecking during $S-$, so that a negative rule seems not to be necessary? Closer inspection reveals the rules set out here to be ambiguous with regard to signal classification. It is clear from work with rats[14] that stimuli are classified by animals along certain dimensions only. The use of the term "$S-$" in these rules leaves open what is the negative signal for the animal—how the negative signal is classified. In all cases of discrimination learning, it has been shown that pecking rate systematically changes as the $S+$ is shifted along the dimension involved in discrimination training. For example, a pigeon performing discriminatively with respect to red as $S+$ and green as $S-$ will show a decline in rate of pecking as the wavelength shifts from red either towards or away from green. It is clear therefore that pecking was occurring with respect to red on the colour dimension. Tests with $S-$, however, reveal differences between training methods. If a discrimination is learned between a red circle ($S+$) and a white vertical line ($S-$) with errors in a go/no go situation, it has been shown that variations in line-angle from the vertical cause increase in pecking, although these variations do not bring the line any closer to the red circle[15]. Thus it seems clear that the $S-$ in the negative rule means the quite specific signal used by the experimenter.

After errorless learning, this effect does not occur. Variations in $S-$ do not alter pecking rate, which remains zero. This is possible only if identification of the $S-$ is made on the basis of absence of $S+$. If the $S-$ is identified in its own right, the pigeon's behaviour will change when $S-$ is changed, as it does after go/no go discrimination learning with errors.

It seems therefore that it is possible to analyse discrimination performance in pigeons according to the nature of the rules governing their behaviour and according to the way in which $S-$ is identified or classified. It is important to separate these two aspects of discrimination situations. An earlier attempt by Jenkins[16] to set out rules governing the pigeon's behaviour was vitiated by a confusion between the rules themselves and the way in which the positive and negative signals were classified. The side-effects of training turn out to be the product of formation of a negative rule, a finding obscured by Jenkins' rules, which all contained negative parts. Forming a negative rule is clearly a difficult process for the pigeon, shown by emotional behaviour during $S-$. Through attention to all aspects of behaviour in these discrimination situations, it is possible to improve our understanding of the behaviour of animals that undergo the various kinds of discrimination training. Such analysis has ideally both conceptual and empirical value. It can clarify our concept of discrimination and provide us, at the same time, with more information about the handling of discrimination problems by animals.

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[1] Ferster, C. B., and Skinner, B. F., *Schedules of Reinforcement* (Appleton, N.Y., 1957).

[2] Spence, K. W., *Psychol. Rev.*, **43**, 427 (1936). Hull, C. L., *Principles of Behaviour* (Appleton, NY, 1943).

[3] Kimble, G. A., *Hilgard and Marquis' Conditioning and Learning*, chap. 12 (Appleton, NY, 1961).

[4] Terrace, H. S., *J. Exp. Anal. Behav.*, **6**, 1 (1963).

[5] Reynolds, G. S., *J. Exp. Anal. Behav.*, **4**, 57 (1961). Bloomfield, T. M., *J. Exp. Anal. Behav.*, **9**, 155 (1966).

[6] Pavlov, I. P., *Conditioned Reflexes* (Oxford University Press, London, 1927).

[7] Honig, W. K., *J. Exp. Psychol.*, **64**, 239 (1962).

[8] Terrace, H. S., *Operant Behaviour: Areas of Research and Application* (edit. by Honig, W. K.) (Appleton, NY, 1966).

[9] Bloomfield, T. M., *J. Exp. Anal. Behav.*, **10**, 151 (1967).

[10] Reynolds, G. S., *J. Exp. Anal. Behav.*, **4**, 57 (1961).

[11] Helson, H., *Adaptation-Level Theory* (Harper and Row, New York, 1964).

[12] Hearst, E., *J. Exp. Anal. Behav.*, **8**, 135 (1965).

[13] Terrace, H. S., *Science*, **140**, 318 (1963).

[14] Sutherland, N. S., *Endeavour*, **23**, 148 (1964).

[15] Terrace, H. S., *Science*, **154**, 1677 (1966).

[16] Jenkins, H. M., *Stimulus Generalisation* (edit. by Mostofsky, D.) (Stanford University Press, California, 1965).

LETTERS TO THE EDITOR

ASTRONOMY

Are the Optical Luminosity Fluctuations of 3C 273 Random ?

WE have shown[1] that a light curve similar to that of the quasi-stellar source *3C* 273 can be generated by a superposition of unit light curves occurring at random times Gudzenko, Ozernoy and Chertoprud[2] have argued (1) that the distribution of the observed luminosities should be expected to be Gaussian on our model, and (2) that the observed distribution is not Gaussian, so that the luminosity curve cannot be regarded as the superposition of random events The purpose of this communication is to question the validity of both arguments and to show that the luminosity record may still be considered to be of random origin

Regarding the first argument, the probability distribution of a shot noise process will not usually be Gaussian, only in the limit when the number of pulses per unit time approaches infinity is the probability distribution rigorously Gaussian[5] In our model, the ratio of the number of pulses per unit time to their "time constant" is about 40, so that it is difficult to say to what extent the distribution on our model should be expected to be Gaussian Furthermore, it should be noted that, if a random variable has a Gaussian distribution, a function of s usually does not Specifically, $\log(s)$, or the astronomical magnitude of s would not have a Gaussian probability distribution

Regarding the second argument, if the observed luminosity is written as

$$\begin{aligned} I(t) &= x(t)\cos 2\pi f_0 t + y(t)\sin 2\pi f_0 t \\ &= \sqrt{x^2(t)+y^2(t)}\,(\cos 2\pi f_0 t + \tan^{-1} x(t)/y(t)) \\ &= R(t)\cos(2\pi f_0 t + \theta(t)) \end{aligned}$$

then, if $x(t)$ and $y(t)$ are independent Gaussian processes so that $I(t)$ is Gaussian, $R(t)$ is a Rayleigh distribution[3] It was argued that the hypothesis of Rayleigh distribution for R calculated from the data may be rejected with 99 per cent confidence and hence that $I(t)$ is not Gaussian distributed

For $R(t)$ to be Rayleigh distributed, it is crucial that $x(t)$ and $y(t)$ be Gaussian and independent The $x(t)$ and $y(t)$ are calculated from the observational record by forming trigonometric sums the coefficients of which are weighted time averages of the observed $I(t)$ Demonstration of the independence of $x(t)$ and $y(t)$ requires that the time over which the averages are made is very large In the case of *3C* 273, if the dominant frequency of about 0 10 yr^{-1} is taken for f_0, the total observation time is only about seven times the supposed periodicity and it seems questionable whether this can ensure the independence of $x(t)$ and $y(t)$

Probably the most straightforward way of testing the Gaussian hypothesis is to apply the χ^2 test[4] We have done this with both the actual luminosity curve and our simulation In the case of the luminosity data, the original photometer data in magnitudes were converted to intensities The samples used in the test must be independent, and so the luminosity curve samples must be separated by an interval of the order of the correlation time which for our simulation is about 1 year Hence for 73 years of observations seventy-three samples were taken The calculated ("observed") value of χ_ν^2, $\hat{\chi}_\nu^2$, and the probability that χ_ν^2 exceeds $\hat{\chi}_\nu^2$, $P(\chi^2 > \hat{\chi}_\nu^2)$, are shown in Table 1 We see that the Gaussian hypothesis for *3C* 273 may be accepted at a 7 per cent confidence level, which is about the same level as that for the simulation which was constructed by a random process We conclude that the observed luminosity record may still be regarded as the result of a high density random process

Table 1 APPLICATION OF THE χ_ν^2 TEST TO THE GAUSSIAN HYPOTHESIS

	ν (degrees of freedom)	$\hat{\chi}_\nu^2$	$P(\chi_\nu^2 > \hat{\chi}_\nu^2)$
3C 273	7	12 9	7 per cent
Simulation	7	14 0	5 per cent

We thank Dr H J Smith for furnishing us with a copy of the original photometer data The work of one of us (M S) was sponsored by a US National Science Foundation grant

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[1] Manwell, T , and Simon, M , *Nature*, 212, 1224 (1966)

[2] Gudzenko, L I , Ozernoy, L M , and Chertoprud, V E , *Nature*, 215, 605 (1967)

[3] Davenport, W B , and Root, W L , *An Introduction to the Theory of Random Signals and Noise* (McGraw–Hill Book Co , NY, 1958)

[4] Cramer, H , *Mathematical Methods of Statistics* (Princeton, 1946)

[5] Papoulis, A , *Probability, Random Variables and Stochastic Processes* (McGraw-Hill Book Co , NY, 1965)

Stimulated Inverse Compton Scattering in Radio Sources

OSTER[1] has claimed that energy losses due to the inverse Compton effect can be considerably larger than was previously thought, when stimulated scattering is taken into account If true, this would seriously hinder the understanding of compact radio sources We wish to point out that Oster's discussion is erroneous and grossly exaggerates the importance of stimulated inverse Compton scattering

We first consider the scattering of radiation by an electron at rest, neglecting recoil The radiation field is described by $N(\varepsilon, \theta, \varphi)$ photons per unit volume, per unit energy interval, per unit solid angle, θ and φ being polar co-ordinates centred on the electron We are neglecting recoil, and so the photon energy is not changed by scattering The rate at which photons of energy ε incident from a small element of solid angle $d\Omega(\theta,\varphi)$ are scattered into an element $d\Omega'(\theta', \varphi')$ is given by

$$c\sigma(\gamma)\,N(\varepsilon, \theta, \varphi)\,[1 + \frac{hc^3}{8\pi\varepsilon^2} N(\varepsilon, \theta', \varphi')]\,d\Omega d\Omega'$$

where χ is the angle between the directions of the incoming and outgoing photons, and $\sigma(\chi)$ is the differential cross-section The second term in the brackets is the number of photons per unit volume of phase space (occupation number), and gives the contribution of stimulated scattering Similarly, the reverse rate is given by

$$c\sigma(\chi)\,N(\varepsilon, \theta', \varphi')\,[1 + \frac{hc^3}{8\pi\varepsilon^2} N(\varepsilon, \theta, \varphi)]\,d\Omega d\Omega'$$

We observe that the net contribution from stimulated scattering is zero

Oster considered the case of relativistic electrons, with Lorentz factor γ, moving through an isotropic radiation field In the rest frame of an electron, the radiation field then consists of an intense beam of angular spread $\sim\gamma^{-1}$ and a weak component from all other directions This argument, which applies to an arbitrary angular distribution of radiation, shows that the stimulated term has no net effect Oster obtained a large contribution from stimulated scattering (even though he neglected recoil) because he included scattering out of the beam, but neglected scattering back into it. The latter represents events where, in the source frame, the electron gains energy from the photon. Although spontaneous scatterings of this type are indeed negligible, the corresponding stimulated scatterings are important because of the high occupation number in the beam, and do in fact cancel the term obtained by Oster.

A more complete analysis, which allows for recoil, shows that stimulated scattering does contribute to the loss rate of relativistic electrons. The relevant formalism is given by Dreicer[2]. The contribution is $\sim \varepsilon/\gamma mc^2$ times smaller than Oster's estimate, however, where ε is the final energy of the relevant photons in the frame of the source. In Oster's example, where $\gamma \sim 100$ and the important frequency range is $\lesssim 10^{11}$ Hz, this factor is $\lesssim 10^{-11}$. Furthermore, for reasonable photon and electron spectra the effect has the opposite sign, and tends to reduce the loss rate.

Stimulated scattering could be important only if the brightness temperature of the radiation field greatly exceeded the kinetic temperature of the relativistic electrons. This is not the case in any of the quasar models referred to by Oster.

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[1] Oster, L, *Nature*, 217, 148 (1968)

[2] Dreicer, H, *Phys Fluids*, 7, 735 (1964)

Profiles of the Unidentified Interstellar Absorption Bands at 4890 Å and 4760 Å at Different Galactic Longitudes

OF the four known diffuse interstellar absorption bands, those at 4430 Å and 6180 Å have already[1,2] had profiles of high photometric accuracy assigned to them, and it has been shown that these profiles are the same over three regions of galactic longitude. A similar analysis is made here for the two known remaining bands at 4890 Å and 4760 Å.

The first of these two bands was discovered independently by Wilson[3] and Butler and Seddon[4], while the second was tentatively identified as interstellar by Merrill, in private correspondence with Beals[5]. Little has been attempted in the investigation of these features because of their weakness and width. The band at 4890 Å is particularly difficult to investigate because it overlaps the stellar lines of Hβ at 4861 Å and HeI at 4922 Å, both of which are strong in the spectra of most stars sufficiently bright and distant to show significant band absorption. The spectrophotometry of early type stars carried out at this observatory[4] assumed the region of this band to be a continuum, until sufficient data were acquired to show this assumption to be untenable. Wilson has published in this series[6] a spectrophotometric atlas of *B*1 stars, in which the distortion of the continuum following on this assumption can be seen. The effect of neglecting the band is to give the appearance of asymmetrical and wide emission to the Hβ profile.

In this investigation, the same collection of data has been used as before[1,2], so that the results may be strictly comparable. There are fifteen supergiant stars of types ranging from *B*2 to *A*0, divided into three groups by galactic longitude. The ranges of longitude are group 1 (Cygnus), $020° < l^{II} < 120°$, group 2 (Perseus), $120° < l^{II} < 145°$, group 3 (Auriga-Gemini), $145° < l^{II} < 190°$. For convenience, the stars used are detailed in Table 1, this table being based on that in ref. 1, with the addition for some of the stars of the β-index of Crawford, and of some individual measures of 4890 and 6180 in the present list of stars, taken from the *Publications* of this observatory. The spectrophotometric measures in each of these three groups have been averaged as before. This process reduces the stellar contribution because of the range of types, and increases the signal-to-noise ratio of the general continuum and the interstellar features.

The measures are shown in Fig 1. It has not been considered worth while to show the individual traces for the three regions. The bottom two traces, labelled m_1-m_2 and m_1-m_3, show the differences between the Cygnus and Perseus regions, and between Cygnus and Auriga-Gemini, the ordinates being to the same scale as the trace "SUM".

The general smooth curvature in the traces is a result of differences in photographic emulsions, as mentioned in the communication on the band at 6180 Å, it will not affect the conclusions reached here. The top trace is of the average of all fifteen stars, again showing some general curvature, which has been eliminated in the trace labelled "SUM". There is an obvious band at 4890 Å. The same cannot be said for the band at 4760 Å, which if present at all is little different from the general noise level.

In the earlier communications, it was shown that the bands at 4430 Å and 6180 Å were completely cancelled out by the differencing process. This reflects a purely fortuitous grouping effect in the original selection of the stars, the grouping depending to a large extent on the use of the 36 in reflector at its limit. The same cancellation has taken place here in the difference m_1-m_3, showing that the profiles in these two regions are equal in size and shape. The other difference, however, shows some sign of an inequality in size. To be more sure of this, all measured points outside the apparent profiles of 4890 Å and that presumed for 4760 Å were averaged in groups of ten, and a least mean squares curve of second degree fitted to these groups. These are the inserted square points. The absorption due to the 4890 Å band is apparently greater in the direction of Perseus than in that of either Cygnus or Auriga-Gemini, and the same

Table 1 STARS USED IN FORMING MEAN SPECTRA

H D No	Name	l^{II}	Type	E_{B-V}	β	W_{4430}eÅ	W_{4890}eÅ	W_{6180}eÅ	
164353	67 Oph	030	$B5I_a$	0.26	2.585	0.78	0.14	0.39	Group 1
198478	55 Cyg	086	$B3I_a$	0.60	2.530	1.24	0.51	0.95	
206165	9 Cep	102	$B2I_b$	0.54	2.545	1.24	—	0.96	
224055	—	116	$B3I_a$	0.91		2.11	1.10	1.52	
225094	—	117	$B3I_a$	0.53		1.44	0.73	1.18	
13267	5 Per	133	$B5I_a$	0.49		1.01	0.85	0.82	Group 2
14134	—	135	$B3I_a$	0.67		1.24	0.61	1.14	
14143	—	135	$B2I_a$	0.76		1.23	—	1.14	
14818	10 Per	135	$B2I_a$	0.55		1.11	—	1.32	
21389	—	142	$A0I_a$	0.56		1.51*	0.74*	1.26*	
31327	—	167	$B2I_b$	0.65		0.95	—	0.96	Group 3
36371	χAur	176	$B5I_{ab}$	0.50		1.49	0.56	0.95	
42087	3 Gem	187	$B2.5I_{ab}$	0.42		0.98	0.71	0.80	
43384	9 Gem	188	$B3I_{ab}$	0.65		1.54	1.03	1.11	
41117	χ²Ori	189	$B2I_a$	0.51	2.502	1.12	—	0.42	

* No allowance made for stellar blends

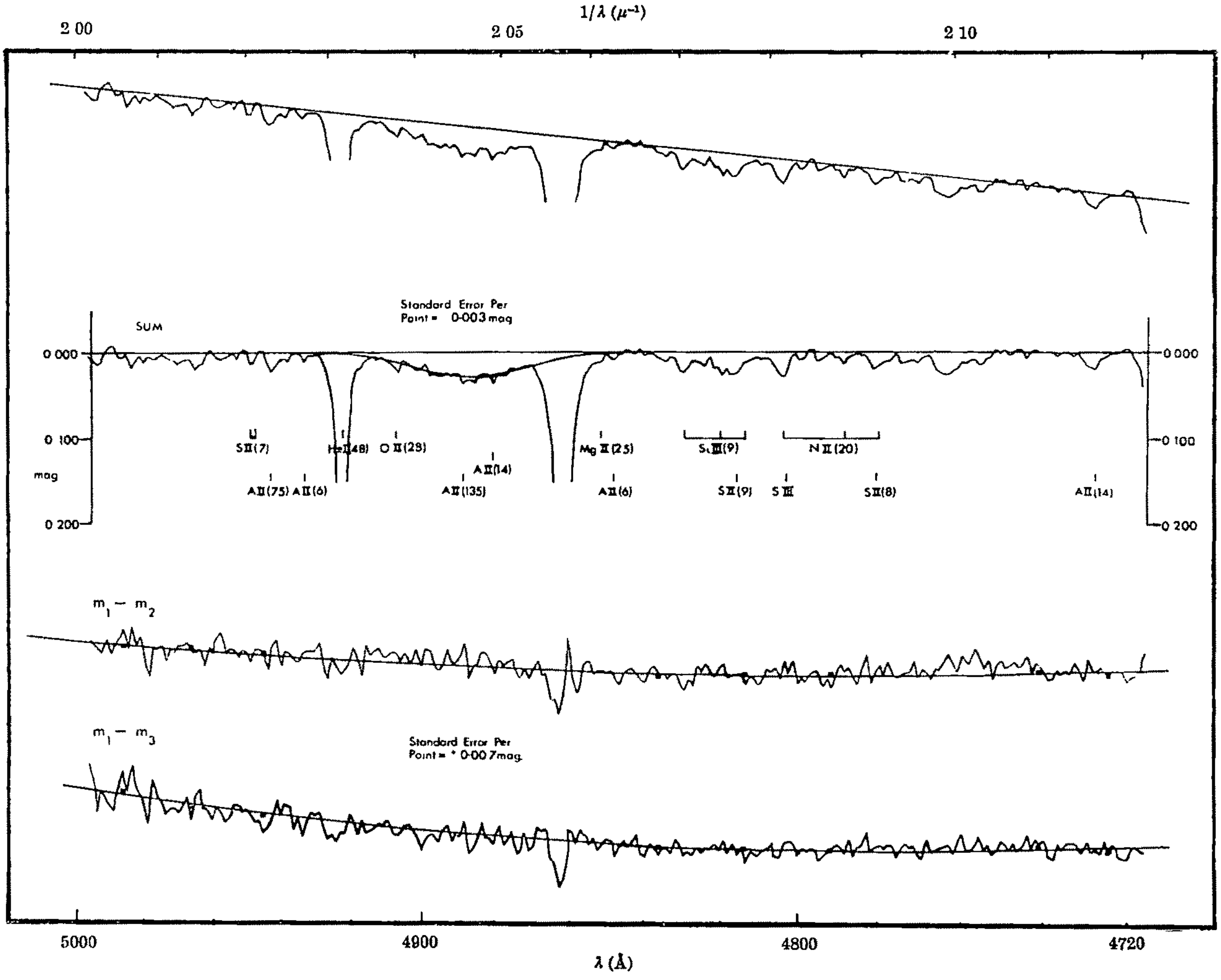

Fig 1 Mean spectrophotometric measures using the stars listed in Table 1

is probably true of the feature at 4760 Å This behaviour is different from that of 4430 Å and 6180 Å, where profile shapes and sizes were the same in all three areas

This difference in the profiles does not indicate any difference in shape, it therefore seems permissible to average all three groups to produce the "SUM" profile Both wings of the 4890 band are obscured, by Hβ on one side and 4922 HeI on the other In the band itself there are some small fluctuations of which the larger are probably real and stellar in origin The individual independent measured points were then averaged in groups of three throughout the possible extent of 4890, omitting only the two strong features and the peaks of the larger, probably real fluctuations These average points are shown in Fig 2 The dotted curve inserted through these points is a simple Gaussian, fitted by least mean squares It is emphasized that a Gaussian curve has been chosen for simplicity, the errors do not justify any elaboration here Nevertheless, the fit is seen to be good, and from the fitting process the centre of 4890 can be given as 4886 6 Å ± 0 9 Å, its half width as 34 9 Å ± 0 8 Å and the equivalent width for this mean group of fifteen stars is 1·08 eÅ. The real error in the last figure is difficult to estimate, but is of the order of ± 0 03 eÅ

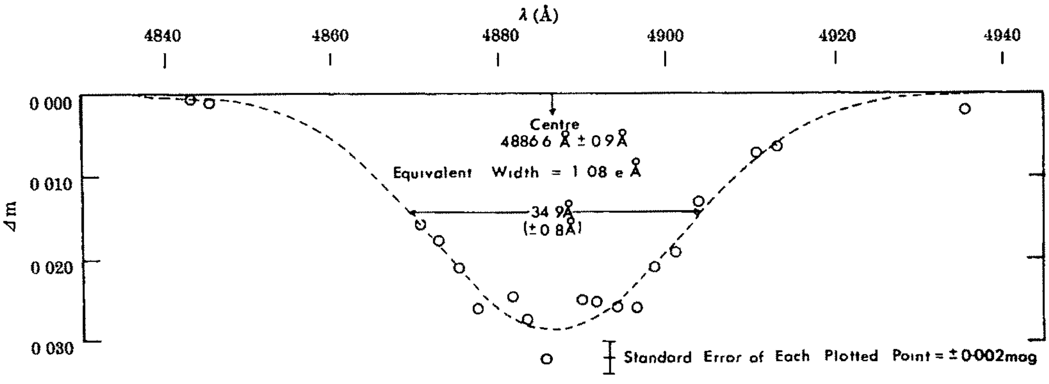

Fig 2 Averaged points throughout the possible extent of the 4890 band

These three parameters are given in Table 2 for each of the diffuse interstellar bands The equivalent widths of 4890 and 6180 are equal and are about half that of 4430 Whereas, however, the half widths of 4430 and 6180 are in the same ratio as their central wavelengths, this does not hold for 4890, which as we have already seen does not have the same profile, nor apparently is it invariant with galactic longitude

Table 2

Nominal wavelength	Central wavelength (Å)	Half width (Å)	Equivalent width (eÅ)
4430	4429 5	21 2	2 18
4890	4886 6	34 9	1 08
6180	6175	29	1 03

Table 1 shows the collected values of equivalent widths of the interstellar bands as found[4,7,8] in the spectrophotometric investigation of early-type stars If a least mean squares fit of these values of strength of 4430 against 6180 and 4890 is made, the two constants of proportionality are found to be 0 68 and 0 52, respectively The former we have seen[2] is to be reduced by a factor of 0 79 to eliminate the contribution of the diffuse line 6203 it then becomes 0 54 The coefficient of correlation in both cases is 0 72 The strengths of 4890 and 6180 are seen by this independent reduction to be equal to each other and to approximately half that of 4430

If it occurs at all, the reported band at 4760 Å is very much smaller than that at 4890 Å There is an indication of a narrower feature at 4760 Å in the mean plot, and a possible band in the Perseus direction that is not present in the Cygnus and Auriga-Gemini regions, but in any case these are too small here to justify further investigation If the reported band at 4760 Å has approximately the same profile as 4890, it is weaker by a factor of 5 at least

The presence of the 4890 absorption band has a small effect on the β-measures of Crawford[9] The whole of the 4890 band is included in his 150 Å broad reference, while his narrow band filters respond to part of the profile. The net result of neglect of the 4890 absorption will be that assigned β values will be too high and this difference will increase with increasing colour excess The size of the difference will depend on the profile of the narrow-band filter, indeed, it will go through zero at a filter half-width of something less than 15 Å, if the filter is centred on Hβ Lack of information about the filters makes it impossible to say with any accuracy how badly the β-index will be affected As a rough estimate, however, the effect will exceed the quoted error of 0 007 for $E_{B-V} = 1\ 0$ in the 30 Å filter case This corresponds to more than 5 per cent of the range of β for any one spectral type

Some evidence has been found[10] that when spectra with heavy 4430 absorption are differenced from others of the same spectral type with small absorption, the residual 4430 profile is slightly asymmetrical, with a steeper slope on the red wing than the blue Because, however, the band considered here at 4890 and that at 6180 are of small equivalent width and greater half-width than 4430, any such asymmetry that might be present in them is not detectable here More accurate photoelectric measures, with averaging and differencing techniques, will be needed for all three bands

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[1] Seddon, H , *Nature*, **214**, 257 (1967)
[2] Seddon, H , *Nature*, **215**, 495 (1967)
[3] Wilson, R , *Astrophys J* , 128, 57 (1958)
[4] Butler, H E , and Seddon, H , *Publ Roy Obs Edin* , 2, 113 (1957)
[5] Beals, C S , *Mon Not Roy Astro Soc* , 102, 96 (1942)
[6] Wilson, R , *Publ Roy Obs Edin* , 2, 1 (1956)
[7] Butler, H E , and Seddon, H , *Publ Roy Obs Edin* , 2, 187 (1960)
[8] Butler, H E , and Thompson, G I , *Publ Roy Obs Edin* , 2, 225 (1961)
[9] Crawford, D L , *Astrophys J* , 128, 185 (1958)
[10] Wickramasinghe, N C , Ireland, J G , Nandy, K , Seddon, H , and Wolstencroft R D , *Nature*, 217, 412 (1968)

Spectral Absorption of Excited Molecules in Iron Aerosols

THE existence of interstellar absorption bands of unknown origin has drawn our attention to absorption by finely dispersed aerosols of solids We have in particular investigated iron aerosols obtained by thermal decomposition of $Fe(CO)_5$ at concentrations of less than ten parts per million in various carrier gases The iron particles resulting from this treatment were between 30 and 200 Å in size These yield an absorption curve which, between 3500 and 6000 Å, agrees well with the theory developed by Mie if the optical constants of bulk iron and the real size of the particles were taken into account With a particular size distribution of the particles the absorption was found to be inversely proportional to the wavelength, as observed in interstellar absorption

Superimposed on this absorption were bands with half-widths between 20 and 100 Å which varied with the carrier gas but which were not found in the absence of iron particles With argon as the carrier gas, the positions of these bands coincided with the Herzberg and Ångstrom bands for carbon monoxide, but, within the range of accuracy, the bands may also be interpreted as triplet transitions because the (0–0)-transition of $d^3\Pi \rightarrow b^3\Sigma^+$ differs from the corresponding transition of the Ångstrom system by less than 25 Å. Evidence which supports the second possibility is provided by the fact that when large amounts of nitrogen are added to the argon carrier gas, the bands of the second positive group of nitrogen were observed together with the Goldstein–Kaplan bands In addition, bands of the green system have been found Although the absorption is small ($k < 10^{-6}$ cm^{-1}), observations are reproducible with an absorption length of 100 m because no strong absorption bands from the ground state of the nitrogen or carbon monoxide molecules are present in the spectral range investigated

The lower states of the observed absorption bands are all attributable to energy levels of 7–9 eV, which implies that the excitation cannot be due either to chemical reactions or to incident photons only In our view, the bands are due to molecules which are adsorbed on the surfaces of the iron particles The excitation might be caused by the large surface energy of the small particles When small particles are combining or surface crystallites are relocated, large amounts of energy may be set free which could be transferred to single gas molecules adsorbed on the surfaces In this way, certain high energy levels can be populated in a manner which differs from that involved in thermal equilibrium Support for this view is provided by the disappearance of the carbon monoxide bands as nitrogen concentration in the carrier gas is increased It must be assumed that the large free enthalpy of adsorption of carbon monoxide on iron particles is outweighed by the large number of the nitrogen molecules which reach the surface

With pure photochemical decomposition of $Fe(CO)_5$ in the same conditions, only the carbon monoxide bands and the well known absorption edge towards the ultraviolet have been found Independently of the carrier gas used, the carbon monoxide bands show up particularly well The aerosol particles obtained in this way were crystallites of iron with a mean diameter of about 10 Å and large flakes of the well known decomposition product $Fe_2(CO)_9$ The strength of the carbon monoxide bands could be explained by the fact that this decomposition produces an especially high concentration of carbon monoxide near the surfaces of the particles

We are at present investigating the excitation associated with particles with a large heat of sublimation—carbon, for example—and are also looking for excitation of metastable states using various carrier gases

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PLANETARY SCIENCE

Electrodynamic Effects of Jupiter's Satellite Io

Jupiter's satellite Io has remarkable control of Jovian decametric radio emission (recently reviewed[1,2]) and also a very high and possibly varying albedo[3,4]

Io's orbit is approximately 6 R_J (Jovian radii), while the magnetosphere itself probably extends[5] beyond 50 R_J The radiation belt responsible for the decametric radio synchrotron emission extends[6] to about 6 R_J so that Io moves deep in the magnetosphere near the belt boundary The magnetosphere rotates with the planet, causing Io to move westwards through the magnetosphere at a speed of about 56 km $\sec^{-1}$ This motion may cause an electromagnetic disturbance which contributes to the radio emission On the other hand, tidal control by Io seems unlikely in view of the absence of any control by the other satellites

The possible form of the electromagnetic disturbance has been discussed by a number of workers Warwick[1] concluded that the disturbance would be minor, while Ellis[7] mentioned the possible importance of the low-velocity hydromagnetic mode There seems little likelihood of a long fan-shaped proboscis[8] or of the direct stimulation of plasma waves[9], because of the gravitational potential barrier[10]

If the satellite's electrical conductivity were very small then it would cause only a minor hydromagnetic disturbance, as does the Moon in its motion through the interplanetary medium (unpublished work of N F Ness, K W Behannon, C S Scearce and S C Canterano)

Irrespective of Io's electrical conductivity, long immersion of the satellite in the steady magnetospheric field must ensure its magnetization The alternative requires a system of electric currents flowing on its surface, driven by an electric field E given by Maxwell's equation $\nabla \times E = -\partial B/\partial t$ and this requires a flow of field lines into Io The only way to provide E continually, and so to demagnetize Io permanently, is to rotate it about an axis perpendicular to the undisturbed field In the absence of such rotation Io's magnetic field strength is 0 05 gauss[1] and flux about 4×10^{15} gauss cm^2

If Io is magnetized and is conducting, then its relative motion through the magnetosphere (V) will have drastic results Field lines passing through Io cannot follow the motion of the external plasma because this would induce excessive electric field $V \times B$ and excessive current in the satellite and in the plasma Sufficient current (j) flows to deform the external field and to provide a force $j \times B$ which slows down the plasma, the field deformation then propagates away The situation is shown in Fig 1a, plasma and field are compressed at the left and diffuse into Io, while field lines diffuse out at the right The effectiveness of Io in creating a disturbance depends on its conductivity σ according to the well known relationship $t_0 \sim \sigma l^2$, where t_0 and l are characteristic time and dimension Putting $l = 3\ 2 \times 10^8$ cm (Io's diameter) and t_0 equal to this distance divided by the relative speed (about 57 s) we find the required conductivity (emu) $\gtrsim 6 \times 10^{-16}$

If the ratio of the velocity V to the hydromagnetic velocity V_A is large, then the disturbance travels "down wind" to provide a magnetotail as for the Earth If the ratio is small, then most of the disturbance travels as hydromagnetic twist or shear waves along the field lines The velocity V_A near Io is probably in the range 10^3–10^4 km $\sec^{-1}$ (refs 1 and 7), and so is much larger than V and far from shock conditions There is little magnetic perturbation in Jupiter's equatorial plane and the main effect is that the whole magnetic force tube frozen into Io (4×10^{15} gauss cm^2) moves bodily with Io as in Fig 1b which shows a meridian plane in Jupiter's magnetosphere and Io's force tube (IFT) The relative motion of Io is out from the plane of the paper, and if we regard the force tube as being rigid then it moves as a whole, executing 'interchange motions" with surrounding force tubes These motions resemble the motions discussed in connexion with the Earth's magnetosphere[11], except for the driving force The whole of IFT moves about the axis of rotation, more or less in meridian planes with the centre passing through Io and the ends dipping into the iono-

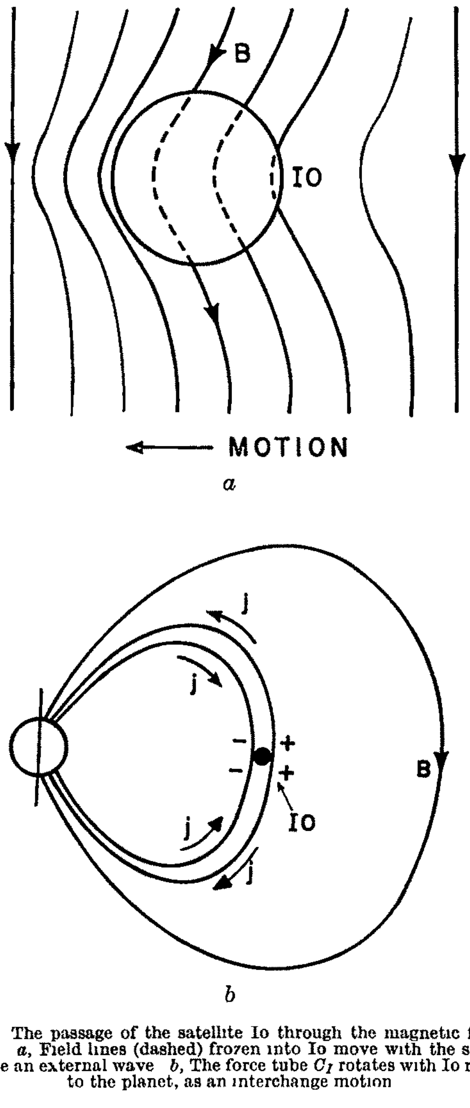

Fig 1 The passage of the satellite Io through the magnetic field of Jupiter a, Field lines (dashed) frozen into Io move with the satellite to create an external wave b, The force tube C_I rotates with Io relative to the planet, as an interchange motion

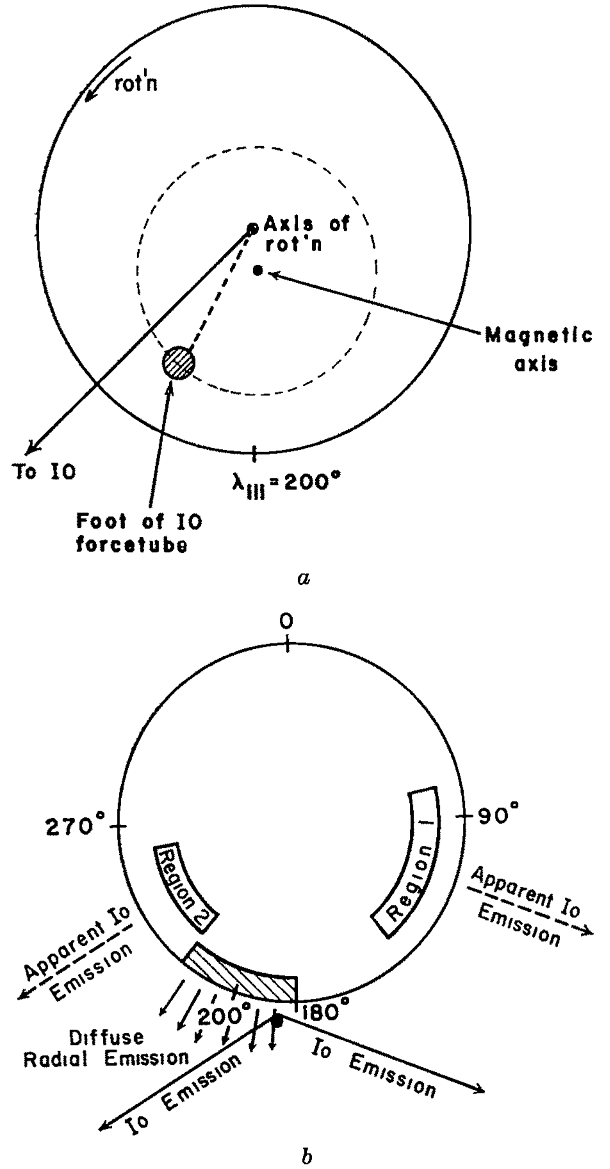

Fig 2 Two plots of Jupiter in Longitude System III *a*, The high latitude ionosphere showing the rotational and magnetic axes and the path (dashed line) of the foot of Io's force tube *b*, A schematic representation of the apparent emission regions of decametric radio bursts and the manner in which most emission may originate from one region

sphere near latitudes 65° N and S The foot of IFT in the northern hemisphere moves around the north magnetic north pole (Fig 2*a*) and, because of the 10° tilt of the magnetic to the rotational axis, excentrically around the latter

Relative motion V creates electric polarization as shown in Fig 1*b*, electrons from the plasma flow to neutralize the positive charge and ions to neutralize the negative charge (because Io cannot emit electrons) Currents (j) flow up and down the sides of IFT and hydromagnetic waves propagate to the ionosphere in each hemisphere, or lower if the lower atmosphere is conducting For maximum effect, Io's electrical conductivity must be such that time $t_0 \sim \sigma l^2$ exceeds the time for the wave to propagate to the ionosphere The velocity of the wave is not evident, but cannot exceed that of the fast mode, which is the Alfvén velocity[12] Elements of the slow hydromagnetic mode, however, can also be present and we will for the present assume a velocity of propagation along IFT of 500 km s^{-1} The disturbance reaches the ionosphere (in each hemisphere) in about 20 min and the required conductivity of Io is about 10^{-14} emu

If Io's conductivity exceeds this value, then its whole force tube, extending from ionosphere to ionosphere, will swing westward relative to the magnetosphere The cross-sectional area of the force tube perpendicular to the direction of motion is about 200 times that of Io and the disturbance correspondingly large, leaving a wake of turbulence and fast particles extending eastward from IFT The bending of the force tube means that the feet are displaced eastward of Io, the time delay of 20 min corresponding to a longitude difference of about 10 degrees Thus any Io related electromagnetic phenomena should be found at longitudes between 0° and ~10° or more eastward of Io

IFT dips into both N and S ionospheres and rotates around the two poles (rotational and magnetic) as shown in Fig 2*a* The horizontal dimensions of the feet are about 150 km and the speed through the ionosphere is about 5 km s^{-1} Collisions between ions carried by the force tube and stationary neutral atoms will heat the ionosphere to a temperature exceeding 1,000° K, considerably above the undisturbed value of about 200° K[13] The tube motion is also likely to cause a shock wave outside the tube and to generate an extensive disturbance

Decametric radio emission from Jupiter is associated with several System III longitude regions and is controlled to a considerable extent by the position of Io in its orbit There is yet no general agreement as to the locations of these regions and positions, but an approximate picture is given in Fig 2*b* Emission probability is highest when the longitude of Io approximates that of the north magnetic pole ($\lambda_{III} \sim 200°$) The sectors marked 1 and 2 and the hatched sector are "apparent" regions of emission in the sense that stronger signals are received during their central meridian passages seen from the Earth[1,2] For none of these regions, however, need we assume that emission is radially away from the centre of the planet, and so there is great flexibility in the interpretation of the data

When Io is at $\lambda_{III} = 200°$ its wake and ionospheric disturbance lie eastward, let us suppose at $\lambda_{III} = 185°$ Radio emission from this region (shown by a large dot) need not be wholly radial and we will assume that some is emitted in two beams directed at about 75° away from the meridian plane of the disturbance Emission which seems to come from regions 1 and 2 is now explained in terms of radiation from Io's disturbance region[14], and does not meet the chief difficulty of having to explain Io's control exercised from a distant part of the magnetosphere[7] The radiation from the hatched sector is presumably in meridian planes and may have a different source mechanism

We speculate that Io's force tube disturbance, either in the ionosphere or magnetosphere, may have the form of a bow wave directed westward Radio emission from the wave front, in both the forward and reverse directions, might be expected, as for type II solar radio bursts[15] If either the beaming or generation processes have increased efficiency at lower rotational latitudes, this would account for enhanced emission near the magnetic pole as may be seen from Fig. 2*a* Lack of influence of the other Jovian satellites on the decametric emission may be caused by a lack of adequate electrical conductivity or by rotation about an axis perpendicular to Jupiter's axis

The motion of IFT may also account for Jupiter's radiation belt The electric potential across IFT is about 10^5 V, and particles accelerated there and drifting inwards under the influence of the complex electromagnetic field[5] will be further accelerated The violent ionospheric disturbance will spread plasma through the magnetic shell traversed by Io as well as providing magnetic disturbances and auroras near latitude 66° The centrifugal force on plasma near the equatorial plane will create a Jovian ring current and lead to interchange motions Finally, the incidence of plasma on the surface of Io may account for its unusual optical properties[3,4]

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[1] Warwick, J W , *Space Sci Rev* , 6, 841 (1967)
[2] Drake, J F , *Univ Iowa Res Rep* , 67-22 (1967)
[3] Frantz, D J , thesis, Univ Iowa (1966)
[4] Binder, A B , and Cruikshank, D P , *Icarus*, 3, 299 (1964)
[5] Piddington, J H , *Univ Iowa Res Rep* , 67-63 (1967)
[6] McAdam, W B , *Planet Space Sci* , **14**, 1041 (1966)
[7] Ellis, G R A , *Radio Sci* , **69D**, 1513 (1965)
[8] Marshall, L , and Libby, W F , *Nature*, **214**, 126 (1967)
[9] Gledhill, J A , *Nature*, **214**, 155 (1967)
[10] Melrose, D B , *Planet Space Sci* , **15**, 381 (1967)
[11] Chang, D B , Pearlstein, L D , and Rosenbluth, M N , *J Geophys Res* , **70**, 3085 (1965)
[12] Piddington, J H , *Geophys J Roy Astro Soc* , **3**, 314 (1960)
[13] Gross, S H , and Rasool, S I , *Icarus*, **3**, 311 (1964)
[14] Dulk, G A , *Icarus*, **7**, 173 (1967)
[15] Kundu, M R , *Solar Radio Astronomy* (Interscience Publishers, New York, 1965)

Detection of a Deep Boundary Current in the Western South Pacific

FROM the distribution of salinity, temperature, oxygen and radio-carbon observed in the deep oceans (deeper than 2,000 m) of the world, it seems that all the deep water in both the North and South Pacific comes from other oceans, entering the Pacific from the south The Tasman Sea is closed to the north at depths greater than 3,000 m, so that any deep current reaching into the North Pacific must lie east of New Zealand The simple linear geostrophic theory of the abyssal circulation proposed by Stommel and Arons[1,2] requires that the source water, "after passing south of New Zealand, flows northward into the Pacific Ocean as a narrow western boundary current along the Tonga–Kermadec Trench". It seems surprising that the source water should move in such a narrow current when it has the full width of the South Pacific within which it might flow, but the dynamics demands weak, southward geostrophic flow over most of the width of the South Pacific, and permits northward flow only in a higher-order boundary current We are not at all certain that such simple dynamics do prevail, and the data have so far been insufficient to reveal the existence or non-existence of this current

Between March 10 and August 2, 1967, we made two long oceanographic sections (Scorpio Expedition) across the South Pacific between Australia and Chile on 43° 15′ S and 28° 15′ S , with 78 and 100 hydrographic stations, respectively In the deep water, temperatures were measured with Kahl reversing thermometers of range − 2° to 60° C on which temperature could be read to ± 0 001° C Salinities were measured on a University of Washington conductivity bridge ('Paquette' model) with precision ± 0 002‰ The work was done aboard the USNS Eltanin, the research vessel of the US Antarctic Research Program

The sections were made partly to fill a great void in the world coverage of oceanographic data, and partly in the belief that with closely spaced, highly precise observations, features not discernible in the sparse older data[3] would be disclosed Chief among these was the expectation that some evidence of the postulated deep western boundary current would be found along the eastern side of New Zealand and the Tonga–Kermadec Ridge—kinematical, through advection of properties such as temperature, salinity and oxygen; and dynamical, through an intensification of the field of horizontal density gradient. No attempt was made to make direct measurements of the velocity field such as those by Swallow and Worthington[4] in a similar current in the North Atlantic

The evidence for the current on the salinity profile at 28° 15′ S consists of a salinity maximum layer with highest values (greater than 34 730‰) compressed within 70 km of the eastern slope of the Tonga–Kermadec Ridge, between the depths of 3,000 and 4,000 m A somewhat thicker (between 2,500 and 4,400 m) and much broader (extending 1,300 km to the east) tongue is delineated by the 34 720‰ contour. Further eastward the salinity at these depths falls to 34 690‰ and increases monotonically with depth The deep salinity maximum is probably the last distinct evidence of the North Atlantic component of this water's origin—the extremum being continuously traceable all the way from the Atlantic sector of the Antarctic to the Pacific sector It seems, however, that the current is not as wide as might be inferred from the 34 720‰ salinity contour, but is essentially confined to the short distance of 70 km adjacent to the Ridge, because the intense horizontal gradient of temperature (and density) is confined to this narrow region The 1·10° C isotherm drops from 4,000 m, where it intersects the eastern slope of the Ridge, to 4,600 m, 70 km to the east A similar slope—downward towards the east—occurs above these levels, but the amplitude of drop diminishes with decreasing depth, thus at 2 00° C the downward drop had diminished to a change from 2,550 m to 2,650 m At 3 00° C the slope is the other way

The current, as computed geostrophically, thus appears to be 70 km wide, to be confined between depths of 2,500 m and 4,500 m (there is always some uncertainty in assigning depths of no motion), and to transport an amount of water northward amounting to 8 to 12×10^6 m³/s, which compares with 12–15×10^6 m³/s as estimated in a steady stage budget of heat, mass, salt and radiocarbon[5]

There was no such intensification of the current observed on the 43° 15′ S section; the zone of sloping isotherms is about 500 km wide (though the vertical displacements are about the same as at 28° 15′ S), and the tongue of salinity greater than 34 730‰ extends 1,100 km east of the New Zealand slope This is presumably caused both by the fact that at this latitude the current is still in transition from the zonal current that feeds it from the south and has not travelled a sufficient distance in latitude to be influenced by the change in Coriolis parameter, and to the fact that the western boundary at this latitude has an extremely gentle and prolonged slope

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[1] Stommel, H , *Deep-Sea Res* , **5**, 80 (1958)
[2] Stommel, H , and Arons, A B , *Deep-Sea Res* , **6**, 217 (1960)
[3] Reid, jun , J L , *The Johns Hopkins Oceanographic Series*, No 2, 85 (1965)
[4] Swallow, J C , and Worthington, L V , *Deep-Sea Res* , **8**, 1 (1961)
[5] Bolin, B , and Stommel, H , *Deep-Sea Res* , **8**, 95 (1961)

Second Additional Theory of Origin of Fiamme in Ignimbrites

Gibson and Tazieff[1] have questioned a widely accepted explanation of dense black clots of glass (fiamme) in ignimbrites A new interpretation of these features is long overdue, for the conventional interpretation of fiamme as flattened and highly compressed lumps of pumice is very difficult to substantiate As they point out, in many ignimbrites there are few if any lumps of pumice in the upper, uncompacted levels that are large enough to produce the dense glassy clots by compression

Gibson and Tazieff interpret fiamme in the Fantale ignimbrite of Ethiopia as unexpanded lumps of volatile-rich "very fluid" glass carried up in the vent during the initial eruption They do not explain why the glass did not vesiculate until a very late stage even though it had a high temperature and volatile content and a low viscosity Perhaps the nucleation of bubbles was retarded in a crystal-poor fraction of the melt

Fiamme are common in certain of the ignimbrites of central Honduras and northern Nicaragua There, too, large pumice lumps are absent or very scarce in overlying levels of the same units Regardless of their pumice content, ignimbrites with prominent horizons of glassy clots seem to be restricted to areas directly underlain by coarse fluvial sediments This relation would suggest that the glass is not primary but a fortuitous secondary feature related to local influx of water

To test this possibility, I have compared two contrasting samples from a Miocene ignimbrite in north-western Nicaragua Analyses are shown in Table 1 No 258 is a black glass lens about 2 m above the chilled base of the unit where it overlies fluvial sand and gravel Under the microscope, the glass appears deep reddish-brown with many perlitic cracks The original vitroclastic texture is faintly preserved by thin streaks of fine opaque dust outlining original shards and fragments The abundance of plagioclase phenocrysts is approximately the same as in the surrounding rocks (about 5 per cent), lithic fragments of andesite, which are very abundant in the host rock, are fewer and smaller in the interior of the glass lenses In some areas, andesitic fragments are completely absent At their margins, the fiamme (as their name implies) extend tongues and feathery streaks into the surrounding material, so that the boundary is much less distinct than it appears on a mesoscopic scale

Table 1 CHEMICAL ANALYSES OF FIAMME AND HOST ROCK

	No. 258	No 260
SiO_2	64 35	65 61
TiO_2	0 92	1 00
Al_2O_3	15 15	14 75
Fe_2O_3	0 66	4 75
FeO	3 26	0 41
MnO	0 09	0 18
MgO	1 12	0 79
CaO	2 56	2 53
Na_2O	3 00	3 84
K_2O	4 95	3 93
H_2O+	3 46	1 16
H_2O-	0 08	0 59
P_2O_5	0 19	0 23
	99 79	99 77

N-258, Dense black glass clot about two metres above base of mesa-forming ignimbrite at Vuelta La Barranca, 3 5 km south of the Honduras border, north-west Nicaragua *N*-260, Dacitic ignimbrite strongly contaminated with xenoliths of andesite Host rock of *N*-258 Analyses were performed by Dr K Aoki

The second specimen, No 260, is taken from an adjacent portion of the same unit, but slightly below the fiamme horizon It is crowded with accidental fragments, chiefly of andesite The glassy portion consists of fine, strongly welded shards outlined by opaque dust Glass in the rims has a lower refractive index and paler colour than the interior of the shards and may be hydrated

It is evident that the two specimens are almost indistinguishable on the basis of their chemical analyses The fiamme has a higher water content and more reduced iron It also appears to have a slightly higher potassium-sodium ratio In view of the extreme heterogeneity of the highly contaminated ignimbrite, the resemblance of the two analyses is revealing It is apparent that the clot is not an uncontaminated glass of the same generation as the shards, but is chemically very similar to the mixture of shards and basic lithic fragments

The geological relations suggest that the glassy clots were formed after the ignimbrite came to rest over a bed of water-saturated sand and gravel Although the immediate base of the sheet was strongly chilled, the interior, which still retained a large amount of heat, absorbed water vapour streaming up through the fractured base Because the rate of inward diffusion of water exceeded the outward diffusion of heat, the water content of the rocks became high enough to depress the melting point until it intersected the steep temperature gradient There followed a brief period of refusion, first of the low-melting portion of the rock and next of the incorporated xenolithic fragments

If this interpretation is correct, it is apparent that analyses of fiamme do not necessarily represent a more uncontaminated portion of an ignimbrite magma, but may approach the composition of the total rock

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[1] Gibson, I L, and Tazieff, H, *Nature*, 215, 1473 (1967)

Fossil Bivalve Shells indicate Longer Month and Year in Cretaceous than Present

ALTHOUGH Wells[1] and Scrutton[2] suggested methods of studying fossils to determine probable changes in day length, few studies have followed these suggestions We have examined fossil bivalve shells to try to determine whether the obvious growth lines on the shells are related to tidal or other environmental phenomena Although the study is continuing, some of the results already obtained are significant

Major growth bands, 1–2 cm wide, can be easily seen on the surfaces of bivalve shells Study of growth in some marine, temperate-water dwelling bivalve species has demonstrated that major growth bands defined by grooves on the shell surface are formed annually[3] These grooves may be distinguished from those formed erratically in response to sudden changes in environment such as temporary removal from water or sudden deep burial of the animal

The major growth bands that can be demonstrated to have formed annually can be seen, when the shells are examined in thin section, to be composed of approximately 300 or more very fine growth laminae These growth laminae are approximately 50μ thick in juvenile shells They attain a thickness of 100 to approximately 300μ in the second annual band A marked decrease in size of these fine growth laminae can be noted in growth bands successively older than the second annual The fine laminae may be as thin as 10μ in the last-formed growth band in shells of old adults

The fine growth laminae are commonly clustered The clusters form ridges and troughs that are discernible on the shell surface In shells of some species, twenty-four clusters of fifteen fine growth laminae occur within each annual band In shells of other species, the fine growth laminae occur in clusters of thirty, and there are twelve such clusters within each annual growth band These clusters of fine growth laminae are suggestive of, in the first case a fortnightly, and in the second case, a monthly, cycle of growth

Some specimens of a species of *Chione* living in Cholla Bay, Sonora, Mexico, were marked and studied closely during a period of more than a month to determine whether there was a relationship between the tidal cycles and shell growth as suggested by the studies of the fine growth laminae of their shells In one test sample, thirty specimens were marked and returned to their natural habitat The specimens were recovered 25 days later and observed to have added a ridge and trough to their shells In a second test, twenty-five specimens were collected from the same area on each of 34 successive days Study of the marginal growth increments of shells collected on successive days indicated that the addition of the ridges is related to the tidal cycle In a third test sample, a count of the number of ridges per shell in 464 specimens showed the number of ridges to be polymodal in distribution The number of modes corresponds with the apparent age of the animal in years determined by counting the number of major growth bands believed to have formed annually Also, the cut-off point for the first mode is 25 These data are interpreted as indicating a fortnightly periodicity of shell growth

The data at hand suggest a relationship between shell growth increments and tidal cycles in at least some bivalves Because this relationship seems to be relatively well founded in at least some living bivalve species, many shells of fossil bivalves have been examined It is possible to recognize the same major bands (believed, and in some cases demonstrated, to have formed annually), the very fine growth laminae and the clusters to ridges and troughs of the fine growth laminae in shells of some fossil species as were seen in shells of living species

In particular, specimens of *Idonearca vulgaris* (Morton) from Late Cretaceous age strata from several North American localities show these features most clearly There are between eight and twelve bands of the type considered to have formed annually on most of the adult shells of *I vulgaris* which were studied (It may be noted here that most bivalves living in wild conditions have a life span of 8–12 yr) The major bands, herein interpreted to have formed annually, were observed to be comprised of ridges and troughs that are clusters of fine growth laminae similar to those seen on living species On the shells studied there is an obvious grouping of twenty-four or twenty-five ridges on each major band Because drawing a precise boundary between the ridges of adjacent growth bands is relatively subjective, the ridges were counted in a continuous series across a number of major bands on each shell. The resulting number of ridges per band were weighted in relationship to the number of bands per shell involved in the count to obtain the mean number of ridges per band This value in all the shells examined is 24 98 with a standard deviation of 0 04 This figure is considered to indicate, using the implications of the studies of shells of living species, that there were 24 98 fortnightly cycles in the Late Cretaceous year If there were 24 98 such cycles in the Late Cretaceous, then there were 12 49 lunar months in the Late Cretaceous year

Many unbroken successions of the fine growth laminae were studied in shells of *Crassatella vadosus*, a species found in association with *I vulgaris* in many deposits The major growth bands in the shells of *C vadosus* are delimited by sharply incised grooves related to abrupt changes in shell curvature Ridges and troughs similar to those observed in *I vulgaris* and in many living species were also seen on shells of *C vadosus* The ridges are composed of fine growth laminae A count of 1,095 fine laminae across thirty-seven ridges in eight different shells of *C vadosus* yielded a weighted average of 29 65 fine laminae per ridge with a standard deviation of 0 18 This average is interpreted to indicate that there were 29 65 days in the month in the Late Cretaceous (compared with 29 53 at present)

Combining the data from this study of Late Cretaceous bivalve shells suggests that there were $29\ 65\times12\ 49=370\ 3$ days in the Late Cretaceous year This figure can be compared with astronomical estimates of the number of days in the year in the past As Wells[1] noted, astronomical data indicate that the Earth's rotation has been slowing down at about 2 s/100,000 yr At the beginning of the Cambrian, day length was about 21 h if the present rate of deceleration has been maintained from the beginning of the Cambrian, about 6×10^8 yr ago[4] Length of day has thus apparently been increasing throughout time and the number of days in the year decreasing Wells[1] indicated, in a plot of the number of days in a year against time in millions of years, that astronomical data lead to an estimated 371 days in the year near the end of the Cretaceous The number of days in the year in the Late Cretaceous obtained from the study of shells of Late Cretaceous bivalves is thus quite close to that derived from astronomical data.

Wells[1] suggested that there were approximately 400 days in the year in about Mid Devonian time, based on his studies of growth increments in tetracorals The number of days in the year in the Middle Devonian derived from astronomical data, assuming the present rate of deceleration in the Earth's rotation has been maintained since the Cambrian, is consistent with the number of days obtained from the study of fossil material The data from both Devonian tetracorals and Late Cretaceous bivalves thus appear to be consistent with those from astronomical evidence The results of this study support Wells's[1] suggestion that the study of fossils may provide a third line of evidence to geophysical and astronomical estimates of the age and history of the Earth

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[1] Wells, J W , *Nature*, 197, 948 (1963)
[2] Scrutton, C T , *Palaeontology*, 7, 552 (1964)
[3] Barker, R M , *Journal of Paleontology* (in the press)
[4] Munk, W H , and MacDonald, G L F , *The Rotation of the Earth*, 250 (Cambridge Univ Press, 1960)

Calcium Carbonate Content of Karst Water in relation to Flow-through Time

MEASUREMENT of the primary porosity of massive limestone gives little indication of the rate of underground movement, which takes place largely along joints and bedding planes There is also the possibility that these zones of water movement can exist as discrete systems and not in a uniformly permeable interconnected lattice[1] Consequently, the permeability of a stratum of any fractured rock is difficult to estimate The purpose of this communication is to report a finding which reinforces the hypothesis that the flow-through time of water in massive limestones might be inferred from seasonal changes in the solute concentration of the water[2]

It has been observed that "karst spring water", supplied from precipitation which has fallen only on limestone and which remains unaffected by dilution by run-off from non-calcareous outcrops, may show a clearly marked correlation between seasonal variations in the amounts of calcium carbonate in solution and antecedent air temperatures The relationship seems to reflect the increased carbon dioxide output in the plant root zone of the soil at higher temperatures[1] The lag between the air temperature midsummer peak and the subsequent peak in the chemically altered water should therefore indicate the flow-through time of the water The approximate length of the lag can be identified by calculating correlation coefficients between the measurements of calcium carbonate content of the karst water, and air temperatures averaged for periods at progressively greater time intervals before each of the sampling days Using a preset interval spanning 3 weeks, the calcium carbonate observations are correlated first with air temperatures for the 0–20 day period before sampling, then with those for 21–41 day period before each sampling day, followed by those for the 42–62, 63–83, 84–104 day and progressively earlier intervals

Between February 1962 and June 1965 karst water samples were collected monthly for a 1 or 2 year period from nearly 100 sampling points in the southern and central Pennines Eighteen of these points, mostly underground, show a very close correlation between antecedent air temperature and the calcium carbonate content of the drainage or cave-seepage water These include all the stations of those analysed so far where the antecedent temperature factor extracts more than 50 per cent of the variance in 12 monthly samples In eight cases this factor accounts for more than 80 per cent of the variance (Table 1).

In the case of the *S*1 seepage in Ingleborough Cave, near Clapham, the correlation coefficients are $r=0{\cdot}76$ for the 21–41 day period, $r=0{\cdot}94$ for the 42–62 day period and $r=0{\cdot}91$ for the 63–83 day period In this case, it seems that the correlation is closest for a longer time lag than indicated by the mid-point for the 42–62 day period In Table 1, therefore, column 5, the time at which the correlation might be closest has been estimated by interpolation based on the degree of correlation shown by the 3 week preset intervals on each side of the interval showing the highest correlation

As Fig 1 shows, the estimated flow-through time seems to determine the mean calcium carbonate content rather closely, regardless of the locality in the Pennines, or whether the water sampled is an underground or surface stream, cave seepage or stagnant pool The demonstration of this relationship is not only of geochemical significance but also supports the argument that the lag between antecedent air temperature and the subsequent correlated changes in calcium carbonate concentration is a reliable measure of the flow-through time in massive limestones

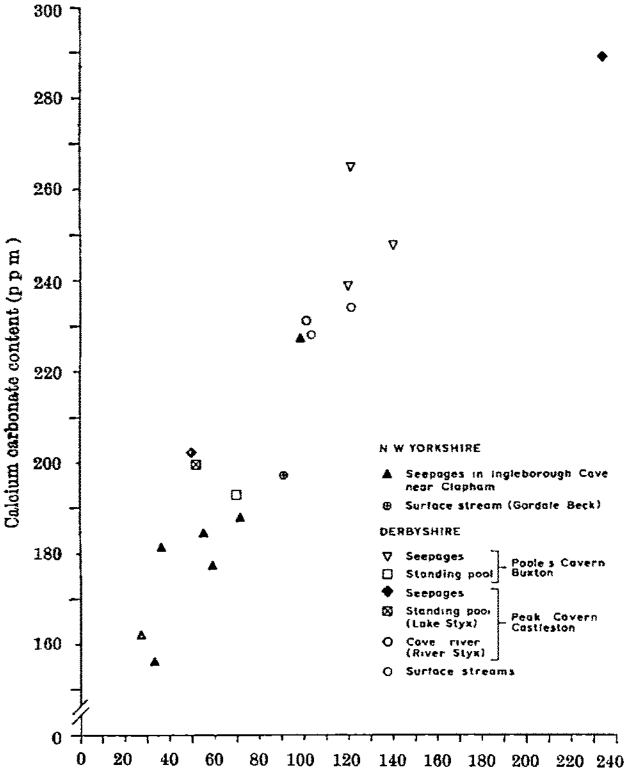

Fig 1 The relationship between estimated flow-through time and mean calcium carbonate content of karst waters from the southern and central Pennines The equation of a straight-line relationship, fitted by the method of least squares, is $y=0{\cdot}667x+152{\cdot}56$ There is, however, no reason to assume either that this is an adequate representation or that a curvilinear relationship would be more accurate

Table 1

1	2	3	4	5	6
Ingleborough Cave	*S*1	42– 62	0·94	59	177
	*S*2	21– 41	0·89	33	156
	*S*3	21– 41	0·94	27	162
	*S*4	42– 62	0·94	54	184
	*S*5	21– 41	0·90	36	181
	*S*8	63– 83	0·90	71	188
	*S*9	84–104	0·85	99	227
Gordale Beck		84–104	0·82	91	197
Poole's Cavern	*S*1	105–125	0·75	120	239
	*S*3	126–146	0·75	140	248
	*S*5	105–125	0·94	121	265
	SP	63– 83	0·90	69	193
Peak Cavern	*SP*	42– 62	0·83	51	200
	*S*2	42– 62	0·79	51	201
	*S*5	231–251	0·79	234	289
River Styx		84–104	0·92	101	231
Crowdwell rising		105–125	0·79	121	234
Bradwell		84–104	0·71	103	228

Key 1, locality, 2, code number, where needed, 3, preset 3 week interval showing closest correlation between antecedent air temperature and calcium carbonate concentrations, 4, correlation coefficients between antecedent air temperature for preset interval shown in column 3 and the calcium carbonate observations, 5, estimated flow-through time, in days, 6, mean calcium carbonate content of karst water (p p m)

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[1] Pitty, A F, *An Approach to the Study of Karst Water*, 59 (University of Hull Occasional Papers in Geography, 5, 1966)

[2] Pitty, A F, *J Hydrol* (in the press)

PHYSICS

Drag Reduction by Bacterial Metabolites

In conditions of turbulent flow, relatively low concentrations of linear, high-molecular weight polymers can greatly reduce the frictional drag of a liquid[1–4] By measuring the pressure drop along a finebore tube in these conditions, the drag-reducing potential of such a polymer solution can be estimated by comparing with the pressure drop for the pure solvent. This is the principle on which the turbulent flow rheometer[5] is based

Only recently have living organisms been shown to produce drag-reducing materials The marine dinoflagellate, *Prorocentrum micans*, and the marine diatom, *Chaetoceros didymus*, have been found to be good friction reducers when they are grown in synthetic media[6–8] When their menstrua were tested for drag-reduction effectiveness, values greater than 25 per cent were obtained Similarly, reductions were achieved by the freshwater algae, *Anabaena floss-aquae*, *Chlamydomonas peterfii* and *Porphyridium aerugineum*, and by extracts of many seaweeds, including *Chondrus crispus*, *Ulva rigida* and *Fucus furcatus*

Little work has been done on bacteria as a source of drag-reducing materials 'Kelzan' (Kelco Company, San Diego, California), a bacterial polysaccharide produced by *Xanthomonas campestris*, is effective a 50 p p m solution reduced friction 36 per cent, a 100 p p m solution, 50 per cent, and a 200 p p m solution, 62 per cent Greater concentrations did not enhance reduction

Because bacteria are ubiquitous and produce diverse metabolites, the purpose of the investigation described here was to study the potential of bacteria as drag reducers

Bacteria were cultivated on a medium (TYGS) composed of the following 5 g of tryptone (Difco), 5 g of yeast extract (Difco), 5 ml of glycerol, 5 g of sucrose,

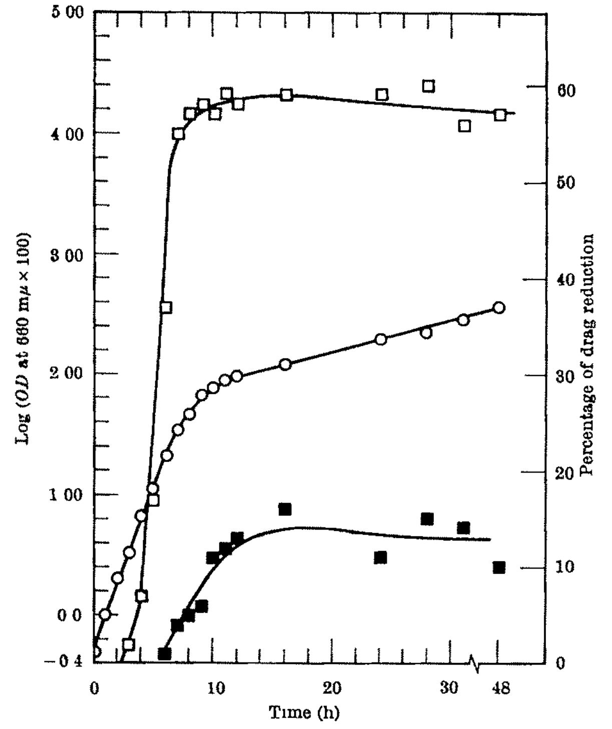

Fig 1 Drag reduction by *Pseudomonas* species in relation to the growth curve (○) A TYGS broth culture was tested for drag reduction full-strength (□) and after a 1 10 dilution (■)

5 g of glucose, 1 g of K_2HPO_4 $3H_2O$, and 1 l of de-ionized water For the cultivation of marine bacteria, K_2HPO_4 $3H_2O$ was replaced by NaCl (20 g) and 'Rila' (Rila Products, Teaneck, N J) marine salts (5 g) Solid media were prepared by the addition of 2 per cent agar (Difco) to the liquid medium The *p*H was adjusted to 7 6 with NaOH and sterilized by autoclaving

Initial studies to demonstrate drag reduction by bacterial metabolites involved agar-grown cultures Each organism tested was inoculated on to a TYGS-agar Petri dish and incubated at room temperature, approximately 25° C Four inoculating loops of each culture were collected for periods up to 7 days, suspended in 10 ml of de-ionized water, and tested immediately for drag reduction effectiveness in a turbulent-flow rheometer Synthetic sea water (35 g of 'Rila' marine salts/l) was used to suspend marine bacteria

Some bacteria that exhibited significant drag reduction capabilities (greater than 5 per cent) are described in Table 1 Bacteria found to be positive friction reducers were observed for the presence of capsules, for moistness, for butter-like consistency and for a pituitous quality (manifested by a colony sticking to the inoculating loop and producing an elastic, string-like extension), because these characteristics indicate the production of extracellular metabolites Capsule formation was discerned by means of indirect staining with india ink

Table 1 DRAG-REDUCING BACTERIA

Organism	Source	Capsule	Colony description
Pseudomonas sp	California State College, Los Angeles	−	Pituitous
Bacillus sp	California State Polytechnic College, Pomona	+	Pituitous, moist
Neisseria sp	California State Polytechnic College, Pomona	−	Pituitous
Achromobacter fisheri	Georgetown University, Washington DC	−	Pituitous
20-PFD-2 (G^- rod)	Antarctic waters	+	Buttery
PWW-E (G^- coccus)	Sewage effluent, Pomona, California	+	Moist
WG-3 (G^- rod)	Laboratory water	+	Moist
NUWC-1 (G^- rod)	Air outside laboratory	−	Pituitous
SLJ-1 (G^- rod)	3 Miles off Scripps pier, La Jolla, California	−	Pituitous, moist
SLJ-2 (G^- rod)	3 Miles off Scripps pier La Jolla, California	+	Buttery
MDFS-1 (G^- rod)	Fish slime from bass	+	Moist
FTTS-1 (G^- rod)	National Physical Laboratory Tow Tank, Feltham, England	+	Moist
FTTS-2 (G^- coccus)	National Physical Laboratory Tow Tank, Feltham, England	+	Moist
TTTS-1 (G^+ coccus)	Admiralty Research Laboratory Tow Tank, Teddington, England	+	Moist

Figs 1, 2 and 3 illustrate the kinetics of friction-reducing metabolite production in relation to the growth curves of species of *Pseudomonas*, *Bacillus* and *Neisseria*,

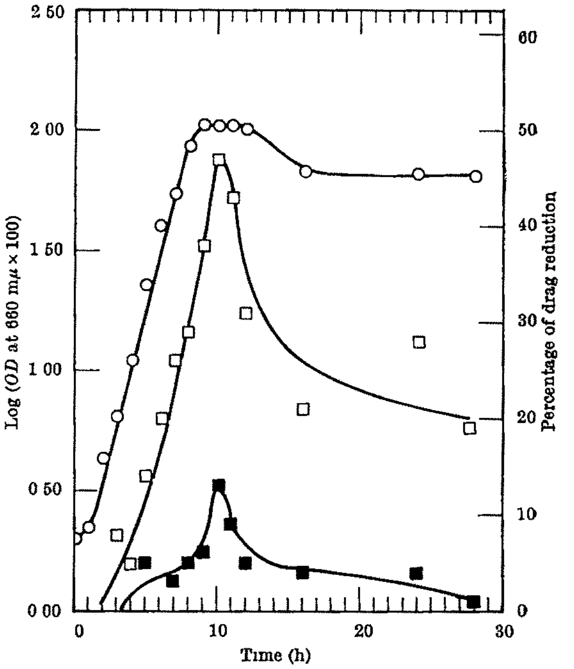

Fig 2 Drag reduction by *Bacillus* species in relation to the growth curve (○) A TYGS broth culture was tested for drag reduction full-strength (□) and after a 1 10 dilution (■)

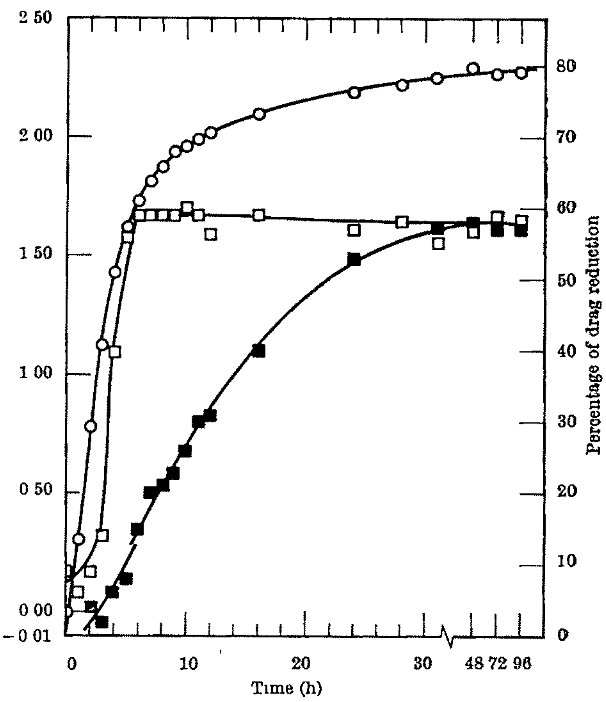

Fig 3 Drag reduction by *Neisseria* species in relation to the growth curve (○) A TYGS broth culture was tested for drag reduction full-strength (□) and after a 1 10 dilution (■)

respectively A sample (20 ml) of broth culture was inoculated into 300 ml of TYGS broth in a 1,000 ml Erlenmeyer flask, which was shaken at room temperature Portions of 5 ml were withdrawn once every hour for 12 h, and then after varying periods Turbidity was estimated at 660 mμ by means of a Bausch and Lomb Spectronic 20' colorimeter equipped with an infrared-sensitive phototube and red filter A 1 10 dilution of each sample was also made and the drag-reduction capabilities of both were estimated in a turbulent flow rheometer Preliminary studies indicated that the presence of bacterial cells had no significant effect on drag reduction

The production of friction-reducing metabolites increased rapidly and concomitantly with logarithmic culture growth (Figs 1, 2 and 3) Following logarithmic growth, however, different trends were noted for each culture At the end of exponential growth, the friction reduction by the *Pseudomonas* culture remained at approximately 58 per cent *Bacillus* species showed a decrease in friction reduction from about 47–20 per cent following exponential growth The 1 10 dilution of the culture showed a similar trend This indicated the degradation or utilization of the drag-reducing substances following logarithmic growth Friction reduction by the *Neisseria* culture remained constant at about 59 per cent after exponential growth Drag reductions from the 1 10 dilutions, however, continued to increase to the 59 per cent level, which indicated the continued production of drag-reducing materials after the logarithmic phase This merely means that the concentration of metabolite in the culture was in excess of that required to give maximum drag reduction

Fig 4 illustrates the effect of shear on drag-reducing materials derived from *Achromobacter fisheri* and *Pseudomonas* species cultures The friction of a 1 10 dilution of the culture after the first and third pass through the rheometer is plotted as a function of the age of the culture Each pass through the instrument subjects the dissolved polymers to shear The metabolites from both bacteria were subject to shear, as indicated by the difference between the curves Earlier research had shown that shear increases drag by decreasing molecular length[5]

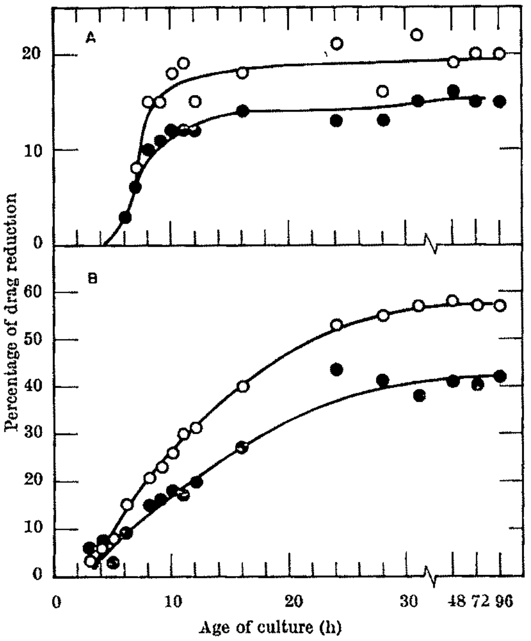

Fig 4 Shear effects on *Achromobacter fisheri* (*A*) and *Neisseria* species (*B*) drag-reducing metabolites in relation to culture age A 1 10 dilution of the culture was tested for drag reduction after the first pass through the rheometer (○) and after the third pass (●) The difference between the curves indicates the effect of shear

The results of this investigation clearly demonstrate that some bacteria can enhance the drag-reducing properties of their culture medium As Table 1 shows, bacteria isolated from many habitats were effective While many bacteria were found to be effective drag-reducers, however, many were not *Aerobacter aerogenes*, *Bacillus cereus*, *Serratia marinorubra*, *Staphylococcus aureus*, *Leuconostoc mesenteroides*, *Escherichia coli* and others were found to be ineffective

No attempt was made to isolate or purify the friction-reducing components of the bacterial cultures in this study High molecular weight extracellular polysaccharides or polysaccharide–protein conjugates, however, are likely causative agents High molecular weight, soluble, linear macromolecules are known to produce friction-reducing effects in turbulent-flow conditions 'Kelzan', a commercially produced bacterial polysaccharide with little branching and an estimated molecular weight of 6×10^6, was very effective Hyaluronic acid, a polymer that consists of repeating units of glucuronic acid-1-β-3-*N*-acetylglucosamine, is a component of some extracellular bacterial polysaccharides[9,10] Purified hyaluronic acid from a mammalian source was shown to reduce drag by more than 30 per cent in a concentration of 200 p p m (ref 5)

Bacteria affixed to phytoplankton, decomposing organic matter or other inanimate or animate objects, or even non-attached bacteria, may have sufficient nutrients available to promote the synthesis of extracellular drag-reducing metabolites These metabolites may have a pronounced effect on hydrodynamic test results, and so the marine designer should take their potential into consideration, as well as the marine diatoms, dinoflagellates, seaweeds and freshwater algae which have also been shown to be important in this respect

The drag-reducing properties of some bacterial macromolecules may make them suitable for quantitative measurements based on drag-reduction estimates This may be a useful procedure in studying extracellular polysaccharides, natural and induced leakage and lysis products, and rates of polymerization and depolymerization Further investigations are needed to explore these possibilities, as well as to examine the effects of other factors on the production of bacterial drag-reducing metabolites *in situ*

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[1] Gadd, G E , *Nature*, 212, 874 (1966)

[2] Hoyt, J W , *Polymer Lett* , 4, 713 (1966)

[3] Hoyt, J W , *Nature*, 211, 170 (1966)

[4] Hoyt, J W , and Fabula, A G , *Fifth Symp on Naval Hydrodynamics*, Bergen, Norway, 947 (ONR, Washington, 1964)

[5] Hoyt, J W , in *Symp Rheology*, 71 (ASME, New York, 1965)

[6] Hoyt, J W , *The Effects of Algal Contaminants on Frictional Resistance*, *Proc Eleventh Intern Towing Tank Conf* , Tokyo (1966)

Hoyt, J W , *The Influence of Polymer-Secreting Organisms on Fluid Friction and Cavitation* (US Naval Ordnance Test Station *TP* 4364, China Lake, California, 1967)

[8] Hoyt, J W , and Soli, G , *Science*, 149, 1509 (1965)

[9] Stacey, M , and Barker, S A , *Polysaccharides of Micro-Organisms* (Oxford University Press, London, 1960)

[10] Wilkinson, J F , *Bact Rev* , 22, 46 (1958)

Observation of Surface Vibration Modes by Stroboscopic Hologram Interferometry

Powell and Stetson[1] have shown that holography can be used to study the vibration modes of a surface The technique they employed was to record a hologram while

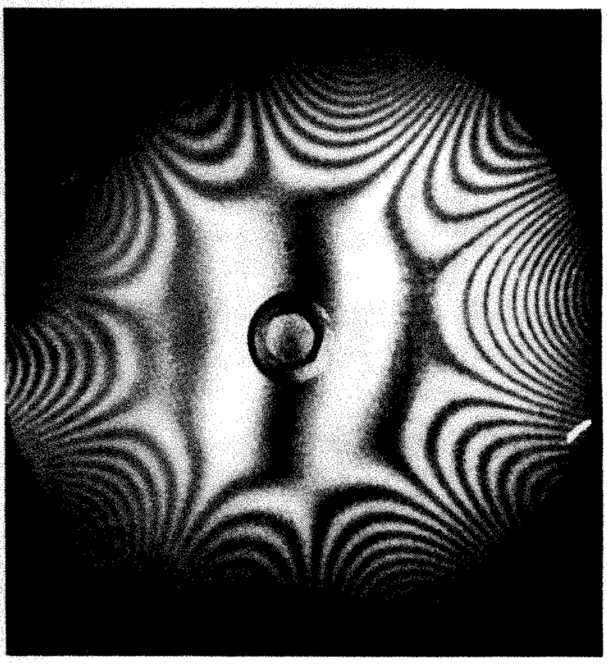

Fig. 1. Interference fringes on a white-painted aluminium disk vibrating at a resonant frequency of 3.074 Hz. Fringe motion is arrested by stroboscopic illumination.

the surface was actually vibrating. The reconstructed image from such a hologram varies in brightness with the total excursion of the surface movement, and gives a kind of "time-averaged" contour map of the vibration amplitude. We wish to report a method of real-time analysis of the surface shape and to demonstrate its advantages.

Interference effects can be obtained between an object and its holographically recorded image if the two are superimposed[2-4], by replacing the processed hologram plate in exactly the same position in which it was recorded, and viewing the object through the hologram plate using the same illuminating conditions. A slight displacement of the surface of the object will then give rise to a pattern of interference fringes, from which can be calculated the degree of displacement of the surface in a direction bisecting the illuminating and viewing directions. The appearance of the fringe pattern is independent of the roughness or otherwise of the surface. For a surface vibrating normal to its plane, the interference fringes will sweep across it in synchronism with the frequency of vibration, so that by pulsing the illumination at this frequency, their motion can be arrested and the distortion of the surface, measured relatively to its rest position, can be deduced.

In experiments on an aluminium disk 180 mm in diameter, bolted centrally to a piezo-electric transducer, a hologram of the stationary disk was first recorded. The laser beam, before being diverged to illuminate the disk, was first focused through a 0·375 mm hole drilled diametrically across a steel spindle 4·5 mm in diameter. In the image reconstruction stage this spindle could be rotated by means of an air-driven turbine to speeds in excess of 100,000 r.p.m. so that the illumination was pulsed at double this frequency (0–3·5 KHz). The transmitted pulse duration was approximately one-twentieth of the time interval between pulses. To achieve synchronization of the vibrating disk with the pulsed illumination, a small fraction of the light from the laser was split off and directed onto the end of the rotating spindle, on which was mounted a small circular front-surfaced mirror faced with a piece of polaroid. Because the laser beam is polarized, the light reflected from this combination of mirror and polaroid will vary periodically in intensity at the same frequency as the pulsing beam (2 cycles per revolution). This reflected light was then focused onto a photodetector, the output of which was amplified and used to drive the transducer. By altering the angle of the plane of polarization of the original synchronizing beam, it was possible to vary the phase of the drive signal relative to the illumination pulses. A photograph of the interference fringe pattern obtained at a resonant frequency of 3,074 Hz is shown.

Vibration analysis by interferometry is in general performed at normal incidence, in which case it has a high intrinsic sensitivity, because successive interference fringes are effectively contour lines spaced at intervals of one-half wavelength amplitude. This is especially advantageous at higher frequencies, where the amplitude of motion may be small. The fringe patterns viewed stroboscopically enable the vibration modes to be examined over the full frequency range and ciné records can be made. In this way sharp resonance conditions can be detected. For example, the resonance of which the interference pattern is shown in Fig. 1 had a very narrow bandwidth, such that the frequency had to be held within 0·5 Hz for it to remain stable. A resonance of this kind would almost certainly be missed if the "time-averaged" hologram method were used. In addition, provided that the pulse duration is a sufficiently small fraction of the periodic time, a greater number of fringes can be viewed stroboscopically. This follows from the intensity distribution of the two types of fringe pattern. For real-time fringes, for an infinitely short pulse duration, the intensity is proportional to $\sin^2(2\pi a/\ )$, while for the "time-averaged" fringes it is proportional to $J_0^2\,(4\pi a/\lambda)$ (where a is the amplitude and λ is the wavelength). The peaks of the latter distribution become progressively smaller with increase of a, but not so the former. By watching the development of the fringe pattern stroboscopically, as either the frequency or amplitude is changed, it is possible to determine the order number of any fringe and so to know the displacement of the surface at that point, whether or not there exists a node.

To follow the waveform of an oscillating surface, one merely has to change the relative phase of the drive signal and pulsing beam. By this means the presence of harmonics can be detected. It is also possible to determine whether different parts of the surface are oscillating in phase or in anti-phase, by giving the surface a slight displacement in the normal direction and noticing the direction in which the fringes move. The stroboscopic method will, of course, also show the presence of a progressive wave motion.

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[1] Powell, R. L., and Stetson, K. A., *J. Opt. Soc. Amer.*, **55**, 1593 (1965).
[2] Stetson, K. A., and Powell, R. L., *J. Opt. Soc. Amer.*, **55**, 1694 (1965).
[3] Collier, R. J., Doherty, E. T., and Pennington, K. S., *App. Phys. Lett.*, **7**, 223 (1965).
[4] Burch, J. M., Ennos, A. E., and Wilton, R. J., *Nature*, **209**, 1015 (1966).

Possible Explanation of Anomalies in Neutral *K* Phenomena

The experimental results usually interpreted as evidence for CP violation in neutral K decay have been found[1] to be inconsistent with the values of all the relevant observables obtained from other measurements. In particular, either the K_L–K_S mass difference or the difference $(\delta_2-\delta_0)$ between two pion–pion scattering phases, as determined from a phenomenological analysis[1] of the former data, has an opposite sign from that obtained by independent measurements of these two quantities. (The measurements of the sign of the mass difference have been summarized in ref. 2, and the independent determination of the sign of $(\delta_2-\delta_0)$ is backed by a considerable body of evidence; see ref. 3.)

This result, taken at face value, cannot be accounted for by any existing CPT-conserving theory of CP violation. Such a possibility can, however, be accommodated within, and in fact was predicted[4] by, the theory in which the effects in question are due not to CP violation but to the interaction of neutral K mesons with external electromagnetic fields. A possible change in sign of the effective K_L–K_S mass difference Δ in the presence of external fields was expressly considered by Spitzer[4] in connexion with equation (9) of his paper. (This equation was derived under the working hypothesis that the proposed interaction contributes primarily to the real mass matrix M, but not to the decay matrix Γ. The result that Δ can change sign is more general than equation (9) and holds even if the above assumption does not, but the form of this equation as well as the stated conditions[4] in which the sign change can occur have to be modified if both M and Γ depend appreciably on the external field.)

The coupling between neutral K and electromagnetic fields leading to such an effect was originally proposed[5] in connexion with other considerations, based on the more general phenomenon of associated production and the observed equality of the domains of validity of this phenomenon and of parity conservation. According to these considerations the two domains are equal because they are physically identical[5,6]. The proposed interaction is invariant under P and C (separately) and is non-minimal. It leads to K^0–$\bar{K}^0$ interconversion in the presence of suitable external fields[4,6] and accordingly modifies the propagation and decay characteristics of the neutral K. For example, the states K_L and K_S that exhibit simple exponential time dependence in such fields, for example, a longitudinal magnetic field, will in general be linear combinations[3] of the states K_1 and K_2 that exhibit such time dependence in the absence of matter and of external fields. Also, the effective mass difference and lifetimes of K_L and K_S will be field dependent[4,6].

The interaction leads to K_1 regeneration from K_2 by external fields[4], which entails reappearance at large distances from the target (even in the absence of matter) of 2π decay modes[4] and, through interference between K_L and K_S, of a charge asymmetry in leptonic decay. Such an asymmetry has recently been observed[7,8]. An additional possible cause of charge asymmetry, if the assumed interaction is present, is that the field-independent and field-dependent parts of the amplitudes for leptonic decay of K^0 transform under CP with a relative minus sign (for a fixed external field). This source of charge asymmetry is almost completely independent of the composition of the neutral K in terms of K^0 and $\bar{K}^0$ and therefore contributes even after the interference term has died out. As will be elaborated later, the field-dependent part of the decay amplitude is linear in the external field, so that it may be possible to vary the degree of asymmetry by changing either the magnitude or the direction of the external field.

The theory predicts that the regeneration rate may saturate in its field dependence, depending on the values of the unknown field-independent parameters. Without detailed knowledge of the field strengths and configurations in the relevant experiments it is not possible to determine these parameters on the basis of existing data. Because of the possibility of saturation, the regeneration effect may not be the most suitable one for a quantitative study of the interaction. Any dependence of, for example, the anomalous 2π decay rate on the longitudinal magnetic field[4] would, however, establish its presence qualitatively. A more promising approach to a quantitative study is likely to be provided by measuring the field dependence of Δ, but it is premature to consider this aspect of the problem until further qualitative information becomes available.

Although the proposed interaction[5,6] depends on the electromagnetic field bilinearly through the pseudoscalar $\mathbf{E}\cdot\mathbf{H}$, it seems unlikely that any of the experiments in question involved external field configuration with a finite value of $\mathbf{E}\cdot\mathbf{H}$. If the effects that have been attributed to CP violation are in fact due to the interaction of the neutral K with external fields, the relevant amplitude for field-induced K^0–$\bar{K}^0$ interconversion or K decay is, to lowest order, almost certainly linear in the external field[4,6]. The origin of such an amplitude is considered here.

The effects resulting from an amplitude for K^0–$\bar{K}^0$ interconversion or K decay which is linear (bilinear) in the external electromagnetic field will be referred to as the linear (bilinear) effects, but it must be kept in mind that the actual dependence of the various observables on external fields is in general considerably more complicated[4,6] than this terminology might suggest.

The linear effects can occur because a virtual photon can couple singly to a neutral K or to some of its decay products through known interactions. One of the photons from the vertex where the electromagnetic field acts bilinearly to interconvert K^0 and $\bar{K}^0$ can thus couple internally while the other is exchanged with the external field.

It should also be mentioned, in connexion with the linear effects, that because a π^0 cannot couple to a single photon, even to a virtual one, the lowest-order field-dependent part of the amplitude for $\pi^+\pi^-$ decay has no counterpart for $2\pi^0$ decay. The field dependence of the latter thus provides a measure of the external-field contribution to the complex mass matrix $(\Gamma+iM)$. (This remains true even in the presence of the bilinear effects, for the matrix elements proportional to $\mathbf{E}\cdot\mathbf{H}$ contribute only to M, not to Γ or to any of the decay amplitudes.) On the other hand, the field dependence of $\pi^+\pi^-$ decay has its origin in both the complex mass matrix and the decay amplitude. Hence the ratio of the partial decay rates of the neutral K into the charged and neutral 2π modes may be field dependent. A study of 2π decay can therefore provide important information on the extent to which the proposed interaction contributes to the decay amplitudes and on its relative contributions to M and Γ.

Finally, there is an aspect of the linear effect that bears on the question of the relative magnitude of the two kinds of amplitudes for leptonic decay. Denote by f and g the field-independent amplitudes

$$K^0\rightarrow\pi^-l^+\nu : f$$
$$\bar{K}^0\rightarrow\pi^-l^+\nu : g$$

and by A and B the field-dependent amplitudes

$$\bar{K}^0\rightarrow\pi^-l^+\nu : A$$
$$K^0\rightarrow\pi^-l^+\nu : B$$

Existing experiments (summarized, for example, in ref. 9) place an upper limit of $\sim 0{\cdot}2$ on the parameter $|g/f|$. Of interest here is the fact that even if g is zero, the proposed interaction can lead to phenomena generally believed to require the presence of both f and g. The point is that the non-minimal coupling interconverts K^0 and $\bar{K}^0$ and therefore, when acting together with the decay characterized by f, it leads to the field-dependent process characterized by A, not to the one characterized by B. Thus the amplitude f and the non-minimal interaction can combine to simulate the presence of the amplitude g. If the field dependence of neutral K phenomena is confirmed experimentally, it becomes imperative that in testing for the possible presence of the amplitude g both the electric and magnetic fields be minimized in the region of interest.

More generally, if the interaction with electromagnetic fields is responsible for the anomalous effects, such as 2π decay and charge asymmetry at large distances from the target, then these can all be completely eliminated by properly shielding the external fields. It is emphasized that such null experiments can serve not only to establish the presence of the proposed interaction, but in addition would dispel a related conceptual question. Specifically, the total absence of evidence for *CP* or *T* violation in any phenomena not involving neutral *K* mesons is very puzzling if the anomalous effects are interpreted as evidence for *CP* violation in neutral *K* decay. If, however, these effects can be accounted for within the framework of *CP* invariance, as discussed here and earlier[4,5], there is then no longer any reason to expect such violation elsewhere.

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[1] Bennett, S., Nygren, D., Saal, H., Steinberger, J., and Sunderland, J., *Phys. Rev. Lett.*, **19**, 997 (1967).

[2] Camerini, U., and Murphy, C. T., in *Proc. Thirteenth Intern. Conf. on High Energy Physics, Berkeley, 1966* (University of California Press, 1967).

[3] Walker, W. D., *Rev. Mod. Phys.*, **39**, 695 (1967).

[4] Spitzer, R., *Nuclear Phys.*, **68**, 691 (1965).

[5] Spitzer, R., *Nuclear Phys.*, **21**, 681 (1961).

[6] Spitzer, R., *Nuclear Phys.*, **47**, 367 (1963).

[7] Dorfan, D., Enstrom, J., Raymond, D., Schwartz, M., Wojcicki, S., Miller, D. H., and Paciotti, M., *Phys. Rev. Lett.*, **19**, 987 (1967).

[8] Bennett, S., Nygren, D., Saal, H., Steinberger, J., and Sunderland, J., *Phys. Rev. Lett.*, **19**, 993 (1967).

[9] Bell, J., and Steinberger, J., *Proc. Oxford Intern. Conf. on Elementary Particles, 1965*, 195 (Rutherford High Energy Lab., 1966).

Characteristic Energy Loss of Electrons transmitted through Argon

Interpretation of kinetic-to-internal energy transfers in many-electron heavy particle ionizing collisions[1,2] has demonstrated the importance of looking for possible large characteristic energy losses in electron scattering. Previous workers[3-5] have studied levels < 100 eV. Most X-ray absorption studies[6] do not use continuum sources but characteristic line sources. Heavy particle collision energy losses might arise not from diabatic curve crossing[7] but from collective oscillation of electrons[8].

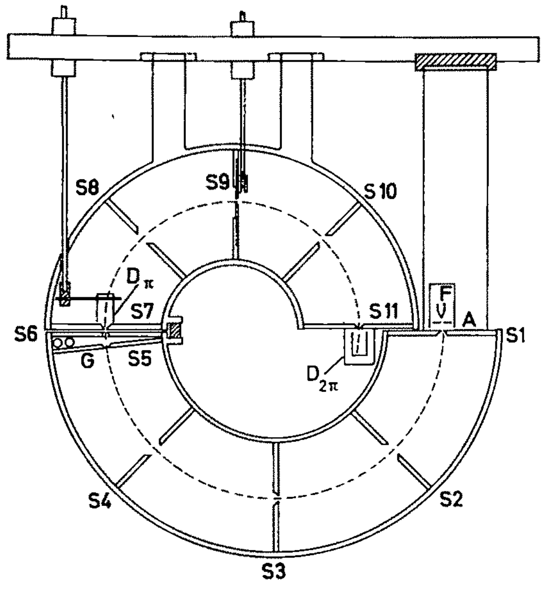

Fig. 1. Schematic diagram of apparatus.

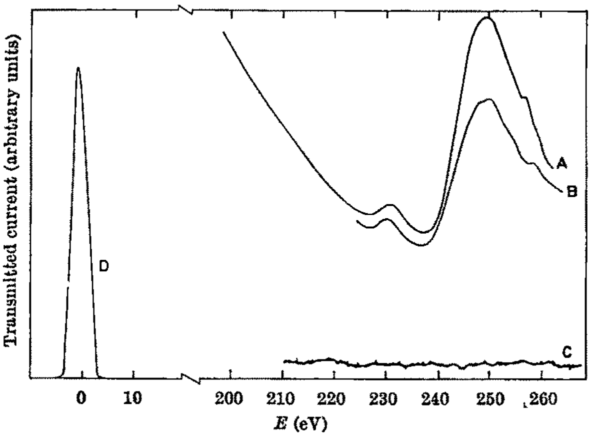

Fig. 2. Part of energy loss (E) spectrum for argon, for forward scattered 1,100 eV electrons. A, B, Typical runs; C, background spectrum in absence of gas; D, elastically scattered beam.

We have conducted a Born–Bethe approximation[9,10] zero angle electron scattering energy loss search for unidentified levels in argon, using a double 180° magnetic electron spectrometer. Because[11] the cross-section $\sigma \propto E^{-3}$, then, for a level $E \sim 200$ eV, σ will be so small that any electron spectrometer must possess high efficiency, at the expense of resolution.

The spectrometer (Fig. 1) is in a uniform magnetic field. Electrons from tungsten filament F are accelerated through anode A up to their full primary energy 1,100 eV at S_1. The 180° magnetic monochromator slits S_2–S_5 are equipotential. Gas at pressure ~0.1 torr is passed into the collision chamber G. After collisions, the electrons passing through S_6 are retarded to an energy appropriate to the 180° magnetic analyser composed of slits S_7–S_{11}, all equipotential. The monochromator and analyser slits are, respectively, on circles of radii 3 in., 2.25 ± 0.0005 in. Widths are as follows: $S_{1,5,6,7,11}$, 0.010 in.; S_3, 0.020 in.; S_9, 0.033 in.; $S_{2,4}$, 0.100 in.; $S_{8,10}$, 0.200 in.

Slit S_9, adjustable radially, was tuned for maximum electron current at zero scattering angle, within resolution 1.5°. There is high order focusing at S_{11}. The Faraday cup D_{π} could be moved into the beam path for tuning the monochromator by magnetic field variation. At constant magnetic field, the analyser was tuned to transmit the unscattered beam to the Faraday cup $D_{2\pi}$, by adjustment of the analyser potential, the scanning of which allows collection of electrons scattered with energy loss.

Fig. 2 shows typical Ar energy loss spectra, around the $L_{2,3}$ edge, for electrons of 1,100 eV initial energy. The measured overall spectrometer resolution ≃3 V. Those of the analyser and monochromator calculated from orbit theory are, respectively, 2.5 and 3.5 V. The spectrum shows excitation and ionization of M shell electrons, $L_{2,3}$ inner shell ionization edges[12] at 247 and 245 eV, and structure at 258 eV resulting from simultaneous ionization of L ($2p$) and M ($3p$) electrons. Auger transitions from this level have been reported[13]. The most interesting feature is the unidentified peak at ~230 eV, for which an oscillator strength ~0.01 is found, normalizing against X-ray data[6]. Excitation of an $n=2$ electron into $n=3$ ($1s^2\,2s^2\,2p^5\,3s^2\,3p^6\,nl$) might account for such an energy. Slater's rules[14] show that all such levels lie within 3 eV of the $L_{2,3}$ edges, and some have been identified in Auger transitions[15] and in X-ray absorption[16]. This identification of the 230 eV level is not possible. Slater's rules also show that for a 230 eV level to be produced by excitation of $n=3$ electrons, all eight must be involved. Reports of this and a similar experiment, using a different type of spectrometer, were given recently[17,18]. Structure around 230 eV showed up clearly in both spectra In scattering through non-zero angles (> 30′) the feature disappeared The measurement of single electron energies in heavy particle ionizing collisions[13] shows no peak arising from this level The inference is that the level, in decaying, distributes its energy among several electrons

We submit that this 230 eV energy loss peak could be caused by the collective excitation of a number of electrons

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[1] Afrosimov, V V, Gordeev, Y S, Panov M N, and Fedorenko, N V, *Soviet Phys Tech Phys*, **9** 1248, 1256, 1265 (1965)
[2] Everhart, E, and Kessel, Q C, *Phys Rev*, **146**, 27 (1966)
[3] Geiger, J, *Z Physik*, **175**, 530 (1963), ibid, **177**, 138 (1964), ibid, **181**, 413 (1964)
[4] Lassettre, E N, Berman, A S, Silverman, S M, and Krasnow, M E, *J Chem Phys*, **40**, 1232 (1964)
[5] Kuyatt, C E, and Simpson, J A, in *Atomic Collision Processes* (edit by McDowell, M R C), 191 (N Holland Pub Co, 1964)
[6] Lukirskii, A P, and Zimkina, T M, *Izv Akad Nauk SSSR Ser Fiz*, **17**, 817 (1963)
[7] Fano, U, and Lichten, W, *Phys Rev Lett*, **14**, 627 (1965)
[8] Amusia, M Y, *Phys Lett*, **14**, 36 (1965)
[9] Bethe, H A, *Ann Physik*, **5**, 325 (1930)
[10] Mott, N F, and Massey, H S W, *The Theory of Atomic Collisions*, third ed, 475 (Oxford University Press, NY, 1965).
[11] Lassettre, E N, and Jones, E A, *J Chem Phys*, **40**, 1208 (1964)
[12] Fine, S, and Hendee, C F, *Nucleonics*, **13**, 36 (1955)
[13] Rudd, M E, Jorgensen, T, and Volz, D J, *Phys Rev*, **151**, 28 (1966)
[14] Slater, J C, *Quantum Theory of Atomic Structure*, 368 (McGraw-Hill, 1960)
[15] Mehlhorn A P, thesis, Univ Karlsruhe
[16] Lukirskii, A P, and Zimkina, T M, *Izv Akad Nauk SSSR Ser Fiz*, **27**, 325 (1963)
[17] Boness, M J W, Hale, G D, Hasted, J B, and Larkin, I W, in *Abstracts Fifth Intern Conf Physics of Electronic and Atomic Collisions*, Leningrad, 1967, 577 (Nauk, Leningrad, USSR)
[18] Afrosimov, V V, Gordeev, Y S, Lavrov, V M, and Shchemelinin, S G, in *Abstracts Fifth Intern Conf Physics of Electronic and Atomic Collisions*, Leningrad 1967, 127 (Nauk Leningrad, USSR)

CHEMISTRY

Mass Spectrometric Analysis of Oxygen

We have recently analysed a sample of medical oxygen by mass spectrometry, and found that the level of carbon monoxide, approximately 1 per cent, far exceeded the toxic level for humans Subsequent investigation of the literature disclosed that this phenomenon is to be expected because of the interaction of oxygen with carbon impurities in the hot tungsten filament of the mass spectrometer electron gun[1-3] In a mass spectrometer, used for general analysis of organic compounds, there is the additional possibility that carbide is formed on the tungsten surface which may then react with oxygen in the system Carbides of rhenium have the advantage of being less stable than those of tungsten and this has led to the widespread use of rhenium filaments Even with a rhenium filament, however, significant quantities of carbon monoxide are produced in oxygen analysis

The construction and use of a windowless photoionization source suitable for the *M S* 9 have been described[4-6] The occurrence of reactions on the filament and the hot ion chamber in the electron bombardment source of the *M S* 9 has been shown and it has also been shown that these effects can be minimized using photoionization[7] Using a newly designed ion source (to be published) permitting simultaneous use of electron and photon beams, the analysis of oxygen has been re-examined

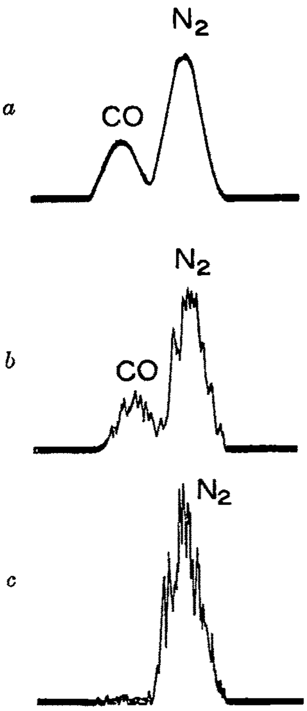

Fig 1 Doublet at m/e = 28 *a*, Electron source 200° C, *b*, photoionization source 200° C, electron energy (E < I P) *c*, photoionization source 20° C

Fig 1*a* shows the mass 28 multiplet observed when oxygen is introduced at 10^{-6} torr into an AEI *M S* 9 mass spectrometer using electron bombardment from a rhenium filament with an ion source temperature of 200° C The nitrogen peak originates from a small impurity in the ordinary tank oxygen used for this experiment The significant carbon monoxide peak largely disappeared when the oxygen flow was stopped and is thus caused by the reaction with the filament and hot ion source Fig 1*b* shows the appearance of the multiplet using photoionization by the helium 584 Å resonance line with the filament also switched on and the ion source ambient temperature at 200° C The electron energy was set at 5 V so that no ionization could occur by electron impact (Switching off the photon beam in these conditions gave zero ion current, thus confirming the absence of stray high energy electrons) Fig 1*b* shows the presence of carbon monoxide as in *a* Fig 1*c* shows the observed ion current at m/e = 28 when photoionization alone is used at a source temperature of 20° C The carbon monoxide has virtually disappeared

The use of photoionization is obviously of advantage where the mass spectrometric analysis of reactive gases is to be undertaken

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[1] Singleton, J H, *J Chem Phys*, **45**, 2819 (1966)
[2] Schlier, R E, *J Appl Phys*, **29**, 1162 (1958)
[3] Becker, J A, Becker, E J, and Brandes, R G, *J Appl Phys*, **32**, 411 (1961)
[4] Brion, C E, *Anal Chem*, **38**, 1941 (1966)
[5] Brion, C E, and Hall, L D, *J Amer Chem Soc* **88**, 3661 (1966)
[6] Beynon, J H, Fontaine, A E, Turner, D W, and Williams, A E, *J Sci Instrum*, **44**, 283 (1967)
[7] Brion, C E, *Anal Chem* **37**, 1706 (1965)

Detection of Oxygen Atoms during Gamma Radiolysis of Carbon Monoxide Gas

IT has been suggested[1] that the yield of carbon dioxide, formed during radiolysis of carbon monoxide gas at atmospheric pressure and ambient temperature, results from the reaction of oxygen atoms with carbon monoxide The principal evidence in support of this is the observation that during proton irradiation G (CO_2) is decreased from 2 to 0 5 (molecules/100 eV) as the dose rate is increased from 5×10^{15} to 10×10^{18} eV ml $^{-1}$ s^{-1} The effect was interpreted in terms of the reaction sequence

$$\begin{aligned} CO &\rightsquigarrow C + O \\ C + CO &\rightarrow C_2O \rightarrow \text{polymer} \\ O + CO &\rightarrow CO_2 \\ O + C_2O &\rightarrow 2CO \end{aligned}$$

This communication describes chemical evidence for the formation of oxygen atoms and an estimate of the yield of these intermediates in irradiated carbon monoxide

Propene was used as an oxygen atom scavenger Cvetanović has shown[2] that $O(^3P)$ atoms generated during the 2537 Å mercury sensitized photolysis of nitrous oxide

$$Hg^* + N_2O \rightarrow N_2 + O(^3P) + Hg$$

react with propene to form chiefly propene oxide and propanal with similar yields, small quantities of acetaldehyde and acetone are also formed Hughes *et al* [3] confirmed this result in a study of the reaction between $O(^3P)$ and solid propene at 77° K

The experimental arrangements were conventional A 17,000 Ci source of cobalt-60 was used and dose rates were determined by ferrous sulphate dosimetry After irradiation, both the scavenger and the reaction products were separated from the unreacted carbon monoxide by slowly pumping the contents of the vessel through a spiral trap half immersed in liquid nitrogen The trap was designed to be fitted to a gas chromatography unit by means of a switch-over valve The reaction products were identified and quantitatively estimated using a Perkin-Elmer model 452 gas chromatograph with a flame ionization detector A 2 m column of polyethylene glycol on 'Chromosorb *P*' and a 2 m pre-column of tetra ethylene glycol dimethylether on 'Chromosorb *P*' were used The pre-column ensured a good separation between the olefine scavenger and the reaction products Quantities of the order of 10^{-8} moles of product could easily be detected The experiments were performed at a dose rate of about 3×10^{13} eV ml $^{-1}$ s^{-1} in vessels of 145 ml capacity at 22° C

Irradiation of carbon monoxide containing propene was found to result in the formation of products characteristic of the reaction of $O(^3P)$ atoms with propene The G values of these products are shown in Table 1

Table 1 γ-RADIOLYSIS OF CARBON MONOXIDE AND PROPENE (0 03–0 185 PER CENT) MIXTURES AT 22° C IN THE PRESSURE RANGE 1–3 3 ATM *G* VALUES OF PRODUCTS

Product	G (molecule 100 eV^{-1})
Propene oxide	0 58 ± 0 09
Propanal	0 61 ± 0 07
Acetaldehyde	0 14 ± 0 04
Acetone	0 08 ± 0 02
Total	1 41 ± 0 22

The G values of the products were found to be independent of pressure in the range studied (1–3 3 atm) and of propene content in the range 0 03–0 185 per cent Yield-dose plots were linear to a total dose of 2×10^{19} eV With the exception of acetone, the same G values were obtained with 2 5 per cent propene mixtures At this mixing ratio G (acetone) was found to be 0 4 ± 0 04 molecules/100 eV

Cvetanović[2] reported difficulty in establishing quantitatively the extent of fragmentation of the primary association complex and suggested that addition products perhaps only account for between 70 and 80 per cent of oxygen atoms consumed One possible source of experimental error cited was the uncertainty of the quantitative recovery of the addition products from the photolysed mixture

The following experiment was designed to determine the relationship between the nitrogen and addition-product yields during the 2537 Å mercury-sensitized photolysis of nitrous oxide–propene mixtures

The experimental arrangements were similar to those described by Cvetanović[2] To avoid possible experimental error caused by preliminary fractionation of the reacted mixture by low temperature distillation, using a Le Roy still the aldehyde formed was directly extracted with water and estimated by its reaction with 2,4-dinitrophenyl hydrazine[4] The yield of product non-condensable at − 196° C was estimated by PVT measurement

The rate of formation of aldehyde, which may be assumed to be substantially propanal, was found to be $(3\ 5\pm0\ 5)\times10^{-6}$ moles/min The rate of formation of the "non-condensable" product was $(6\ 90\pm0\ 33)\times10^{-6}$ moles/min It can be assumed that the propanal yield is equal to the propene oxide yield Thus it can be concluded that the "non-condensable" product yield was substantially nitrogen and that all the $O(^3P)$ atoms produced during the photolysis reacted with the propene to form carbonyl and epoxide products

It has been shown that during gamma radiolysis of mixtures of carbon monoxide and propene when the propene content is less than 0 2 per cent, the yield ratios of carbonyl, epoxide and ketone products are similar to those found by Cvetanović[2] for the reaction of $O(^3P)$ with propene Furthermore, the yield of $O(^3P)$ atoms can be taken to be the sum of the product yields shown in Table 1—that is, $G[O(^3P)] = 1\ 41\pm0\ 22$, which accounts for about 75 per cent of the carbon dioxide yield

The mechanism of formation of the atoms and the electronic state in which they are initially formed are at present uncertain

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[1] Anderson, A R , Best, J V F , and Willett, M J , *Trans Faraday Soc* , **62**, 595 (1966)

[2] Cvetanović, R J , *Canad J Chem* , **36**, 623 (1958)

[3] Hughes, A N , Scheer, M D , and Klein, R , *J Phys Chem* , **70**, 798 (1966)

[4] Johnson, G R A and Scholes, G , *Analyst*, **79**, 217 (1954)

Formation of $O_2(^1\Delta_g)$ in a Photochemical System involving Ozone

ELECTRONICALLY excited oxygen in the $^1\Delta_g$ state exists in the upper atmosphere, and a recent review[1] discusses several mechanisms for its formation Energy considerations alone suggest that the photochemical formation of $O_2(^1\Delta_g)$ involves absorption of the Sun's radiation by ozone[2] Singlet molecular oxygen in both $^1\Sigma_g^+$ and $^1\Delta_g$ states is known to react with ozone[3–5], so that the reactions of these species are probably of importance in atmospheric chemistry as well as being of considerable interest in studies of reaction kinetics Circumstantial evidence has accumulated which indicates that $O_2(^1\Delta_g)$ is produced on the photolysis of ozone[6,7] although direct observation of

$O_2(^1\Delta_g)$ has not hitherto been made in the photochemical system This communication describes the identification of $O_2(^1\Delta_g)$ by detection of the "infrared atmospheric" band of O_2 in emission at 1 27μ in a photochemical system involving ozone

The general technique of using a flow system to study low-level "photochemiluminescence" has previously been described[8] A reactant is irradiated in a part of the flow system upstream and optically isolated from the observation region. For the present experiments, three mercury resonance ($\lambda = 2537$ Å) lamps surrounded a quartz irradiation zone, and the observation chamber was a 5 l 'Pyrex' bulb with a gold reflecting layer evaporated onto its inside surface An RCA 7102 photomultiplier operated at −80° C was used as a radiation detector in conjunction with a chopper, amplifier and phase-sensitive detector to give good signal-noise discrimination Various wavelength bands could be isolated by means of narrow-band interference filters

Early experiments[8] on the ozone system were complicated by the observation of photochemiluminescence of the $NO + O_3$ reaction resulting from traces of N_2O_5 present in the ozone Extensive purification of the ozone reduces the $NO + O_3$ emission sufficiently to allow a small correction for it to be applied to any intensity observed at 1 27μ The ozone used usually contained less than 10 per cent oxygen as the only significant impurity. Radiation was observed at 1 27μ on photolysis of ozone to which molecular oxygen had been added. The emission did not appear on addition of nitrogen or argon, nor when oxygen was added after irradiation A search was made for radiation at 0 70μ, 0 76μ and 0 87μ, but no emission except that attributable to the weak residual $NO + O_3$ photochemiluminescence could be observed in any experiment Irradiation at $\lambda = 2537$ Å of molecular oxygen in the absence of ozone does not produce any detectable photochemiluminescent emission Fig 1 shows the variation of corrected intensity at 1 27μ with pressure of oxygen added at several typical ozone concentrations For some experimental conditions, the intensity observed at 1 27μ was more than 100 times that which could be accounted for by the residual $NO + O_3$ photochemiluminescence, and the emission is consistent with the production of $O_2(^1\Delta_g)$

The necessity of the presence of molecular oxygen for the appearance of the emission leads to certain conclusions about the mode of excitation of $O_2(^1\Delta_g)$ The primary photochemical process in the decomposition of ozone has often been suggested to be

$$O_3 + h\nu \rightarrow O_2(^1\Delta_g) + O(^1D) \quad (1)$$

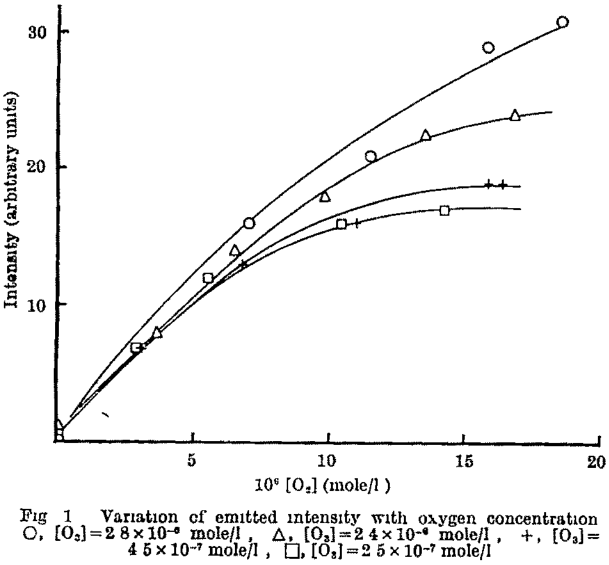

Fig 1 Variation of emitted intensity with oxygen concentration ○, $[O_3] = 2\ 8 \times 10^{-6}$ mole/l , △, $[O_3] = 2\ 4 \times 10^{-6}$ mole/l , +, $[O_3] = 4\ 5 \times 10^{-7}$ mole/l , □, $[O_3] = 2\ 5 \times 10^{-7}$ mole/l

Badger *et al* [9] have shown that the rate of spontaneous emission from $O_2(^1\Delta_g)$ is increased in the presence of molecular oxygen, although their results suggest that much greater pressures of oxygen than we employed would be necessary to give a marked enhancement of the transition probabilities An alternative explanation of the results is that the overall process represented by equation (1) is inefficient at the low pressures employed, and becomes more efficient with increasing pressure. Although there is some evidence in corroboration of this suggestion[10], addition of gases other than oxygen does not increase the observed emission intensity

A more plausible source of $O_2(^1\Delta_g)$ which is consistent with the results involves energy transfer in a secondary process It has been well substantiated that $O(^1D)$ is formed in the primary step in the photolysis of ozone, and one of the simplest excitation mechanisms for $O_2(^1\Delta_g^-)$ would be

$$O(^1D) + O_2(^3\Sigma_g^-) \rightarrow O_2(^1\Delta_g) + O(^3P) \quad (2)$$

as suggested by Vallance-Jones and Gattinger[11] for the formation of $O_2(^1\Delta_g)$ in the atmosphere Although the maximum concentration of $O(^1D)$ appears to lie at a considerably greater altitude in the atmosphere than the region from which the infrared atmospheric band originates, there is no reason to suppose that the rate of formation of $O_2(^1\Delta_g)$ will be greatest where the concentration of $O(^1D)$ is greatest Indeed, Bates[12] points out that at least one source of $O(^1D)$ in the atmosphere

$$3O(^3P) \rightarrow O_2 + O(^1D) \quad (3)$$

is likely to be most important at altitudes not inconsistent with those from which the atmospheric bands originate Because atomic oxygen persists in the atmosphere at night, either as ^1D or ^3P, the occurrence of reaction (2) would explain the observation of the infrared atmospheric band in the night airglow as well as in the dayglow

Young and Black[13] have reported an experiment involving the photolysis of oxygen at 1470 Å. Emission was observed at $\lambda = 0\ 76\mu$ and ascribed to $O_2(^1\Sigma_g^+)$ formed by the process

$$[O(^1D) + O_2(^3\Sigma_g^-) \rightarrow O_2(^1\Sigma_g^+) + O(^3P) \quad (4)$$

The rate of reaction (4) is important, because the reaction could account for the persistence of the atmospheric band at 0 76μ in the night airglow We did not observe emission from $O_2(^1\Sigma_g^+)$ in the ozone photolysis system, and consideration of the relative sensitivity of our apparatus for detection of $O_2(^1\Sigma_g^+)$ and $O_2(^1\Delta_g)$ suggests that reaction (4) is at least 10^4 times slower than reaction (2) if both reactions occur A kinetic study of the ozone photochemiluminescent system is being undertaken in order to attempt to elucidate further the excitation mechanisms, and to establish the efficiencies of reactions (2) and (4)

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[1] Wayne, R P , *Quart J Roy Met Soc* , **93**, 69 (1967)
[2] Houghton, J T , *Proc Roy Soc* , A, **288**, 545 (1965)
[3] Clyne, M A A , Thrush, B A , and Wayne, R P , *Nature*, **199**, 1057 (1963)
[4] Kaufman, F , and Kelso, J R , *Disc Faraday Soc* , **37**, 26 (1964)
[5] March, R E , Furnival, S G , and Schiff, H I , *Photochem Photobiol* , **4**, 971 (1965)
[6] Norrish, R G W , and Wayne, R P , *Proc Roy Soc* , A, **288**, 200 (1965)
[7] DeMore, W B , and Raper, O F , *J Chem Phys* , **44**, 1780 (1966)
[8] Wayne, R P , *Photochem Photobiol* , **5**, 889 (1966)
[9] Badger, R M , Wright, A C , and Whitlock, R F , *J Chem Phys* , **48**, 4345 (1965)
[10] Wayne, R P , and White, I F , *Berichte* (in the press, 1968)
[11] Vallance-Jones, A , and Gattinger, R L , *Plan Space Sci* ,**11**, 961 (1963)
[12] Bates, D R , *The Physics of the Upper Atmosphere* (edit by Ratcliffe, J A), 228 (Academic Press, New York, 1960)
[13] Young, R A , and Black, G , *J Chem Phys* , **47**, 2311 (1967)

Complex Formations between Iso and Syndiotactic Polymethylmethacrylate in Solutions

LIQUORI[1,2] showed the occurrence of a possible "stereocomplex" in dissolved mixtures of iso and syndiotactic polymethylmethacrylate (PMMA) in a ratio of 1/2 i/s PMMA and, on the basis of X-ray diffraction on the solid mixtures, suggested its possible structure[1] After Liquori's first publication in 1965, we supposed that such a stereocomplex could also be shown by viscosity, light-scattering and refractive index measurements of solutions of isomer mixtures and by infrared spectral analysis of solid mixtures The syntheses of the iso and syndiotactic[3,4] isomers (I and S) are described in ref 5 The molecular weights and nuclear magnetic resonance tacticities are given in Table 1 The preparation of the solutions is described in ref 5 Chloroform is used as solvent

Table 1

PMMA	$\bar{M}_v$	Tacticity values[6] (Varian $A60$)				
		I	H	S	$\bar{n}_i$	$\bar{n}_s$
I	75,000	94	5	1	40	2
S	339,000	5	27	68	3	7

$\bar{M}_v$ is calculated from $[\eta] = 4\ 8 \times 10^{-5}\ \bar{M}_v^{0\ 8}$ (ref 3) where at 25° C $[\eta]$ is the intrinsic viscosity of solut ons in chloroform

From the viscosity measurements, made with a Ubbelohde viscosimeter, the reduced specific viscosity was calculated as

$$\eta^{\rm spec}_{\rm red} = (t_c - t_o)/t_o\ c\ ({\rm dl\ /g})$$

A Sofica 40,000 A was used to measure the light-scattering value V_v in mA, which is the intensity of the vertical polarized scattered light transmitted at right angles from a vertical polarized primary beam From the infrared spectra, registered with a Hitachi *EPI-G* after Goode[3], we calculated Goode's J-value for each mixture The refractive index measurements and measurements on other isomer mixtures are recorded in ref 5.

The curves in the graph clearly show an anomaly not only at the weight ratio 1/2 i/s PMMA mentioned by Liquori but also at the 2/1 i/s ratio Because the curve of the J-value (which is related to movement of the α-CH_3 groups) also shows inflexions at the same two ratios, these α-CH_3 groups must be involved in the complex conformations It is evident that the two special interactions appear independently of the isomer molecular weights or tacticities[5], and we expect the stereocomplexes not to be large structures We also proved[5] that the 21 Å interference in the X-ray spectra of the PMMA stereoisomers, used by Liquori[1] for structure calculation, is a parasitic one caused by a wavelength of the residual "white X-rays" These investigations lead us to doubt the structure and occurrence of one single stereocomplex proposed by Liquori Careful examination of the measured points in graph 2 of Liquori's second publication[2] indicates that he, too, measured, without recognition, both special interactions

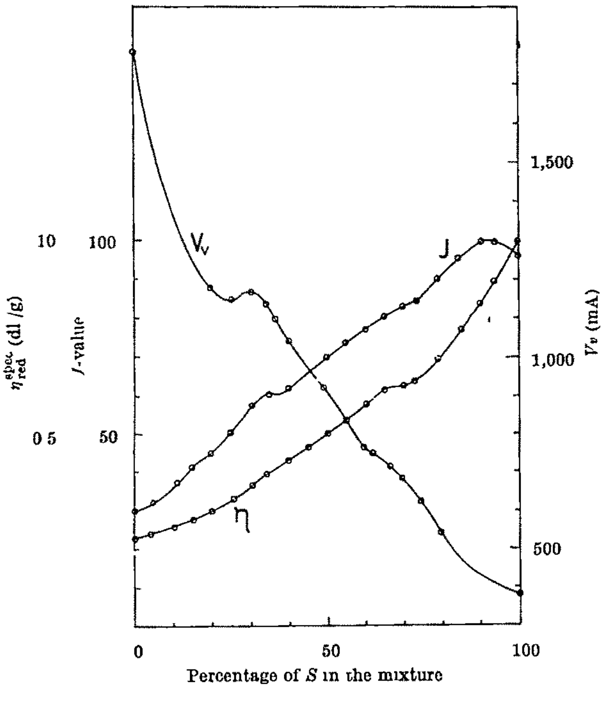

Fig 1 V_v, J-value and the reduced specific viscosity as a function of the weight percentage of the syndiotactic isomer in the mixtures Solution concentration 0 60000 g/dl

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[1] Liquori, A M, *et al*, *Nature*, **206**, 358 (1965)
[2] Liquori, A M, *et al*, *J Polymer Sci*, **4B**, 943 (1966)
[3] Goode, W E, *et al*, *J Polymer Sci*, **46**, 317 (1960)
[4] Belgium Pat No 566,713 (1958)
[5] van den Berg, W B, thesis, Eindhoven (1967)
[6] Braun, D, *et al*, *Makromol Chem*, **51**, 15 (1961)

Stereoselective Interaction of Amino-acids and Metal Complexes

STEREOSELECTIVE effects frequently occur[1] in the formation and reactions of optically active co-ordination compounds The formation of ions of the type [Co(L-dipeptide)$_2$]$^-$ shows[2,3] very marked stereoselectivity which may be attributed to the three point attachment[4] of the dipeptide acting as a terdentate ligand, giving a puckered chelate ring Similarly, peptides of the type gly-Lα, α being an amino-acid, react[5] with the ion D[Coen$_2$(H_2O)(OH)]$^{2+}$ more rapidly than with its enantiomer, through three-point attachment. Related three-point attachment can also occur with amino-acids containing a polar side chain For example, we recently demonstrated[6] that L-glutamate ion reacts specifically with DL[Coen$_2$(H_2O)$_2$]$^{3+}$ giving D(+)[Coen$_2$(Lglu)]$^+$.

We report some stereoselective interactions of basic amino-acids with metal complexes of 1,2-diamine-tetra-acetate ions which probably also involve three-point bonding For example, resolution of the DL[Co(EDTA)]$^-$ ion is achieved when L-histidine is added to its acidic solution in aqueous ethanol The optically pure diastereoisomer [L-hisH_2] (+)$_{5461}$[CoEDTA]$_2$ 2H_2O crystallizes first Other Lα amino-acids with basic side chains (for example, L-arginine) can be used, although crystallization proceeds less readily [Co(PDTA)]$^-$ and other similar anions may also be resolved using L-histidine The present route provides a much simpler resolution of [Co(EDTA)]$^-$ than commonly used[7] which involves an initial resolution of a DL-*cis*-dinitrobisethylenediaminecobalt(III) salt DL-Histidine may also be resolved by using its diastereoisomeric salts with (+) or (−) [CoEDTA]$^-$

Related to this convenient new resolution is a novel resolution of lysine using [Co(EDTA)]$^-$ or the *trans*-1,2-diaminocyclohexane-*NNN′N′*-tetra-acetatocobaltate(III) anion When a solution of dodecatungstophosphoric acid is slowly added to a concentrated solution of K (−)$_{5461}$ [Co(EDTA)] (1 mole) and DL-lysine hydrochloride (2 moles), the initial precipitate is the tungstophosphate of

D-lysine If sufficient dodecatungstophosphoric acid is added, then its L-lysine salt is eventually precipitated

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[1] Dunlop, J H , and Gillard, R D , *Adv Inorg Chem Radiochem* , 9, 185 (1966)
[2] Gillard, R D , Harrison, P M , and McKenzie, E D , *J Chem Soc* , A, 618 (1967)
[3] Payne, N C , thesis, Univ Sheffield (1967)
[4] Dunlop, J H , Gillard, R D , and Payne, N C , *J Chem Soc* , A, 1469 (1966)
[5] Allen, D E , and Gillard, R D , *Chem Comm* , 1071 (1967)
[6] Dunlop, J H , Gillard, R D , Payne, N C , and Robertson, G B , *Chem Comm* , 874 (1966)
[7] Dwyer F P , and Garvan, F L , *Inorg Syntheses*, 6, 192 (1960)

BIOCHEMISTRY

Relationship between Concentrations of Human Chorionic Gonadotrophin in Plasma and Cerebrospinal Fluid

Human chorionic gonadotrophin (HCG) has been detected by various bioassay procedures in the cerebrospinal fluid of pregnant and non-pregnant subjects with a high concentration of the hormone in the plasma and urine[1-5] It was not detected in the cerebrospinal fluid when the plasma and urine concentrations were lower In agreement with these earlier workers, McCormick[6] and Tashima *et al* [7] concluded that HCG did not appear in the cerebrospinal fluid until a high threshold value, equivalent to a urinary excretion rate of about 250,000 IU of HCG/day, was exceeded

The opportunity to obtain cerebrospinal fluid during normal pregnancy rarely arises, but patients with trophoblastic tumours also have HCG in their body fluids and this permits study of the plasma–cerebrospinal fluid relationship for this hormone We report here radioimmunoassay estimates of corresponding concentrations of HCG of plasma and cerebrospinal fluid in twelve subjects who had trophoblastic tumours but who did not have any evidence of metastases in the central nervous system The observations indicate that for a thousand-fold range of concentrations there is no evidence of a threshold effect and that the concentration of HCG in the cerebrospinal fluid, determined immunologically, is directly proportional to that in the plasma

Details of the radioimmunoassay have been described earlier[8-10] Both HCG and luteinizing hormone (LH) are estimated and approximately 1 ml of the sample fluid is required Twenty-four hour urine samples were collected with merthiolate (100 mg) as a preservative Cerebrospinal fluid obtained by lumbar puncture was stored at − 15° C without anticoagulant or preservative Plasma samples were collected at the time of lumbar puncture with 2 mg/ml of ethylenediamine tetraacetic acid (EDTA) as anticoagulant

Gonadotrophic activity was not detectable in the cerebrospinal fluid of ten hospital patients with normal plasma concentrations of LH who underwent lumbar puncture in the course of investigations for various complaints Total cerebrospinal fluid protein concentrations of the patients with choriocarcinoma were determined by a turbidometric method using sulphosalicylic acid, and all were normal ($<$ 40 mg/100 ml) The corresponding concentrations of HCG in plasma and cerebrospinal fluid are shown in Table 1 and are plotted on a double logarithmic scale in Fig 1 The log HCG cerebrospinal fluid and log HCG plasma are significantly correlated ($P < 0$ 001—two-sided tests) and the data are adequately represented by the simple relationship concentration of HCG in cerebrospinal fluid = 0 0057 × concentration of HCG in plasma The results demonstrate a close correlation between the concentrations of HCG in plasma and cerebrospinal fluid, determined immunologically, for a range of plasma concentrations of 0 28–280 IU/ml

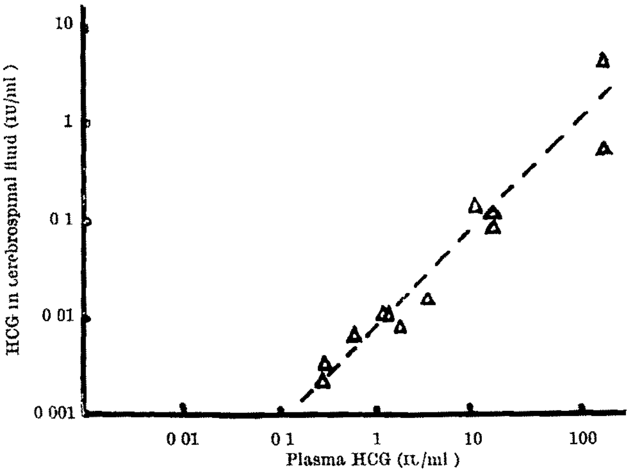

Fig 1 Relationship between concentration of HCG in plasma and spinal fluid

Table 1 CONCENTRATIONS OF HCG IN PLASMA AND CEREBROSPINAL FLUID OF PATIENTS WITH TROPHOBLASTIC TUMOURS

Case No	HCG (IU/ml)		Plasma/
	Plasma	Cerebrospinal fluid	Cerebrospinal fluid
69	0 320	0 003	106
111	0 280	0 002	140
132	280 000	3 550	79 0
133	1 520	0 009	169
149	17 600	0 088	200
153	18 000	0 094	192
157	12 000	0 127	94 5
161	2 170	0 007	311
164	1 880	0 009	209
169	0 700	0 006	117
191	270 000	0 450	600
193	3 620	0 015	240

The discrepancy between our findings and the suggestion, from results of bioassay studies, that a threshold effect limits the entry of HCG into the cerebrospinal fluid at low plasma concentrations could be accounted for if much of the HCG entering the cerebrospinal fluid lost its biological but not its immunological activity It is known that the biological activity of HCG can be lost without a parallel loss of immunological activity as a result of heating or exposure to neuraminidase which modifies the charge of the molecule (unpublished work of Wilde, Orr and Bagshawe) We have, however, no evidence that degradation occurs in the cerebrospinal fluid or that there is selective transport of immunologically active, biologically inert molecules The mean plasma cerebrospinal fluid ratio of 177 1 found by radioimmunoassay in this work can be compared with a ratio of 187 1 obtained by bioassay[6] after HCG was infused into a nephrectomized dog It is also clear that the bioassay procedures used were too insensitive to detect the small amount of HCG present in the cerebrospinal fluid when the plasma concentration was relatively low and the proposition of a threshold effect may be attributed to failure to recognize fully the consequences of this insensitivity

The relatively low concentration of HCG in the cerebrospinal fluid is typical of proteins in general[12], and if equilibration between the compartments is slow, rapid changes in the plasma concentration will not be reflected promptly in the cerebrospinal fluid concentration The presence of HCG producing tumours in the central nervous system may also disturb the plasma cerebrospinal fluid ratio and observations on patients with intracranial metastases will be reported later

Although differences between HCG produced by tumours and the normal placenta have been suspected[13], these have not been confirmed by later work[14,15], and we have found no reports of a disturbance of plasma–cerebrospinal fluid transport in normal pregnancy or in patients with trophoblastic tumours outside the central nervous system On present evidence it therefore seems likely that the plasma cerebrospinal fluid relationship for HCG reported here also obtains in normal pregnancy

We are not aware that similar plasma–cerebrospinal fluid relationships have been established for any other substance, although it may also apply to insulin Margolis and Altszuler[16] have recently examined the concentration of insulin in plasma and cerebrospinal fluid by radioimmunoassay during insulin infusion in the dog When their data are plotted on a double logarithmic scale a similar linear relationship is seen between the concentrations of this hormone in plasma and cerebrospinal fluid

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[1] Ehrhardt, K , *Med Klin* ,**27**, 426 (1931)
[2] Zondek, B , *J Amer Med Assoc* ,**108**, 607 (1937)
[3] Zondek, B , *J Obstet Gynaec Brit Emp* , **49**, 397 (1942)
[4] Mathieu, A , *Amer J Obstet Gynec* , **37**, 654 (1939)
[5] Vesell, M , and Goldman, S , *Amer J Obstet Gynec* **42**, 272 (1944)
[6] McCormick, J B , *Obstet Gynec* , **3**, 58 (1954)
[7] Tashima, C K , *J Clin Endocrinol Metab* , **25**, 1493 (1965)
[8] Wilde, C E , Orr, A H , and Bagshawe, K D , *Nature*, **205**, 191 (1965)
[9] Bagshawe, K D , Wilde, C E , and Orr, A H , *Lancet*, i, 1118 (1966)
[10] Wilde, C E , Orr, A H , and Bagshawe, K D , *J Endocrinol* , **37**, 23 (1967)
[11] Wide, L , *Acta Endocrinol* , 41, Suppl 70 (1962)
[12] Davson, H , *Physiology of the Cerebrospinal Fluid* (J and A Churchill, Ltd , London, 1967)
[13] Reisfelt, R A , Bergenstal, D M , and Hertz, R , *Arch Biochem Biophys* , **81**, 456 (1959)
[14] Lewis, J , Dray, S , Genuth, S , and Schwartz, H S , *J Clin Endocrinol* , **24**, 197 (1964)
[15] Wilde, C E , and Bagshawe, K D , *Gonadotrophins Physiochemical and Immunological Properties*, Ciba Foundation Study Group, No 22 (edit by Wolstenholme, G E W , and Knight, J) (J and A Churchill, Ltd , London, 1965)
[16] Margolis, I , and Altszuler, N , *Nature*, **215**, 1375 (1967)

Glycogen Synthetase Activity in Blood Platelets

Leloir *et al*[1-3] found the key enzyme in glycogen synthesis to be UDP glucose α1-4 glucan-4-glucosyl transferase (E C 2 4 1 11), also called glycogen synthetase The enzyme has been extensively studied in liver[1-3] and muscle[4,5], and has been identified in blood leucocytes[6]

The results presented here indicate that pig blood platelets have a significant glycogen synthetase activity, and some general properties of the crude enzyme are reported

Pig blood was collected on acid citrate dextrose medium (B formula of the National Institutes of Health) and maintained at 0° C until the platelets had been separated The blood was centrifuged at 1,200 r p m for 10 min in an International PR_2 refrigerated centrifuge (rotor No 259), the plasma was collected and run at 2,300 r p m for 5 min and the supernatant was used as a source of platelets which were isolated by centrifugation at 15,000 r p m for 20 min in a refrigerated Sorvall centrifuge (rotor *SS* 34) The platelets were washed three times in saline to which was added 1 mM versene, *p*H 7 5 The final pellet was resuspended in twice distilled water or in a medium made up of 1 mM *tris*, *p*H 7 5, 0 25 M sucrose and 1 mM versene, *p*H 7 5 All the centrifugations were performed with plastic tubes Examination of the platelet suspension by phase contrast microscopy indicated that no aggregation had occurred during preparation The homogenate was prepared by treating the platelet suspension in a tissue grinder fitted with a rotating 'Teflon' pestle for 5 min at 0° C

Glycogen synthetase activity was measured by a method similar to that described by De Wulf and Hers[7], and the amount of radioactivity incorporated into glycogen from UDP-glucose labelled with carbon-14 was determined The assay mixture contained 2 mg of shellfish glycogen, 0 2 μmole of UDP-glucose, 5,000–6,000 c p m of ^{14}C-UDP-glucose (Radiochemical Centre, Amersham) and 4 μmoles of glycylglycine, *p*H 8 4, in some cases, 0 5 μmoles of glucose-6-phosphate was added in a volume of 0 05 ml The reaction was initiated by adding 0 1 ml of homogenate, the mixture was incubated at 37° C, and the reaction was stopped by the addition of 0 5 ml of cold 10 per cent TCA A volume (0 05 ml) of a 10 per cent glycogen solution was then added and the total glycogen precipitated and collected in the way described by De Wulf and Hers[7] Radioactive samples (0 5 ml) from the various tests were added to vials containing 10 per cent naphthalene, 1 per cent 2,5-diphenyloxazole, 0 025 per cent 1,4-*bis*-2-(5-phenyloxazolyl) benzene and 20 per cent ethanol in dioxan, and the radioactivity was then counted in a Nuclear Chicago liquid scintillation counter Glycogen was determined by the anthrone method[8] and proteins were measured by the method of Lowry *et al* [9]

Homogenates of pig blood platelets can catalyse the incorporation of radioactivity from ^{14}C-UDP-glucose into glycogen Within certain limits, the reaction is linearly proportional to the amount of homogenate (Fig 1) and to the time of incubation (Fig 2) Addition of glucose-6-phosphate greatly increases the enzyme activity (Figs 1 and 2) At a concentration 3 3 mM, a three to six-fold increase has been observed in different preparations Half the maximal activation is obtained with a concentration of glucose-6-phosphate of about 0 8 mM

Fig 3 illustrates the effect of changing the concentration of UDP-glucose The K_m value in the presence of glucose-6-phosphate is $5\ 5 \times 10^{-4}$ M The sugar phosphate

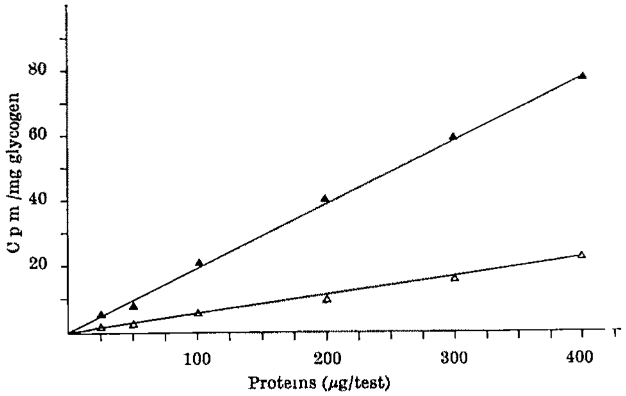

Fig 1 Dependence of the reaction rate of glycogen synthetase on enzyme concentration The amount of enzyme preparation added was varied as indicated The assay mixture was incubated for 20 min at 37° C without glucose-6-phosphate (△—△) or in the presence of 0 5 μmoles of glucose-6-phosphate (▲—▲)

causes a striking increase in the V value but does not considerably affect the K_m value for UDP-glucose The optimum *p*H for the platelet enzyme was found to be about 8 5 in the presence of glucose-6-phosphate Glycogen synthetase of pig blood platelets is chiefly associated with structure sedimenting at high speed in 0 25 M sucrose The enzyme is purified seven to nine-fold in the sediment obtained by centrifugation of the homogenates at 105,000*g* for 60 min Homogenates of pig platelets maintained at 0° C or at – 20° C retain their glycogen synthetase activity for at least 1 month without any significant change, purified preparations obtained by centrifugation (105,000*g* pellet) are less stable and lose a significant part of their activity after 24 h at 0° C or at – 20° C

Mercaptoethanol at a concentration of 0 05 M stimulates the total activity of the enzyme Table 1 shows the results obtained on a partially purified enzyme (105,000*g* pellet) The thiocompound significantly increases the activity of the enzyme when tested in the presence of glucose-6-phosphate, but the effect is less marked in the absence of the sugar phosphate Preincubation of the preparation for 60 min at 30° C in the presence of mercaptoethanol does not significantly change the effect of the compound

Table 1 EFFECT OF MERCAPTOETHANOL ON THE GLYCOGEN SYNTHETASE ACTIVITY OF BLOOD PLATELETS

Preincubation time (min)	C p m /mg glycogen With glucose-6-phosphate		Without glucose-6-phosphate	
	– M–SH	+ M–SH	– M–SH	+ M–SH
0	142	183	40	44
60	127	178	36	43

0 1 ml of enzyme (105,000*g* pellet) containing 1 36 mg of protein is preincubated at 30° C for 0 or 60 min in the presence or absence of 5×10^{-3} M mercaptoethanol (M–SH) The assays are then performed with or without the addition of 3 3 mM glucose-6-phosphate for 20 min

The general properties of the glycogen synthetase in pig blood platelets are comparable with those of glycogen synthetase found in other tissues, in particular with liver and muscle enzymes[1-4] As with these enzymes, UDP-glucose-transglucosylase activity in pig blood platelets is at least partially dependent on the presence of glucose-6-phosphate, its optimum *p*H is slightly alkaline and the K_m value for UDP-glucose is comparable with that found by Leloir and Goldemberg[3] for crude preparations of liver and muscle Moreover, after centrifugation the platelet enzyme is chiefly recovered in a 105,000*g* pellet with which the platelet glycogen is probably associated

Rosell-Perez and Larner using a dog muscle preparation[10] have found that mercaptoethanol promotes the conversion of glycogen synthetase from a form which is dependent on glucose-6-phosphate to a form independent of that compound On the other hand, according to these authors, total activity of glycogen synthetase of frog and toadfish muscle is stimulated by mercaptoethanol in the presence of glucose-6-phosphate without conversion from one form to another The platelet enzyme behaves in the same way, total activity being increased by the thiocompound without any apparent conversion of the enzyme

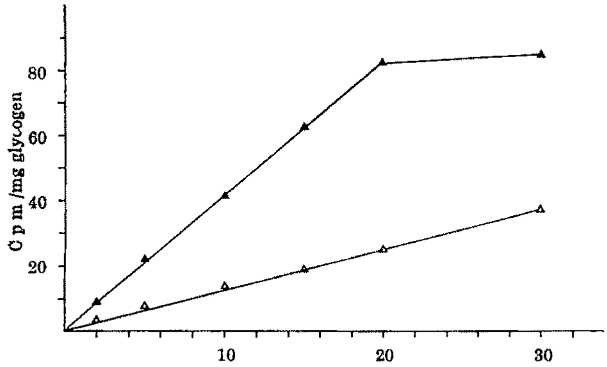

Fig 2 Time course of the glycogen synthetase reaction The times were as indicated The tests were performed at 37° C with 0 1 ml of a homogenate containing 5 mg of protein/ml without glucose-6-phosphate (△—△) or in the presence of 0 5 μmoles of glucose-6-phosphate (▲—▲)

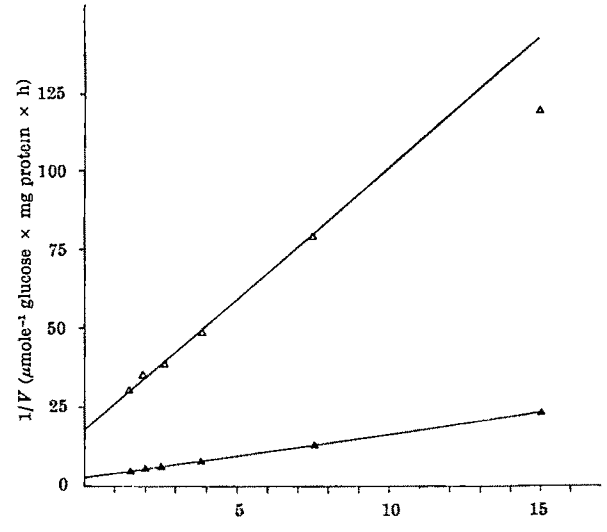

Fig 3 Reciprocal plot of the dependence of UDP-glucose concentration on thrombocyte glycogen synthetase without glucose-6-phosphate (△—△) or in the presence of 0 5 μmoles of glucose-6-phosphate (▲—▲) The tests were performed with 0 1 ml of a homogenate containing 5 mg of protein/ml

Glycogen seems to be one of the principal energetic materials of thrombocytes[11] The enzymes involved in its metabolism are thus of particular interest with respect to the general metabolism of blood platelets Most of the enzymes of glycolysis[11] and phosphorylase[12] have been identified in these blood constituents Our results show that thrombocytes can also synthesize their own glycogen and do not depend completely on glycogen obtained from the megakaryocytes

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[1] Leloir, L F , and Cardini, E C , *J Amer Chem Soc* , 79, 6340 (1957)
[2] Leloir, L F Olavarria, M J , Goldemberg, J S , and Carminatti, H , *Arch Biochem Biophys* , 81, 508 (1959)
[3] Leloir, L F , and Goldemberg, H S , *J Biol Chem* , 235, 919 (1960)
[4] Villar-Palasi, C , and Larner, J , *Biochim Biophys Acta*, 30, 449 (1958)
[5] Larner, J , Villar-Palasi, C , and Richman J D , *Ann NY Acad Sci* , 82, 345 (1959)
[6] Nakai, S G , and Craddock, G Ch , *Biochim Biophys Acta*, 121, 195 (1966)
[7] De Wulf, H , and Hers, G H , *Eur J Biochem* , 2, 50 (1957)
[8] Hassid, Z W and Abraham, S , in *Methods in Enzymology* (edit by Colowick S P , and Kaplan, N O), 3, 35 (Academic Press, Inc , New York, 1957)
[9] Lowry, O H , Rosenbrough, N J , Farr, A L , and Randall, R J , *Biol Chem* , 193, 265 (1951)
[10] Rosell-Perez, M , and Larner, J , *Biochemistry*, 3, 81 (1964)
[11] Gross, R , in *Platelets Their Role in Hemostasis and Thrombosis* (Trans Conf , Chapel Hill, North Carolina, USA (1966) (*Thrombos Diathes Haemorrh* , Suppl)), 143 (F K Schattauer-Verlag, Stuttgart, 1967)
[12] Scott, B R , *Blood*, 30, 321 (1967)

Formation of Melatonin and 5-Hydroxyindole Acetic Acid from ^{14}C-Tryptophan by Rat Pineal Glands in Organ Culture

THE rat pineal gland contains large amounts of serotonin[1] and the enzymes tryptophan hydroxylase[2] and aromatic l-amino-acid decarboxylase[3] which synthesize this indoleamine from tryptophan. We have reported that rat pineals in organ culture retain the ability to produce serotonin from its amino-acid precursors tryptophan and 5-hydroxytryptophan[4]. Most of the serotonin normally formed in the pineal is oxidized to 5-hydroxyindole acetic acid[5] by the enzymes monoamine oxidase[6] and aldehyde dehydrogenase. In addition, a portion of the indoleamine is *N*-acetylated[7] and then converted to melatonin by hydroxyindole-*O*-methyl transferase[8], an enzyme present in mammals only in the pineal gland[9]. By using isotopically labelled tryptophan as the substrate for our organ culture system, we have observed that such preparations can transform the serotonin produced to two end products: 5-hydroxyindole acetic acid (5HIAA) and melatonin.

Sprague–Dawley female rats weighing 160–180 g each were killed by neck fracture, and their pineal glands were removed and clotted to the walls of a Wasserman tube as described before[4]. Nutrient medium (0.5 ml)[4] containing ^{14}C-tryptophan or ^{14}C-5-hydroxytryptophan was added, and the tubes were sealed with a rubber stopper and incubated for 2 days at 37° C. Control cultures were frozen immediately after the addition of the nutrient medium or were incubated without a pineal gland. At the end of the incubation period, the media were frozen, samples were then assayed for their contents of total radioactivity, ^{14}C-serotonin, ^{14}C-melatonin and ^{14}C-5HIAA as follows. A 0.1 ml sample of the medium was transferred to a glass-stoppered centrifuge tube containing 2 ml of 0·1 M borate buffer, *p*H 10, and the ^{14}C-melatonin was extracted into 6 ml of chloroform. After centrifugation, a 1 ml sample of the aqueous phase was transferred to a second glass-stoppered centrifuge tube and the ^{14}C-serotonin was extracted into 6 ml of a mixture of butanol and chloroform (1 : 3). The rest of the aqueous phase in the first tube was removed by suction. The chloroform was washed with 2 ml of borate buffer, *p*H 10, and 4 ml was transferred to a counting vial and evaporated. The radioactivity was measured in a liquid scintillation spectrophotometer after the addition of phosphor. The chloroform-extractable radioactivity was identified as ^{14}C-melatonin by chromatography in three systems (ascending paper chromatography, butanol : acetic acid : water, 8 : 2 : 2; ascending paper chromatography, isopropanol : ammonium hydroxide : water, 8 : 2 : 2; thin-layer chromatography, chloroform : methanol, 94 : 6). The butanol : chloroform mixture in the second centrifuge tube was washed with 2 ml of borate buffer, *p*H 10, and 4 ml. of the washed mixture was transferred to a vial and evaporated; its radioactivity was counted after the addition of phosphor. This radioactive material was identified as ^{14}C-serotonin using the chromatographic systems already described. A further 0.1 ml of the culture medium was transferred to a glass-stoppered centrifuge tube containing 1 ml of 0.2 M hydrochloric acid and the ^{14}C-5HIAA was extracted into 6 ml of ethyl ether. The ether layer was transferred to another centrifuge tube, and the 5HIAA was re-extracted into 2 ml of 0.1 M buffer, *p*H 7.9. The ether layer was removed by suction, and the aqueous layer was shaken again with 6 ml of ether. The ether layer was discarded, the aqueous layer was acidified with 1 ml of 1.0 M hydrochloric acid, and the radioactivity was re-extracted into a final 6 ml of ether. A 4 ml sample of the organic phase was transferred to a vial and evaporated to dryness; its radioactivity was measured after the addition of phosphor. The radioactive material was shown to be ^{14}C-5HIAA, using the chromatographic system already described. The recoveries of serotonin, melatonin and 5HIAA were approximately 80 per cent, 90 per cent, and 50 per cent, respectively, and were relatively constant from experiment to experiment. The sum of the labelled serotonin, melatonin and 5HIAA extracted from each culture tube was taken as an estimate of the total amount of serotonin produced in that tube. Each treatment group contained four culture tubes.

Pineal organs were incubated with ^{14}C-tryptophan (10^{-4} M, 460,000 c.p.m.) or ^{14}C-5-hydroxytryptophan (2.5×10^{-4} M, 460,000 c.p.m.) for 2 days. The culture tubes were held stationary or were rotated in a roller wheel.

In all conditions studied, considerable amounts of serotonin, melatonin and 5HIAA were formed. The mean synthesis of each indole from ^{14}C-tryptophan in roller tubes was similar in four separate experiments. When tryptophan was used as the substrate, rotation was associated with an increase both in the total serotonin formed and in its conversion to 5HIAA. Pineals in stationary tubes transformed 0.81 ± 0.07 per cent of the ^{14}C-tryptophan into its three indole metabolites. Similar organs in roller tubes converted 2.03 ± 0.17 per cent of the amino-acid to serotonin, melatonin or 5HIAA. Most of this difference was represented in the serotonin and 5HIAA fractions; the concentrations of ^{14}C-melatonin in the media did not differ between groups of stationary and rotated tubes (Table 1). When pineals were incubated with ^{14}C-5-hydroxytryptophan instead of tryptophan, the total ^{14}C-serotonin formed was not increased by rotating the culture tubes: 2.01 ± 0.21 per cent of the radioactivity in the media from stationary cultures was serotonin, melatonin or 5HIAA, as compared with 2.24 ± 0.22 per cent in the media from roller tubes. The formation of ^{14}C-5HIAA, however, was significantly enhanced in the roller tubes (Table 1). These results indicate that the metabolism of tryptophan by rat pineal glands in organ culture proceeds as follows:

$$\begin{tikzcd} & & & \text{melatonin} \\ \text{tryptophan} \arrow[r] & \text{5-hydroxytryptophan} \arrow[r] & \text{serotonin} \arrow[ur] \arrow[dr] & \\ & & & \text{5HIAA} \end{tikzcd}$$

The formation of serotonin from tryptophan (but not from 5-hydroxytryptophan) involves the action of an oxidative enzyme, tryptophan hydroxylase. Similarly, the production of 5HIAA (but not melatonin) from serotonin involves successive oxidations of the amine to an aldehyde and then to an acid. Only those three of the foregoing reactions which involve the action of oxidative enzymes were suppressed by stationary incubation (Table 1). In striking contrast, the remaining three enzyme reactions which do not involve oxidation proceeded at the same rate in both stationary and rolling cultures. Thus the most likely explanation for increased synthesis of serotonin and 5HIAA (but not melatonin) in the roller tubes is that rotation makes more oxygen available to the cells, thereby facilitating the actions of the oxidative enzymes but not altering the rates of the non-oxidative reactions.

The metabolism of pineal indoles is influenced by the activity of the sympathetic nerves to the pineal[10]. The development of an *in vitro* system in which all the pineal enzymes involved in indole metabolism remain operative should facilitate studies on the biochemical mechanisms

Table 1. BIOSYNTHESIS OF SEROTONIN, MELATONIN AND 5HIAA FROM ^{14}C-AMINO-ACIDS BY RAT PINEAL GLANDS IN ORGAN CULTURE

Substrate	Tubes rotated	^{14}C-Serotonin	^{14}C-Melatonin	^{14}C-5HIAA	Total
^{14}C-Tryptophan	No	0.17	0.23	0.41	0.81
	Yes	0.74*	0.27	1.02*	2.03*
^{14}C-5-Hydroxytryptophan	No	0.99	0.29	0.73	2.01
	Yes	0.65	0.19	1.40*	2.24

Groups of four culture tubes each containing a single rat pineal were incubated with ^{14}C-tryptophan (10^{-4} M, 460,000 c.p.m.) or ^{14}C-5-hydroxytryptophan (2.5×10^{-4} M, 460,000 c.p.m.) for 2 days at 37° C.

* $P < 0.01$ differs from stationary group.

by which nerve impulses control pineal parenchymal function This system might also provide a relatively simple mechanism for testing the effects of drugs and hormones on the biosynthesis of serotonin and related compounds

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[1] Quay, W B , *Gen Comp Endocrinol* , 1, 3 (1963)
[2] Lovenberg, W , Jequier, E , and Sjoerdsma, A , *Science*, **155**, 217 (1967)
[3] Snyder, S H , Axelrod, J , Fischer, J E , and Wurtman, R J , *J Pharmacol Exp Ther* , **147**, 371 (1965)
[4] Shein, H , Wurtman, R J , and Axelrod, J , *Nature*, **213**, 730 (1967)
[5] Quay, W B , *Proc Soc Exp Biol and Med* , **115**, 710 (1964)
[6] Wurtman, R J , Axelrod, J , and Phillips, L S , *Science*, **142**, 1071 (1963)
Weissbach, H , Redfield, B G , and Axelrod, J , *Biochim Biophys Acta*, **43**, 352 (1960)
[5] Axelrod, J , and Weissbach, H , *J Biol Chem* , **236**, 211 (1961)
[9] Axelrod, J , MacLean, P D , Albers, R W , and Weissbach, H , in *Regional Neurochemistry* (edit by Kety, S S , and Elkes, J), 307 (Pergamon Press, Oxford, 1961)
[10] Wurtman R J , Axelrod, J , and Fischer, J E *Science*, **143**, 1325 (1964)

MICROBIOLOGY

Enzootic Rabies in Rodents in Thailand

RABIES virus has occasionally been isolated from feral rats[1] and yet rodents are not considered to be a significant reservoir of sylvatic rabies[2] These conclusions are based on negative results of surveys of small mammal species in areas in which rabies is endemic[2,3] The isolation of rabies virus from a field rat, *Bandicoota indicus*, in the course of routine diagnostic procedures prompted a survey of several small mammals in Thailand, a country where rabies is endemic When it was certain that the virus was present in a number of *Bandicoota indicus*, it then became important to determine whether overt disease occurred and if virus was being transmitted as an inapparent infection in the species The results of the survey and preliminary data on the epidemiology of rabies in *Bandicoota indicus* are the subject of this report

Rodents and other small mammals were trapped in rural farm and forest areas and around small towns in central and south-eastern provinces of Thailand between August 1 and December 31, 1966 The animals were identified, their heads were removed, frozen in dry ice and shipped to the laboratory Initially only *B indicus* and *B bengalensis* were retained for examination, but later all trapped animals were tested for rabies

The fluorescent antibody technique (FAT) as described by Goldwasser and Kissling[4] was utilized with slight modifications to make the initial diagnoses The modifications consisted of the use of a commercially prepared anti-rabies fluorescein dye conjugate, equine origin (Baltimore Biological Laboratories), and rhodamine in a 1 100 dilution as a background stain[5]

Positive control slides, interspersed among the survey samples, were prepared from weanling mice which had been inoculated with street virus FAT positive specimens were confirmed by intracranial inoculation of weanling mice Most specimens negative by FAT were discarded without further testing, but a few were inoculated along with the FAT positive specimen

Homogenates of brain tissue from all specimens giving a positive fluorescent antibody test and selected specimens giving a negative reaction (10–20 per cent brain suspension in buffered saline, pH 7 2, prepared using a Ten Broeck tissue homogenizer) were each inoculated intracranially into six weanling white mice (0 3 ml per mouse) Mice were observed daily for symptoms of rabies Animals showing terminal signs of rabies were killed and examined by the fluorescent antibody test

One isolate from each species was identified in a serum neutralization test Serial ten-fold dilutions of the isolate (2×10^{-1}–2×10^{-8}) were prepared from first passage mouse brain Dilutions of virus (0 5 ml per dilution) were then incubated with an equal volume of hyperimmune equine anti-rabies serum (Lederle) or with normal horse serum for 1 h at 37° C The serum–virus mixtures were then inoculated intracranially into weanling mice (0 03 ml per mouse) The mice were observed daily for 28 days for signs of rabies

For the susceptibility tests ten immature bandicoot rats were inoculated intramuscularly in the quadriceps region and subcutaneously in the neck region (0 05 ml per site) with a 20 per cent suspension which consisted of a composite of all the bandicoot isolates after first mouse brain passage The suspension contained $10^{6\ 6}$ weanling mouse LD_{50}/0 03 ml as computed by the Reed–Muench method[6] Inoculated animals were caged individually and observed daily for symptoms of rabies Those found dead or killed when moribund were examined by the fluorescent antibody test and mouse inoculation tests to confirm the cause of death

Rabies virus was isolated from several species of wild rodents as shown in Table 1 The percentage of positive cases was highest in *B indicus* Ten of the 126 examined (7 9 per cent) were positive both to the fluorescent antibody test and to mouse inoculation Only seven of 375 (1 9 per cent) *B bengalensis*, a closely related species, were found to be positive

All isolates were confirmed by mouse inoculation Mice injected intracranially with suspensions from brains which gave a positive fluorescent antibody test usually began to show symptoms of rabies 10–14 days after inoculation Symptoms and death rarely occurred between 5 and 10 days One hundred and fifteen specimens negative to the test were confirmed by mouse inoculation (M I) Several negative specimens were included with each group of positives There was 100 per cent correlation between the fluorescent antibody test and mouse inoculations Hyperimmune equine anti-rabies serum had a log neutralization index of 3 0 or greater when tested against isolates from each species

Table 2 shows the results of susceptibility tests Five of the ten rats injected parenterally with the homologous virus isolate died, and four showed typical symptoms of rabies There was an average incubation period of 20 6 days and a range of 14–33 days One apparently normal rat was killed 78 days after inoculation and virus was

Table 1 RODENT AND SMALL MAMMAL SURVEY FOR RABIES

Species	No examined	No positive	% positive
Bandicoota indicus	126	10	7 9
Suncus murinus	30	2	6 7
Rattus rajah	16	1	6 3
Rattus norwegicus	192	9	4 7
Rattus rattus	58	2	3 5
Rattus exulans	236	6	2 5
Bandicoota bengalensis	375	7	1 9
Mongoose	2	0	0 0
Rattus berdemorei	2	0	0 0
Totals	1,037	37	3 6

Table 2 INFECTIVITY OF RABIES VIRUS FOR *Bandicoota indicus*

Incubation period (days)	Symptoms	Duration of illness (days)	Fluorescent antibody test results	Mouse inoculation results
14	Lethargy, paralysis	2	Positive	Positive
16	Lethargy, paralysis	5	Positive	Positive
19	None noted	0	Positive	Positive
21	Lethargy, paralysis	2	Positive	Positive
33	Ruffled hair, lethargy, paralysis	3	Positive	Positive
78*	None noted	0	Positive	Positive
113*	None noted	0	Negative	Negative
212*	None noted	0	Negative	Negative
212*	None noted	0	Negative	Negative
212*	None noted	0	Negative	Negative

The parenteral route of inoculation was used
* Animal was killed

isolated from both brain and salivary gland One rat killed after 113 days and three after 212 days after inoculation were negative to both FAT and M I

The well known century cyclic nature of rabies raises the important question Why does the incidence increase dramatically in endemic areas ? WHO experts suggest that the answer must lie in the wildlife reservoir The information reported here and the studies by Svet-Moldavskaya[7] indicate that rabies virus infections may be chronic or inapparent in some species of rats The fact that one apparently healthy rat was shown to have rabies virus in the brain and salivary glands 78 days after inoculation suggests that inapparent infections can occur naturally

The high percentage of rabies positive specimens in *B indicus* is surprising The possibility that the occurrence of rabies in the rodent population results from exposure to stray dogs and cats in an area where the disease incidence is high cannot be dismissed Another possible means of introduction of the disease into the rat population might be from fruit and insectivorous bats in the area Their close proximity in natural habitats such as temples, caves and heavily forested areas lends credence to this suggestion The incidence of rabies in some species of bats in certain areas of Thailand has been shown to be as high as 3 per cent[8] The oral route in infected tissue or food must also be considered according to a recent report by Soave[9]

Rabies has been diagnosed in six different species of rodents in Thailand Approximately 8 per cent of *Bandicoota indicus* tested were positive, but the percentage was much lower in other species of wild rodents Thirty-seven of 1,037 specimens of rodents or other small mammals were examined and found to be positive A preliminary experiment revealed that one apparently healthy *B indicus* killed 78 days after inoculation with a massive dose of rabies virus had virus in brain and salivary tissue The evidence presented here suggests that the bandicoot rat may be an inapparent carrier of rabies virus infections Rabies virus was also isolated from two of thirty common house shrews, *Suncus murinus*

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[1] Hull, T G , in *Diseases Transmitted from Animals to Man* (edit by Hull, T G), 903 (Chase Thomas, Springfield, Illinois, 1963)

Tierkel, E S , in *Diseases Transmitted from Animals to Man* (edit by Hull, T G), 323 (Chase Thomas Springfield, Illinois, 1963)

[3] Dimson, A , and Leach, N , *Amer J Pub Health*, **30**, 267 (1940)

[4] Goldwasser, R A , and Kissling, R E , *Proc Soc Exp Biol and Med* , **18**, 219 (1958)

[5] Dean D J , in *Laboratory Techniques in Rabies*, second ed (edit by WHO Com on Rabies), 67 (WHO, Geneva, Switzerland, 1966)

[6] Reed L J , and Muench, H , *Amer J Hyg* , **27**, 493 (1938)

[7] Svet-Moldavskaya, I A , *Acta Virol* , **2**, 228 (1958)

[8] Smith, P C , Lawhaswasdi, K , Vick, W , and Stanton, J S , *Nature*, **216**, 384 (1967)

[9] Soave, A *Amer J Vet Res* , **27** 44 (1966)

Genetics and Cultural Properties of Mutants of *Salmonella typhimurium* lacking Glucosyl or Galactosyl Lipopolysaccharide Transferases

THE somatic (0) lipopolysaccharide of smooth salmonella strains contains an inner part or core, believed to be the same in all species, this consists of a heptose-containing "backbone", with short side-chains which contain glucose, galactose and *N*-acetylglucosamine and are thought to have the structure shown in Fig 1 (ref 1) The outer, O-specific part of lipopolysaccharide (absent in rough strains) is made up of many repeat units of an oligosaccharide the composition of which determines the O antigens of the strain These O-specific chains are probably attached to the distal ends of the core side-chains This and the following communication describe mutants in glycosyl-transferring enzymes concerned in the biosynthesis of the side-chains of the core

Mutants lacking the enzyme UDP-galactose-4-epimerase are unable (in the absence of exogenous galactose) to syn-

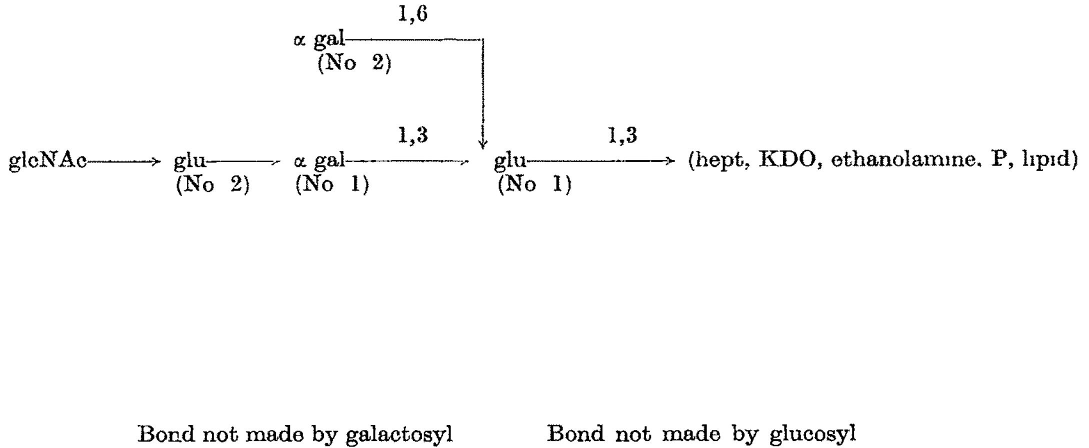
1,6
α gal
(No 2)
1,3
1,3
glcNAc
glu
(No 2)
α gal
(No 1)
glu
(No 1)
(hept, KDO, ethanolamine, P, lipid)
Bond not made by galactosyl transferase I (*rfaH*) mutants
Bond not made by glucosyl transferase I (*rfaG*) mutants

Fig 1 Structure of side-chain of *Salmonella* lipopolysaccharide core glu, Glucose, gal, galactose, hept, heptose, KDO, 3-deoxy-D-manno-octulosonic acid, glcNAc, *N*-acetylglucosamine, P, phosphate

Table 1 PHAGE RESISTANCE PATTERNS OF TRANSFERASE-DEFICIENT AND OTHER DERIVATIVES OF *Salmonella typhimurium* STRAIN *LT*2

Strain	Action of phage* *P*22 and 9*NA*	Felix O	6*SR* and φ*X*-174	*C*21	*Br*60 *Ffm* and *P*221	*Br*2
Smooth[a]	+	+	−	−	−	−
rfb[b] (=*rouB*)	−	+	+	−	+	+
Galactose epimerase[c]	−	−	−	+	+	+
Glucosyl transferase[d]	−	−	−	+	+	−
Galactosyl transferase[e]	−	− or ±	−	+	+	+

[a] Smooth parent strain *SL*1027, and its *pmi* derivatives *SL*1028 and *SL*1030 (tested on mannose-supplemented medium)

[b] Pattern of rough parent strains *TV*119 (*rfb*-430) and *TV*149 (*rfb*-433)

[c] Pattern of various mutants lacking UDP-4-galactose epimerase

[d] Pattern of *SL*1004 (*rfa*-571), *SL*1005 (*rfa*-572) and *SL*1063 (*rfa*-654), isolated from *TV*119 (*rfb*), *SL*1006 (*rfa*-653), from *TV*149 (*rfb*), *SL*1059 (*rfa*-494), from *SL*1028 (*pmi*), *SL*1061 (*rfa*-488) and *SL*1062 (*rfa*-489), from *SL*1030 (*pmi*), and *SL*1032 (*rfa*-471), from *SL*1027 (wild-type)

[e] Pattern of *SL*1033 (*rfa*-481) and *SL*1060 (*rfa*-487), both derived from *SL*1030 (*pmi*) *SL*1060 was resistant, *SL*1033 partly sensitive, to Felix O phage

* Effect of phage (10^8 plaque-forming U/ml) on surface inoculated nutrient agar (mannose-supplemented, in the case of *pmi* strains)

thesize UDP-galactose, the donor of the galactose components of lipopolysaccharide, and so they make a polysaccharide with incomplete core side-chains, lacking the galactose units and all the sugars which normally lie distal to them[2,3] When UDP-galactose is added to a broken-cell preparation of such a mutant, galactose is transferred to the lipopolysaccharide[4,5] We thought that mutants lacking the enzyme or enzymes which effect this transfer would make lipopolysaccharide similar to that of the galactose-epimerase-negative mutants Such transferase-negative mutants would, however, retain the wild-type ability to ferment galactose, which ability, because it involves the epimerization of UDP-galactose to UDP-glucose, is absent in epimerase-negative mutants We found that galactose-epimerase-negative mutants of *S typhimurium* strain *LT*2 had a distinctive pattern of resistance and susceptibility to a battery of smooth-specific and rough-specific phages—which, presumably, utilize lipopolysaccharide as their site of adsorption to the bacteria (Table 1) Unlike other non-smooth mutants, including *rouA* (=*rfa*) and *rouB* (=*rfb*) mutants[6] and semi-rough mutants of classes *C* and *D* (ref 7), they were sensitive to phage *C*21, which in *Escherichia coli* strain *K*12 attacks only mutants unable to synthesize UDP-galactose or UDP-glucose[8] We therefore looked for *LT*2 mutants which were sensitive to phage *C*21 but able to ferment galactose, in the expectation that they would be galactosyl-transferase-negative In some experiments we used as parent strains *TV*119 and *TV*149, *LT*2 mutants of the type *rouB* (=*rfb*) which cannot synthesize the O-specific distal part of the side-chain and are sensitive to Felix anti-O phage[9] and to the rough-specific phage 6*SR* (ref 10), neither of which attacks galactose-epimerase mutants Among spontaneous mutants selected for resistance to a mixture of these two phages, we encountered four which were *C*21-sensitive but galactose-fermenting We also attempted to isolate mutants of this sort from a genetically marked *LT*2 smooth line, *SL*1027, of genotype *metA trpB H*1-*b H*2-*e,n,x fla str xyl*, and from two phosphomannose-isomerase-negative (*pmi*) mutants of it Mutants of the latter class make incomplete lipopolysaccharides unless grown with exogenous mannose[11], the *pmi* mutants were grown on mannose-supplemented medium The phenotypically smooth cultures were exposed to a mixture of virulent phages comprising *P*22*c*$_2$ (ref 12), which attacks only smooth cultures, Felix O phage and, in some experiments, the rough-specific phage 6*SR* Six *C*21-sensitive galactose-fermenting mutants were thus obtained The cultural and phage-resistance properties of these presumed galactosyl-transferase-negative mutants were tested on mannose-supplemented media in the case of strains lacking phosphomannose isomerase All ten strains had some of the cultural properties of rough mutants they grew in broth as a deposit, leaving a clear supernatant fluid and agglutinated in 0 9 per cent, but not 0 2 per cent, sodium chloride Such suspensions were not agglutinated by undiluted anti-04 antiserum in slide tests Their pattern of susceptibility to phages divided the mutants into two classes (Table 1) All resembled galactose-epimerase mutants in that they were resistant to the smooth-specific phage *P*22 and to 9*NA* (a smooth-specific phage serologically and otherwise unrelated to *P*22, personal communication from P Gemski), resistant to the rough-specific phages 6*SR* and φ*X*-174, but sensitive to *C*21 and to the rough-specific phages *P*221 (ref 13) *Ffm* (ref 14) and *Br*60 (phage 60 of Brandis[15]) Eight mutants were completely resistant to Felix O phage and to the rough-specific phage *Br*2 (phage 2 of Brandis[15]) which attacks epimerase mutants The other two mutants, *SL*1033 and *SL*1060, were sensitive to *Br*2, *SL*1060 was completely resistant, *SL*1030 partly resistant, to Felix O phage

Biochemical examination of the ten mutants by Dr M J Osborn (see following communication) showed that the two mutants sensitive to phage *Br*2 as expected lacked a galactosyl transferase, but that the eight mutants resistant to phage *Br*2 had normal galactosyl transferase activity, they instead lacked the enzyme which transfers to glucose-deficient lipopolysaccharide the glucose unit which lies proximal to the galactose units of the core side-chains The sugar composition of the lipopolysaccharide from the two classes of mutant and their abilities to accept glucose or galactose in the presence of extracts of wild-type cells confirmed these conclusions

To map the mutated loci of the glucosyl and galactosyl transferase mutants, we mated some of them with smooth Hfr sublines of *LT*2 (ref 16) Recombinants from various selections after purification were scored for all segregating characters, their glycosyl transferase character being inferred from their sensitivity to appropriate phages, phage-pattern always segregated as though determined by a single locus The glucosyl transferase mutant *SL*1032 was first mated to *LT*2 Hfr*A* (ref 17) which injects its chromosome clockwise, with *ile* as the leading locus (Fig 2) None of the seventy recombinants selected for possession of the donor marker *trpB*$^+$ had the smooth character of the Hfr parent We infer that the glucosyl transferase locus does not lie between the point of origin of this Hfr and the last donor locus represented, *his* We then used Hfr*B*2 (ref 16) which injects clockwise from a

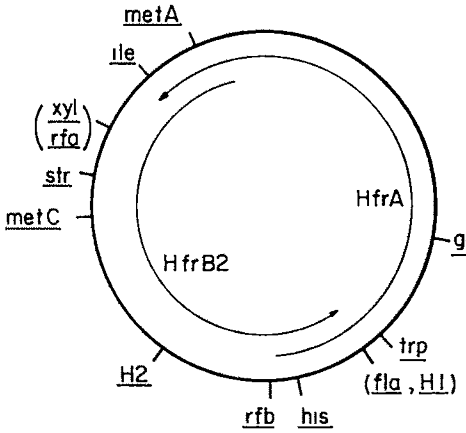

Fig 2 Linkage map of *S typhimurium* (distances approximate) and Hfr donors used, with their points of entry and approximate extents of chromosome transfer (loci bracketed, order unknown) *met*, *trp*, *his*, *ile*, Requirement for methionine, tryptophan, histidine, isoleucine, *gal*, *xyl*, fermentation of galactose, xylose, *fla*, flagellation, *H*1, *H*2, phase-1 and phase-2 flagellar antigens, *str*, streptomycin-resistance, *rfb* (=*rouB* of ref 6), defect in biosynthesis of O-specific part of lipopolysaccharide, *rfa* (=*rouA* of ref 6, and *rfaG* and *rfaH* of this communication), defect in synthesis of lipopolysaccharide core

point between *trp* and *fla* In the cross Hfr*B*2→*SL*1032 more than 85 per cent of recombinants selected for acquisition of the donor *metA*$^+$ and more than 85 per cent of those selected for possession of the donor *xyl*$^+$ had the smooth character of the donor, and were completely resistant to phage *C*21 and the other rough-specific phages active on *SL*1032 Analysis of the number of crossovers required to account for the observed recombinant classes indicated that the transferase locus mapped between *str* and *metA* Taken together with the negative result of the cross with Hfr*A*, the data show that the transferase locus of *SL*1032 is closely linked to *xyl* and lies between *str* and the point of origin of Hfr*A* (Fig 2) but do not show on which side of *xyl* it is Two further glucosyl transferase mutants, *SL*1061 and *SL*1062, and the two galactosyl transferase mutants, *SL*1033 and *SL*1060, were similarly crossed to Hfr*B*2 In all four crosses 89 per cent or more of recombinants selected for possession of the *xyl*$^+$ and *metA*$^+$ loci of the donor had also its transferase-positive character, as indicated by their phage resistance pattern This suggests that in these four mutants also the site of mutation is close to *xyl* Several *LT*2 rough mutants inferred to be able to make O-specific material but unable to attach it to lipopolysaccharide core (because this material was absent in lipopolysaccharide but accumulated in a non-sedimentable fraction) were mapped in the *str-ile-metA* region by Subbaiah and Stocker[6] and were named *rouA* mutants Probably the genes affected in their *rouA* mutants and the glucosyl and galactosyl transferase genes now reported all form part of a cluster of genes concerned with the synthesis of the lipopolysaccharide core Because there are several such genes, and to accord with the principles of the suggested uniform nomenclature for bacterial genetics[18], we now propose to call mutants with defects in such functions *rfa*, instead of *rouA*, and we assign the symbol *rfaG* to the gene for the transferase which adds the proximal glucose unit (glucose No 1) to lipopolysaccharide, and the symbol *rfaH* to the gene for the transferase which adds a galactose unit (galactose No 1) to the 3 position of glucose No 1 of lipopolysaccharide

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[1] Luderitz, O , Staub, A M , and Westphal, O , *Bact Rev* , **30**, 192 (1966)
[2] Fukasawa, T , and Nikaido, H , *Genetics*, **46**, 1295 (1961)
[3] Nikaido, H , *Proc US Nat Acad Sci* , **48**, 1337 (1962)
[4] Nikaido, H , *Proc US Nat Acad Sci* , **48**, 1542 (1962)
[5] Osborn, M J , Rosen, S M , Rothfield, L , and Horecker, B L , *Proc US Nat Acad Sci* , **48**, 1831 (1962)
[6] Subbaiah, T V , and Stocker, B A D , *Nature*, **201**, 1298 (1964)
[7] Naide, Y , Nikaido, H , Makela, P H , Wilkinson, R G , and Stocker, B A D , *Proc US Nat Acad Sci* , **53**, 147 (1965)
[8] Shedlovsky, A , and Brenner, S , *Proc US Nat Acad Sci* , **50**, 300 (1963)
[9] Felix, A , and Callow, B R , *Brit Med J* , ii, 127 (1943)
[10] Kay, D , *J Gen Microbiol* , **27**, 201 (1962)
[11] Rosen, S M , Zeleznick, L D , Fraenkel, D , Wiener, I M , Osborn, M J , and Horecker, B L , *Biochem Z* , **342**, 375 (1965)
[12] Levine, M , *Virology*, **3**, 22 (1957)
[13] Yamamoto, N , and Anderson, T F , *Virology*, **14**, 430 (1961)
[14] Brandis, H , *Z Bakt Parasitkde*, I (Orig), **165**, 305 (1956)
[15] Brandis, H , *Z Bakt Parasitkde*, I (Orig), **199**, 185 (1966)
[16] Sanderson, K E , and Demerec, M , *Genetics*, **51**, 896 (1965)
[17] Zinder, N D , *Science*, **131**, 924 (1960)
[18] Demerec, M Adelberg, E A , Clark, A J , and Hartman, P E , *Genetics*, **54**, 61 (1966)

Biochemical Characterization of Mutants of *Salmonella typhimurium* lacking Glucosyl or Galactosyl Lipopolysaccharide Transferases

Previous work[1–5] on the mechanism of biosynthesis of the somatic (0) lipopolysaccharide of *Salmonella typhimurium* has identified four enzymes involved in synthesis of the core region of the polysaccharide*

$$\text{UDP-Glucose} + \text{Glu-deficient LPS} \xrightarrow{\text{glucosyl transferase I}} \text{Glu-LPS} \qquad (1)$$

$$\text{UDP-Galactose} + \text{Glu-LPS} \xrightarrow{\text{Galactosyl transferase I}} \alpha\text{-Gal-1,3-Glu-LPS} \qquad (2)$$

$$\text{UDP-Glucose} + \alpha\text{-Gal-1,3-Glu-LPS} \xrightarrow{\text{Glucosyl transferase II}} \text{Glu-}\alpha\text{-Gal-1,3-Glu-LPS} \qquad (3)$$

$$\text{UDP-}N\text{-Acetylglucosamine} + \text{Glu-}\alpha\text{-Gal-1,3-Glu-LPS} \xrightarrow{N\text{-Acetylglucosaminyl transferase}} \text{GlcNAc-Glu-}\alpha\text{-Gal-1,3-Glu-LPS} \qquad (4)$$

The tetrasaccharide sequence so formed makes up the distal portion of the core region (Fig 1, preceding communication) and is linked through the proximal glucosyl unit to the heptose-containing polysaccharide backbone It should be noted that the complete core structure contains a second galactosyl residue linked as an α-1,6 branch to the proximal glucose unit[6] The enzyme (galactosyl transferase II) responsible for addition of this galactose to the chain has not yet been demonstrated in a cell-free system A lipopolysaccharide (glu-deficient LPS) which is able to act as acceptor of glucosyl residues in reaction (1) was obtained from a mutant strain lacking phosphoglucose isomerase (*SL*797) (ref 7) This organism is deficient in biosynthesis of UDP-glucose and is therefore unable to carry out reaction (1) *in vivo* As a result, an incomplete lipopolysaccharide is formed, the polysaccharide components of which contain only the constituents of the backbone (heptose, KDO, ethanolamine and phosphate) Similarly, mutants which lack UDP-galactose-4-epimerase and fail to synthesize UDP-galactose are unable to carry out reaction (2) and subsequent steps Such organisms produce an incomplete lipopolysaccharide which terminates at the first glucosyl residue of the core (compare Fig 1, preceding communication), and serves as acceptor of galactosyl residues in the isolated galactosyl transferase I system[1–3]

In mutants deficient in biosynthesis of a nucleotide sugar, the defect in lipopolysaccharide synthesis is a secondary phenomenon resulting from a lack of the required substrate, rather than from absence of the glycosyl transferase enzyme The present report is concerned with biochemical characterization of two classes of mutants lacking glucosyl transferase I and galactosyl transferase I, respectively These mutants were isolated by Wilkinson and Stocker (see previous communication) as galactose-positive strains sensitive to phage *C*21. Of ten isolates examined, eight have been found to lack glucosyl transferase I activity (reaction (1)), while two contain normal concentrations of this enzyme but are deficient in galactosyl transferase I (reaction (2))

The mutants could be divided into two classes on the basis of lipopolysaccharide composition Lipopolysaccharide was isolated from each of the mutants by extraction with aqueous phenol, and purified by precipitation with magnesium chloride[1] The sugar composition was examined qualitatively by acid hydrolysis and paper chromatography In eight strains (*SL*1004, *SL*1005, *SL*1006, *SL*1032, *SL*1059, *SL*1061, *SL*1062 and *SL*1063) heptose, KDO and glucosamine (from lipid *A*) were the only sugars observed Neither glucose nor galactose could be detected, and O-specific sugars (mannose, rhamnose and abequose) were also absent These results were confirmed by quantitative analysis of lipid-free polysaccharide preparations obtained from three of these strains (Table 1) The polysaccharides contained normal amounts of heptose and KDO, but no detectable glucose, galactose or glucosamine The composition of these lipopolysaccharides was very similar to that of the mutant unable to synthesize UDP-glucose[7] In contrast, both glucose and galactose were present in the polysaccharides isolated from *SL*1033 and *SL*1060 (Table 1) however, the molar ratio of these sugars relative to heptose was approximately 50 per cent of that observed in the polysaccharide of *TV*119, an *rfb* (=*rouB*) mutant which forms the complete core structure

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* LPS, Lipopolysaccharide, glu, glucose, gal, galactose, GlcNAc, *N*-acetylglucosamine, KDO, 3 deoxy-D-manno octulosonic acid

Table 1 CARBOHYDRATE COMPOSITION OF THE POLYSACCHARIDES OF GAL$^+$ C21^s MUTANTS

Mutant strain	Heptose	KDO	Molar ratio Glucose	Galactose	Glucosamine
*SL*1004	1 0	0 42	0 04	0 02	0 02
*SL*1005	1 0	0 51	0 02	0 02	0 02
*SL*1032	1 0	0 56	0 02	0 02	0 02
*SL*1033	1 0	0 54	0 56	0 59	0 05
*SL*1060	1 0	0 44	0 50	0 66	0 08
rfb (*TV*119)	1 0	0 53	1 12	0 89	0 47

Lipid-free polysaccharides were prepared by mild acid hydrolysis of lipopolysaccharide[15] KDO was determined by the semicarbazide method[16], and heptose by a previously described modification[15] of the cysteine-sulphuric acid reaction[17] Analyses for glucose, galactose and glucosamine were performed after acid hydrolysis of the polysaccharide (1 *N* hydrochloric acid, 3 h, 100° C) Glucose and galactose were determined with glucose and galactose oxidase, and hexosamine by the method of Reissig *et al* [18]

Table 2 ACCEPTOR ACTIVITIES OF MUTANT LIPOPOLYSACCHARIDES IN THE SOLUBLE GLYCOSYL TRANSFERASE SYSTEMS

Source of lipopolysaccharides	Acceptor activity (mμmoles of sugar incorporated) Glucosyl transferase I	Galactosyl transferase I
*SL*1004	1 00	0 08
*SL*1005	1 62	0 04
*SL*1006	1 19	0 05
*SL*1032	1 36	0 01
*SL*1059	1 24	0 17
*SL*1061	0 96	0 05
*SL*1062	0 89	0 12
*SL*1063	0 74	0 01
*SL*1033	0 09	1 82
*SL*1060	0 21	2 40
EI-1 (*pgi*$^-$, *gal-epi*$^-$)	0 72–1 54	0 07–0 19
G-30 (*gal-epi*$^-$)	0 06–0 18	1 54–2 17

Purified lipopolysaccharides were employed as acceptors in the standard assay system of Rothfield and Horecker[6] Comparable amounts of acceptor (8–11 mμmoles, as heptose) were employed in each case Standard acceptors for glucose (strain *EI*-1) and galactose (strain *G*-30) were included in each experiment, and the figures given show the range of values observed with these lipopolysaccharides *EI*-1 is a double mutant lacking both phosphoglucose isomerase and UDP-galactose-4-epimerase[4,8] The enzyme fraction consisted of the 105 000*g* supernatant fraction obtained from strain *EI*-1, this preparation contains both glucosyl and galactosyl transferase activity Incubations were continued for 60 min in order to give maximal levels of incorporation of both sugars

Table 3 SOLUBLE TRANSFERASE ACTIVITIES

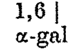
Enzyme source	Specific activity (mμmoles/mg/30 min) Glucosyl transferase I	Galactosyl transferase I
*SL*1004	0 05	4 16
*SL*1005	0 04	2 74
*SL*1006	0 09	3 12
*SL*1032	0 02	2 30
*SL*1059	0 12	2 94
*SL*1061	0 11	2 62
*SL*1062	0 07	2 53
*SL*1063	0 03	2 42
*SL*1033	1 63	0 42
*SL*1060	1 24	0 30
EI-1	1 53–2 54	2 16–4 73

The 105,000*g* supernatant fraction was prepared from the indicated strains by the method of Rothfield and Horecker[8] Strain *EI*-1 is a mutant of *S typhimurium* lacking both phosphoglucose isomerase and UDP-galactose-4-epimerase, and has been used in this laboratory as a standard source of both soluble glycosyl transferase enzymes The enzyme fractions were assayed as described by Rothfield and Horecker[8] Glucose-deficient lipopolysaccharide employed as acceptor in the glucosyl transferase assay was isolated from *EI*-1 The galactose-deficient lipopolysaccharide of the UDP-galactose-4-epimerase mutant, *G*-30, was employed as acceptor in the galactosyl transferase assay

The ability of the purified mutant lipopolysaccharides to act as *in vitro* acceptors of glucosyl and galactosyl residues in reactions (1) and (2) was tested using the soluble glucosyl transferase I and galactosyl transferase I systems described before[8–10] The results are summarized in Table 2 As expected, those lipopolysaccharides which lacked glucose were active as acceptors in the glucosyl transferase I reaction, and their activity was equal to that of the chemically similar lipopolysaccharide produced by a phosphoglucose isomeraseless mutant which is unable to synthesize UDP-glucose The lipopolysaccharides of this group did not accept galactose, presumably because they lacked the glucosyl residues which are necessary for attachment of galactose The lipopolysaccharides of *SL*1033 and *SL*1060 showed the opposite pattern of acceptor activity. In spite of the fact that these lipopolysaccharides already contained 50 per cent of the normal amount of galactose, they were highly active as acceptors in the galactosyl transferase I system Both the rate of galactose incorporation and the total amount incorporated were indistinguishable from that obtained with the lipopolysaccharide of a UDP-galactose-4-epimeraseless mutant (*G*-30), which is completely devoid of galactose The lipopolysaccharides of *rfa* (=*rouA*) and *rfb* mutants, which contain the normal complement of galactose, did not accept galactose in the soluble transferase system The composition of the lipopolysaccharides of *SL*1033 and *SL*1060, together with their acceptor activities in the galactosyl transferase reaction, suggested that those strains might lack galactosyl transferase I (which catalyses addition of galactose to the 3-position of glucose), but retain a second, independent activity specifically transferring galactose to the 6-position of glucose (galactosyl transferase II)

Determination of soluble transferase activities in extracts of the mutant strains confirmed that the two classes were deficient in glucosyl transferase I and galactosyl transferase I, respectively (Table 3) No significant glucosyl transferase activity was detected in the 105,000*g* supernatant fractions obtained from the eight mutants the lipopolysaccharide of which lacked glucose Essentially normal concentrations of soluble galactosyl transferase activity were found in these organisms By contrast, the glucosyl transferase I activities of *SL*1033 and *SL*1060 were within the normal range, but the soluble galactosyl transferase activity of these strains was only 10–15 per cent of the normal level The available evidence indicates that the galactosyl transferase activity observed in cell-free systems results in addition of galactose predominantly if not exclusively, to the 3-position of glucose, and it appears to be this enzyme activity which is lacking in *SL*1033 and *SL*1060 It is not yet clear whether the low residual levels of galactosyl transferase activity observed in extracts of these strains represent the α-6-galactosyl transferase (transferase II) or reflect leakiness in the transferase I defects There is evidence that *in vivo* the block in lipopolysaccharide biosynthesis is not absolute, which suggests that in both strains the defects are somewhat leaky

Mutants of *SL*1033 and *SL*1060 lacking galactosyl transferase I would be expected to produce an incomplete polysaccharide of the type



$$\begin{array}{l}\text{glu—[hep, KDO, PO}_4\text{, ethanolamine]}\\ 1{,}6\ |\\ \alpha\text{-gal}\end{array}$$

Evidence for this structure was obtained by characterization of the oligosaccharide products of partial acid hydrolysis of the polysaccharides Lipid-free polysaccharide fractions derived from *SL*1033 and *SL*1060 were hydrolysed as previously described[11] with 60 per cent formic acid (45 min at 100° C), and the products were separated by preparative paper chromatography (Whatman No 3 *MM* paper, descending chromatography in ethyl acetate acetic acid H_2O (3 1 3) for 96 h) As a control, parallel experiments were also carried out with the polysaccharide of an *rfa* mutant (*TV*161), which contains both galactosyl residues The hydrolysates of the *SL*1033 and *SL*1060 polysaccharides showed a single major oligosaccharide product with an R_F identical to that of melibiose (α-galactosyl-1,6-glucose) This material was also present as a major component in the hydrolysate from the *rfa* strain The disaccharides from the three polysaccharides were indistinguishable from each other, and from melibiose, in chromatography in four solvent systems, and in borate electrophoresis The reduced disaccharides also had the same electrophoretic mobility as melibitol in sodium molybdate buffer, *p*H 5 Analytical data confirmed the identification as α-galactosyl-1 6-glucose (*a*) each oligosaccharide contained equimolar amounts of glucose and galactose (as determined by enzyme assay with glucose and galactose oxidase after acid hydrolysis), (*b*) reduction of the oligosaccharides with $NaBH_4$ led to destruction of more than 90 per cent of the glucose, with quantitative recovery of galactose, (*c*) treatment with coffee bean α-galactosidase resulted in quantitative hydrolysis of the oligosaccharide with liberation of corresponding amounts of free glucose and galactose, (*d*) periodate oxidation of the reduced disaccharides gave 0 93 to 1 17 moles of formaldehyde/mole of disaccharide

No fraction corresponding to the other possible galactose disaccharide, α-galactosyl-1,3-glucose, was detected in the partial acid hydrolysates of any of these strains This galactosyl linkage has previously been found to be extremely acid-labile[3] The hydrolysate of the *rfa* polysaccharide, however, contained a minor component in the trisaccharide area which was tentatively identified as the branched trisaccharide

α-gal-1,3-glu
1,6 |
α-gal

This component was entirely absent from the hydrolysates of *SL*1033 and *SL*1060 Although not conclusive, the results provide some additional support for the hypothesis that the α-galactosyl-1,3-glucose linkage is lacking in these mutants

The glucosyl and galactosyl transferaseless mutants, like *rfa* strains, form incomplete core polysaccharides which presumably lack the sites required for attachment of the O-specific side chains Beckmann and her colleagues[12] have shown that, although the lipopolysaccharides of *rfa* mutants are devoid of O-antigen, these strains produce a separate polysaccharide chemically and immunologically similar to the O-specific side chains of the lipopolysaccharide (O-specific hapten) The hapten was not found in *rfb* mutants, in which the lesion directly affects synthesis of the O-antigen chain Examination of the mutants lacking glucosyl or galactosyl transferase for the presence of O-specific hapten is summarized in Table 4 The hapten-containing fraction was obtained from the aqueous phenol extract after removal of lipopolysaccharide by magnesium chloride precipitation, as described in the legend The presence of the O-specific sugars (mannose, rhamnose and abequose) in this fraction was taken as the criterion for the presence of hapten As expected, no hapten was detected in transferase-negative strains derived from an *rfb* parent. Large amounts of this material, however, were found in *SL*1032, a glucosyl-transferase-negative strain derived directly from the wild type The lipopolysaccharide of *SL*1032 was entirely free of O-specific sugars The two galactosyl transferase mutants, *SL*1033 and *SL*1060, and three of the strains lacking glucosyl transferase are double mutants which also lack phosphomannose isomerase Except when provided with an external supply of mannose, these organisms are unable to synthesize GDP-mannose, a required precursor of O-antigen mannose[11] When the five strains were grown on mannose-supplemented medium, O-specific hapten was recovered in large amounts Chromatographic analysis of the lipopolysaccharide fractions obtained from mannose-grown cells also showed in each case the presence of small amounts of the O-specific sugars This suggested that the transferase defects in these strains might be somewhat leaky In order to obtain more quantitative information, strains *SL*1060 (galactosyl-transferase-deficient) and *SL*1061 and *SL* 1062 (glucosyl-transferase-deficient) were grown in the presence of ^{14}C-mannose, and the relative amounts of radioactivity in lipopolysaccharide and the haptenic fraction determined After phenol extraction of the cell envelopes, the total polymer fraction (that is, lipopolysaccharide plus hapten) was isolated by gel filtration on 'Sephadex *G*-25', and hapten and lipopolysaccharide were separated by filtration on 'Sephadex *G*-200' Lipopolysaccharide is excluded from the gel, while the haptenic material is recovered in the partially included fraction Ultracentrifugation and chromatography on DEAE cellulose confirmed identification of the two fractions The amount of ^{14}C-mannose recovered in the lipopolysaccharide fraction, expressed as percentage of total radioactivity incorporated into polymeric form, was 22 per cent in *SL*1060, 19 per cent in *SL*1061 and 12 per cent in *SL*1062 In parallel experiments carried out with a phosphomannose isomeraseless mutant (M_2) (ref 11) with normal core transferase function, more than 98 per cent of the total ^{14}C-mannose incorporated into polymeric form was recovered as lipopolysaccharide, and only trace amounts in hapten The total incorporation of ^{14}C-mannose into O-antigen, however, was seven to ten times greater in M_2 than in the mutants deficient in glucosyl or galactosyl transferase Thus, the lipopolysaccharide of the transferase-deficient strains actually contained only 1–2 per cent of the normal amount of mannose

Table 4 PRESENCE OF HAPTENIC O-ANTIGEN

Genotype	Strain	Hapten
rfa	*TV*161	+
rfb	*TV*119	0
rfb, Glu transferase neg	*SL*1004, *SL*1005, *SL*1006, *SL*1063	0
Glu transferase neg	*SL*1032	+
pmi⁻, Glu transferase neg	*SL*1059, *SL*1061, *SL*1062	
	Minus mannose	0
	Plus mannose	+
pmi⁻, Gal transferase neg	*SL*1033, *SL*1060	
	Minus mannose	0
	Plus mannose	+

Cell envelope fractions prepared from 10 g wet weight of cells were extracted with 45 per cent phenol at 70° C (ref 1) Lipopolysaccharide was precipitated from the aqueous phase with magnesium chloride (ref 1) The supernatant fractions were concentrated and the bulk of the RNA was removed by precipitation at *p*H 3 0 The supernatant material was deionized and low molecular weight material was removed by passage through 'Sephadex *G*-25' Samples of the excluded fractions were hydrolysed in 0 1 N hydrochloric acid for 15 min at 100° C for detection of abequose, and in 1 N hydrochloric acid for 3 h at 100° C for detection of other sugars The sugar composition was determined by paper chromatography in *n*-butanol pyridine water (6 4 3)

The results indicate that formation of the O-specific hapten is a general property of mutants which are blocked in biosynthesis of the core region of the lipopolysaccharide The incomplete lipopolysaccharides made by such mutants therefore presumably lack a structure required for attachment of the O-specific chains Studies on the mechanism of biosynthesis of O-antigen (refs 13 and 14 and unpublished work of Osborn and Kent) have provided some support for the hypothesis that the hapten represents a precursor of the O-specific side chains of lipopolysaccharide, but have not entirely excluded the possibility that this material is formed as a side product only in conditions in which the normal biosynthetic sequence is disturbed

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[1] Osborn, M J , Rosen, S M , Rothfield, L , and Horecker, B L *Proc US Nat Acad Sci* , **48**, 1831 (1962)

[2] Nikaido, H , *Proc US Nat Acad Sci* , **48**, 1337 (1962)

[3] Rosen, S M , Osborn, M J , and Horecker, B L , *J Biol Chem* , **239**, 3196 (1964)

[4] Rothfield, L , Osborn, M J , and Horecker, B L , *J Biol Chem* , **239**, 2788 (1964)

[5] Osborn, M J , and D'Ari L , *Biochem Biophys Res Commun* , **16**, 568 (1964)

[6] Sutherland, I W , Luderitz, O , and Westphal, O , *Biochem J* , **96**, 439 (1965)

[7] Fraenkel, D , Osborn, M J , Horecker, B L , and Smith, S M , *Biochem Biophys Res Commun* , **11**, 423 (1963)

[8] Rothfield, L , and Horecker, B L , *Proc US Nat Acad Sci* , **52**, 939 (1964)

[9] Rothfield, L , and Takeshita, M , *Biochem Biophys Res Commun* , **20**, 521 (1965)

[10] Rothfield, L , and Pearlman, M , *J Biol Chem* , **241**, 1386 (1966)

[11] Zeleznick L D , Rosen, S M , Saltmarsh-Andrew, M , Osborn, M J , and Horecker, B L , *Proc US Nat Acad Sci* , **53**, 207 (1965)

[12] Beckmann, I , Subbaiah, T V , and Stocker, B A D , *Nature*, **201**, 1299 (1964)

[13] Weiner, I M , Higuchi, T , Rothfield, L Saltmarsh-Andrew, M , Osborn, M J , and Horecker, B L , *Proc US Nat Acad Sci* , **54**, 228 (1965)

[14] Osborn, M J , and Weiner, I M , *Fed Proc* , **26**, 70 (1967)

[15] Osborn, M J , *Proc US Nat Acad Sci* , **50**, 499 (1963)

[16] Umbarger, H E , and Magasanek, B , *J Amer Chem Soc* , **74**, 4253 (1952)

[17] Dische, Z , *J Biol Chem* , **204**, 983 (1953)

[18] Reissig, J L , Strominger, J L , and Leloir, L F , *J Biol Chem* , **217**, 1959 (1955)

PHYSIOLOGY

Membrane Surface Specialization of Blood Platelet and Megakaryocyte

Recent evidence has indicated the importance of the surface of blood platelets in initiating the chain reaction in relation to platelet aggregation, and the formation of haemostatic plugs and thrombi[1-3] During an investigation of the coating of the platelet surface, we noticed an elaborate surface structure which differs from that of other blood cells (unpublished results)

Platelet-rich plasma and platelet concentrates were prepared as follows 450 ml and 10 ml of whole blood were drawn from normal human and rat, respectively, with sodium citrate or heparin as anticoagulant The blood was centrifuged at 1,300 and 1,800 r p m for 15 min at room temperature to obtain platelet-rich plasma and platelet concentrate, respectively Samples (3 ml) of each, in a conical centrifuge tube, were fixed with osmium[4] Another 3 ml of each was fixed with ruthenium red, as suggested by Luft[5] and Kelly[6] The final sediment was dehydrated in a series of graded concentrations of acetone and embedded in 'Epon' Thin sections were cut on a Porter-Blum ultramicrotome and examined with a Hitachi electron microscope

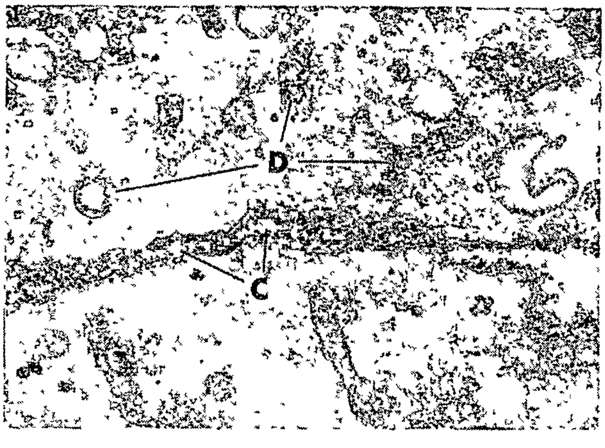

Fig 1 Two blood platelet surfaces in contact show dense extraneous coat across the midline of the micrograph and identical pattern in demarcation tubules (*D*) *C*, Space surrounded by surface patches Ruthenium red preparation stained with uranyl and lead (×*c* 65,800)

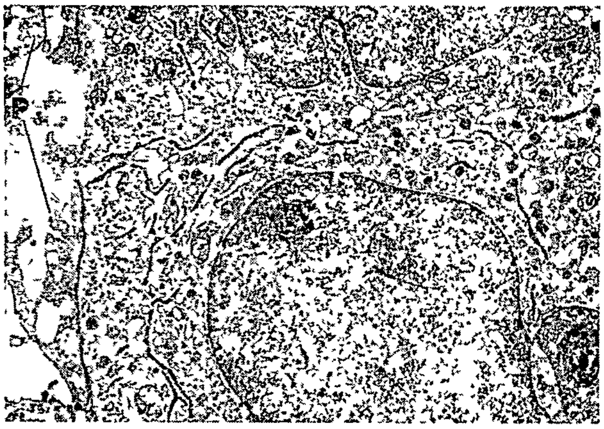

Fig 2 A portion of megakaryocyte shows selective staining of demarcation system The left edge of the picture shows detaching platelets (*P*) Ruthenium red preparation, otherwise unstained (×*c* 3,950)

Bone marrow from several rats (10 days and 1 yr old) was prepared for electron microscopy as follows one limb was injected through the femoral artery with the combination of glutaraldehyde fixative in cacodylate buffer containing ruthenium red The other limb was injected similarly with either 6 per cent glutaraldehyde in cacodylate buffer[7] only, or buffered osmium tetroxide The marrows were then processed identically

In contrast to the platelets and megakaryocytes, none of the other blood cells showed obvious extraneous coating in ruthenium red preparations The platelets showed distinct dense patches on the outer dense layer of the unit membrane as shown in Fig 1 The circulating blood platelet surface was studded with ruthenium red stained material, which varied in size between 50 and 300 Å (average 100 Å) and was irregularly globular There was also noticeable variation in staining, suggesting heterogeneity in composition The interior cytoplasm of the platelets contained many tubular and vesicular profiles which were identical in appearance to the plasma membrane (Fig 1*D*) but inverted or inside-out No other structures showed staining comparable to the extraneous coating

The bone marrow showed exclusive and dramatic staining of megakaryocyte (Fig 2) in the region of the intracellular demarcation system and in the intercellular adherent material at the interface between the megakaryocytes and other bone marrow cells The megakaryocytes (Fig 2) showed almost identical ruthenium red stained material at the surface of developing platelets The cytoplasm of megakaryocytes showed numerous vesicles and tubules which again revealed intense staining with ruthenium red These vesicles and tubules are identical in structure with the membrane of the circulating blood platelets, and coincide precisely with the demarcation system which occurs in the development, maturation and separation of platelets[8]

Furthermore, the midzone of the exoplasm of Heidenhain showed numerous myelin figures—reported earlier as lipoid granules (lecithin)[9]—intermingled with the demarcation tubules The myelin figures were associated with a microglobular structure, particularly toward its surface lamellae, suggesting micro-emulsification of myelin figures

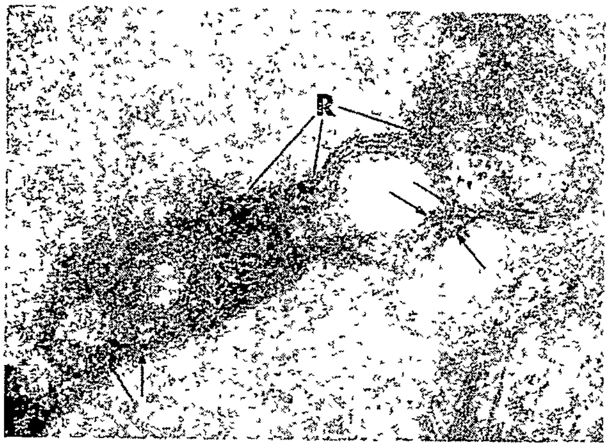

Fig 3 Part of the demarcation tubules in Fig 2 shows electron-dense ruthenium red stained material (*R*) surrounding the myelin figures Arrows indicate developing microglobular structure of phospholipid micromicelles Ruthenium red preparation, otherwise unstained (×c 131,600)

in relation to phospholipid micelles, measuring 10–30 Å, and apparently embedded in mucopolysaccharide present within the demarcation tubules (Fig 3) This specialized type of chemical assembly suggests a possible site of localization of coagulation factors[10,11]

Ruthenium red reacts strongly with chondroitin sulphate and so our finding supports the existence of a mucopolysaccharide, probably a chondroitin sulphate, in the extraneous coat, this has been reported previously from chemical analysis although no precise localization was found[12,13] This structural elaboration suggests that blood platelets have a specialized function in aggregation and haemostasis, they would rapidly form an agglutinated platelet mass when altered by various factors, such as *p*H, ionic strength, size and shape of interactants, and so on[14–16], besides foreign surfaces, especially collagen fibrils[17–19] This seems to support the suggestion of a "masking" and "unmasking" mechanism of functionally active phospholipid micelles[20–22]

Furthermore, the patchy layer of acid mucopolysaccharide on the surface seems to be very interesting when compared with the red blood cell surface Morgan has suggested that the stability of the erythrocyte plasma membrane is probably related to either an enzyme mechanism or mucopolysaccharide patches at the surface[23] We have not so far encountered such specialization of the surface of the erythrocyte in our preparations

The structural integrity of specifically combined phospholipid micelles, mucopolysaccharide and serum proteins (Fig 1*C*) suggests that the triggering of normal and abnormal adhesiveness depends on the "plasmatic milieu" produced

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[1] Born, G V R , *Ann Roy Coll Surg Eng* , 36, 200 (1965)
[2] Marcus, A J , *J Clin Invest* , 45, 14 (1966)
[3] Erickson, R B , and Cintron, J R , *Thromb Diath Haemorrh* , 18, 80 (1967)
[4] Millonig, G , *Electron Microscopy*, 2, 8 (Academic Press, New York, 1962)
[5] Luft, J H , *Electron Microscopy*, 2, 65 (Maruzen Co , Tokyo, 1966)
[6] Kelly, D E , *J Cell Biol* , 28, 51 (1966)
[7] Sabatini, D D , *et al* , *J Cell Biol* , 17, 19 (1963)
[8] Yamada, E , *Acta Anat* , 29, 267 (1957)
[9] Ciaccio, C , *Folia Haematol* , 7, 321 (1909)
[10] Marcus, A J , in *Advances in Lipid Res* (edit by Paoletti, R , and Krichevsky, D), 4, 1 (Academic Press, New York, 1966)
[11] Marcus, A J , and Zucker, M , *The Physiology of Blood Platelets* (Grune and Stratton, New York, 1965)
[12] Odell, jun , T T , and Anderson, B , *Proc Soc Exp Biol and Med* , 94, 151 (1957)
[13] Anderson, B , and Odell, jun , T T , *Proc Soc Exp Biol and Med* , 99, 765 (1958)
[14] Silver, M J , *Amer J Physiol* , 209, 1128 (1965)
[15] Davidson, E A , *Seventh Intern Cong Biochem* , 2, 67 (Tokyo, 1967)
[16] Kirschmann, C E , thesis, Hebrew Univ , Jerusalem (1960)
[17] Bounameaux, Y , *Thromb Diath Haemorrh* , 1, 209 (1957)
[18] Hovig, T , *Thromb Diath Haemorrh* , 9, 264 (1963)
[19] Nakao, K , *et al* , *Exp Mol Pathol* , 6, 213 (1967)
[20] Sharp, A A , and Path, M C , *New Eng J Med* , 272, 89 (1965)
[21] Surgenor, D M , and Wallach, D F H , in *Blood Platelets* (edit by Johnson, S A , *et al*) (Little, Brown and Co , Boston, 1961)
[22] Bezkorovainy, A , and Rafelson, jun , M E , *J Lab Clin Med* , 64, 212 (1964)
[23] Morgan, W T J , *Exp Cell Res* Suppl 1 228 (1949)

Simultaneously Recorded Oscillations in Membrane Potential and Isometric Contractile Force from Cardiac Muscle

IN 1943, Bozler[1,2] reported unusual sinusoidal oscillations in membrane potential and contractile tension which occurred immediately after relaxation of a normally triggered contraction in turtle heart muscles bathed in calcium solutions of high concentrations Reiter[3–5] observed similar contractile oscillations (which he called "aftercontractions") in mammalian myocardium in a variety of conditions that enhance contractility Contractile oscillations have been shown to be unaccompanied by propagated action potentials[4,6] Kaufmann's group, however, detected slow corresponding oscillatory waves in membrane potential similar to those described by Bozler, and they assumed that the contractile oscillations are initiated by electrical oscillations

Oscillatory contractile phenomena in cardiac muscle have now been studied in detail[3–5,7,8], but a similarly detailed analysis of oscillatory electrical phenomena and the relation of electrical oscillations to contractile oscillations has not been made In this communication, we report some preliminary observations for simultaneously recorded electrical and contractile oscillations in guinea-pig left atrial preparations in a variety of conditions The results indicate that there is a direct coupling between electrical and mechanical activity in cardiac muscle in these conditions

The atria were isolated from animals of either sex weighing 470–810 g Each preparation was mounted horizontally in a 'Plexiglas' muscle chamber surrounded by a thermostatically controlled water jacket One end of the preparation was impaled on a strain gauge ('Grass *FT*-03') lever arm The other end was fixed to the terminus of an adjustable rod, the movement of which provided the establishment of the resting length of the muscle All preparations were continuously perfused at a rate of about 3 ml /min with a solution composed of 154 mM sodium chloride, 5 6 mM potassium chloride, 7 1 mM sodium bicarbonate, 11 2 mM dextrose and 5 0 or 10 0 mM calcium chloride as indicated Intracellular membrane potentials were recorded by conventional microelectrode techniques[9] Transduced muscle contractions and membrane potentials were displayed on a 'Tektronix 565' oscilloscope and photographed with a 'Tektronix' oscilloscope camera, or were recorded with a 'Grass' direct writing oscillograph

Each preparation was initially driven at a basal stimulus interval of 1,000 ms at a temperature of 30° C for a period of not less than 60 min. After being stabilized the tissue was perfused with a high concentration calcium solution (10 0 mM) and the temperature of the bath was lowered at a rate of about 1°/min to 20° C or less (minimum of

10° C) Oscillations in membrane potential (electrical oscillations) and isometric contractile tension (mechanical oscillations) were then elicited or "unmasked" by stimulating the preparation with repeated trains of impulses (40 impulses per train) at stimulus intervals of 400, 500, 630, 800 or 1,000 ms, alternated with (*a*) periods of quiescence, or (*b*) periods of stimulation at a constant stimulus interval of 2,000 ms In this communication, oscillations (electrical as well as mechanical) recorded during quiescent periods are termed "post-stimulation oscillations", and oscillations recorded between stimulated contractions are referred to as "inter-stimulation oscillations"

Electrical and mechanical oscillations were observed in a total of fifteen atrial preparations Firm criteria were established for transmembrane recordings considered suitable for analysis of oscillatory electrical phenomena at high sensitivities These criteria were uniform action potential configuration (that is, smooth repolarization phase and constant AP amplitude) during the train of driven beats, and a noise level of less than ± 1 mV All records not meeting these criteria were rejected

Representative examples of electrical and mechanical oscillations recorded from guinea-pig atrium are shown in Fig 1 At all times the recorded mechanical oscillations were found to be identical with those previously described for guinea-pig atria in similar conditions[3,7,8] Furthermore, the electrical oscillations observed in this study were generally comparable with those reported by Kaufmann *et al*[6] In each case the oscillatory wave was smooth and sinusoidal, with the electrical peak generally preceding the mechanical peak In the quiescent period after a series of stimulated contractions (post-stimulation oscillations, Fig 1, *A* and *B*) the electrical and mechanical oscillation peaks were followed by a slow curvilinear recovery toward resting conditions Multiple oscillation peaks generally occurred in each trace The number of these varied between two and seven and appeared to be directly dependent on the amplitude of the first oscillation in the trace Two extra beats (stimulated) are interpolated into the quiescent period in Fig 1*A* Each elicited a small oscillatory electrical and mechanical response but did not alter the overall pattern of the slow recovery phase Oscillations occurring between a series of stimulated beats following a change in driving rate (interstimulation oscillations) are presented in Fig 1*C* The amplitude of both the electrical and mechanical oscillation progressively decreases with oscillation number (that is, between the first and last oscillation of the series) The change in oscillation amplitude as a function of oscillation number has been plotted for another experiment in Fig 2, which also shows concurrent changes in oscillation rise time These data make it evident that changes in electrical oscillatory phenomena closely parallel those in mechanical oscillatory phenomena This relationship holds for quantitative as well as qualitative changes Temperature has been shown to be a very important determinant of mechanical oscillatory activity[7] Fig 3 illustrates the effect of this variable on simultaneously recorded electrical and mechanical oscillations As temperature is decreased the amplitude of both the electrical and mechanical oscillations increases, again in a closely parallel fashion

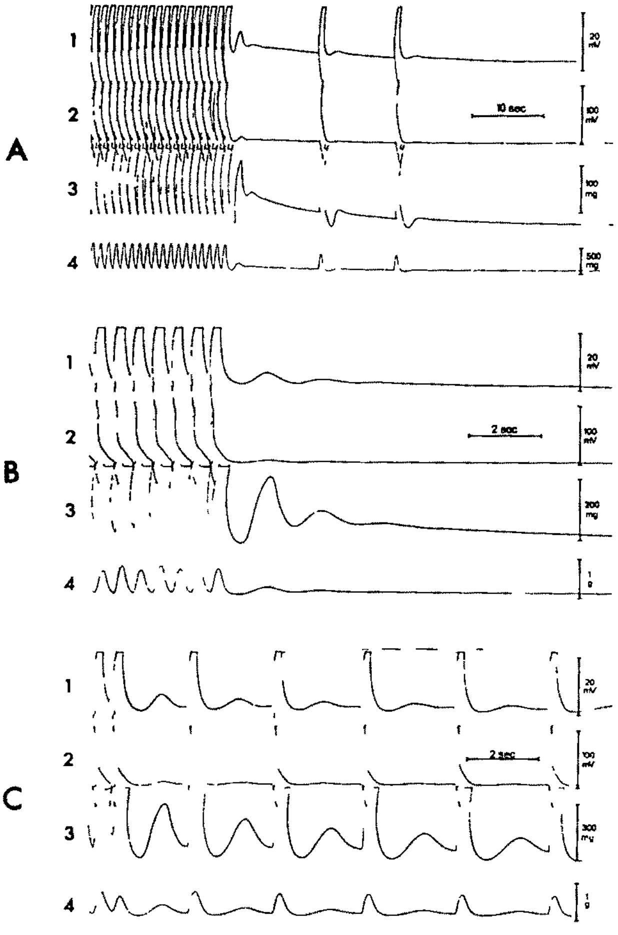

Fig 1 Photograph of oscillations in membrane potential and isometric contractile tension recorded from guinea-pig left atrium with a direct writing oscillograph The temperature was 18° C The calcium concentration of the bath was 10 0 mM *A* and *B* show oscillations occurring during a quiescent period (post-stimulation oscillations) following a train of forty driven contractions at a 630 ms stimulus interval *C* shows oscillations occurring between stimulated contractions (interstimulation oscillations) following a change of stimulus interval of 500–2,000 ms Two extra beats occur during the quiescent period of *A* Numbers on left indicate polygraph channel (1) high sensitivity electrical, (2) low sensitivity electrical, (3) high sensitivity mechanical, (4) low sensitivity mechanical

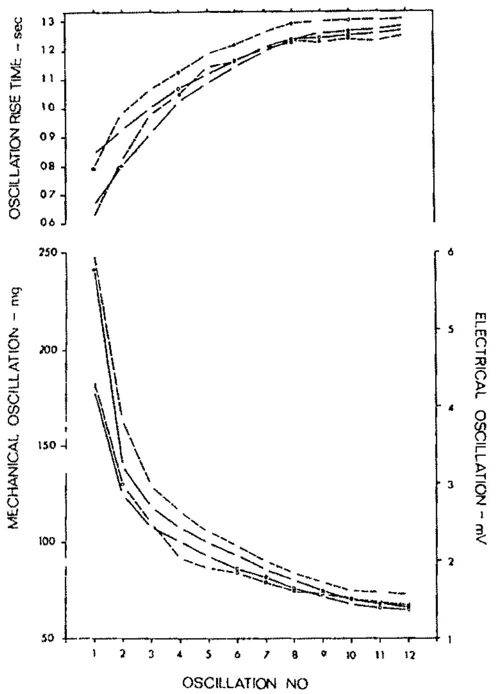

Fig 2 Relation between oscillation amplitude (lower), oscillation rise time (upper), and oscillation number in a series of interstimulation oscillations Oscillation rise time and amplitude measured between origin and peak of oscillation Guinea-pig left atrium was used (——) Mechanical oscillations, (– – – –) electrical oscillations Stimulus interval of preceding train 500 ms (●), and 630 ms (○) The temperature was 20° C The calcium concentration of the bath was 10 0 mM

A primary purpose of this work was to establish an initial correlation between oscillatory electrical changes at the cardiac cell membrane and oscillatory contractile responses within the cell Previous efforts to relate electrical activity with mechanical activity in muscle, both

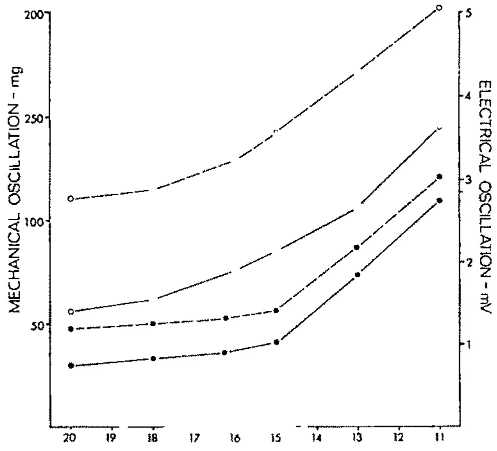

Fig 3 Effect of change in temperature on electrical (– – – –) and mechanical (——) oscillation amplitude Guinea-pig left atrium was used Stimulus interval of preceding train 630 ms (○), and 800 ms (●) The calcium concentration of the bath was 10 0 mM

skeletal and cardiac, have been chiefly concerned with attempting to correlate changes in resting or action potentials with contractile changes, and usually very little correlation has been found (see ref 10) In the present work with guinea-pig atria, we have found good correlation between membrane electrical activity and developed tension in the form of oscillatory electrical and contractile responses

In the interpretation of these results, two important questions immediately arise are the electrical oscillations artefacts arising from the mechanical oscillations and, if not, do the electrical oscillations elicit, and in some way control, the time course of the mechanical oscillations ? That the electrical oscillations are not artefacts is suggested by the smoothness and remarkable repeatability of oscillatory changes in membrane potential This idea is supported by the fact that the electrical and mechanical events can be altered in parallel, with oscillation amplitude inversely related to rise time in the case of the electrical as well as the mechanical oscillations A third, more indirect piece of evidence is suggested by the fact that electrical oscillatory phenomena are not unknown in excitable tissues, in addition to the previously mentioned examples in cardiac muscle[1,2,6], electrical oscillations of various forms have been documented for nerve tissue[11], smooth muscle[12] and skeletal muscle[13] in a variety of conditions At this stage of the investigation no answer can be given as to the mechanisms involved in electrical and mechanical oscillatory phenomena It is clearly evident, however, that there is a close relationship between the two events

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[1] Bozler, E , *Amer J Physiol* , **138**, 273 (1943)
[2] Bozler, E , *Amer J Physiol* , **139**, 477 (1943)
[3] Reiter, M , *Biochem Pharmacol* , **8**, 37 (1961)
[4] Reiter, M , *Arch Exp Pathol Pharmak* , **242**, 497 (1962)
Reiter, M , *Arch Exp Pathol Pharmak* , **245**, 551 (1963)
[6] Kaufmann, R , Fleckenstein A , and Antoni, H , *Pfluger's Archiv* , **278**, 435 (1963)
[7] Katzung, B , *J Cell Comp Physiol* , **64**, 103 (1964)
[8] Braveny, P , Sumbera, J , and Kruta, V , *Arch Intern Physiol Biochem* , **74**, 169 (1966)
[9] Brady, A J , and Woodbury, J W , *Science*, **123**, 100 (1956)
[10] Sandow, A , *Pharmacol Rev* , **17**, 765 (1965)
[11] Shanes, A M , *Pharmacol Rev* , **10**, 249 (1958)
[12] Bülbring, E , and Burnstock, G , *Brit J Pharmacol* , **15**, 611 (1960)
[13] Falk, G , and Landa, J , *Amer J Physiol* , **198**, 289 (1960)

Distribution of Ethanol between Plasma and Erythrocytes in Whole Blood

When capillary and venous blood samples were collected simultaneously from volunteers who had taken varying quantities of alcohol by mouth, certain discrepancies were noted in their alcohol content Further investigation revealed that when added to human blood *in vitro* ethanol was distributed unevenly between the plasma and the red cells[1] If a similar uneven distribution could be demonstrated *in vivo* when alcohol was absorbed from the gut, it might help to explain the differences between capillary blood and venous blood It might also help to explain the observed discrepancies in the blood breath ratios and urine blood ratios reported by different authors

Accordingly twenty volunteers were invited to drink varying quantities of alcohol in the form of whisky or gin during a period of up to 1 h A blood sample of approximately 20 ml was then withdrawn from a superficial vein on the forearm Part of the sample was used to derive the alcohol content of whole blood by duplicate analysis using the Nickolls modification[2] of the Cavett method, a second small fraction was used to determine the haematocrit value The remainder of the sample was placed in a glass test tube sealed with a ground-glass stopper and spun at 2,000 r p m for 20 min at room temperature in a Griffin–Christ centrifuge (type *UJ* 3) After centrifuging, enough plasma and packed red cells were removed separately by pipette to allow duplicate analyses Again the alcohol content was determined by the Nickolls modification of the Cavett method By means of the haematocrit value and a correction factor for trapped plasma[3] the alcohol content of the whole blood sample was calculated from the plasma and erythrocyte contents for comparison with the measured value

Table 1 ALCOHOL CONTENT IN PLASMA, ERYTHROCYTE AND WHOLE BLOOD SAMPLES FROM TWENTY VOLUNTEERS, TOGETHER WITH THE RATIOS OF DISTRIBUTION

		Alcohol content (mg/100 ml)				Ratios	
Subject (1)	Haematocrit (2)	Whole blood (3)	Plasma (4)	Red blood cells (5)	Calculated whole blood (6)	Plasma red blood cells (7)	Plasma whole blood (8)
1	39	114	127	93	114	1 37/1	1 11/1
2	45	148	181	96	143	1 89/1	1 22/1
3	39	58	68	46	59	1 48/1	1 17/1
4	46	87	101	74	89	1 36/1	1 16/1
5	42	70	83	52	70	1 60/1	1 19/1
6	42	64	78	54	68	1 44/1	1 22/1
7	37	44	50	33	44	1 52/1	1 14/1
8	47	41	49	31	41	1 58/1	1 20/1
9	45	41	49	35	43	1 40/1	1 20/1
10	41	48	58	39	50	1 49/1	1 21/1
11	44	37	46	29	38	1 59/1	1 24/1
12	29	43	48	38	45	1 26/1	1 12/1
13	43	60	69	47	59	1 47/1	1 15/1
14	46	40	49	31	41	1 58/1	1 23/1
15	40	21	24	16	21	1 50/1	1 14/1
16	48	167	189	134	163	1 41/1	1 13/1
17	45	68	75	48	63	1 56/1	1 10/1
18	45	66	89	45	69	1 98/1	1 35/1
19	50	52	62	35	49	1 77/1	1 19/1
20	46	54	62	38	51	1 63/1	1 15/1

The values obtained are set out in Table 1 from which it is obvious that the alcohol content of plasma was consistently higher than that of the erythrocytes by a mean factor of 1 54 (*S D* 0 176) with a range from 1 26 to 1 98 Similarly the alcohol content of the plasma was consistently higher than that of the corresponding whole blood In this instance, the plasma : whole blood ratio was 1 18 1 (*S D* 0 057) with a range of 1 10 to 1 35 No significant difference between the measured values and the calculated values for the alcohol content of whole blood was observed ($P > 0\ 9$)

Expressed in another way, it can be shown that the distribution of alcohol between the plasma and red blood cells of a given blood sample has a ratio of about 2 1 (mean of twenty samples 2 07 1, *S D* 0 365, range 1 53 to 3 09)

The idea that there is a difference in the distribution of alcohol between the plasma and the erythrocyte components of whole blood is not new In 1923, Miles[4] demonstrated in ten volunteers that the alcohol concentration in the plasma was between 20 per cent and 25 per cent higher than that in whole blood On the basis of these measurements, he calculated that the alcohol content of the plasma component was about twice that of the red cell component of a given blood sample Our results, obtained by direct measurement, are in broad agreement with the calculated values given by Miles

The difference in alcohol content between plasma and red blood cells can be attributed to the uneven distribution of water between plasma and cells, as suggested by the work of Polonovski, Lindenberg and Rabussier[5] on dogs and later confirmed by that of Gruner[6] on man Gruner showed that alcohol and water had the same uneven distribution between plasma and cells His ratio of 1 15 1 is slightly lower than our figure of 1 18 1, but this can probably be explained by the small size of his series of four subjects

The significance of this observation is that it invalidates the use of such accepted standards as the urine blood ratio and the blood breath ratio for the determination of blood alcohol levels When the distribution of alcohol has reached equilibrium in the body, the tissue levels, including those of the lung and kidney, reflect the concentration in the plasma In particular, the tension, and therefore the amount, of alcohol in the alveolar air is in equilibrium with the tension in the plasma which in turn is directly related to the plasma concentration But because the plasma concentration is substantially higher than the red cell concentration the whole blood concentration must vary inversely with the haematocrit for any given plasma concentration and therefore for any given breath concentration Thus it follows that the blood breath ratio and similarly the urine blood ratio cannot be constant even in one individual but must always be dependent on the haematocrit once equilibrium has been achieved

This problem would be eliminated if plasma breath and urine plasma ratios were used

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[1] Payne, J P , Foster, D V , Hill, D W , and Wood, D G L , *Brit Med J* , **3**, 819 (1967)

[2] Nickolls, L C , *Analyst*, **85**, 840 (1960)

[3] Chaplin, jun , H , and Mollison, P L , *Blood*, **7**, 1227 (1952)

[4] Miles, W R , *J Pharmacol* , **20**, 265 (1923)

[5] Polonovski, M , Lindenberg, B A , and Rabussier, H , *CR Acad Sci* , **232**, 1595 (1951)

[6] Grüner, O , *Dtsch Z Ges Gerichtl Med* , **46**, 10 (1957)

Variations in Embryospecific Globulins of Human Blood Sera in Foetuses and the Newborn

The use of immunochemical methods for studying the composition of foetal human blood serum proteins led to the discovery of, in addition to the known alpha foetoprotein[1-8], other embryospecific globulins with electrophoretic mobility of beta$_1$ (refs 5, 6, 9) and beta$_2$ globulins[9] Alpha foetoprotein is a relatively constant component of foetal blood serum Embryospecific beta$_1$ globulin was discovered in about 75 per cent[9,10] and embryospecific beta$_2$ globulin in 50 per cent of the cases[9]

In 1965 (ref 9), variations of human blood sera were classified with respect to three embryospecific globulins It was interesting to find at what stage of embryogenesis it is possible to discover group variations of foetal sera and whether these variations remain until the end of intrauterine development

Immunochemical investigations of group variations in embryospecific globulins have been carried out on the sera of 6–10 week embryos (medical interruption of pregnancy), 11–30 week foetuses (spontaneous interruption) and 40 week newborns Two hundred and sixty samples of foetal blood sera from one hundred embryos, sixty foetuses and one hundred newborns were studied

Antisera against embryospecific globulins were prepared by repeated reimmunization[6] of rabbits with pooled foetal serum The serum used as the antigen was obtained from 10–30 week foetuses Rabbit antisera against foetal serum were adsorbed by a surplus of pooled human serum obtained from healthy donor adults Detailed descriptions and identification of embryospecific alpha globulin (ESA$_1$ globulin), beta$_1$ globulin (ESB$_1$ globulin), and beta$_2$ globulin (ESB$_2$ globulin) have been given before[9,11]

Foetal sera were tested in identical conditions, by the Ouchterlony method[12] modified by Khramkova and Abelev[13] This modification permits a comparison of an antigen with the antigen of a standard test system, and can reveal an antigen even if it is in a "below threshold" concentration Some selected fractions of globulins of pooled human foetal serum, prepared by electrophoresis on agar gel, were used as the antigens of test systems for determining components of each embryospecific antigen

Each of the embryospecific globulins is found equally often in the sera of embryos, foetuses and newborns[14], the analyses (Tables 1 and 2) did not register the stage of pregnancy ESA$_1$ globulin, the alpha foetoprotein of Bergstrand and Czar[1], is a more constant antigen component in the foetal sera than ESB$_1$ globulin and ESB$_2$ globulin (Table 1) The embryospecific globulins, migrating in the region of beta globulins, may be distinguished not only by electrophoretic mobility (Fig 1), but by their relation to the Schiff reaction It was shown[11] that ESB$_1$ globulin is a typical glycoprotein

The characteristics of foetal sera with respect to three embryospecific globulins are given in Table 2 Sera of group 1, containing all the known embryospecific globulins, were observed more frequently than any other groups The sera of group 2, in which there is a characteristic lack of ESB$_2$ globulin, were found only half as often as sera of group 1 Quantitative relationships between groups 3 and 4 are similar The sera in which there is ESB$_1$ globulin but not ESA$_1$ globulin (groups 5 and 6) were observed in about 2 per cent of the cases Sera from which all the known embryospecific globulins are absent or which contain only ESB$_2$ globulin are found very seldom

Immunochemical investigations of three embryospecific globulins made it possible to subdivide foetal sera into at least eight groups If variations of foetal sera are discovered at an early stage of human embryogenesis and remain up to the end of the period of intrauterine life, genetic control of the synthesis of each embryospecific globulin may be considered

Table 1 EMBRYOSPECIFIC GLOBULINS IN FOETAL HUMAN SERA

Embryospecific antigen components	Total amount of foetal sera in analyses	Positive result	
		Number	Per cent
ESA$_1$ globulin	260	248	95 4
ESB$_1$ globulin	260	194	74 6
ESB$_2$ globulin	260	150	58 4

Table 2 CHARACTERISTICS OF HUMAN FOETAL SERA WITH RESPECT TO THREE EMBRYOSPECIFIC GLOBULINS

Group number	Globulins* present	Number of sera observed with group characteristics	Per cent
1	1–2–3	121	46 4
2	1–2–0	63	24 2
3	1–0–0	44	16 7
4	1–0–3	19	8 1
5	0–2–3	5	1 9
6	0–2–0	5	1 9
7	0–0–3	1	0 4
8	0–0–0	1	0 4

*1—ESA$_1$ globulin, 2—ESB$_1$ globulin, 3—ESB$_2$ globulin

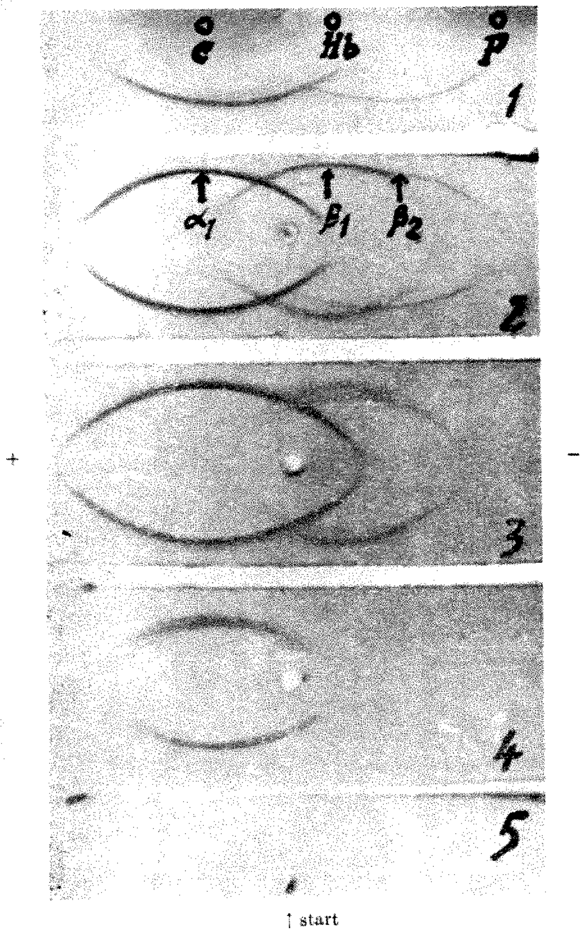

Fig. 1. Immunoelectrophoresis of foetal human sera. Precipitation lines obtained by development with adsorbed antiserum against human foetal serum proteins. Group variations of the embryospecific globulins (Table 2): 1, (1–0–3); 2 and 3, (1–2–3); 4, (1–0–0); 5, adult human serum. The zone of migration: c, colour T-1824; Hb, haemoglobin; p, pyronine.

It will be interesting to find out if antibodies are produced in women during pregnancy where mother and foetus belong to different groups. In such cases some embryospecific globulins would have become heterogenic antigens for the mother. Analogous views relating to stadiospecific antigens can be found in the early literature[15].

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[1] Bergstrand, C. G., and Czar, B., *Scand. J. Clin. Lab. Invest.*, **8**, 174 (1956).

[2] Goldo, A., Casado, J.-P., and Talavera, R., *Arch. Franç. Pediat.*, **16**, 954 (1959).

[3] Bodman, J., *Clin. Chim. Acta*, **4**, 103 (1959).

[4] de Muralt, G., and Roulet, D. L. A., *Helv. Paediat. Acta*, **16**, 517 (1961).

[5] Vasileisky, S. S., and Yablokova, V. I., *Buill. Eksp. Biol. Med.*, **57**, 4, 52 (1964).

[6] Tatarinov, Yu. S., *Vopr. Med. Khim.*, **10**, 6, 584 (1964); *Fed. Proc.*, **24**, 916 (1965).

[7] Masopust, J., *Rev. Czech. Med.*, **8**, 214 (1962).

[8] Gitlin, D., and Bosmann, M., *J. Clin. Invest.*, **45**, 1826 (1966).

[9] Tatarinov, Yu. S., *Vopr. Med. Khim.*, **2**, 3, 98 (1965).

[10] Vasileisky, S. S., and Annenkov, G. A., *Vopr. Antropology (Moscow)*, **18**, 102 (1964).

[11] Tatarinov, Yu. S., *Vopr. Med. Khim.*, **13**, 1, 37 (1967).

[12] Ouchterlony, O., *Lancet*, **256**, 346 (1949).

[13] Khramkova, N. I., and Abelev, G. I., *Buill. Eksp. Biol. Med.*, **52**, 12, 107 (1961).

[14] Tatarinov, Yu. S., Masyukevich, V. N., Mesnyankina, N. V., and Parfenova, L. F., *Akush. i. Gin.*, **43**, 8, 20 (1967).

[15] Vjasov, O. E., *Immunology of Embryogenese*, Moscow (1962).

In vivo Detection of Circulating Gas Emboli associated with Decompression Sickness using the Doppler Flowmeter

THE ultrasonic Doppler flowmeter[1] has been used to measure blood flow in several animal species[2,3] and man[4,5] by monitoring the frequency shift produced in the scattering of ultrasonic waves by moving blood cells. The extreme sensitivity of this instrument to air bubbles in the circulating fluid is a familiar nuisance encountered during *in vitro* testing and calibration procedures. We have exploited this characteristic for the *in vivo* detection of circulating gas emboli in an animal decompressed rapidly from a hyperbaric environment.

The transducer used in this experiment consists of two piezoelectric crystals mounted opposite each other in a rigid cuff which encircles the vessel. One crystal is electrically excited at a fixed frequency, producing ultrasonic waves which propagate into the vessel at an angle relative to the vessel axis. Waves backscattered from the moving cells cause sympathetic vibrations of the second crystal, producing an electrical signal of a slightly different frequency. The two crystal signals are combined to form an amplitude modulated signal which is then demodulated, producing an audio frequency signal the amplitude of which is a function of the amount of backscattered energy and the frequency of which is directly proportional to flow velocity.

Because of the disparate acoustic properties of plasma and air, the interface between plasma and a gas embolus is a highly efficient scattering surface. A circulating gas embolus thus produces an artefact on the flow signal which may have an amplitude many times greater than the flow background. Such an artefact can be detected by ear even when electronic discrimination is difficult, having a characteristic sound best described as a "chirp".

Because of interest in the miniature swine as a physiological analogue to man, this species was selected for hyperbaric investigations which include evaluation of the Doppler flowmeter as a detector of circulating gas emboli. A thoracotomy was performed on a 60 kg male Hanford miniature swine[6] and the Doppler flow transducer was placed on the caudal vena cava about 8 cm cranial to the diaphragm. After a 3 week recovery period the animal was fitted with a harness assembly on which was fastened the crystal exciter, crystal receiver/demodulator and telemetry transmitter circuits[7]. The blood flow audio signal was telemetered from within the hyperbaric chamber to a receiver outside, thus permitting the animal to move about without danger of lead entanglement.

Using an atmosphere of air the chamber pressure was varied according to a schedule (Fig. 1) consisting of compression to 5·3 atm. gauge (equivalent to about 53 m of sea water), maintenance at that level for approximately 1 h, rapid (6 min) decompression to zero atmospheres gauge, recompression to the original level, and stepwise decompression according to a therapeutic schedule[8] which was admittedly inadequate considering the observed signs of decompression sickness. Flow audio was recorded on magnetic tape throughout the experiment.

The first embolic event, defined as an unmistakably characteristic artefact on the flow audio, was detected 2 min after beginning rapid decompression, or at about 3 atm. gauge chamber pressure. The rate and loudness of these events increased as decompression continued. Within 5 min of the completion of decompression the embolic events had become largely confluent and obliterated most of the flow background. There were momentary periods of total loss of signal probably indicating total inhibition of ultrasound transmission by gas in the vessel. Oscilloscope observations of the tape recorded audio indicated that the peak voltage of most embolic events was at least twice that of the flow background. Using

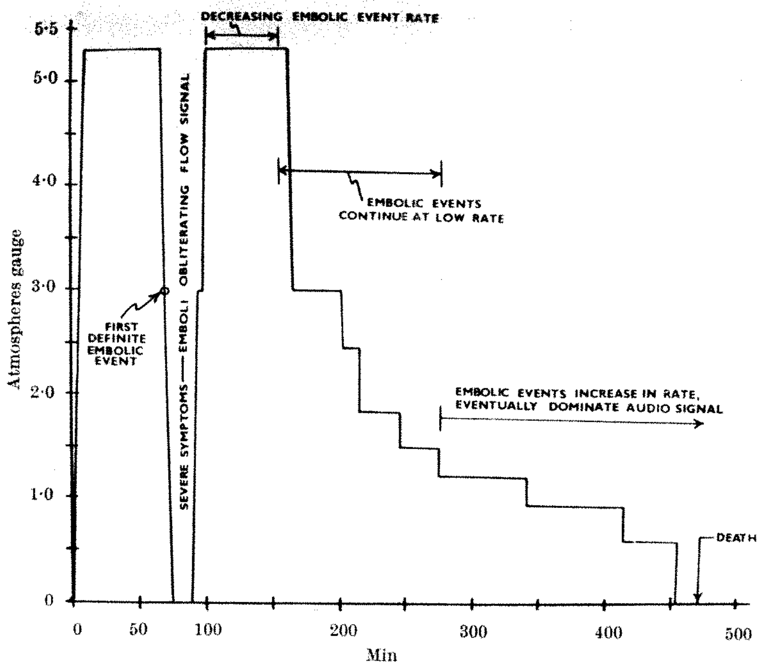

Fig. 1. Chamber pressure against time. Occurrence of embolic events, defined in the text, is described qualitatively.

a triggered sweep with the threshold set above the maximum flow signal, and connecting the vertical gate pulse output to a pen recorder, embolic events could be displayed as shown in Figs. 2 and 3.

The animal was removed from the chamber after decompression and allowed to move freely about the room. Its gait was hesitant and mildly ataxic, with evidence of carpal pain. Increasingly severe ataxia and partial paraplegia appeared within minutes, followed closely by complete collapse. Clonic convulsions and unconsciousness occurred 11 min after completion of decompression.

The animal was returned to the chamber and recompressed to the original level of 5·3 atm. gauge. After about 1 h at this level, only occasional embolic events were detected. There was no significant change in embolic event rate during therapeutic decompression to the 1·22 atm. gauge level, except for occasional embolic "showers" which followed movement of the animal. At the 1·22 atm. gauge level the embolic event rate gradually increased until their signals again obliterated most of the flow background, and continued at this rate to the completion of therapeutic decompression. The animal died about 20 min. later; immediate post-mortem examination showed the entire venous system to be filled with frothy blood.

The conditions of this pilot experiment were extreme, but suggest the potential usefulness of the technique in milder situations. Transcutaneous transducers capable of measuring blood flow in superficial vessels have been in use for some time[4], making the technique immediately applicable to humans. Should circulating gas emboli be detectable by this method before the onset of the subjective symptoms presently used as indicators of decompression sickness, contemporary decompression schedules may require re-evaluation or it can be argued that any gas embolus, regardless of size, is potentially dangerous. The experimental animal will continue to be valuable in more fundamental studies using implanted transducers, such as monitoring gas embolus clearance or

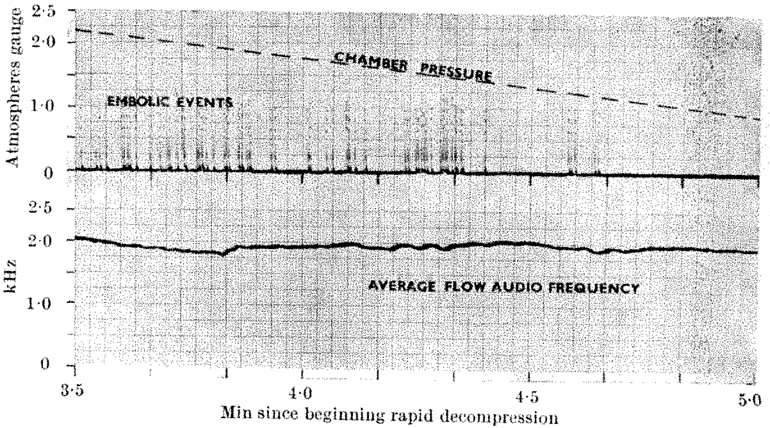

Fig. 2. Simultaneous recording of embolic events and average flow audio frequency during rapid decompression. Chamber pressure is indicated in the upper chart.

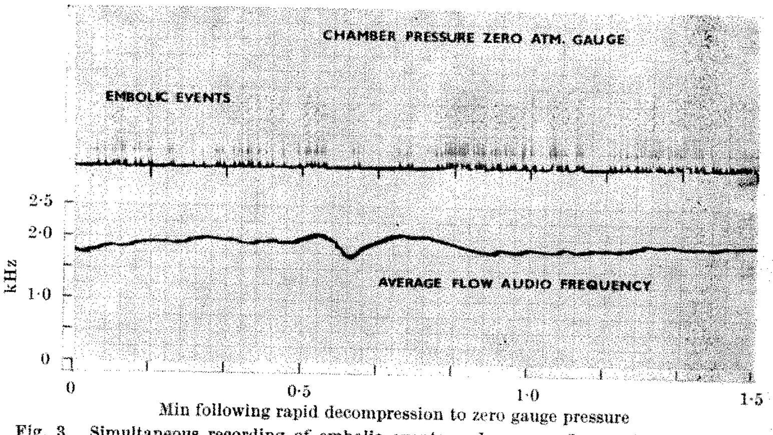

Fig. 3. Simultaneous recording of embolic events and average flow audio frequency immediately after rapid decompression. Note the increased rate of embolic events and absence of appreciable change in vena cava flow rate.

evolution by selected organs The ultimate value of the technique depends on the sensitivity attainable Because this apparatus is designed for measurement of blood flow, changes in design may increase its efficiency for detection of gas emboli The use of artificial emboli of known size should facilitate efforts in this direction as well as aid in the development of more refined discrimination techniques which may permit some degree of quantitation of embolic density or size or a combination of the two

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[1] Franklin, D L, Schlegel, W A, and Rushmer, R F, *Science*, 134, 564 (1961)

[2] Van Citters, R L, and Franklin, D L, *Proc Ann Conf on Eng in Med and Biol*, 9, 27 7 (1967)

[3] Gillis, M F, *Proc Ann Conf on Eng in Med and Biol*, 9, 33 6 (1967)

[4] Strandness, jun, D E, and Sumner, D S, *Proc Ann Conf on Eng in Med and Biol*, 9, 27 5 (1967)

[5] Benchimol, A, *Proc Ann Conf on Eng in Med and Biol*, 9, 27 6 (1967)

[6] Bustad, L K, Horstman, V G, and England, D C, in *Swine in Biomedical Research*, 769 (Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington, 1966)

[7] Franklin, D L, Watson, N W, Pierson, K E, and Van Citters, R L, *Amer J Med Electronics*, 5, 24 (1966)

[8] *U S Navy Diving Manual*, 1 6 2, NAVSHIPS 250-538 (Superintendent of Documents, US Government Printing Office, Washington, 1963)

Secretion of 3β-Hydroxypregn-5-en-20-one (Pregnenolone) by the Adrenal Gland

IN a previous communication[1] it was reported that a compound behaving in a manner similar to pregnenolone in several paper and gas chromatographic systems is secreted by the adrenal gland of the dog and pig The same compound was later found in extracts of adrenal glands of these species and of rats[2]

Further evidence for the identity of this compound was obtained by analysis in an LKB 9000 gas chromatograph–mass spectrometer (*GC–MS*) The compound was isolated from adrenal venous blood collected from a female dog under chloralose anaesthesia The purified blood extract was chromatographed first in the E_2B system of Eberlein and Bongiovanni[3], to separate pregnenolone from the principal gluco- and mineralocorticoids, and then in the E_1 system (Eberlein and Bongiovanni[3]) The eluate of the pregnenolone region of this chromatogram was applied to a 3 8 per cent *SE*-30 column (120 cm, 230° C) of an F and M gas chromatograph fitted with a flame ionization detector (260° C) a peak with a retention time of 0 61 relative to that of cholestane was observed (Fig 1) This retention time was equal to that of authentic pregnenolone After acetylation both the peak observed in the blood extract and that of authentic pregnenolone changed their relative retention time to 0 91

A portion of the purified extract of blood was then injected into the *GC–MS* (fitted with a 1 per cent *SE*-30 column at 240° C) The mass spectrogram obtained (Fig 2*a*) shows that the compound has a molecular weight of 316 The spectrum is in satisfactory agreement with that of authentic pregnenolone (Fig 2*b*) Further evidence for the identity of the steroid isolated from adrenal venous blood with pregnenolone was obtained after forming its trimethylsilyl ether The mass spectrogram of this derivative is shown in Fig 3*a* It is in good agreement with the spectrogram of the trimethylsilyl ether of authentic pregnenolone obtained under the same conditions (Fig 3*b*) The molecular weight of the ether is 388 The retention time of the ether formed from authentic pregnenolone on a 1 per cent *SE*-30 column at 240° C was 0 42 in relation to that of the trimethylsilyl

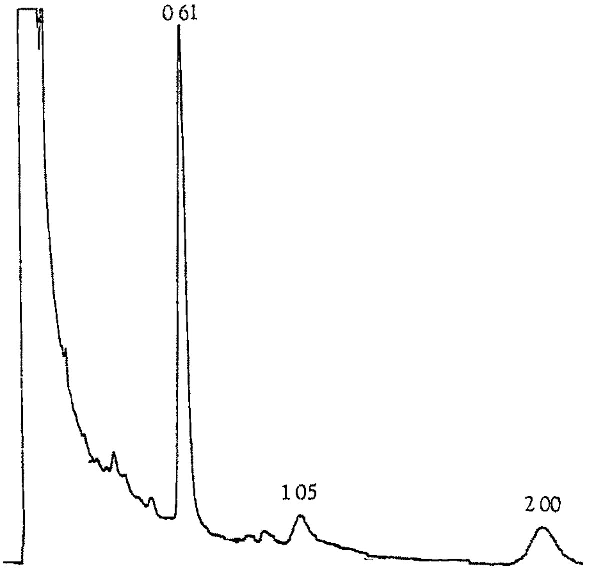

Fig 1 Gas chromatograph tracing of extract from dog adrenal venous blood collected for 30 min and chromatographed in E_2B and E_1 Region with R_F value of pregnenolone eluted, eluate dried, dissolved in 40 μl of ethanol, 1 μl applied to column (range 1, attenuation 8) The number is the retention time relative to that of cholestane Authentic pregnenolone had a relative retention time of 0 61

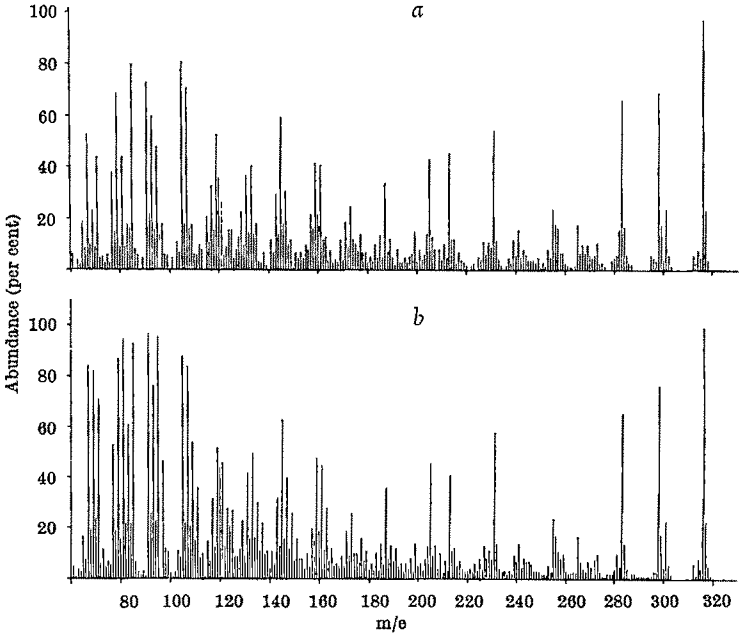

Fig 2 Line diagrams of mass spectrograms obtained with the LKB 9000 gas chromatograph–mass spectrometer (1 per cent *SE*-30 column) *a*, Pregnenolone, separated from dog adrenal venous blood *b*, Authentic pregnenolone

Table 1 SECRETION OF 3β-OH-PREGN-5-EN-20-ONE (PREGNENOLONE) BY THE LEFT ADRENAL OF AN ADULT FEMALE DOG (20 KG) UNDER CHLORALOSE ANAESTHESIA

Experimental conditions	Adrenal secretion rates (μg/g adrenal/h)		Adrenal blood concentrations (μg/100 ml)	
	Pregnenolone	Cortisol	Pregnenolone	Cortisol
Pituitary intact, 30 min after cannulation of adrenal vein	112	715	33 5*	212
1 h after hypophysectomy	6	166	1 9	53
During infusion of ACTH (0 3 mμ/min/kg)	82	690	30 7	260
After ACTH infusion (150 min after hypophysectomy)	4	140	1 4	50

Adrenal venous blood was collected for periods for 30 min from the cannulated left adreno-lumbar vein (Purification and assay as described in ref 1)

* The concentration of pregnenolone in arterial blood collected simultaneously was 0 17 μg/100 ml

ether of cholesterol The same retention time was found for the trimethylsilyl ether of the compound extracted from blood

Pregnenolone was found to be a regular secretion product of the adrenal gland of dogs, puppies and piglets In the dog, it was secreted at an average rate of 100 μg/g adrenal/h in conditions of stress This was about one-tenth of the secretion rate of cortisol Its concentration in adrenal venous blood was in the order of 30 μg/100 ml, whereas its concentration in arterial blood under the same conditions was less than 0 3 μg/100 ml Table 1 shows the dependence of the adrenal secretion of pregnenolone on the presence of the pituitary gland It can be seen that hypophysectomy caused a fall in the secretion of pregnenolone by more than 90 per cent Its concentration in adrenal venous blood, however, was still ten times greater than in arterial blood collected before hypophysectomy This observation indicates that pregnenolone is secreted under resting conditions

The release of pregnenolone from the adrenal gland is not only a function of its concentration in the adrenal tissue In experiments on piglets, decreasing the haematocrit to 4 per cent by infusing dextraven during adrenal blood collection caused a severe fall in the rates of secretion of corticosteroids and also of their concentrations in adrenal tissue The adrenal concentration of pregnenolone was not decreased, however In spite of this, its rate of secretion fell by 85 per cent

The *GC–MS* data were recorded using the LKB 9000 instrument purchased from an SRC grant to Drs C J W Brooks and G Eglinton, Department of Chemistry, University of Glasgow We thank Dr C J W Brooks for arranging for the analysis of the blood extract on this instrument and for evaluating the spectrograms, and also Miss Janice Malcolm who carried out the *GC–MS* analysis, and Miss H Humphrys who drew the line diagrams

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[1] Heap, R B, Holzbauer, M, and Newport, H M, *J Endocrinol*, 36, 159 (1966)

[2] Holzbauer, M, and Newport, H M, *J Physiol*, 193, 131 (1967)

[3] Eberlein, W R, and Bongiovanni, A M, *Arch Biochem Biophys*, 59, 90 (1955)

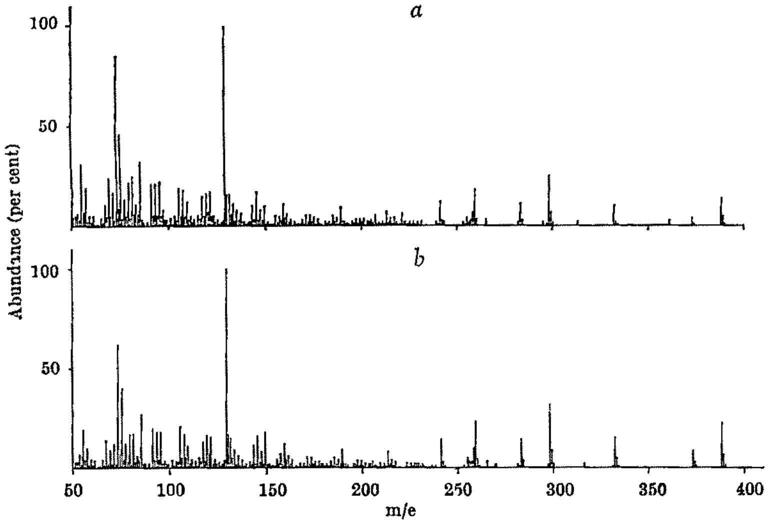

Fig 3 Line diagrams of mass spectrograms obtained with the LKB 9000 gas chromatograph–mass spectrometer (1 per cent *SE*-30 column) *a*, Trimethylsilyl ether of pregnenolone extracted from dog adrenal venous blood *b*, Trimethylsilyl ether of authentic pregnenolone

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Comparative Ultrafiltrability of Calcium and Strontium in Human Plasma

UNTIL recently there have been few and conflicting reports about the comparative ultrafiltrability of calcium and strontium in human plasma Some report that there is no difference[1], others that strontium filters to a greater extent[2] The lack of further experimental data on this subject prompted us to look for differences in the ultrafiltrability of these elements

Ten patients, studied at the time of bone scanning, were injected intravenously with 1–3 mCi of strontium-87m This was obtained from an yttrium-87–strontium-87m generator with 0 005 per cent citric acid, the eluate was sterilized and adjusted with sterile saline to isotonicity with blood plasma Blood samples were taken in anaerobic conditions from the other arm 5–10 min after injection Anaerobic ultrafiltration was carried out on fresh plasma by the method of Lavietes[3] as modified by Smarsz[4] Plasma calcium (Ca_T) and ultrafiltrate calcium (Ca_{UF}) were determined by a standard method[5], and the amounts of strontium-87m in the plasma (Sr_T) and plasma ultrafiltrate (Sr_{UF}) were measured in a scintillation spectrometer, 'Tracerlab *SC* 78', with a counting error not greater than 1 3 per cent The percentage ultrafiltrability of calcium and strontium was calculated from the following formula

$$\text{Percentage ultrafiltrability} = \frac{\text{ultrafiltrable value}}{\text{total value in equal volume}} \times 100$$

The results are shown in Table 1 The percentages of ultrafiltrable calcium and strontium were similar and the ratio of these percentages was almost unity We conclude that the distribution of calcium and strontium in the plasma is similar

These results are of particular importance in calcium–strontium discrimination studies They imply that there is no renal discrimination of strontium against calcium in the glomerulus during filtration, because the filtered plasma water contains the same percentages of calcium and strontium Thus plasma specific activity and the corresponding body retention of radiostrontium can be used instead of radiocalcium to calculate the bone accretion rate, for no real difference in the rate of skeletal uptake of these elements has been found[6]

We thank Dr D J Silvester for the strontium generators, Dr C Smarsz for the ultra-

Table 1 PLASMA CONCENTRATIONS AND ULTRAFILTRABILITY OF CALCIUM AND STRONTIUM

Patient	Ca_T mg/100 ml	Ca_{UF} mg/100 ml	%	Sr_T c p m × 1,000	Sr_{UF} c p m × 1,000	%	Ca_{UF}-Sr_{UF} %	$\frac{Ca_{UF}}{Sr_{UF}}$
1	8 90	5 87	66 0	35 38	18 76	53 0	+13 0	1 25
2	8 77	5 94	67 7	20 75	12 97	62 5	+5 2	1 08
3	8 77	4 76	54 3	33 52	18 49	55 2	−0 9	0 98
4	8 71	4 57	52 5	31 47	18 69	59 4	−6 9	0 88
5	8 84	4 44	50 2	31 64	14 24	45 0	+5 2	1 12
6	9 00	4 89	54 3	26 85	13 91	51 8	+2 5	1 05
7	9 07	5 08	56 0	10 18	6 00	58 9	−2 9	0 95
8	10 51	6 14	58 4	18 60	12 56	67 5	−9 1	0 87
9	9 97	6 37	63 9	6 73	4 48	66 6	−2 7	0 96
10	9 42	5 79	61 5	12 88	7 52	58 4	+3 1	1 05
Mean	9 20	5 39	58 5			57 8	+0 7	1 02
±S E M	±0 190	±0 224	±1 90			±2 18	±2 06	±0 036
p							NS	

filtration apparatus and for advice on its use, and Mrs K Rogalska for technical assistance

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[1] Harrison, G E , Raymond, W H A , and Tretheway, H C , *Clin Sci* ,14, 681 (1955)

[2] Samachson, J , and Lederer, H , *Proc Soc Exp Biol and Med* , 98, 867 (1958)

[3] Lavietes, P H , *J Biol Chem* ,120, 267 (1937)

[4] Smarsz, C , *Polskie Archiwm Med Wewn* , 38, 767 (1967)

[5] Bett, I M , and Fraser, G P *Clin Chim Acta*, 4, 346 (1959)

[6] Shimmins, J , Smith, D A , Nordin B E C , and Burkinshaw, L , *Strontium Metabolism*, 149 (Academic Press, London and New York, 1967)

Environmental Temperature and Plasma Electrolyte Regulation in the Carp, *Cyprinus carpio*

WHILE the ability of the freshwater teleost to control body fluid electrolyte composition has been appreciated primarily in terms of neuromuscular and related membrane-dependent phenomena, an increasing body of evidence points to an equally important role in the regulation of intermediary metabolism[1] Control of water-electrolyte balance in these animals involves adjustment of a variety of passive and metabolically-dependent transfer processes operating at phase boundaries within the body fluid system, and at the several sites at which exchange with the environment is possible Regulation is also influenced by the mass transport activities of the cardiovascular and respiratory systems, the primary functions of which prompt both endosmosis and electrolyte efflux

Adaptation to changes in environmental temperature frequently imposes marked variations in oxygen requirements Satisfaction of these might, *a priori*, be expected to entrain stresses demanding adjustment of the ionoregulatory apparatus In addition, the complexities of the overall regulatory mechanism provide scope for expression of any differential thermosensitivities existing among component elements Despite this, surprisingly little attention has been directed toward this facet of teleostean ionic regulation During a study of systemic aspects of the thermoacclimatory process, we have tried to determine the relationship between temperature and regulatory ability in a typical freshwater eurytherm, the common carp (*Cyprinus carpio*)

Experimental stocks of the animals were obtained by seining and trapping in local waters Holding and experimental facilities and analytical procedures have been outlined before[2] Assessment of regulatory ability was based on determination of plasma osmolarity, and the concentrations of the principal blood electrolytes (sodium, potassium, calcium, magnesium, chloride and phosphate) in groups of animals acclimatized to 4°, 17°, 27° and 33° C for intervals of not less than three weeks

The results of the investigation are summarized in Table 1 It is apparent that both the set-points of regulation (as judged by sample means) and the precision of regulation (as judged from sample variability) are modified by temperature In some cases, the relationships are non-linear Thus plasma sodium concentrations were maximal at 27° C, with a mean concentration of about 9–10 mmoles/l greater than those observed at 4° C and 33° C By contrast, concentrations of chloride increased steadily from a mean of 115 6 mmoles/l at 4° C to 137 0 mmoles/l at 33° C Coupled with this the precision of chloride regulation diminished markedly at higher temperatures Phosphate concentrations increased sharply between 4° C and 17° C, and then diminished slowly, but showed no great change in variability Plasma calcium also increased with temperature from 1 94 mmoles/l at 4° C to 2 54 mmoles/l at 33° C Potassium and magnesium concentrations, on the other hand, displayed no consistent variation in either set-point or precision at the different acclimation temperatures employed

Determinations of blood *p*H were not included in the investigation It is apparent, however, that the ratio *p*Cl/Σ (cation) increased with temperature Taking into account the relationship between phosphate concentration and temperature, and assuming the condition of body fluid electroneutrality, this observation suggests that the concentration of some anionic species, presumably bicarbonate, increases at lower temperatures Accordingly, the occurrence of a condition of essential alkalosis might be predicted in cold-acclimated specimens, an interpretation consistent with the findings of a recent study on acid-base balance in this species[3]

The data suggest a fall in plasma osmolarity at increased temperatures, with a pronounced drop between 27° C and 33° C In contrast, the sum of all cations plus chloride steadily rose with temperature An increasing contribution of non-electrolyte to total osmolarity at lower temperatures is suggested The nature of the materials involved is uncertain Values for total plasma protein levels indicate that it was not protein

While no similar studies on cyprinid fishes appear to have been carried out, information relating plasma composition to environmental temperature is available for a number of salmonids The data are, however, somewhat inconsistent Hickman *et al*[4] report an inverse relationship between plasma sodium and temperature in rainbow trout, while neither Gordon[5] (working on brown

Table 1 PLASMA COMPOSITION OF THERMALLY ACCLIMATED CARP

	Acclimation temperature			
	4° C	17° C	27° C	33° C
Size (g)	478 9 ±16 6 (15)*	453 4 ±16 6 (15)	409 4 ±12 3 (14)	393 1 ±23 0 (15)
*p*Osm (mOsm/l)	277 ± 1 (13)	274 ± 3 (13)	270 ± 4 (9)	257 ± 2 (14)
*p*Na (mM/l)	127 9 ± 1 1 (15)	130 3 ± 1 4 (15)	137 3 ± 2 3 (13)	128 2 ± 1 4 (14)
*p*K (mM/l)	2 74 ± 0 13 (14)	2 93 ± 0 11 (15)	2 39 ± 0 19 (13)	2 77 ± 0 13 (14)
*p*Ca (mM/l)	1 94 ± 0 04 (15)	2 12 ± 0 10 (15)	2 44 ± 0 10 (13)	2 54 ± 0 09 (14)
*p*Mg (mM/l)	1 19 ± 0 02 (14)	1 23 ± 0 03 (15)	1 21 ± 0 05 (13)	1 13 ± 0 03 (14)
*p*Cl (mM/l)	115 6 ± 0 9 (15)	125 2 ± 1 8 (15)	122 2 ± 2 6 (13)	137 0 ± 5 5 (14)
pPO_4 (mg/100 ml)	3 7 ± 0 3 (14)	6 9 ± 0 3 (15)	6 7 ± 0 3 (13)	6 0 ± 0 2 (15)
Σ (cation) + Cl (mM/l)	249 9 ± 1 4 (13)	261 7 ± 2 8 (15)	262 5 ± 4 4 (11)	276 4 ± 6 4 (11)
Cl/*Σ* (cation)	0 86 ± 0 01 (13)	0 91 ± 0 01 (15)	0 85 ± 0 02 (11)	1 01 ± 0 06 (11)
*p*Protein (g/100 ml)	1 8 ± 0 1 (15)	2 2 ± 0 1 (14)	2 3 ± 0 1 (9)	2 1 ± 0 1 (15)

* Mean ± one standard error (sample size)

trout) nor Houston *et al* [2] (in investigations on rainbow trout) observed any consistent variation in this ion Hickman *et al* describe an increase with temperature in the chloride levels of 8° and 16° C-acclimated trout A similar situation was noted by Houston *et al* in summer fish of the same species, but not in winter animals A relationship of this type does not appear in the brown trout[5] Increases in plasma potassium were observed by both Gordon and Houston *et al* at elevated temperatures A slight drop in the plasma concentration of this ion was, however, recorded by Hickman *et al* Similarly, the studies on rainbow trout disagree with respect to the relationship between calcium levels and temperature Both rainbow[2,4] and brook trout[6] display increases in total phosphate levels at higher temperature

The significance to be attached to the differences observed in these studies is uncertain It would appear, however, that both the set-points and precision of ionic regulation can be altered by temperature acclimation Nevertheless, recent studies on thermal shock effects[4,7] suggest that the animals are capable of some considerable degree of compensation The functional importance of this is clear with regard to sensory-neuromotor activity, and may be important in terms of metabolic adjustment to temperature as well[1,8]

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[1] Bygrave, F L , *Nature*, **214**, 667 (1967)
[2] Houston A H , Reaves, R S , Madden, J A , and DeWilde, M A *Comp Biochem Physiol* (in the press)
[3] Rahn, H , *Ciba Found Symp , Development of the Lung*, 3 (1966)
[4] Hickman, C P , McNabb, R A , Nelson, J S , Van Breeman, E D , and Comfort, D , *Can J Zool* , **42**, 577 (1964)
[5] Gordon, M S , *J Exp Biol* , **36**, 227 (1959)
[6] Phillips, A M , *Prog Fish Cult* , Jan 22 (1962)
[7] Heinicke, E A , and Houston, A H , *J Fish Res Board Can* , **22**, 1455 (1965), Reaves, R S , Houston, A H , and Madden, J A , *Comp Biochem Physiol* (in the press)
[8] Prosser, C L , *Arctic Aeromedical Laboratory, Ft Wainwright, Alaska, Symp No 2*, Comparative Physiology of Temperature Regulation, 1 (1962) Hochachka, P W , and Hayes, F R , *Can J Zool* , **40**, 261 (1962)

Pyrogenicity of 'Pyromen'

Wyllie, Limbosch and Nyhus[1], in commenting on the results of Baume, Nicholls and Baxter[2], state that the inhibition of gastric secretion by lipopolysaccharide "was clearly described in 1950" by Blickenstaff and Grossman[3] Blickenstaff and Grossman used 'Pyromen', a commercially available pyrogen (Nesset *et al* [4]), which is very impure and only partially characterized It was for this reason that their results were not discussed by Baume *et al* [2] in their brief communication

'Pyromen' is an extract of *Pseudomonas* obtained from autoclaved cells by tryptic digestion[4] The material represented 25–35 per cent of the cell mass Analyses for nucleic acids, protein and ash accounted for approximately 80 per cent of cell dry weight and 50 per cent of the extract The authors[4] then assumed that the remainder represented pyrogenic polysaccharide From newer methods of extraction it is concluded that lipopolysaccharide represents 1–2 per cent of cell mass rather than the 20 per cent inferred by Nesset and co-workers

The actual amount of lipopolysaccharide in the preparation of 'Pyromen' is difficult to deduce From the chemical analyses no conclusions are possible Studies on pyrogenicity indicated a three-fold increase in activity compared with whole cells which could mean the presence of 5 per cent lipopolysaccharide, but the pyrogenic dose was at least one thousand times greater than that for the purified lipopolysaccharide used by Baume *et al* [2]

From its biological properties, we may presume that 'Pyromen' contains some lipopolysaccharide but, unlike Wyllie *et al* [1], conclude that there is no evidence that the lipopolysaccharide component was responsible for the inhibition of gastric secretion In contrast, Baume *et al* [2] used a purified lipopolysaccharide which has been fully characterized[5], and was made available in a water soluble form[6] This left no doubt that the effects on gastric acid secretion were caused by the lipopolysaccharide

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[1] Wyllie, J H , Limbosch, J M , and Nyhus, L M , *Nature*, **215**, 879 (1967)
[2] Baume, P E , Nicholls, A , and Baxter, C H , *Nature*, **215**, 59 (1967)
[3] Blickenstaff, D , and Grossman, M I , *Amer J Physiol* , **160**, 567 (1950)
[4] Nesset, N M , McLallen, J , and Anthony, P Z , *J Amer Pharm Ass* , **39**, 456 (1950)
[5] Taylor, A , Knox, K W , and Work, E , *Biochem J* , **99**, 53 (1966)
[6] Knox, K W , Cullen, J , and Work, E , *Biochem J* , **103**, 192 (1967)

Permeability and Excitation of Insect Muscle

The membrane of most nerve and muscle cells is assumed to be more permeable to potassium ions than to sodium ions in the resting condition and the potential observed approaches that of a potassium electrode as predicted by the Nernst equation The plasma membrane becomes much more permeable to sodium ions during excitation, and the potential across the membrane reverses, producing a positive overshoot of the action potential The skeletal muscles of herbivorous insects, however, have unusual bioelectric properties and have been studied by Wood[1–4] and Huddart[5–7] Analysis of the haemolymph and myoplasm of Orthoptera, Dictyoptera and Lepidoptera reveals an unusual ionic distribution[8] and it is suggested that the observations of the resting and action potentials fail to agree with the values predicted by the Nernst equation and with the classical studies of excitable cells For example, frequently no positive overshoot to the action potential has been recorded It has been suggested that active transport of chloride ions may contribute to the resting potential[4], and that an inward movement of sodium[9] or magnesium ions[1,9] may participate in the action potential Our present knowledge in this field has been reviewed by Aidley[9]

If sodium ions enter the cell during the action potential some system must be present to remove them A sodium–potassium–magnesium–ATPase has recently been found in the plasma membrane of many tissues of other species and is believed to be associated with active cation transport[12] A study of the enzyme properties of the muscle membrane of herbivorous insects could represent the first step in establishing which ions move and carry the ionic current during excitation

Cockroach (*Periplaneta americana*) coxal muscle was therefore studied to determine whether a sodium–potassium–magnesium–ATPase was present in a microsomal preparation The muscle microsomal fraction was prepared according to experimental details given elsewhere[10] except that the isolation medium contained 10 mM EDTA ATPase activity was determined as the inorganic phosphorus liberated[11] in 15 min at 25° C, the reaction being linear during this period Other conditions of incubation are given in Table 1, in which the results of two representative experiments are summarized It can be seen that the magnesium–ATPase activity constitutes much of the total activity, addition of either sodium ions or potassium ions increases this activity, although sodium ions have been found to be consistently less effective than potassium ions. When sodium and potassium ions are, however, added together, little or no synergistic action was found; indeed, higher concentrations of sodium ions inhibited the potassium–magnesium–ATPase activity Strophanthin-*G* at high concentration (10^{-4} M) had only a small inhibitory effect on the activity that was stimulated by the addition of monovalent cations Thus there is little genuine sodium–potassium–magnesium–ATPase (with an obligatory requirement for both sodium and potassium ions[12]) in this preparation and, furthermore, we have found that the monovalent cation-stimulated fraction of the ATPase activity is suppressed by extraction with DOC, a treatment which usually increases the ratio of sodium–potassium–magnesium–ATPase magnesium–ATPase activity in mammalian tissues[12]

These results can be directly compared with those obtained with a microsomal preparation from cockroach nerve cord[13], where a genuine sodium–potassium–magnesium–ATPase, which is sensitive to strophanthin-*G*, constitutes 75 per cent of the total ATPase activity Thus while the monovalent cation-stimulated ATPase found in the muscle microsomal preparation might be concerned with the active transport of cations, its properties are clearly very different from those of the nerve cord from the same animal, and also from those described in mammalian tissues[14] (although compare frog muscle[15]). It is therefore possible that there is little requirement for the active outward extrusion of sodium ions across the muscle membrane, and the view that sodium ions do not enter during excitation is supported by the observation that $[\mathrm{Na}_i^+]>[\mathrm{Na}_o^+]$ in the muscle of some species of insects[7].

There may well be a wide variation in the underlying mechanisms accompanying excitation of muscle in herbivorous insects, but the following hypothesis is tentatively advanced to account for some of the observed phenomena. THE RESTING POTENTIAL in *Bombyx* muscle fibres sodium ions (as well as potassium ions) are more concentrated intracellularly than in the haemolymph[7] (compare squid and vertebrates) while chloride ions are more concentrated in the haemolymph[7] This ionic imbalance is presumably maintained by organic anions inside the fibre, and possibly assisted by organic cations in the haemolymph[16] It seems clear that the resting potential is the resultant of the differential permeability of the membrane to potassium and chloride ions[17], although sodium ions may have an additional effect, augmenting the potential where $[\mathrm{Na}_i^+]>[\mathrm{Na}_o^+]$ and reducing it when the concentration gradient is established in the opposite direction.

THE ACTION POTENTIAL could be the result of (i) An inward movement of sodium ions in some species, although such an explanation is not tenable for muscles (for example, *Sphinx*, *Telea*, *Bombyx*, *Actias*) where $[\mathrm{Na}_i^+]>[\mathrm{Na}_o^+]$ (ref 7). The atypical and low specific activity of the monovalent cation-stimulated ATPase of the cockroach muscle (where $[\mathrm{Na}_o^+]>[\mathrm{Na}_i^+]$ (ref. 3)) also suggests that the movement of sodium ions may contribute little to the action potential (ii) An inward movement of magnesium ions The magnesium–ATPase found in the microsomal preparations in these experiments would then act in the subsequent active transport of these ions outwards Such a hypothesis is also unlikely to be the complete explanation (*a*) $[\mathrm{Mg}_o^{2+}]$ is low in some species (only 1 4 mequiv /l in *Anabrus*)[18], (*b*) the action potential of *Carausius* persists when $[\mathrm{Mg}_o^{2+}]$ is reduced to zero[1], (*c*) there is evidence that some of the $[\mathrm{Mg}_o^{2+}]$ is bound to organic molecules in the haemolymph[19]; and (*d*) in *Calliphora* larvae, no exchange of magnesium ions takes place when the muscle membrane permeability is artificially increased in heat death (personal communication from T F Davison)

We therefore suggest that, after excitation, the action potential is produced by a transient, simple decrease in permeability of the muscle membrane to the diffusible ions (potassium, chloride and possibly in some species sodium), so that the membrane is depolarized and the resting potential approaches zero Such a system requires no form of active transport We visualize the magnesium–ATPase found in our preparation as being a contractile protein which is sited at the membrane pores and which controls the diameter/charge relationships there[10,20] There would therefore be a change in potential across the muscle membrane at the multiterminal endings, and excitation would spread decrementally to the local sarcoplasmic reticulum. The change in membrane potential possibly initiates muscle contraction by a displacement of bound calcium ions from the endings of the sarcoplasmic reticulum

Such a hypothesis is almost certainly not the complete explanation of the complex events observed during the excitation of these muscles, but the production of a depolarization by a decrease in permeability would serve to explain many of the phenomena observed in these insects. Where an active membrane response, with a small positive overshoot, is superimposed on this basic endplate potential, however, other ionic factors must be involved This may well be an inward movement of magnesium or calcium ions

The permeability of all excitable cells is controlled by a dynamic system, but in most excitation is achieved by opening "gates" for sodium ions to enter[20] Our suggestion for the membrane of the muscle of herbivorous insects involves a more fluid control of passive permeability which controls both the resting potential and the changes in permeability during excitation It is interesting that temperature changes have small effects on the resting potential of crab and frog muscles ($Q_{10} = 1\ 06$), whereas the Q_{10} for cockroach muscle is higher at 1 296 (ref 21). This marked effect of temperature is much higher than would be predicted by a simple application of the Nernst equation

Communication in the nervous system of these insects, however, necessitates genuine, non-decremental, propa-

Table 1. ATPASE ACTIVITY OF A MICROSOMAL PREPARATION FROM COXAL MUSCLE OF THE COCKROACH (*Periplaneta americana*)

Experiment	Ionic conc (mM) Mg	Na	K	Pi liberated	Effect of addition of alkali metal ions	Experiment	Ionic conc (mM) Mg	Na	K	Strophanthin-*G* (10^{-4} M)	Pi liberated	Effect of addition of alkali metal ions	Strophanthin-sensitive activity
A 1	4	–	–	523	–	*B* 1*a*	4	–	–	–	507	–	–
2	8	–	–	598	–	1*b*	4	–	–	+	474	–	33
3	–	–	10	18	–	2*a*	4	–	20	–	828	321	–
4	–	–	50	17	–	2*b*	4	–	20	+	764	–	31
5	–	10	–	23	–	3*a*	4	10	20	–	843	336	–
6	–	50	–	25	–	3*b*	4	10	20	+	729	–	81
7	4	–	10	714	191	4*a*	4	20	20	–	876	369	–
8	4	–	50	762	239	4*b*	4	20	20	+	710	–	133
9	4	10	–	607	84	5*a*	4	50	20	–	790	283	–
10	4	50	–	707	184	5*b*	4	50	20	+	716	–	41
11	4	10	10	740	217	6*a*	4	100	20	–	760	253	–
12	4	50	10	730	207	6*b*	4	100	20	+	657	–	70
13	4	10	50	813	290								

Enzyme activity expressed as mμmoles of inorganic phosphate liberated/mg of protein/min Effect of the addition of alkali metal ions is shown as the additional enzyme activity found above that in the presence of 4 mM magnesium ions alone (Experiments *A*1 or *B*1*a*) No correction is made for the small activity found in the presence of sodium or potassium ions alone (experiments *A* 3–6) Enzyme activity sensitive to strophanthin-*G* is calculated as [a–b–(1a–1b)] Conditions 2 mM ATP (*tris* salt), 50 mM *tris*-HCl (*p*H 7 2), 25° C gated transmission and therefore a typical permeability increase (probably to sodium ions) can be predicted in nerve cells[22] An appropriate transport sodium-potassium-magnesium-ATPase has been identified in cockroach nerve cord[13] The need for this form of excitation may explain the presence of the sheath and compartments around the insect nerve cord, which serve to isolate it in its appropriate ionic micro-environment[23,24]

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[1] Wood, D W , *J Physiol* ,138, 119 (1957)
[2] Wood, D W , *Comp Biochem Physiol* , 4, 42 (1961)
[3] Wood, D W , *Comp Biochem Physiol* , 9, 151 (1963)
[4] Wood, D W , *Comp Biochem Physiol* ,15, 303 (1965)
[5] Huddart, H , *Comp Biochem Physiol* ,18, 131 (1966)
[6] Huddart, H , *Arch Int Physiol Biochim* , 74, 592 (1966)
[7] Huddart, H , *Arch Int Physiol Biochim* , 74, 603 (1966)
[8] Rockstein, M , *The Physiology of the Insecta*, 3 (Academic Press, New York, 1964)
[9] Aidley, D J , *Adv Insect Physiol* , 4, 1 (1967)
[10] Bowler, K , and Duncan, C J , *J Cell Physiol* , 70, 121 (1967)
[11] Fiske, C H , and Subbarow, Y , *J Biol Chem* , 66, 375 (1925).
[12] Skou, J C , *Progr Biophys* ,14, 131 (1964)
[13] Grasso, A , *Life Sci* , 6, 1911 (1967)
[14] Heinz, E , *Ann Rev Physiol* ,29, 21 (1967)
[15] Duggan, P F , *Biochim Biophys Acta*, 99, 144 (1965).
[16] Levenbook, L , and Hollis, jun , W W , *J Insect Physiol* , 6, 52 (1961)
[17] Usherwood, P N R , *J Physiol* ,191, 29P (1967)
[18] Pepper, J H , Donaldson, F T , and Hastings, E , *Physiol Zool* , 14, 470 (1941)
[19] Carrington, C B , and Tenney, S M , *J Insect Physiol* , 3, 402 (1959)
[20] Duncan, C J , *The Molecular Properties and Evolution of Excitable Cells* (Pergamon Press, Oxford, 1967)
[21] Kerkut, G A , and Ridge, R M A P , *Comp Biochem Physiol* , 3, 64 (1961)
[22] Treherne, J E , *J Exp Biol* , 42, 1 (1965)
[23] Treherne, J E , *J Exp Biol* , 42, 7 (1965)
[24] Treherne, J E , and Maddrell, S H P , *J Exp Biol* , 46, 413 (1967)

PSYCHOLOGY

New Method of Testing Long-term Retention with Special Reference to Amnesic Patients

A specific isolated and severe defect of memory, with preservation of other intellectual functions, including span of attention, may occur as the result of localized brain pathology In amnesic patients the memory defect is so marked that performance on tests of recent memory declines sharply within a matter of minutes[1]

Because normal subjects show excellent retention on a test which we devised, it seemed interesting to find out whether amnesic patients would show day to day retention on it This communication describes the technique and reports results showing day to day retention in a series of amnesic patients The findings are considered in relation to some theoretical issues

Milner[2] reported that maze learning in one severely amnesic patient with bilateral hippocampal lesions was impaired on the easiest form of the test, that there was some learning within a test session on one day, and some evidence of retention between days In contrast she also reported that a motor skill, mirror drawing, was learnt at a normal rate So far, however, there has been no clear demonstration of day to day retention of pictorial and verbal material by severely amnesic patients

The subjects of this experiment were six patients with a severe and circumscribed memory defect, and five patients with peripheral nerve lesions who had no known

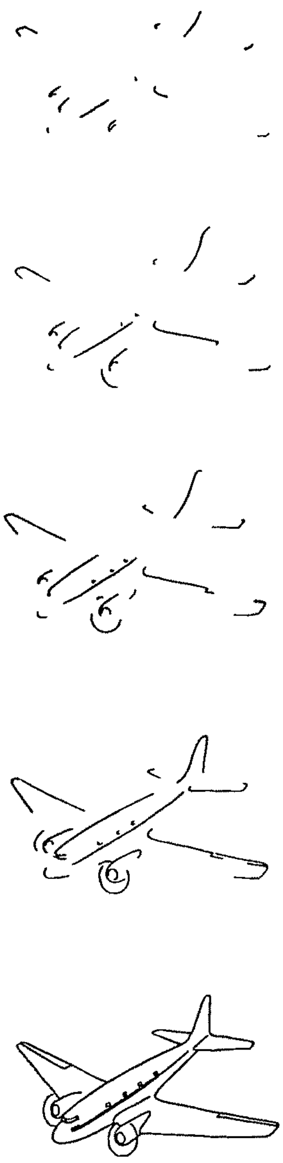

Fig. 1. Example of incomplete picture (from Gollin's[4] series)

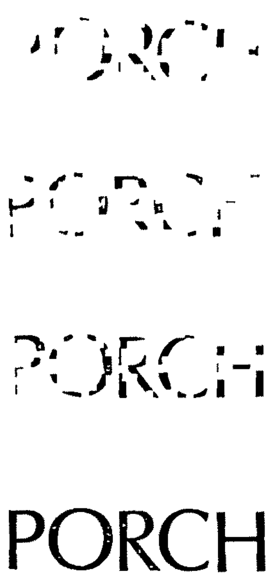

Fig 2 Example of incomplete word

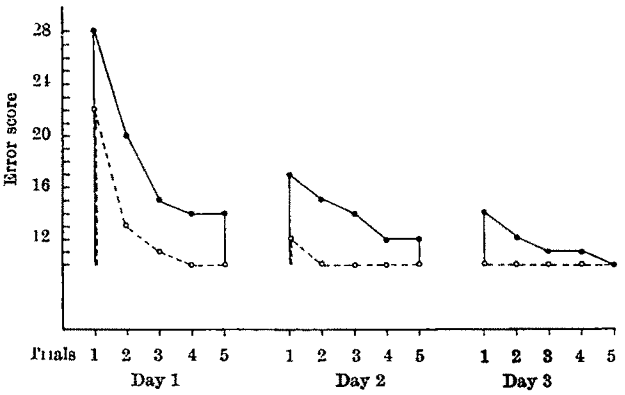

Fig 3 Mean error scores for each trial, for each patient group, on the incomplete picture test ●—●, Amnesics, ○ - - - ○, controls

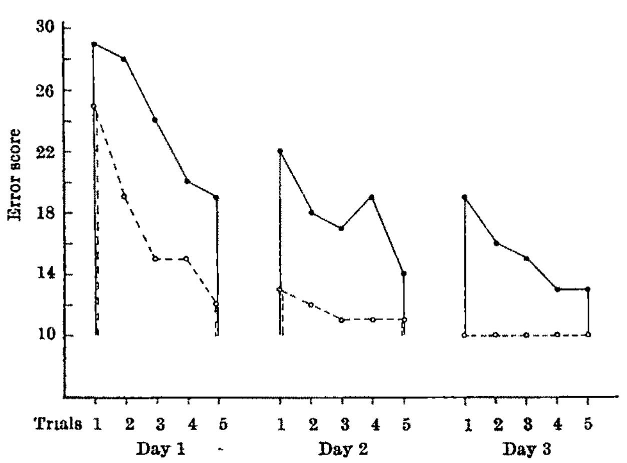

Fig. 4 Mean error scores for each trial, for each patient group, on the incomplete word test ●—●, Amnesics, ○ - - - ○, controls

cerebral disease The six amnesic patients all had intelligence quotients not less than average, as had the control group, and the ages of the two groups were also matched The amnesic patients were all unable to recall day to day events and had retrograde amnesias which, as far as could be determined clinically, varied in length from 10 to 30 yr Five of these amnesics were patients with alcoholic Korsakoff psychoses, and the other (N T) became severely amnesic after a right temporal lobectomy This case has been described in detail by Dimsdale, Logue and Piercy[3]

In 1960, Gollin[4] prepared a graded series of fragmented drawings There are five versions of each drawing, the first being very incomplete, and the fifth being a complete representation of the object, with three intermediate versions (Fig 1) Ten sets were used for this experiment

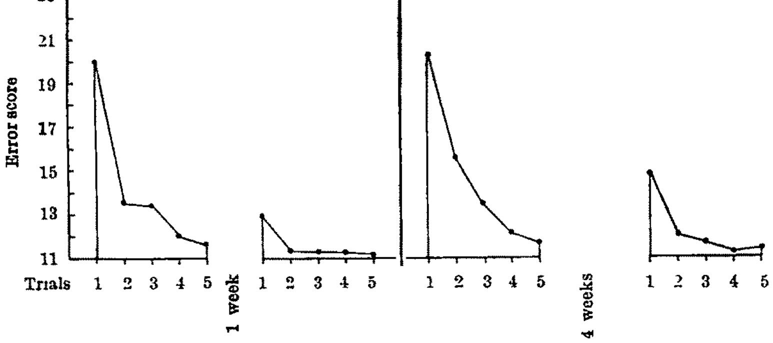

Fig 5 Mean error scores for each trial in normal subjects Group I ($N = 8$) were re-tested after 1 week, and Group II ($N = 10$) after 4 weeks

We also prepared a series of graduated incomplete words Five letter words, all of high frequency in the Thorndike–Lorge word count, were photographed through patchwork filters of varying density Ten sets were prepared, with three incomplete versions of each word (Fig 2)

A testing situation was devised in which the recognition of incomplete pictures and words could be used as a measure of learning and retention The most incomplete drawing was presented first, and then the succeeding versions, until there was a correct recognition or identification A recognition error score was established on the first trial and the series was immediately repeated and the reduction in errors noted. All subjects were tested on 3 successive days, five trials were given on each day The total error score on each trial was recorded The ten sets of each test were always presented in the same order, each subject doing the incomplete pictures first The incomplete word test was done by two amnesics and two controls immediately after the incomplete picture test Two amnesics and two controls were further tested, starting on the third day, using the same procedure with an equivalent but new series of incomplete pictures

The mean score for each trial for each group of patients on the incomplete picture test is shown in Fig 3, and for the incomplete word test in Fig 4 It is evident that learning proceeded rapidly and smoothly in both control subjects and amnesic patients, although the amnesics were slower The controls showed almost perfect retention 24 and 48 h later The amnesic group, although impaired in comparison with the control group, nevertheless showed retention on the second and third days For both types of material the scores obtained on the second and third days were better than at the starting point of the previous day, that is, in re-learning some saving was demonstrated

Two amnesic and two control patients were tested on a new but equivalent series of incomplete pictures The performance was comparable with their original scores in terms of initial speed of learning and retention between days It cannot therefore be argued that the improvement within days and the retention between days reflect a non-specific sensitization for similar types of material—that is, that the subjects simply became better at "guessing" independently of the specific content of the test

Retention and re-learning over an interval of 1 week and 4 weeks in a group of eighteen normal subjects for incomplete words (eleven sets) is shown in Fig 5, which demonstrates that retention is good over relatively long periods One amnesic patient (N T) was re-tested on the incomplete pictures after an interval of 3 months, comparing the summed error scores for the first five trials on each occasion, there was a saving of 20 per cent after 3 months

In amnesic patients, the striking aspect of the results is the excellence of retention between days The words, all of high frequency in the Thorndike–Lorge test, were also used in more conventional tests of recall and recognition, and very poor retention was found after delays as short as 5 min[5]

Meanwhile, it is clear that evidence for some (if incomplete) retention from day to day can be found in severely amnesic patients, and therefore the characterization of their defect as a rapid decay of traces is not easy to maintain, and the hope[6] that the rapid forgetting curves of amnesic patients could be used to generate the shape of the short-term trace seems forlorn in the face of the evidence reported here

Significant retention from day to day by amnesic patients has been reported for motor tasks, simple stylus mazes and stories[1,2] and now for the present type of task The question whether these tasks have anything in common other than being favourable for good retention in normal subjects remains to be seen Measurable retention is certainly not restricted to examples in which each learning trial can be encompassed by the immediate memory span, as suggested by Milner[2] for her simple stylus maze, because our perceptual tasks exceeded the immediate memory span.

Talland[1] and Williams[7] used a similar approach to investigate recognition in patients with cerebral dysfunction, and found that pre-exposure to fragmented drawings or "perceptual prompts" improved performance There are several features which distinguish this type of test from more conventional memory tests, and at this stage one can only speculate as to which factors are important in accounting for the difference in performance Further work is planned to elucidate this question. It is, however, worth mentioning that in addition to the rapidity and uniformity in learning this task, patients find it a much less exacting test than more conventional ones They treat it more as a "guessing game" than a formal test of memory. The test might therefore commend itself for use with a broader class of patients

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[1] Talland, G A , *Deranged Memory* (Academic Press, 1965)

[2] Milner, B , in *Analysis of Behavioural Change* (edit. by Weiskrantz, L) (Harper and Row, 1968, in the press)

[3] Dimsdale, H , Logue, V , and Piercy, M., *Neuropsychologia*, 1, 287 (1964).

[4] Gollin, E. S , *Percept Mot. Skills*, 11, 289 (1960)

[5] Warrington, E. K , and Weiskrantz, L , *Neuropsychologia* (1968, in the press)

[6] Weiskrantz, L , in *Amnesia* (edit. by Whitty, C W , and Zangwill, O L) (Butterworth, 1966)

[7] Williams, M , *J. Neurol. Neurosurg. Psychiat.*, 16, 14 (1953).

CYTOLOGY

Mitotic Activity in the Primate Subependymal Layer and the Genesis of Gliomas

Globus and Kuhlenbeck[1,2] drew attention to the presence beneath the lining of the cerebral ventricles of a distinct cell layer, which they named the subependymal cell plate They demonstrated it in the brain of human infants, showed it to contain primitive cells and stressed its possible role in the development of certain paraventricular neoplasms Various workers[3–5] have shown the persistence of a subependymal layer in man from foetal to adult life, but except for explaining the origin of "subependymomas"[6] the idea that this zone plays a part in the genesis of gliomas has met with little support

A mitotically active and well-defined subependymal layer is present in mammalian brains throughout life Allen[7] and Bryans[8] described mitoses in the lateral ventricular wall of adult rats Smart[9] used autoradiography to demonstrate proliferation of subependymal cells in the young mouse and their transformation into mature neuroglia, and Altman[10] has shown this to occur in cats Fischer[11] examined the brains of dogs aged up to 17 yr and found subependymal mitotic activity, and this phenomenon is thus common to different species at all ages

According to Kershman[12] the subependymal plate is a source of neurones and glia in early life In the embryo it is formed from the ependyma as a broad layer of neuroblasts and spongioblasts and it decreases in thickness in later foetal life, persisting after birth only as a thin, discontinuous lamina. In adult rodents the subependymal plate is situated in a rostral and dorsal position, with an extension into the olfactory lobe[9], its cells exhibit morphological (unpublished work of W. B Blakemore) and histochemical[13] signs of immaturity, show frequent mitotic figures and can divide and migrate away from the ventricle[10]

To what extent is the human subependymal layer comparable with this active and proliferative plate? Although many of the cells underlying the infant's ventricular surface are refractory to glial stains and are therefore considered to be immature[2], the presence of mitoses in this region has not been shown after birth Mitotic figures are seldom if ever seen in post-mortem cerebral tissue or non-tumour biopsy material from the brain[14].

An important reason for this is that fixation by immersion, the method commonly employed, allows many mitoses which have begun before death to go to completion, because diffusion of fixative through a piece of tissue may take longer than the mitotic cycle, which lasts between about 30 and 150 min in mammalian species[15]. In 1960 Cammermeyer[16] introduced perfusion fixation into neuropathological practice, with this technique virtually instantaneous brain fixation can be achieved and post-mortem changes can be reduced to a minimum This has led, among other things, to a reassessment of concepts of cell multiplication in the brain Lapham[17] provided evidence to support the idea that reactive neuroglia divided by amitosis· while this may occur, it is now recognized that mitosis is the usual method of division in glia[18,19].

The apparent absence of mitoses in the human brain is an obstacle to the equating of the rodent with the primate subependymal layer, as well as to the acceptance of Globus and Kuhlenbeck's theory of the origin of certain gliomas, and so this study was carried out on the brains of monkeys fixed by perfusion Sections were examined microscopically for evidence of cell proliferation in the subependymal region

Five young (less than half grown) monkeys were killed with 'Nembutal' and decapitated, and their brains were immediately perfused through the carotid arteries with 1 per cent formol-acetic acid solution, perfusion continued for 30–40 min Coronal brain slices were made through the head of the caudate nucleus, the rostral part of the thalamus, the anterior commissure and the optic chiasma, and five paraffin sections of each slice, stained with haematoxylin and eosin, were scanned with a × 90 oil-immersion objective for mitoses in the paraventricular region. Sections of midbrain and hindbrain from one monkey were also examined

The findings were similar in all five animals A distinct subependymal layer is present in the monkey as a discontinuous band of cells beneath the surface of the ventricles, separated from the ependyma by a fibre layer of variable thickness It is most marked anteriorly in the lateral ventricles and is tenuous and sometimes difficult to recognize posteriorly and in the wall of the third and fourth ventricle and the aqueduct In addition to astrocytes and microglia the layer contains cells of an unusual type, with nuclei measuring 5·0–6 0μ × 3 5–4 5μ and profuse finely stippled chromatin without marginal clumping or a definite nucleolus, as in microglia These cells are most numerous in two prolongations of the subependymal layer in the anterior part of the brain, one dorso-lateral and spreading from the angle of the lateral ventricle between the undersurface of the corpus callosum and the caudate nucleus, and the other ventral and passing below the head of the caudate to the olfactory area Here the cells are often in nests, which are sometimes related to small blood vessels

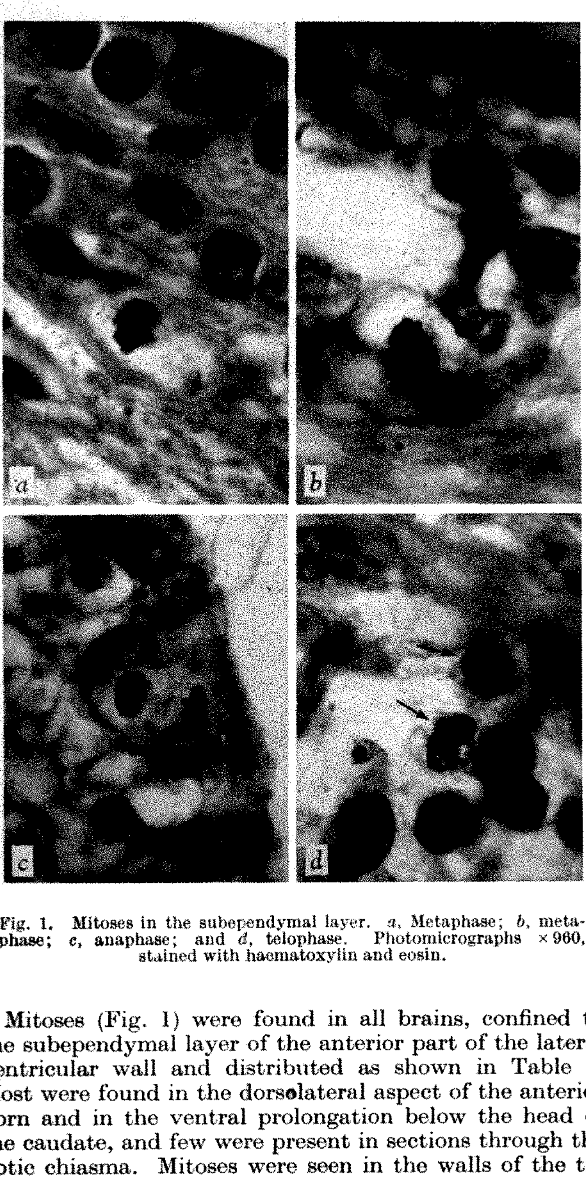

Fig. 1. Mitoses in the subependymal layer. *a*, Metaphase; *b*, metaphase; *c*, anaphase; and *d*, telophase. Photomicrographs × 960, stained with haematoxylin and eosin.

Mitoses (Fig. 1) were found in all brains, confined to the subependymal layer of the anterior part of the lateral ventricular wall and distributed as shown in Table 1. Most were found in the dorsolateral aspect of the anterior horn and in the ventral prolongation below the head of the caudate, and few were present in sections through the optic chiasma. Mitoses were seen in the walls of the tip of the temporal horn in three monkeys.

Table 1. DISTRIBUTION OF MITOSES IN THE SUBEPENDYMAL LAYER

Monkey	Sex	Weight (kg)	No. of mitoses in lateral ventricular wall					
			Dorsal	Lateral	Medial	Ventral	Temporal	Total
Patas 1	M	2·9	2	12	0	2	1	17
Patas 2	M	2·6	3	9	0	6	1	19
Patas 3	M	2·5	2	3	1	9	0	15
Patas 4	F	2·5	0	11	0	2	1	14
Cynomolgus	?	2·5	0	2	0	7	0	9

These findings suggest that the rodent and primate subependymal layers are homologous. Their distribution is similar, with thickenings of the periventricular layer extending between the caudate nucleus and the corpus callosum and in the wall of the remnant of the obliterated olfactory ventricle, rostral in the rodent[9] and ventral in monkey and man. In addition they have similar histology and contain, besides active astrocytes, characteristic immature cells with small dark nuclei, distinguishable ultrastructurally from microglia (unpublished work of W. B. Blakemore) and frequently lying in small nests[20]. The number of mitoses per section is considerably smaller in the monkey than in the rat. This may reflect the relatively diffuse nature of the subependymal layer in the primate and explain why Noetzel and Rox[20] did not find selective uptake of tritiated thymidine in this zone in rhesus monkeys.

Does similarity in structure imply similarity of function? In newborn rats[21] and mice[9] subependymal cells divide, migrate and mature into neurones and glia. An autoradiographic study of adult rats (my unpublished work) provides evidence that astrocytes, oligodendroglia and microglia are derived from the cells of the subependymal layer, which are thus primitive and pluripotential. The weight of the rat's brain increases throughout life[22] and the marked subependymal proliferation in rodents may be an indication of ever-increasing glial number. In man, adult brain weight is reached by the time of adolescence[23] and the relatively low mitotic index may be a sign of a smaller degree of neuroglial proliferation. The pluripotential nature of the subependymal cells suggests that they could be a possible source of different histological varieties of glioma, in particular those tumours in a paraventricular situation and "butterfly" gliomas of the corpus callosum. A scrutiny of these neoplasms in the light of the present findings may lead to a reappraisal of Globus and Kuhlenbeck's theory.

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[1] Globus, J. H., and Kuhlenbeck, H., *Arch. Pathol.*, **34**, 674 (1942).
[2] Globus, J. H., and Kuhlenbeck, H., *J. Neuropathol.*, **3**, 1 (1944).
[3] Rydberg, E., *Acta Pathol. Microbiol. Scand.*, Suppl. 10 (1932).
[4] Schwarz, H., Goolker, P., and Globus, J. H., *Amer. J. Dis. Child.*, **43**, 889 (1932).
[5] Opalski, A., *Z. Ges. Neurol. Psychiat.*, **149**, 221 (1934).
[6] Scheinker, I. M., *J. Neurosurg.*, **2**, 232 (1945).
[7] Allen, E., *J. Comp. Neurol.*, **22**, 547 (1912).
[8] Bryans, W. A., *Anat. Rec.*, **133**, 65 (1959).
[9] Smart, I., *J. Comp. Neurol.*, **116**, 325 (1961).
[10] Altman, J., *Anat. Rec.*, **145**, 573 (1963).
[11] Fischer, K., *Acta Neuropathol.*, **8**, 242 (1967).
[12] Kershman, J., *Arch. Neurol. Psychiat.*, **40**, 937 (1938).
[13] Friede, R., *J. Comp. Neurol.*, **116**, 229 (1961).
[14] Spielmeyer, W., *Histopathologie des Nervensystems*, **1** (Springer, Berlin, 1922).
[15] Baserga, R., *Cancer Res.*, **25**, 581 (1965).
[16] Cammermeyer, J., *Exp. Neurol.*, **2**, 379 (1960).
[17] Lapham, L. W., *Amer. J. Pathol.*, **41**, 1 (1962).
[18] Sjöstrand, J., *Z. Zellforsch.*, **68**, 481 (1965).
[19] Kreutzberg, G. W., *Acta Neuropathol.*, **7**, 149 (1966).
[20] Noetzel, H., and Rox, J., *Acta Neuropathol.*, **3**, 326 (1964).
[21] Altman, J., and Das, G. D., *J. Comp. Neurol.*, **126**, 337 (1966).
[22] Donaldson, H. H., *Mem. Wistar Inst. Anat.*, No. 6 (1915).
[23] Coppoletta, J. M., and Wolbach, S. B., *Amer. J. Pathol.*, **9**, 55 (1933).

BIOLOGY

Integumental Pigments of the Millipede, *Polydesmus angustus* (Latzel)

So far little is known about the tissue pigments of the myriapods[1], except that there is xanthommatin or a nearly related ommatin in some iulids[2] and a water-soluble, violet pigment in some opisthogoneates[3]. The polydesmids, although rather closely related to the iulids, contrast sharply in morphological details and colour, which prompted this investigation of the integumental pigments of *P. angustus*. These differ from those of *Iulus* even more than anticipated.

The animals were eviscerated and divided into pieces two or three metameres long. Treatment with such solvents as acetone indicated that there were no significant amounts of common lipochromes and so the same sequence of extractants was used as for the tissue pigments of leeches[4]: that is, water, 0·1 N HCl, N HCl and finally N HCl in absolute methanol. These extractants seemed to extract with increasing efficiency each of the three pigments recognized; MeOH–HCl eventually removed all detectable colour from the carcass.

Two chief pigments were found, both pink in colour, as well as a yellow minor constituent. An intense crimson fluorescence in ultraviolet light was associated with one of the pink pigments, which seems to be coproporphyrin. The other pink pigment is probably a derivative of this porphyrin, such as a porphyran (metalloporphyrin) or a complex of this with a nitrogen compound (for example, a haemochrome); it retains the Soret absorption band but lacks the ultraviolet fluorescence. Porphyrins and their derivatives have not previously been found as integumental pigments in arthropods. Usually there was no trace of fluorescence in the living animal so that the porphyrin is probably protein-bound *in situ*.

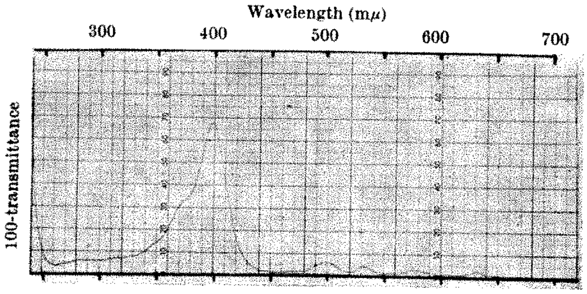

Fig. 1.

The principal peaks in its absorption spectra (Fig. 1) in various solvents were as follows: 283, 403, 551 and 593 mμ for N HCl; 282, 398, 498, 530, 572 and 627 mμ for diethyl ether; 401, 499, 533, 568 and 625 mμ for chloroform; 382, 396, 498, 534, 563 and 614 mμ for N KOH. Some of these values differ in detail from published figures for coproporphyrin[5–7], but the colour is that of coproporphyrin and the "HCl-numbers" of the free porphyrin and its methyl ester were found to be 0·1 and 1·8 per cent, respectively, which are near the expected values for coproporphyrin.

The porphyran was best isolated by partitioning into carbon tetrachloride from methanolic HCl, or into ether from aqueous HCl by cautiously adding sodium acetate, insufficient to drive up the free porphyrin. In CCl_4 its absorption spectrum (Fig. 2) showed peaks at 335, 402, 528 and 567 mμ, that at 402 mμ being a typical Soret, much sharper and more intense than the others, and virtually in the same position as that of the porphyrin in chloroform. The positions of the other peaks, however, differed by 10 mμ, or more, and that at 567 mμ was higher than that at 528 mμ. In ether the Soret peak was at 398 mμ, again in the same position as that of the porphyrin, and the others were at 526 and 564 mμ. In both solvents there was also a peak at 333 mμ. The suspicion that it is a derivative of the porphyrin, of the type suggested, was confirmed by the appearance of the typical porphyrin ultraviolet fluorescence on treatment with concentrated H_2SO_4. The absorption spectrum in dilute H_2SO_4 was virtually identical with that of the native porphyrin in HCl. The artefact porphyrin partitioned into ether, using the sodium acetate method, and in this solvent the spectrum was again identical with that of the native porphyrin.

The porphyran closely resembled protoferrihaem[4] in partitioning directly into CCl_4 from methanolic HCl, without adding water, and iron could be detected, using *iso*-amanol to increase the sensitivity of the familiar ammonium thiocyanate test[8]. This *Polydesmus* pigment therefore is probably a coproferrihaem derivative, if not the ferrihaem itself. It differs from protoferrihaem[4] in retaining, scarcely shifted or otherwise modified, the Soret peak of the free porphyrin. The high solubility in such a relatively apolar solvent as CCl_4 rules out any very hydrophil nitrogenous conjugant, particularly any large molecule such as a protein.

This association between a porphyrin and its non-fluorescent derivative, as integumental pigments, resembles that between protoporphyrin and its ferrihaem in earthworms[4], where the porphyran sometimes contributes most of the visible colour. The present derivative, however, is almost as delicately coloured as the free porphyrin, as indicated by its very low peaks in the visible range (Fig. 2). In the tissues of polychaetes, again, porphyrins in association with their iron porphyrans are very common[7], and the copro III pair are perhaps the most widespread.

It is difficult to free the *Polydesmus* porphyrin completely of the porphyran, although the latter is relatively easily obtained quite free of the porphyrin; that is, of the red ultraviolet fluorescence. After two passages from HCl solution to ether and back, with thorough washing at each stage, there was still demonstrable iron present, although the amount did decrease by a large factor at each stage. Also at each stage non-fluorescent pigment was left behind. Protoporphyrin appears to be more easily cleared of protoferrihaem.

The yellow pigment remained in aqueous media after driving out the porphins, and it was possible to partition it into butanol, carbon tetrachloride and ether. It had a relatively sharp peak in light absorption at 459 mμ, in all of these solvents and in methanolic HCl, and it may also have spectral features in the ultraviolet range. A moderate blue-white ultraviolet fluorescence was associated with it, and furthermore it showed some resemblance to a water-soluble yellow pigment extracted from *Hirudo* and other leeches[4], which may be a porphyrin derivative. The present pigment may therefore be related to the two porphins. The peak in light absorption at 459 mμ is near that of xanthommatin[2], but this pigment did not show the characteristic redox colour change of the ommatins. Reciprocally the iulids examined have yielded very little porphyrin—in fact, less than most invertebrates—whereas *Polydesmus* has an unusually high content. The two diplopods thus further emphasize the fact that the integumental pigments of animals vary more than most classes of biochemical materials, particularly in the lower taxa. For comparative biochromatology, it is therefore not enough to study a few species in each phylum.

Like the other tracheate arthropods, *Polydesmus* has no evident circulatory pigment and the presumption must be that its integumental porphins are synthesized in the same pathway as are the cytochromes and other tissue haemoproteins. This pathway does not usually produce such large amounts of by-products and the polydesmid integumental pigments would seem to have become an end in themselves.

In conclusion, the integumental pigments of *Polydesmus* are (*a*) coproporphyrin, (*b*) a non-fluorescent derivative

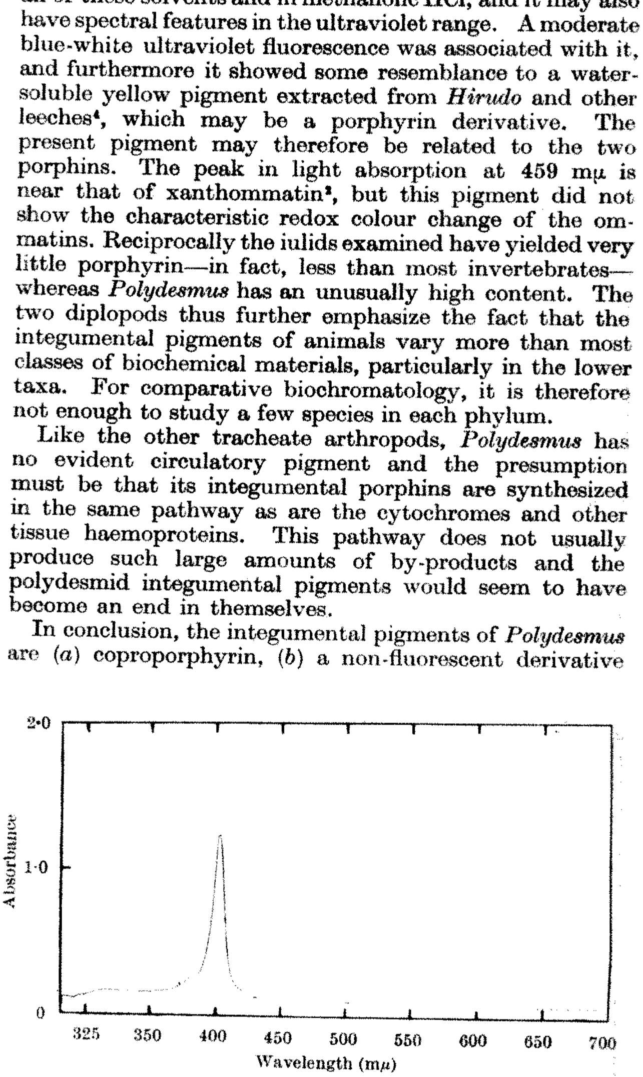

Fig. 2.

soluble in carbon tetrachloride, and (*c*) a yellow pigment, possibly a derivative of the same metabolic pathway.

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[1] Fox, H. M., and Vevers, G., *The Nature of Animal Colours* (Sidgwick and Jackson, London, 1960).
[2] Needham, A. E., and Brunet, P. C. J., *Experientia*, **13**, 207 (1957).
[3] Needham, A. E., *Comp. Biochem. Physiol.*, **1**, 72 (1960).
[4] Needham, A. E., *Comp. Biochem. Physiol.*, **18**, 427 (1966).
[5] Lemberg, R., and Legge, J. W., *Haematin Compounds and Bile Pigments* (Interscience, New York, 1949).
[6] Kennedy, G. Y., and Dales, R. P., *J. Mar. Biol. Assoc. UK*, **37**, 15 (1958).
[7] Mangum, C. P., and Dales, R. P., *Comp. Biochem. Physiol.*, **15**, 237 (1965).
[8] Charlot, G., and Bezier, D., *Quantitative Inorganic Analysis* (trans. by Murray, R. C.) (Methuen, London, 1957).

Familial Behaviour of *Tilapia* (Pisces, Cichlidae) and its Implications

Two types of familial organization are known in *Tilapia* and in cichlid fishes in general[1]. In the biparental type, which is considered to be the more primitive[2,3], both partners participate in digging the nest and defending the territory. The eggs are deposited on the ground ("substratum spawners") and both parents take care of them and of wrigglers and fingerlings for days or even weeks. Hundreds of young form a group which stays within the limits of the territory, and which is kept together by special movements of the parents. The bond between the parents is very strong[4,5].

In most oral incubators, different males in a number of territories are successively visited by different females, whose eggs are thus fertilized in small batches[6]. As soon as a male has emitted his milt, the eggs are picked up in the female's mouth and she leaves the territory and retreats from the spawning ground to another area where she incubates the eggs orally. When they have absorbed their yolk sacs, the fingerlings can swim about freely, but they stay in a group near the female, following all her movements and retreating into her mouth when there is danger. By this time this familial group has generally reached shallow waters sheltered by plants.

Recent work[7] has revealed something of the nature of the bond between the young and their parent(s). Batches of young fish, reared without parents, were exposed at different times to moving models. At about the age at which the rejection normally occurs the young of both *T. mossambica* Peters and *T. macrochir* Blgr., both of which are maternal brooders, immediately showed an orientation and following response when exposed to the moving model. This response was established at the time when the yolk sac was nearly reabsorbed (9–12 days after spawning) and was maintained until the time at which the young usually become independent of their mother (30 days after spawning). If, however, the first exposure was made later (22–23 days after spawning), it was impossible to evoke a positive reaction; on the contrary, fear and escape behaviour occurred. This indicates that there is a precocious sensitive period for the establishment of the familial attachment which suggests that young are really imprinted in maternal-brooders.

In species of the biparental familial type (*T. guineensis*) territorial behaviour is shown until the young become independent; fingerlings do not show such an innate disposition to form a precocious attachment to the first moving object they meet. They can learn to follow a model, but only after several hours' exposure, so that it is impossible to determine the learning process responsible for the establishment of the attachment. Finally, in *T. galilaea* the young react towards the moving model in a manner similar to *T. guineensis*. From an ethological point of view, this species is intermediate between the two principal ethological groups: both parents incubate the eggs and the fry orally, but they are territorial until the young become independent.

In substratum spawners, the young are born and develop at the place where their parents met, and they seem to be more linked to the territory and the substratum itself than to their parents. On the other hand, with females which incubate their young orally the young do not know the habitat where their parents met, for they are born away from the spawning ground. It is essential therefore for the young to be strongly linked to the incubating parent so that they can follow this parent when it moves.

We can assume that the acquisition of oral incubation and both peregrinating behaviour in the incubating parent and imprinting in the young represent a progressive step resulting in a greater independence of the fish from one type of habitat. This facilitates adaptive radiation, which in turn facilitates speciation. Most *Tilapia* species are known to be oral incubators, and only a few are substratum spawners[2,3].

In African waters where water level is periodically lowered, as in artificial lakes producing hydroelectric power, the substratum spawners are often obliged to leave their territory, whereupon the eggs and the fry are destroyed and dried up. If they are free-swimming fingerlings, they stay within the limits of the territory and do not follow the retreating parents. On the other hand, there is no danger to the oral incubators, because the females leave the spawning grounds in search of deeper waters with their eggs and fry in their mouth and retreat with their fingerlings[8]. We know that the water levels of several African natural waters, such as Mweru Wantipa[9], are sometimes lowered; oral incubator species are better armed to face these fluctuations. With this in mind it has been suggested that ponds or artificial lakes which show fluctuations of water level should be charged with oral incubators only[8].

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[1] Baerends, G. P., and Baerends-Van Roon, J. M., *Behaviour*, Suppl. 1 (1950).
[2] Lowe, R. H., *Proc. Zool. Soc.*, **132**, 1 (1959).
[3] Thys, D. E. F., *Bull. Séances ARSOM*, **3**, 570 (Brussels, 1963).
[4] Voss, J., and Ruwet, J. Cl., *Ann. Soc. Roy. Zool. Belg.*, **96**, 2, 145 (1966).
[5] Ruwet, J. Cl., and Voss, J., *Bull. Soc. Roy. Sci. Liège*, **35**, 11, 778 (1966).
[6] Ruwet, J. Cl., *Behaviour*, **20**, 3, 242 (1963).
[7] Destexhe, F., and Ruwet, J. Cl., *Ann. Soc. Roy. Zool. Belg.*, **97**, 3, 161 (1967).
[8] Ruwet, J. Cl., *Rev. Zool. Bot. Afric.*, **66**, 3, 243 (1962).
[9] Brelsford, W. V., *The Northern Rhodesian Journal*, 3 (Nov. 11, 1955).

Mitotic Chromosomes of *Glossina pallidipes* Austen

THE chromosomes of several species of tsetse-fly have been studied by Itard[1]. These include *Glossina tachinoides* Westwood and *Glossina morsitans orientialis* Vanderplank (*Glossina morsitans morsitans* Westwood in the terminology adopted by Itard). *Glossina morsitans orientalis* is one of the two main tsetse-flies which occur in Rhodesia, and so I thought it worthwhile for comparison to study the other, *Glossina pallidipes* Austen. The *G. m. orientalis* material used by Itard originated in Rhodesia.

Glossina pallidipes puparia were supplied by Dr R. J. Phelps and G. J. W. Dean of the Agricultural Research Council in Rhodesia. This material originated from collections of puparia from natural pupation sites near Kariba Township in the Zambezi Valley. The puparial contents were thus aged from 24 h (the time from the last collection to the arrival of the puparia at the laboratory) upwards, although the exact age of

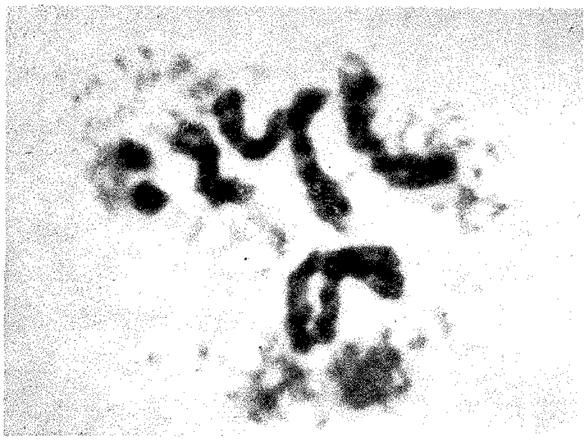

Fig. 1. Normal chromosomes of *Glossina pallidipes* from brain of puparial contents. Two large pairs, one medium and one small, are present. (× 2,500.)

puparia was unknown. The procedure of Itard[1] was followed, except that propionic acid replaced acetic acid wherever the latter had been used, staining being allowed to proceed overnight and the material mounted in 2 : 3 lactic acid : propionic acid.

Good results were obtained up to a month after fixation of the brains in propio-alcohol, a factor which could be useful when collections are made in remote areas.

Glossina pallidipes has four pairs of chromosomes: two long, one medium and one short (Fig. 1). All of the three larger chromosome pairs are metacentric, an interesting departure from the situation in *Glossina tachinoides* and *Glossina morsitans orientalis*, where one of the three larger pairs is said to be sub-acrocentric.

The average absolute lengths of the chromosomes are given in Table 1. The two larger chromosome pairs are treated together (described later). Table 1 also shows the relative lengths of the chromosomes, which provide a better basis for the comparison of chromosomes than do the absolute lengths. The relative length of a chromosome is given by the actual length expressed as a percentage of the total diploid chromosome length.

The small and medium pairs of chromosomes in *Glossina pallidipes* are easily recognizable by their size. The similar lengths of the two large pairs make it impossible to distinguish between them with certainty. One is usually longer than the other, but variation is such that one cannot be sure that the longer is always the same chromosome. It has not been possible to detect any constant morphological feature to aid in identification. The banding which shows up in occasional figures (see Figs. 1 and 2) might be of use in this way if it could be routinely shown up in preparations. The frequency distribution of the lengths of fifty-eight large chromosomes from eighteen figures derived from seven puparial contents is shown in Table 2.

Table 1. LENGTHS OF *G. pallidipes* CHROMOSOMES

	Chromosome:	small pair	medium pair	large pairs
Actual length (μ)	Mean (n)	1·68 (27)	5·72 (31)	7·80 (58)
	Standard error	0·1106	0·2189	0·3264
Relative length (%)	Mean (n)	3·7 (30)	12·3 (36)	16·9 (64)
	Standard error	0·2821	0·2607	0·2470

Table 2. RELATIVE LENGTH FREQUENCY DISTRIBUTION OF LARGE CHROMOSOME PAIRS

Class	13·0–13·9	14·0–14·9	15·0–15·9	16·0–16·9	17·0–17·9	18·0–18·9	19·0–19·9	20·0–20·9	21·0 21·9	22·0–22·9	23·0–23·9
No.	4	8	8	15	8	13	5	0	2	0	1

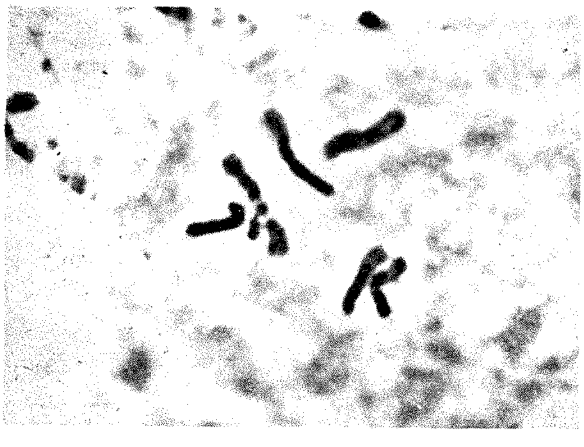

Fig. 2. Unusual chromosome complement from brain of puparial contents of *Glossina pallidipes*. Only three large chromosomes are normal, the fourth is clearly sub-acrocentric. Between the sub-acrocentric chromosome and the nearest large metacentric chromosome can be seen the single small chromosome; the other member of the pair is absent. (× 2,000.)

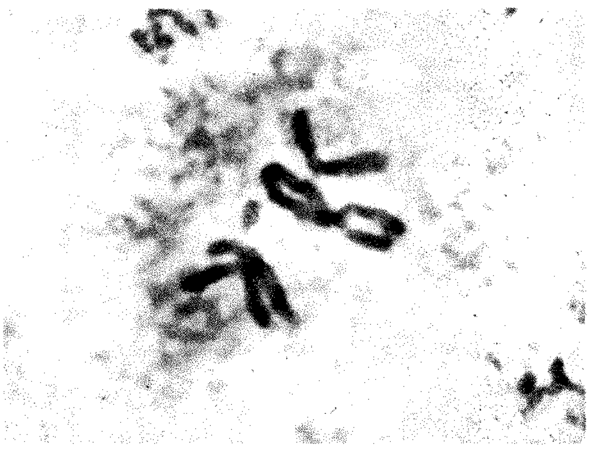

Fig. 3. Unusual chromosome complement from brain of puparial contents of *Glossina pallidipes*. Only one member of the small pair of chromosomes is present. One member of the medium pair is masked by the other which lay above it. (× 2,500.)

There is a hint of bimodality, which may be shown to be real if large numbers of chromosomes were measured. This was not undertaken because the results of such an exercise would not help in identifying the large chromosomes in any one figure, even if bimodality were established. The measurements of the two largest pairs of chromosomes have thus been lumped and the chromosomes treated as if of equal length. This seems to be the only way of dealing with these chromosomes at present.

Some variability seems to occur in the chromosomes of *Glossina pallidipes*. Two unusual chromosome complements are shown in Figs. 2 and 3. Each of these was seen in numerous figures and cannot be a result of some accident in preparation of the material. Fig. 2 shows a complement in which one member of the small chromosome pair is missing, and one member of a large pair is a sub-acrocentric chromosome. A similar situation was seen in another brain, except that both small chromosomes were in this case present. Although this could clearly be seen, no figure gave satisfactory photographs for illustration. In Fig. 3 is seen an otherwise normal complement where one member of the small chromosome pair is missing. (The apparent lack of a medium chromosome is a result of the screening of one member of the pair by the other lying above it.) Itard[1] mentions an instance (an abortive "pupa") in *Glossina morsitans orientalis* where an extra small chromosome was present. Three unusual chromosome complements in the comparatively small sample carefully investigated (many of the field-collected puparia were of unsuitable age; completely satisfactory preparations from twenty-five puparia, each showing a number of figures, comprised the actual sample size) indicate that considerable variability in chromosomal complement and structure might be found to exist in *Glossina pallidipes*.

Itard[1] has shown that there is a difference in chromosome number between *Glossina morsitans orientalis* ($2n = 10$) and *Glossina tachinoides* ($2n = 6$) which belong, respectively, to the "*morsitans*" and "*palpalis*" groups[2]. The present study has shown that the chromosome number ($2n = 8$) of *Glossina pallidipes* ("*morsitans*" group) differs from both. There are thus differences in chromosome number between species belonging to the same group as well as between species belonging to different groups. The work on these three species thus indicates that there might perhaps be found to be less correlation between chromosome morphology and the taxonomic entities comprising the tsetse-fly groups than one might have hoped. More data, however, are necessary before conclusions can be drawn.

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[1] Itard, J., *CR Acad. Sci.*, **263**, 395 (1966).

[2] Buxton, P. A., *The Natural History of Tsetse Flies* (Lewis, London, 1955).

Accumulation of Dry Matter by *Chrysanthemum morifolium* after Flower Removal

Sweet and Wareing[1] have recently discussed the role of plant growth in controlling the rate of photosynthesis by increasing the translocation of assimilates from the leaves. We have worked with *Chrysanthemum morifolium* grown as a flowering pot plant[2] in conditions in which the flowers became an important sink for assimilates. We have examined the effect of removing these sinks on the accumulation of dry matter and our results seem to illustrate a system in which the rate of accumulation of dry matter is not affected by the removal of an important sink.

Rooted cuttings of the cultivar 'Bright Golden Anne' were potted into John Innes No. 1 compost on May 18, 1967, and raised in a glasshouse under 8 h days at a minimum night temperature of 15·5° C. One week after potting, the growing point and young leaves were removed from all plants to induce lateral growth. After a further 2 weeks the lateral growths were reduced to three per plant and the axillary buds were removed from each lateral growth to leave a single terminal flower per branch.

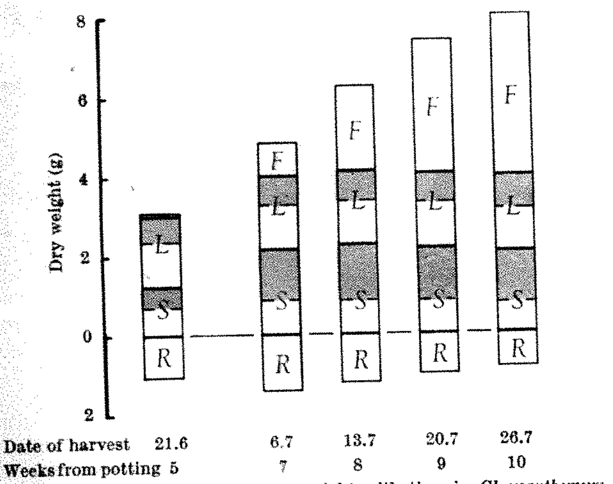

Fig. 1. The distribution of dry weight with time in *Chrysanthemum morifolium* cv 'Bright Golden Anne'. Means of five plants on each occasion. Shaded areas, branch; white areas, main axis. *F*, Flower; *L*, leaf; *S*, stem; *R*, root.

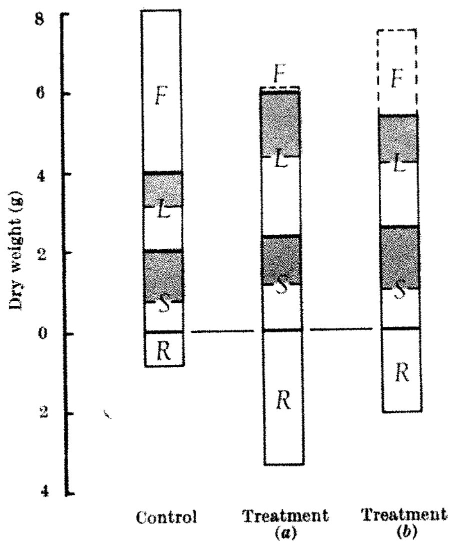

Fig. 2. The distribution of dry weight after flower removal in *Chrysanthemum morifolium* cv 'Bright Golden Anne'. Means of five plants. Control harvested July 26, 1967. Treatment (*a*): Flowers removed June 24, 1967, plants harvested July 27, 1967. Treatment (*b*): Flowers removed July 14, 1967, plants harvested July 27, 1967. Symbols as in Fig. 1. The dotted line encloses the weight of flower removed.

Five plants, chosen at random, were removed on each of five occasions (after 5, 7, 8, 9 and 10 weeks from potting up) and divided into leaf laminae, stems plus petioles, flowers, and roots. The leaves and stems were further subdivided into main axis and branch components. The fresh and dry weights of each of these fractions were measured together with stem length, and an assessment of flower development was made using the scale which we outlined earlier[2]. The data from these harvests are presented in Fig. 1 from which it is clear that as the terminal flowers developed they became almost the only site for the net accumulation of dry matter and the weight of the vegetative parts remained relatively constant. We also modified this pattern of accumulation by two treatments involving removal of flowers. In treatment (*a*) all three terminal flower buds were removed on June 24 when they were less than 15 mm in diameter (flower stages 2–3) and before they were the sole centres for the accumulation of dry matter. In treatment (*b*) terminal flowers were removed on July 14 when the flowers were expanding rapidly (flower stage 6–7) and when a pattern of diversion to flowers only was clearly established. It is worth noting that because all axillary buds had been removed there was no capacity for new aerial growth. Five plants were removed from each of these treatments on July 27 and divided as previously described. The data are presented in Fig. 2, together with the results obtained on July 26 from the plants with flowers.

These data show that if flowers were removed then an alternative existing sink assumed predominance and material was diverted in particular to the roots and to a lesser extent to the leaves. If the weights of flowers at the time of removal were added to the total weights of the flowerless plants at July 27, then the gross total of dry matter formed was similar in all cases. The leaves were almost fully expanded at the times when flowers were removed and at the end of the experiment the total leaf areas were similar in all three treatments.

The measurements of stem length in Table 1 show that the removal of the developing flower at stage 2 (treatment (*a*)) caused a cessation of stem extension although

Table 1. EFFECT OF FLOWER REMOVAL ON GROWTH OF THE LATERAL STEMS

Treatment	Harvest dates	Length (cm)	Weight (mg)	Wt./unit length (mg/cm)	Flower stage
With flowers	21.6.67	9·0	178	19·8	2·0
With flowers	6.7.67	15·4	435	28·2	5·5
With flowers	13.7.67	16·1	469	29·1	6·6
With flowers	20.7.67	16·2	429	26·5	8·0
With flowers	26.7.67	15·9	421	26·5	8·7
(*a*) Flowers removed June 24, 1967	27.7.67	11·0	404	37·8	—
(*b*) Flowers removed July 14, 1967	27.7.67	15·5	519	33·5	—

Means of three laterals per plant, five plants on each occasion.

the accumulation of dry matter continued and at the final harvest the dry weight of the lateral stems was similar to that of the controls with flowers. The weight of unit length of stem was therefore increased considerably. Thus extension growth was terminated by flower removal and did not seem to be affected by a continuing supply of dry matter. In the control plants, extension of lateral stems ceased when the flowers had reached flower stage 6 and the net accumulation of dry matter stopped at about the same time.

The evidence therefore suggests that when sinks are removed from the chrysanthemum without altering the area of the leaf surface (although the weight per leaf and possibly the thickness are changed), then there is no marked depression in the rate of accumulation per unit area of leaf surface and in the long term no great differences are observed in the total dry weight produced. Although short term changes may have occurred, the chief effect has been to direct materials to an alternative sink. A similar effect has been noted by Edelman[3] in the Jerusalem artichoke in which, in normal conditions, the growth of the aerial organs ceases and photosynthates are diverted to stem tubers. If these stem tubers are removed, then an alternative sink becomes predominant and the dry matter accumulates in and distends the fibrous roots. Thus the main change induced by the surgical removal of the principal sink has again been in the distribution pattern.

Although the total dry weights in Fig. 1 are similar, the total dry weight of plants with flowers was slightly less than the gross total for plants without flowers. This difference was probably the result of a combination of two factors. First, shading of the leaves by the opening flowers during the period from approximately July 13 to July 26 and, second, a reduction in the respiratory load of the treated plants because of the removal of flowers. This would give the maximum benefit to treatment (*b*) in which the weight of flowers removed exceeded 2 g.

In an earlier experiment, the rooted cuttings were potted into John Innes No. 1 compost on September 22, 1966. The cultural treatments were as described previously and the flowers were removed at flower stage 2–3 (November 17, 1966) when their contribution to total respiration was probably small. The plants were harvested after only 2 weeks of further growth (December 1) when the florets were just beginning to expand rapidly (flower stage 6) but before any appreciable shading of the leaf surface had occurred. In these conditions, the total dry matter produced by treated and untreated plants was the same after only a fortnight of further growth (Table 2). The distribution pattern had altered, however, and the roots had again become the most important sink for assimilates.

We conclude that growth in the chrysanthemum (in the restricted form we have studied) has been controlled by the rate of photosynthesis. The removal of the most important sink (flowers) permits the other sinks to control the distribution.

Table 2. DRY WEIGHTS (g) OF PLANT PARTS AT HARVEST

	Leaf	Stem	Root	Flower	Flower removed	Total
With flowers	1·69	1·77	0·77	1·02	—	5·24
Without flowers	2·04	1·83	1·26	—	0·16	5·29
Difference	0·36	0·06	0·49			

Means of five plants. Potted September 22, 1966. Flowers removed November 17, 1966. Harvest December 1, 1966.

We thank Professor O. V. S. Heath and Professor P. F. Wareing for discussion.

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[1] Sweet, G. B., and Wareing, P. F., *Nature*, **210**, 77 (1966).
[2] Cockshull, K. E., and Hughes, A. P., *Nature*, **215**, 780 (1967).
[3] Edelman, J., in *The Growth of the Potato*, 135 (Butterworths, London, 1963).

ANTHROPOLOGY

Fossil Hominoid Vertebra from the Miocene of Uganda

THE Miocene site of Moroto II in Karamoja, Uganda, was first described by Bishop and Whyte in 1962 (ref. 1). The only primate specimens so far recorded from this site are dental and cranial remains that have been assigned to *Proconsul major*, Le Gros Clark and Leakey[2,3]. Among fragmentary specimens from Moroto II in the Uganda Museum, Kampala, eight can be shown to be part of the vertebral column of a chimpanzee-sized ape. Four of the fragments, although collected in different years, combine together to make an almost complete lumbar vertebra—the first recorded not only for the *Proconsul* group but for any dryopithecine. The specimens are: *U.M.P.*67.28—an almost complete middle lumbar vertebra; *U.M.P.*68.05—a badly eroded middle lumbar vertebral body; *U.M.P.* 68.06—a last thoracic vertebral body; *U.M.P.*68.07—the laminae and base of spine of a lumbar vertebra; and

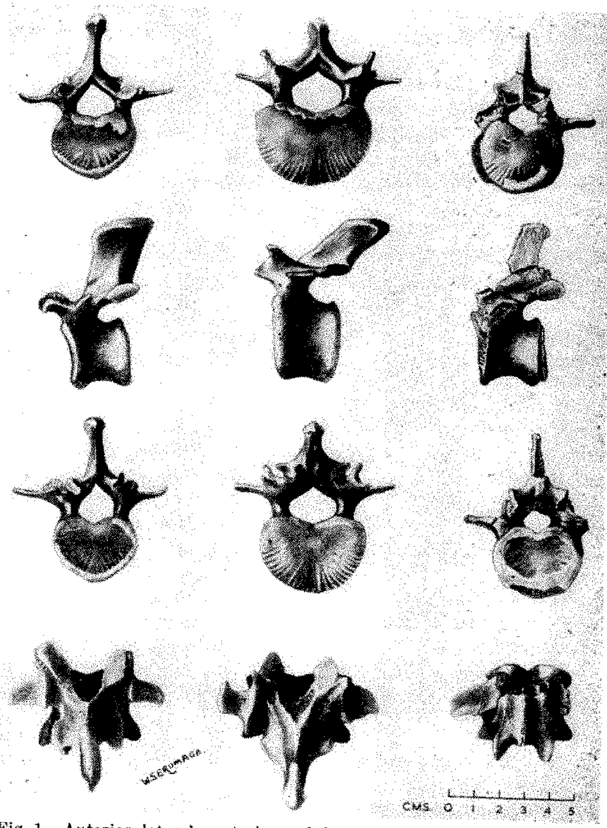

Fig. 1. Anterior, lateral, posterior and dorsal views (from above downwards) of first lumbar vertebrae of a chimpanzee (left) and a juvenile gorilla (middle) compared with the Moroto vertebra (right).

*U.M.P.*68.08—the laminae and base of spine of a thoracic vertebra.

The almost complete vertebra lacks the anterior articular processes, the right transverse process, and the tip and both anterior and posterior borders of the spine. The body is robust and presents reniform anterior and posterior surfaces, with the anterior surface being more nearly circular than the posterior. The posterior surface shows a well defined and fused ring epiphysis (as seen in *Homo* and the modern pongids but not in monkeys), but the anterior epiphysis has been mostly broken away to reveal the radiating rugae on the body proper. There is a slight ventral roughening for the longitudinal ligament. The body is distinctly "waisted", expanding anteriorly and posteriorly but not dorsally in the midline. This expansion accentuates the reniform outline of the anterior and posterior surfaces, narrows the vertebral foramen anteriorly and makes the posterior vertebral notch shallow dorso-ventrally. The pedicles are extremely thick and elongated antero-posteriorly, with the result that the posterior vertebral notch is shallow antero-posteriorly and the vertebral foramen further narrowed. The ventral part of a small anterior vertebral notch is evident on the left. The transverse process is triangular in section with a dorsal flattened surface, and springs from near the base of the pedicle. The posterior articular processes are short and nearly parallel. The articular facets are almost circular in outline and face ventro-laterally at an angle of about 60° to each other. They are gently convex transversely and almost totally overhang the posterior border of the body. The spine is long, slender and directed slightly posteriorly. A slight thickening at the dorsal extremity is taken to represent the base of the tubercle. Although both the anterior and posterior borders are eroded or missing, it is clear that the spine was a thin triangle in section, the apex anteriorly. There are two conspicuous pits at either side of the base of the spine at the anterior border of the laminae and two elliptical depressions on the neural surfaces of the laminae which represent the attachment of strong ligamenta flava. The anterior notch between the laminae is small and does not extend posteriorly to the extent seen in modern pongids. Measurements are given in Table 1.

The complete lumbar vertebra is of the same size as those of an adult male chimpanzee (*Pan troglodytes*). The only cranial remains of any primate from the site are those of *Dryopithecus* (*Proconsul*) *major*, which was the size of a large male chimpanzee, so it seems reasonable to assign these vertebral remains to the same species. In most important respects the lumbar vertebra resembles those of *Pan* and *Gorilla*, but possesses some hominid features. In the very narrow vertebral foramen, the extent and small size of the anterior laminar notch and the steepness of the articular surfaces, this specimen resembles the condition seen in *Homo* rather than in the pongids. In the long, thin spine and the thin transverse process, the Moroto vertebra resembles those of *Pan*. In the stoutness and position of the pedicles and in the detailed marking and positions of the pits for the ligamenta flava this vertebra is much more like those of *Gorilla*. There are therefore quite eclectic resemblances to modern hominoid vertebrae to be seen in the Moroto vertebra, but none of them would necessarily preclude *Dryopithecus* (*Proconsul*) *major* from being a direct ancestor of the genus *Gorilla*, a relationship which Simons and Pilbeam[4] support. Leakey[5], although not regarding the Moroto hominoid as *Dryopithecus* (*Proconsul*) *major*, nevertheless accepts the possibility of it being ancestral to *Gorilla*. It is hoped that a more detailed study will throw light on the varied resemblances of this vertebra.

Table 1. MEASUREMENTS (*U.M.P.*67.28)

	mm
Max. transverse diam. ant. surface of body	34·0
Max. dorso-ventral diam. ant. surface of body	29·0
Max. transverse diam. post. surface of body	37·0
Max. dorso-ventral diam. post. surface of body	27·0
Max. antero-posterior length of body	29·5
Total dorso-ventral diam. vertebra	64·0
Max. width vertebral foramen	11·0
Max. height vertebral canal	8·0

The other specimens are too fragmentary to add many data at this stage.

We thank the trustees and the curator, Mr C. Sekintu, of the Uganda Museum, and Dr W. W. Bishop for their kindness and assistance.

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[1] Bishop, W. W., and Whyte, F., *Nature*, **196**, 1283 (1962).

[2] Allbrook, D., and Bishop, W. W., *Nature*, **197**, 1187 (1963).

[3] Bishop, W. W., *Nature*, **203**, 1327 (1964).

[4] Simons, E. L., and Pilbeam, D. R., *Folia Primat.*, **3**, 81 (1965).

[5] Leakey, L. S. B., in *Classification and Human Evolution* (edit. by Washburn, S. L.), **32** (Aldine, 1963).

APPLIED SCIENCE

Wear of Carbon Fibre Reinforced Polymers in a High Vacuum Environment

THE friction and wear properties of carbon fibre reinforced polymers in dry conditions of sliding in air have recently been reported by Lancaster[1,2]. His findings, that the fibres reduced the wear rate and coefficient of friction of almost all the polymers studied, have promoted considerable interest in these materials. In addition, the resulting improvements in strength and stiffness properties suggest that they are attractive for use as self-lubricating components in aerospace engineering. The wear performance of a variety of these composites in a high vacuum environment has been investigated in this laboratory. It has been found that the addition of fibres can be effective in reducing the wear rate of the polymer in vacuum, but it is also evident that whereas in air rates of wear tend to decrease with increasing fibre content, in a vacuum high fibre concentration (40–50 per cent) is definitely deleterious to the wear performance of the composite.

Wear rates were determined by means of a pin and cylinder type wear rig. Hemispherically tipped cylindrical pins $\frac{1}{8}$ in. in diameter were machined from the polymer test specimens and loaded radially against a $\frac{3}{4}$ in. diameter stainless steel (*EN58BM*) cylinder, polished to 6 μ in. centre line average. This apparatus was enclosed in a bakable vacuum test chamber of 'Pyrex' glass and stainless steel construction, capable of being pumped to pressures in the region 10^{-9} to 10^{-8} torr. Vacuum processing was in accordance with high vacuum technique and contamination of surfaces was minimized by the use of oil-free pumping systems.

The wear rates obtained in vacuum for a number of different composites are given in the second column of Table 1. The carbon fibres in each composite were oriented randomly and are specified in weight percentage concentrations. All tests were conducted at the same sliding speed, 70 cm/s, and applied load, 120 g.

The extent to which the carbon fibre can reduce the specific wear rate of the polymer in vacuum is demonstrated in the case of acetal homopolymer where the reduction is approximately three orders of magnitude compared with the unfilled material. The wear rate for a polytetrafluoroethylene (PTFE)–glass fibre–molybdenum disulphide composite is also included in Table 1, because this is a self-lubricating material commonly used in vacuum

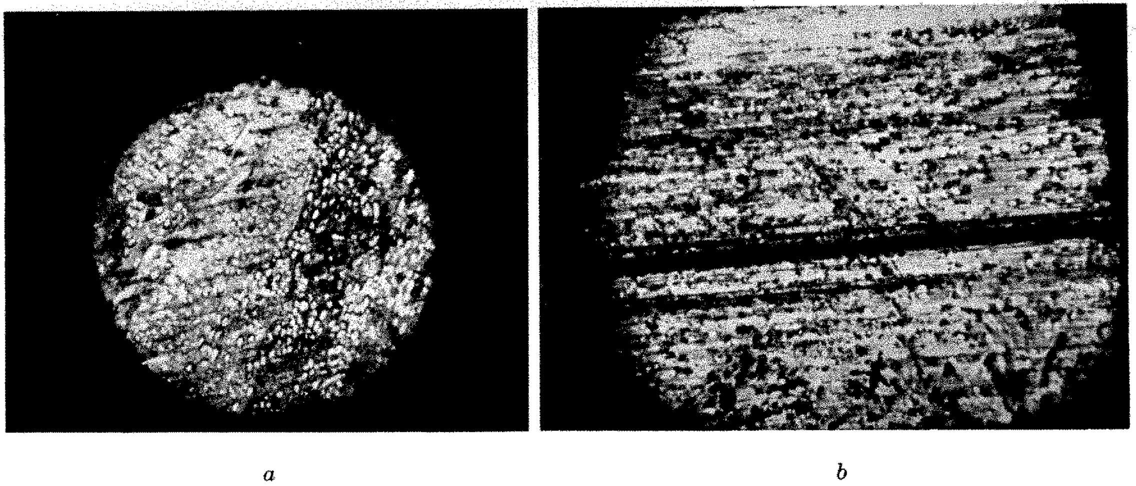

Fig. 1. Photomicrographs of the wear scars produced on riders of polyimide reinforced with 50 per cent by weight high modulus carbon fibres, sliding against *EN*58*BM* stainless steel (*a*) in air, (*b*) in vacuum of 10^{-8} torr. Load, 120 g; speed, 70 cm/s. (× 200.)

applications and therefore provides a convenient yardstick for comparison. Two of the carbon fibre composites have been found to exhibit greater wear resistance than this material.

Wear rates for the various composites were also determined in air. These results are presented in the third column of Table 1. The wear rates measured in air were usually substantially lower than for the same composites in vacuum. It is notable that for the lowest concentration of fibre used, that is, 15 per cent in acetal copolymer, there was virtually no difference in performance in the two environments, whereas the greatest differences were observed for the highest (45 per cent and 50 per cent) carbon fibre content materials. High fibre contents therefore tend to give a low wear rate in air but a high wear rate in vacuum. These findings are consistent with the established behaviour of carbons and graphites independently of the polymers. It is well known (see, for example, refs. 3 and 4) that carbons lose their lubricating ability in vacuum or dry atmospheres, becoming severely abrasive. It seems that at low concentrations any abrasive action attributed to the carbon fibres does not degrade the wear performance of the composite, and may even be beneficial in aiding the establishment of a thin, friction-reducing transfer film on the metallic surface. At high concentrations, however, the carbon abrasion becomes dominant in the wear mechanism with consequent degradation in the wear performance of the specimen.

Table 1. SPECIFIC WEAR RATES OF CARBON FIBRE FILLED POLYMERS AGAINST *EN*58*BM* STAINLESS STEEL IN VACUUM OF 10^{-8} TORR

Composite	Specific wear rate ($cm^3/cm/kg \times 10^{-10}$) Vacuum	Air
Acetal homopolymer + 25 per cent carbon fibre	0·06	0·03
Acetal copolymer + 15 per cent carbon fibre	0·13	0·14
Acetal copolymer + 45 per cent carbon fibre	0·58	0·025
PTFE + 25 per cent carbon fibre (Type 2)*	0·89	0·26
Nylon + 25 per cent carbon fibre	1·50	0·18
Polyimide + 50 per cent carbon fibre	230	0·01
PTFE + glass fibre + MoS_2	0·30	0·16
Acetal homopolymer (unfilled)	74	1·9

* Type 2, high strength fibres: ultimate tensile strength (UTS) $\simeq 410 \times 10^3$ lb. in.$^{-2}$; elastic modulus $\simeq 32 \times 10^6$ lb. in.$^{-2}$. Remainder, high modulus fibres: UTS $\simeq 280 \times 10^3$ lb. in.$^{-2}$; elastic modulus $\simeq 54 \times 10^6$ lb. in.$^{-2}$.

Table 2. INFLUENCE OF COUNTERFACE ON WEAR OF COMPOSITES IN AIR

	Specific wear rate ($cm^3/cm/kg \times 10^{-10}$) Stainless steel (*EN*58*BM*)	Mild steel (*BS*15)	Mild steel* (*BS*15)
Acetal copolymer + 15 per cent carbon fibre	0·14	2·4	0·9
Nylon + 25 per cent carbon fibre	0·18	0·9	2·0

*After Lancaster (personal communication).

Visual evidence supporting this conclusion is presented in Fig. 1, which shows photomicrographs of the scar areas produced on specimen pins of polyimide + 50 per cent carbon fibre after sliding in air and vacuum. The nature of the wear surface produced is clearly different in the two environments. Whereas the in-air sliding has resulted in a smooth, polished surface, in which the ends of the carbon fibres are themselves smooth and clearly defined, the scar produced in vacuum is severely scored and shows evidence of carbon "dusting".

There is an apparent disagreement of about one order of magnitude between the in-air wear rates presented here and those reported previously[1]. But it should be noted that different substrates were employed in the two investigations—stainless steel in the former and mild steel in the latter. On re-determining the results for acetal copolymer and nylon composites against mild steel (*BS*15) it was confirmed that these materials exhibited significantly greater rates of wear against mild steel than against stainless steel. These results are presented in Table 2, in which the values obtained by Lancaster (private communication) using a mild steel counterface are also given for comparison.

It is clear that the counterface material can play an important part in determining the wear of these composites in air. The wear mechanism in vacuum appears to involve the transfer of composite material to the metal surface. Inspection of the cylinder specimens after a sliding test showed that in most cases a thin visible track of transferred material had been formed and in the case of polyimide, containing 50 per cent carbon fibre, gross transfer was observed.

This work was carried out as part of a programme supported by the Ministry of Technology. We thank Dr J. K. Lancaster of the Royal Aircraft Establishment, Farnborough, for his co-operation in supplying the range of sample materials studied and general interest in the work. The materials discussed are the subject of patent application No. 33570/66 by R. C. Fullerton-Batten, J. K. Lancaster, L. N. Phillips and G. Wood, "Carbon Fibre-Resin Composite Bearing Materials".

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[1] Giltrow, J. P., and Lancaster, J. K., *Nature*, **214**, 1106 (1967).
[2] Lancaster, J. K., *RAE Tech. Rep. No. 66378* (Dec. 1966).
[3] Savage, R. H., and Schaeffer, D. L., *J. App. Phys.*, **27**, 136 (1956).
[4] Buckley, D. H., and Johnson, R. L., *ASLE Trans.*, **7**, 91 (1964).

BOOK REVIEWS

AGE OF FACTS

American Science in the Age of Jackson

By George H Daniels Pp viii + 282 (New York and London Columbia University Press, 1968) 72*s*

The aim of this original book is to characterize a particular aspect of a specific society the attitude towards science in the United States of America between 1815 and 1845 Mr Daniels is not interested in scientific achievement and this is just as well, for, as he is aware, and as the men of his period lamented, there were few notable contributions to science from early nineteenth century America. The heroic age of colonial science represented by Benjamin Franklin, the two John Winthrops, or David Rittenhouse, all Fellows of the Royal Society, had ended with the emergence of the new American nation; only Asa Gray and James Dwight Dana, both still young men in 1845, were to become FRS (in 1873 and 1884, respectively) among the many men whose ideas and work are described here

Admirably, Mr Daniels sees his subject in perspective and neither makes nor wishes to make great claims for the men whose ideas he analyses The active scientists were a serious and professional group, nearly all American born, nearly all associated with education—most college graduates, and the majority employed in the growing number of colleges and universities as teachers of science, though many lacked formal training By analysing journal articles Mr Daniels concludes that American science in this period was, on the whole, specialized, not specifically utilitarian, and by no means largely devoted to natural history

A considerable part of this book is devoted to an examination of published views on scientific method, from which it appears that American philosophers and theologians wrote much on the subject Mr Daniels finds the preferred scientific method of this period an extreme "Baconianism", a reverence for what was taken to be Bacon's (and Newton's) exaltation of fact at the expense of theory, a tendency to decry hypothesis as fanciful and to demand the collection of ever more facts To some extent this was justified Darwin and Hooker deplored the free speculation of *The Vestiges of the Natural History of Creation* every bit as much as Asa Gray Many of the subjects investigated by American scientists—the theory of storms, the aurora borealis, the geological structure of their continent—could hardly proceed without more facts, and the first theories were indeed wildly speculative Further, fact was safer than theory, which often led to heartbreaking controversy And, as Bacon himself had recognized, factual science is more democratic than theoretical science, because it is more readily understood by all One very obvious reason for this excessive empiricism was American intellectuals' fear of European "materialism", ironically they saw Baconianism as a conservative force, where European conservatives like De Maistre saw it as an agent of revolution Another reason was the peculiarly theological tone of the American intellectual scene American colleges and universities were not secular, on the contrary, they were highly sectarian and, as college teachers, scientists were expected to accept the tenets of the sect associated with their institutions A scientist unconcerned with religion would find employment difficult All geological speculation was therefore particularly suspect; it is no wonder that Darwin created such a storm in the New World

By his choice of subject Mr Daniels has avoided many of the pitfalls usual in a national history of science Unfortunately he has neglected European science of the period excessively So he does not find it odd that, in 1819, a Boston physician deplored the "overthrow" of Lavoisier's "system" and hence he misinterprets the complaint of a professional chemist (Robert Hare, not a great theoretician) that non-chemists could not understand his work. Both these remarks are signs of what Mr Daniels instances elsewhere, a growing degree of professionalization. That Benjamin Rush was praised for expounding mechanistic physiology in 1768 whereas a physician named Emmet was censured for doing the same thing in 1822 merely reflects events in Europe the decline in popularity of Haller's views and the dominance of the French school which followed the doctrines of Bichat, whose definition of "life" is given here as original with Elisha Bartlett in 1844 The works of Bichat and Richerand were printed in Philadelphia in this period, and were widely popular Indeed, in view of the fact that European travel and study were still uncommon, the concepts of American scientists reflect those of the main stream of European science remarkably well That writers on science were unaware of this is not surprising, nor that they were less well prepared than the scientists for the tremendous growth of science in America after 1845 It is to be hoped that Mr Daniels will analyse the later period in the same way as he has here analysed the earlier one For he has illuminated many interesting aspects of the American scientific scene

Marie Boas Hall

CALCIUM AND REPRODUCTION

Calcium in Reproductive Physiology

A Comparative Study of Vertebrates By K Simkiss (Modern Biological Studies.) Pp xiv + 264 + 9 plates (London Chapman and Hall, Ltd , New York Reinhold Publishing Corporation, 1967) 60*s* net

Dr Simkiss's capability as a reviewer of the literature is well illustrated in his new book on calcium regulation throughout the vertebrates Whereas in his previous articles in *Biological Abstracts* only specific topics of the subject were touched on, in the present book the entire scope of contemporary thinking on the hormonal regulation of calcium balance has been treated The author has done himself an injustice by entitling the book *Reproductive Physiology*, thus implying that it deals solely with the role of calcium ions in the reproductive process In fact, the content is much greater and covers most aspects of the controlling mechanisms of calcium levels in animals, as well as a more specialized consideration of excess calcium demand during the reproductive period

The first part of the book is devoted mainly to the general mechanisms of calcium regulation in different classes of vertebrates and deals with the role of calcified tissues, the digestive system including the liver, and the kidney in the adjustment of calcium balance and serum calcium concentrations The effects of the thyroid–parathyroid complex, the sex steroids and vitamin D on these organs are also discussed The author has rightly emphasized the non-infallibility of some of the experimental approaches in these studies, particularly those which attempt to extrapolate results from short-term measurements into long-term balance effects Although there has been no attempt to describe the experimental techniques in detail, the principles behind them have been adequately treated and their usefulness evaluated

The second part of the book is a survey of the regulation of calcium during ovulation, egg-shell formation, pregnancy and embryonic growth, as the case may be, in different classes of tetrapods It terminates with a stimulating chapter of conclusions and speculations As the author has admitted, this book is theoretical rather than applied and caters primarily for zoologists and comparative physiologists Nevertheless, it is very informative owing to the inclusion of large numbers of tables and figures, so that for the applied physiologist and veterinary and medical scientists who are interested in calcium metabolism it serves as a useful handbook On the whole, the literature covered is quite complete It illustrates the amazing paucity of information on the regulation of calcium in vertebrates other than the tetrapods With the recent discovery of (thyro)-calcitonin and its identity as the hormone secreted by the ultimobranchial bodies, the subject of hormonal regulation of calcium balance is bound to attract more and more attention. This book is a timely review of the past literature and stands out as a milestone to mark the beginning of perhaps a new era.

B Lofts

PHENOLS OXIDIZED

Oxidative Coupling of Phenols

(Organic Substances of Natural Origin a Series of Monographs, Vol. 1) By W. I Taylor and A R Battersby Pp. xiv+387 (London Edward Arnold (Publishers), Ltd, New York· Marcel Dekker, Inc, 1967) 260*s* net.

Phenols and phenol ethers represent a large proportion of the benzenoid-aromatic compounds which are of such widespread occurrence in nature Many of the more complex compounds, for example, alkaloids, tannins, lignins, and many quinonoid pigments are clearly the result of the oxidative coupling of two or more phenolic nuclei, and so much work on this subject has been done in the past few years that the time is ripe for the publication of a monograph on the subject.

It may be said straight away that the present monograph is extremely good It consists of a number of reviews on the most important aspects of the oxidative coupling of phenols written and edited by internationally recognized experts Each chapter is self-contained and there is surprisingly little overlap or duplication between one chapter and another. The text is profusely illustrated with beautifully printed structural formulae.

The first chapter (ninety-four pages, 424 references) is by H Musso and deals concisely with the general problem of phenol coupling in terms of mechanism, formation of C–C and C–O aromatic bonds, and so on. A particularly valuable feature is the comprehensive table of products formed by phenols (mostly "unnatural" ones) when oxidized by various reagents There is also a table of phenoxyls investigated by electron spin resonance methods The second chapter (twenty-three pages, sixty-six references) by A I Scott describes "Some Natural Products Derived by Phenol Oxidation" and gives a glimpse of the variety of naturally occurring phenolic products It whets the appetite for the specialized chapters which follow In the third chapter (fifty-seven pages, 134 references), A R Battersby discusses "Phenol Oxidations in the Alkaloid Field". Since the original suggestions on the possible biogenesis of morphine and other alkaloids by Robinson in 1931, it has been shown that over 200 of the more than 2,000 known alkaloids can be accommodated within the framework of the oxidative coupling of phenols For many alkaloids the detailed pathways of biosynthesis have been unravelled by feeding labelled intermediates to plants as well as by carrying out *in vitro* experiments In the fourth chapter (thirty-five pages, 105 references), entitled "Biochemical Aspects of Oxidative Coupling of Phenols", B R Brown summarizes our present, all too scanty knowledge of the enzymes which catalyse phenol-coupling The fifth chapter (thirty-eight pages, fifty-seven references), by D. W. Cameron and Lord Todd, gives a fascinating account of the aphin pigments The sixth chapter (seventy-nine pages, 277 references), by J M Harkin, and the seventh chapter (thirty-six pages, sixty-three references), by K Weinges and R Spanig, deal respectively with the industrially important, highly irregular polymer known as lignin and with "Lignans and Cyclolignans", which are essentially dimeric derivatives of propenylphenols

All chemists and biochemists interested in phenols and in phenolic natural products should read this monograph It is therefore most unfortunate that the price puts it beyond the range of all but a few individuals and the wealthier libraries

J. F W McOmie

VIRUSES IN THE LABORATORY

Virological Procedures

By J Mitchell Hoskins. Pp xiv+358 (London· Butterworth and Co (Publishers), Ltd, 1967) 70*s*

This book has been written for those concerned with the laboratory diagnosis of virus infections of man, with particular reference to the problems existing in the British Isles. The author considers that a virus diagnostic laboratory should have certain essential services available, and that these services should be used in different ways to meet the particular requirements involved in diagnosing infections with different viruses. Following this logical approach, the book is divided into five sections The first and largest deals with basic laboratory facilities ranging from considerations of the design of suitable accommodation, through technical points concerning equipment, washing and sterilization procedures, to more detailed accounts of the uses of experimental animals embryonated eggs and tissue cultures in virology The preparation of monkey kidney cell cultures is described as an instance of primary cell cultures, and the handling o HeLa cells is used to illustrate the uses of cell lines The treatment of diploid cell cultures and of organ culture methods is less detailed, although useful in the context o the book The information on the uses of laboratory animals and of embryonated eggs is presented clearly with appropriate illustrations

The second section is concerned with the isolation o viruses, starting with the collection of clinical materia and describing the various ways in which virus isolation may be attempted Virus strains successfully isolated must then be identified, and the third section deals with this problem, indicating some of the clues which may assist the virologist in his task, and concluding with methods for the identification of specific viruses

Some virus infections are diagnosed on the basis o serological evidence, and the fourth section is concerned with serological procedures Recommended methods fo preparing and standardizing diagnostic antigens an reference antisera are given, with details of the ways i which complement fixation, haemagglutination-inhibition neutralization and other serological tests are carried out The fifth section suggests the methods of choice fo investigating different groups of viruses This material i presented in the form of tables summarizing criteri which are considered useful in diagnosing infections du to particular groups of viruses, for example the picorna viruses and the myxoviruses This whole section is shor and while tabulation has many advantages the presence of forty-nine footnotes to the table on myxoviruses is no an attractive feature The book concludes with appendixe giving formulae of commonly used media, buffers an stains, and tables of logarithms and antilogarithms

This fairly compact volume contains much that will b of great practical value in a virological laboratory Th author has been selective, and not everyone will agre with his selection on points of detail A major criticism is the complete absence of references to original sources, apart from a list of twelve books recommended for supplementary reading J S. PORTERFIELD

BIOLOGY OF DREAMING

The Biology of Dreaming

(A Monograph in the Bannerstone Division of American Lectures in Living Chemistry) By Ernest Hartmann Pp xiii+206 (Springfield, Illinois Charles C. Thomas, Publisher, 1967) $9 75

SLEEPING and dreaming are of increasing interest to a range of research workers from widely differing basic backgrounds The information explosion of the past decade, due to energetic sleep research, has led to a situation where research is now reported in almost every journal at some time or another It can therefore be very difficult at times to find what is being thought currently about some aspects of this research Many sleep researchers are aware of this difficulty and have produced reviews, usually contained within suitable journals or more massive books

Dr Hartmann has taken on the task of describing present ideas on the biological, clinical and some psychological aspects of the problem of dreaming

He describes the basis for the experimental studies of sleep—although his illustrations in this section have not come out well It is also most unfortunate that after pointing out—admirably—that the neurophysiological state in which most dreaming has been found has already twenty-two names, he adds his own—the "D or dreaming state" The fact that he goes to pains to review at various points the data that dreams have been collected from the other type of sleep, now christened the "S state", emphasizes the semantic difficulty. Surely it is time we agreed on universal terminology

After a short but comprehensive account of the "dream deprivation" experiments and discussion of the demand for the "D state", the author gives a very neutral account of the controversies of the possible chemical basis for this state. There are very full accounts of the association of dreaming with mental illness despite the apparent gaps and conflicts in this area Association of the "D state" with physical illness is also interesting and I enjoyed his discussion of a possible connexion between the "D state" and psychosomatic illness, although our ignorance in this area is made apparent

The biological importance and function of the "D state" are dealt with without bias at the expense of over-inclusion at times

Obviously in this book there are instances where the current knowledge has outstripped the ideas present when the book was written, but these examples are minor

I enjoyed the book and despite its short length and very comprehensive data did not get the feeling of compression The bibliography is good

It is, however, an expensive volume, much of which will be dated rapidly I would see it as a good review which, made available by libraries, will carry its ideas to a wide range of people who otherwise might find difficulty in finding this material in the journals J I EVANS

ECONOMIC ANTHROPOLOGY

Themes in Economic Anthropology

Edited by Raymond Firth Pp x+292 (London Tavistock Publications, Ltd , 1967) 45*s* net

CONTEMPORARY economic theory is becoming increasingly divorced from reality and its arguments have assumed largely the character of futile intellectual *jeux d'esprit.* In so far as this volume is representative for the prevailing trend in economic anthropology—which it ought to be, considering that it is based on the collective wisdom of two scores of anthropologists assembled in conference in 1965—it seems that many economic anthropologists are well on their way towards adopting the most deplorable features of contemporary economics—choice of deliberately abstruse aspects of their subjects and their obscurantist treatment

The language of some of the essays in this book is admittedly still that of Marshall, Keynes and Malinowski, but others are dominated by the economic terminology invented for the purpose of deterring the layman from taking an interest That the participants of the conference, while at pains to familiarize themselves with the teachings of sophisticated contemporary economists, missed out their elementary lessons seems to be indicated by the fact that none of those who pooled their resources at the conference corrected Mary Douglas when she attributed to Adam Smith a frequently quoted remark by John Stuart Mill about the difficulties of barter between a tailor and a baker

Disregarding that minor error, Mary Douglas's essay on "Primitive Rationing A Study in Controlled Exchanges" is a contribution of outstanding interest But in spite of being an eminently lucid presentation of a subject of essentially practical interest, it must be classed among the intellectual *jeux d'esprit* For the author uses her remarkable erudition, incisive analysis and clarity of exposition for a *tour de force* to prove an utterly absurd thesis—that primitive moneys serve the purpose of rationing She failed to produce a single valid argument in support of her thesis But the superb skill with which she presented her basically false case compels admiration, mingled as it is with regret for the waste of such a superb display of scholarship in the service of such an undeserving case

Nothing could indicate more clearly the possibility of defending a wrong thesis in an impressive and convincing way than the fact that Mrs Douglas managed to convert such a formidable scholar as Raymond Firth, at any rate to the extent that, instead of rejecting her contention out of hand, he suggested in his opening essay that the analogy of primitive moneys to ration coupons offered "an intriguing set of criteria for discussion" (page 18) Had Professor Firth examined closely enough the factual evidence provided by Mrs Douglas I am sure he would have discovered that her ethnological instances are either perplexingly irrelevant or they prove exactly the opposite of what she seeks to prove.

Rationing is a system to ensure egalitarian distribution, while the evidence offered by Mrs Douglas conclusively reaffirms the generally known fact that the operation of primitive monetary systems results in increasingly unequal distribution. Such effect is inherent in these systems. This is certainly the case of the system on which she writes on the basis of first-hand knowledge, the use of raffia cloth units by the Lele In the course of her field research in that community, embodied in her excellent monograph *The Lele of Kasai*, she found that most raffia cloth—which could be produced by anyone—tended to accumulate in the hands of a small number of rich old men I could quote scores of instances of moneys operating in primitive communities that produced a similar effect It puzzles me beyond words how a highly intelligent anthropologist like Mary Douglas can possibly imagine that such a system makes for egalitarianism And since it does exactly the opposite, how can it be called rationing ?

Mrs Douglas stated that among the Lele every man could issue his own ration coupon to himself I failed to discover in her essay the strange process of reasoning by which she succeeded in convincing herself that virtually unlimited production of ration coupons "no more makes nonsense of the rationing analogy than it makes nonsense to speak of money when the unit of currency is freely produced and marketed" (page 131). I agree that the free production of the wampum and tobacco currencies by North Americans during the early colonial period does not make nonsense of their description as moneys, even though these currencies did reduce themselves to nonsense eventually as a result of their extreme depreciation. After all, bad money is nonetheless money for being bad. But it is unmitigated nonsense to label as rationing a system under which everybody is in a position to increase the number of his coupons at will. Such a system is not rationing, it is the very negation of rationing. Unlike free money, it would not merely reduce itself to nonsense through its misuse over a period—it would be logically, necessarily and inevitably nonsense from the very outset because it conflicts with the basic principles of rationing.

Mrs Douglas emphatically rejects the idea that raffia cloth performs monetary functions among the Lele. In my *Primitive Money* (revised edition, page 434), I tried to prove on her own evidence that it does play a limited part as a medium of exchange. However, whether raffia cloth is money is largely a matter of definition and there is room for two opinions about it. But no conceivable definition of rationing could possibly fit to the part played by raffia cloth.

When she gets away from her fixed idea Mrs Douglas can and does make valuable contributions to the subject of money. "Money in the international economy" she says, "is very like money in the primitive economy where no sovereign authority is in control" (page 139). This and some other shrewd observations do indeed offer a basis for stimulating discussion. They make me deplore it all the more that she has not turned her wide knowledge of primitive moneys and her original analytical mind to worthier use than her attempt to prove the unprovable.

The other essays in the volume include an introductory essay on "Themes in Economic Anthropology", by Raymond Firth, "One Economist's View of the Relationship between Economics and Anthropology", by Leonard Joy, "Economic Anthropology: One Anthropologist's View", by Ronald Frankenberg, "Economic Analysis and Economic Man", by Percy S. Cohen, "Economic Spheres in Darfur", by Fredrick Barth, with an essay commenting on it by Leonard Joy, "The Structure of Decision-Making among Indians of Colombia", by Sutti Ortiz, "Productive Efficiency and Customary Systems of Rewards in Rural India", by Scarlett Epstein, and "Traditional Groups and New Economic Opportunities in Rural Yugoslavia", by Lorraine Baric. Between them these essays cover a very wide sphere. It is a pity that between them they devote relatively little space to the task that could reasonably be expected to receive the utmost attention of present-day anthropologists—investigation of the problem arising from the sudden change from primitive to modern economy.

As a result of the violently sudden contact between backward and advanced communities during and since the war in various parts of Africa, Asia and Oceania, changes which usually occur very gradually have occurred very suddenly. Modern money, modern systems of production and modern market economy have been forced on backward communities in a matter of years, before they have become ready for such changes. The modern institutions are being applied, therefore, by communities whose minds are still primitive. This striking anomaly has given rise to interesting situations which ought to be investigated very thoroughly. They have been investigated in a number of communities by individual field workers during the past ten years or so. There have appeared several very valuable monographs dealing with the sudden transition from primitive to modern economy in individual communities. What is needed is an adequate summing up of their findings.

Admittedly, there are occasional references in this volume to such problems, but I have looked in vain for a systematic analysis of the evidence produced by field research workers. Is this because economic anthropology, like economic theory, has come to make it a point of honour to keep aloof from realities? Some of the contributors deem it more important to try to prove that Karl Marx was right than to find an answer to the interesting problems of the transition period of our days. All in all, although this volume contains much that is valuable and interesting, it has failed to fill the most important gap that exists in the literature on economic anthropology.

PAUL EINZIG

OBITUARIES

Professor J. D. Boyd

PROFESSOR JAMES DIXON BOYD died in Cambridge, after a long illness, on February 7, 1968. Born in the United States of America, he was taken to Northern Ireland at an early age and educated at the Royal Belfast Academical Institution and Queen's University, Belfast. His undergraduate career was a brilliant one, and he could obviously have made a niche for himself in any branch of medicine. He was, in fact, attracted to work in the department of anatomy at Belfast, and became one of the brightest stars assembled in that department by Professor Thomas Walmsley. After a Rockefeller fellowship, he went to Cambridge in 1935, where he became a university lecturer and Fellow of Clare College. By this time he had already acquired what was to be his principal interest, and on his appointment at the extremely early age of 31 to the chair of anatomy at the London Hospital Medical College, he had begun the collection of sectioned human embryos, second only to that of the Carnegie Institution, to which he was to add continuously during his period in London, and after his appointment to the chair of anatomy in Cambridge in 1951. His textbook on human development, known familiarly to generations of students as "Hamilton, Boyd and Mossman", has become a standard text and will long survive to mark both the depth of his scholarship and the long and fruitful collaboration he enjoyed with Professor W. J. Hamilton.

Although Dixon Boyd was an excellent administrator and one of the best and most enthusiastic of lecturers, his friends will remember him firstly as a microscopist, because on entering his room it was characteristic to see him bent over a microscopic preparation from which he would turn with his cheerful smile. He made a large number of contributions to the study of embryology, clearing up a wide range of problems relating to structures such as the carotid body, the pharyngeal pouches and the ganglia of the sympathetic nervous system. The subject in which he took most interest was the placenta and its formation. He quickly realized the potentiality of the electron microscope in studying this structure and made valuable discoveries as to its contents.

In some respects Dixon Boyd seemed to belong to an age earlier than the present, for he never allowed his own specific research interests to dominate his position as head of a department. He was one of the best-read men of his time, both within and outside his subject. His knowledge of the anatomical literature was so extensive that he was often consulted by experts, who went away inevitably with something new they had gleaned from his rich store. During the past few years when his health was sadly failing, he was engaged with his old friend and colleague, W. J. Hamilton, in the preparation of what is clearly designed to be a classic monograph on the placenta. Those who knew Dixon Boyd well will mourn the fact that he did not live to see its publication, but they will also know that, by his standards, to complete an important piece of scientific work was enough, praise from others for what he had done with his whole heart and mind was ever supererogatory.

DAVID WOOLLAM

University News . London

Mr H. J. Godwin, reader in pure mathematics at the University College of Swansea, has been appointed to the chair of statistics and computer science tenable at Royal Holloway College, and Professor A A Walters, professor and head of the Department of Econometrics and Social Statistics in the University of Birmingham, has been appointed to the Cassel chair of economics with special reference to money and banking tenable at the London School of Economics and Political Science The title of professor of immunology has been conferred on Dr I M Roitt, in respect of his post at the Middlesex Hospital Medical School

Appointments

Captain W E Morris has been appointed director of Royal Naval Aircraft and Helicopter Development in the Ministry of Technology, in succession to Mr L H. G Sterne

Dr M Shafqat H Siddiqi, chairman of the Pakistan Council of Scientific and Industrial Research, has been appointed the first overseas member of the Tropical Products Institute Advisory Committee

Announcements

The Council of the Royal Society of Edinburgh has awarded the Keith Prize for the period 1965–67 to Dr A J Haddow, senior lecturer in epidemiology in the University of Glasgow, for his paper "The Natural History of Yellow Fever in Africa" published in the *Proceedings* of the society during the period of the award

The Center for Theoretical Studies of the University of Miami has announced the establishment of the J Robert Oppenheimer Memorial Prize for significant contributions to the fields of theoretical physics, chemistry, biology, mathematics and the philosophy of science The prize, which consists of a gold medal, a citation and a cash award, is to be a tribute to the late Professor Oppenheimer, who, as a member of the Scientific Council, was instrumental in the founding of the centre Further information concerning this prize can be obtained from the Scientific Council, Center for Theoretical Studies, University of Miami, PO Box 9055, Coral Gables, Florida 33124

Meetings

Vacuum Congress, April 17–19, Manchester (The Secretary, Joint British Committee for Vacuum Science and Technology 47 Belgrave Square, London SW1)

Physics of Thin Films, April 22–24, Southampton (The Meetings Officer, The Institute of Physics and The Physical Society, 47 Belgrave Square, London SW1)

Annual Frequency Control Symposium, April 22–24, Atlantic City, New Jersey (Director, Electronic Components Laboratory, US Army Electronics Command, Fort Monmouth, New Jersey 07703)

The Scanning Electron Microscope—The Instrument and Its Applications, April 30–May 1, IIT Research Institute, Chicago (Dr Om Johane, IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616).

Time-dependent Effects in Polymeric Systems, May 10–11, University of Bradford (The Registrar, University of Bradford, Bradford 7)

Myeloproliferative Disorders of Animals and Man, May 20–23, Richland, Washington (Dr W J Clarke, Symposium on Myeloproliferative Disorders of Animals and Man, P O Box 999, Richland, Washington 99352)

CORRESPONDENCE

Geology in British Universities

Sir,—If geology is taken to mean the science of the Earth, then most university geology departments in Britain do not teach it Instead they offer a range of semi-independent, specialized courses within the field, from which the student is expected to construct his own view of the Earth The main reason seems to be repeated subject segmentation resulting from an accelerating increase in published data Departments at time of formation have been traditionally divided into branches or subject segments, with one teacher for each. Examples are palaeontology, stratigraphy and mineralogy (Fig 1). As data accumulate it eventually becomes clear that a segment will have to split, the original teacher taking one part and a new teacher the other For example, palaeontology might split into macro- and micro-palaeontology. The process can continue indefinitely

The system has three defects First, each subject segment is usually, though not always, separated from its neighbours by a subject gap—or even a crevasse In order to bridge this, those responsible for adjacent segments would need to be familiar with the topics covered by the other, and both would need to agree on a method of presenting the relationship. Such co-operation is rarely successful and is usually not attempted

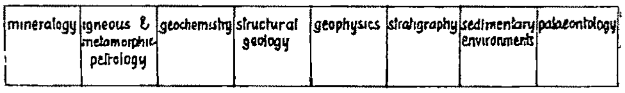

Fig 1 Example of the traditional segmentation of geology

The second defect is that the simple segment structure tends to be self-perpetuating For most departmental heads the temptation to split an already existing segment rather than to create an entirely new one seems to be irresistible The effect on departmental research, necessarily closely related to segment structure, is obvious. Hence, developments during the past fifteen years, and particularly since the IGY in 1957–58, which have altered the very structure of the subject, are relatively neglected Geology as now usually taught is largely confined to the study of the exposed portions of the continental rifts This was inevitable in the past when knowledge of the Earth was gained from direct examination of its land surface Then, the only known way to link observations was to place them either in time sequence, or in descriptive groups (for example, minerals) But it is now becoming increasingly clear that at any given time events in the crust are closely connected both to one another, and to those in the upper mantle and probably also the core. They cannot any longer be treated in isolation as if they were parts of a series of independent Markov chains (for example, local stratigraphic columns).

The third and perhaps most serious defect of the present system is that virtually no research and little teaching is directed towards synthesis of geological information at any level above that of the segment Even within segments the historico-descriptive approach is commonly regarded as adequate The result is a rising mountain of undigested data Hence the necessity for repeated segment splitting and increasing specialization The necessity is largely illusory, the real need is for condensation of data into working hypotheses with a high degree of independence in space and time The fallibility of such hypotheses is recognized, but it does not destroy their central importance to the development of the subject Without them the recitation of geological history, with ever increasing precision, becomes an end in itself

Two excuses are usually advanced for the lack of synthesis in geology The first is the difficulty of dealing with the huge volume of data and the consequent danger of

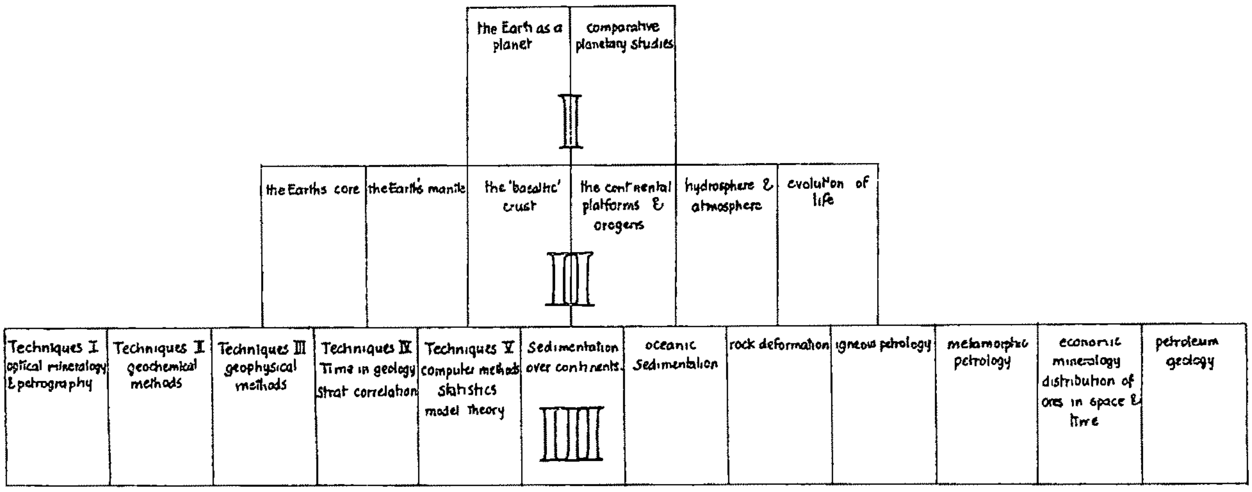
the Earth as a planet
comparative planetary studies
I
the Earths core
the Earth's mantle
the 'basaltic' crust
the continental platforms & orogens
hydrosphere & atmosphere
evolution of life
II
Techniques I optical mineralogy & petrography
Techniques II geochemical methods
Techniques III geophysical methods
Techniques IV Time in geology Strat correlation
Techniques V computer methods statistics model theory
Sedimentation over continents
oceanic Sedimentation
rock deformation
igneous petrology
metamorphic petrology
economic mineralogy distribution of ores in space & time
petroleum geology
III

Fig 2 Suggested three-tier hierarchy of subject segments Subjects shown are merely examples The number of tiers is variable, but two is a minimum

superficiality. The second is the lack of suitable people to do the work However, the need is not for synthesis in the encyclopaedic style of Suess[1] or Gignoux[2], but rather the use of codified data to derive such things as natural classifications of rocks and to define sedimentary, metamorphic, geochemical and tectonic crustal regions The construction of a nested hierarchy of simple quantitative models ultimately embracing the whole Earth can then follow. A similar approach has been suggested by Kosygin and Voronin[3] The contention of superficiality also overlooks the fact that the nature of problems changes with the scale of investigation (cf Haggett *et al*[4]) Thus, the petrographer working with a microscope does not dismiss field studies as superficial even though they are carried out on a scale at least five orders of magnitude greater

The lack of workers experienced in synthesis is certainly real However, there are probably more frustrated synthesists in British geology departments than might be suspected from listed research topics The one certain way to utilize them and to generate new interest, and hence research, in synthesis is to create a demand for it

As an alternative to the existing organizational structure in geology a three-tier hierarchical scheme is proposed (Fig 2) in which scale plays a key part and teaching is process oriented rather than descriptive The chief advantage of this scheme over the traditional one (Fig 1) is that the relationship between subject segments in any tier is dealt with by teachers in the next higher one—without, incidentally, the need for detailed collaboration of teachers either within or between tiers Such a scheme would provide an effective antidote to the cancer of specialization. It also puts much greater emphasis on the rapidly growing areas of the subject, which is essential if universities are to equip students for a working life during which the total amount of geological knowledge may be expected to increase sixteen-fold

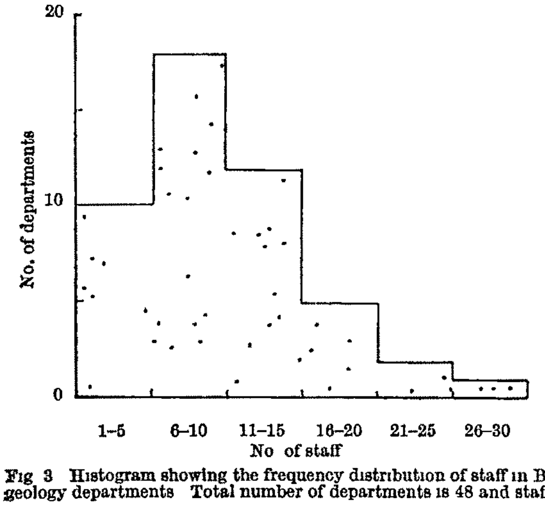

Fig 3 Histogram showing the frequency distribution of staff in British geology departments Total number of departments is 48 and staff 484

An analysis of geological research in British universities and colleges[5] shows that only 2 per cent falls within tiers I and II in Fig 2 Of this, almost half is accounted for by one university—the only one which, to judge from its calendar, has succeeded in establishing a viable Earth sciences department An analysis of the number of academic staff in British geology departments[5] shows a skewed distribution with a mode in the range 6–10 (Fig 3) This is almost certainly too small to support a three-tier hierarchy of the kind shown in Fig 2 Such a scheme would, however, become a theoretical possibility if the number of geology departments was halved and their modal size doubled. Whether they actually succeeded in introducing the scheme would, of course, depend on their willingness and ability to reorganize Better utilization and access to equipment and library resources could be an important by-product of the proposed concentration of geological effort

Needless to say, the voluntary liquidation of half the geology departments in Britain baffles the imagination. It is more probable that geophysically oriented planetary science departments will spring up independently of classical geology They will recognize that the crust of the Earth is the most sensitive meter of events in the interior over the greater part of geological time And the geologists will find their data incorporated into planetary schemes which they have had no part in constructing, partly because they have declined to attempt large-scale synthesis themselves and partly because they have insufficient knowledge of pure science Geology itself will then have been reduced to the status of a technique A similar fate may be awaiting other subjects the conceptual framework of which is still historico-descriptive

I am grateful to several fellow-workers for their thoughtful comments on early drafts of this communication

Yours sincerely,

A D STEWART

Department of Geology,
University of Reading

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[1] Suess, E , *The Face of the Earth*, translated by Sollas, H B C. (Oxford University Press, 1904–24)

[2] Gignoux, M , *Stratigraphic Geology*, translated by Woodford, G G (Freeman, San Francisco, 1955)

[3] Kosygin, Yu A , and Voronin, Yu A , *Int. Geol Rev* , 9, 828 (1967)

[4] Haggett, P , Chorley, R J , and Stoddart, D R , *Nature*, 205, 845 (1965)

[5] Department of Education and Science, *Scientific Research in British Universities and Colleges, 1966–7*, 1, 42 (1967)

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 11

Society of Chemical Industry, Microbiology Group (joint meeting with the Colloid and Surface Chemistry Group, at 14 Belgrave Square, London, SW1), at 5 30 p m —Dr D A Haydon "Some Recent Developments in the Study of Biomolecular Lipid Films"

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I Mech E , and the Institute of Marine Engineers, at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Meeting on "Roll Stabilisation and Auto Pilots in Marine Engineering Application"

Monday, March 11—Thursday, March 14

Institute of Metals (at Church House, Great Smith Street, London, SW1)—Spring Meeting

Tuesday, March 12

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 2 30 p m and 5 30 p m —Discussion Meeting on "Metrication"

Royal Institution (at 21 Albemarle Street, London, W1), at 3 p m — Professor Ronald King "Experiments—or Asking Nature Questions" (Lecture for Preparatory School Pupils from Schools in London and the Home Counties To be repeated on March 13 and 14)

Institution of Civil Engineers (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "Ground and Materials Investigations for Road Schemes—Needs and Methods" introduced by Mr P A Green

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Fault Diagnosis and Maintenance"

University of London (in Mechanical Engineering Lecture Theatre "A", Imperial College of Science and Technology, London, SW7), at 5 30 p m — Professor Maria Luisa Bonelli (Florence) "Introduction The Scientific Renaissance in Florence" *

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Mr H C Cox and Mr A E Norman "The Design of Automobile Door Latches"

Wednesday, March 13

Royal Institution, History of Science Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Mr Frank Greenaway "Convergent and Divergent Research—Some Historical Contrasts" *

University of London (in Mechanical Engineering Lecture Theatre "A" Imperial College of Science and Technology, London, SW7), at 5 30 p m — Professor Maria Luisa Bonelli (Florence) "The Personality of Galileo" *

Society of Environmental Engineers (in the Mechanical Engineerin Department, Imperial College, London, SW7), at 6 p m —Mr B G Toms "Applications of Digital Computers in Environmental Engineering"

University of London (at the Institute of Archaeology, 31–34 Gordon Square, London, WC1), at 6 p m —Mr D Leigh "Sources of Maritime Archaeology—Problems of Conservation of Underwater Discoveries" *

Institute of Information Scientists (at Knightway House, 20 Soho Square, London, W1), at 6 15 p m —Mr L Corbett and Mr P J Gallagher "The Use of Commercially Available Computer Tapes for Current Awareness Services"

Wednesday, March 13—Thursday, March 14

Institution of Mechanical Engineers, Tribology Group (at 1 Birdcage Walk, Westminster, London, SW1)—Symposium on "Experimental Methods in Tribology"

Thursday, March 14

Linnean Society of London (at Burlington House, Piccadilly, London, W1), at 5 p m —Dr R Melville and Mrs F A Wrigley "Fenestration in *Monsteradeliciosa* and Its Bearing on the Morphogenesis and Colour-Patterns of Leaves", Mr T Reynolds "Seed Germination"

Mineralogical Society (at the Geological Society of London, Burlington House, Piccadilly, London, W1), at 5 p m —Scientific Papers

Institute of Petroleum (at 61 New Cavendish Street, London, W1), at 5 30 p m —Mr K R Williams "The Challenge to the Gasoline Engine"

Institution of Civil Engineers, Transportation Engineering Group (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "Measures for Improving Pedestrian Safety" introduced by Mr J T Duff

University of London (in Mechanical Engineering Lecture Theatre "A", Imperial College of Science and Technology, London, SW7), at 5 30 p m — Professor Maria Luisa Bonelli (Florence) "The Accademia del Cimento" *

University of London (in the Botany Theatre, University College London, Gower Street, London WC1), at 5 30 p m —Professor J de Ley (Ghent). "Molecular Taxonomy, a New Parameter on Biological Classification" *

Institution of Electronic and Radio Engineers, Education Group (at 8–9 Bedford Square, London, WC1), at 6 p m —Mr Harley R Sykes "Engineers must Manage or be Managed"

Thursday, March 14—Friday, March 15

Royal Society (at 6 Carlton House Terrace, London, SW1), at 10 30 a m daily—Discussion Meeting on "Triggered Enzyme Systems in Blood Plasma" organized by Professor R G Macfarlane, FRS

Friday, March 15

Association of Applied Biologists (in the Lecture Theatre of the British Museum (Natural History), Cromwell Road, London, SW7), at 10 30 a m — Symposium on "New Insecticides and Their Application in Public Health and Industry"

Royal Institution, Photochemistry Discussion Group (at 21 Albemarle Street, London, W1), at 1 p m —Professor D W Setser "Unimolecular Reactions and Vibrational Energy Transfer Studies with Chemically Activated Haloethanes" *

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I Mech E , and the Institute of Measurement and Control, at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Flow Measurement of Airborne Powders" opened by Mr F J Hiorns *et al*

Society of Chemical Industry, Plastics and Polymer Group (joint meeting with the South Western Section, at the College of Technology, Plymouth), at 5 30 p m —Mr G A R Matthews "Recent Advances in Extrusion"

University of London (in the Botany Theatre, University College London, Gower Street, London WC1), at 5 30 p m —Professor J de Ley (Ghent) "Evolution of Bacteria" *

Royal Institution (at 21 Albemarle Street, London, W1), at 9 p m — Professor Melvin Calvin, For Mem RS "From Molecule to Mars".

Saturday, March 16

Inner London Education Authority (at the Horniman Museum, London Road, Forest Hill, London, SE23), at 3 30 p m —"The Pulse of Africa" (Colour film on the birds, reptiles and mammals of Africa Co-Producer Pierre Koep)*

Monday, March 18

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 10 a m —Colloquium on "Threshold Extension Techniques"

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, SW1), at 5 p m —Mr J A R Bates "A Review of Governmental Control in Various Countries"

University of London (at the Royal Veterinary College, Royal College Street, London, NW1), at 5 p m —Professor J R Buchanan (Pennsylvania) "Radiology of the Canine Heart" *

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Class Structure of the British Single-phase House Service Meter" opened by Mr A J Baggott

University of London (in the Gustave Tuck Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Professor Horst Kunze (Director General of the German State Library, East Berlin) "The Training of Scientific Librarians and Documentalists in Eastern Germany" *

Institution of Electronic and Radio Engineers, Instrumentation and Control Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 6 p m —Professor H H Rosenbrock "Control Past, Present and Future" (Inaugural Meeting)

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 6 p m —Professor Owen Lattimore "China To-day—Some Social Aspects" (first of three Cantor Lectures)

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturers (2) (Grade II or III) in the Department of Civil Engineering —The Registrar and Secretary, The University, Bristol (March 14)

Demonstrator in Pathology—The Registrar, The University, Sheffield (March 22)

Lecturer or Assistant Lecturer (with special qualifications or interests in one of the following fields low temperature, including solid state, laser physics, X-ray crystallography) in Experimental Physics—The Secretary, University of St Andrews, College Gate, St Andrews, Fife (March 23)

Research Assistant (with a first- or second-class degree in some branch of engineering or physics) in the Department of Applied Mathematics for research in rheology—The Registrar, University College of Wales, Aberystwyth (March 23)

Chair of Experimental Physics at Birkbeck College—The Academic Registrar (A), University of London, Senate House, London, WC1 (March 28)

Chair of Physics at University College, Nairobi (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, WC1 (March 31)

Lecturer (with good academic qualifications, equipped to undertake supervision of work for higher degrees, and preferably some teaching experience) in Educational Psychology—The Registrar, The University, Keele, Staffordshire (March 31)

Assistant Lecturer in Biochemistry—The Secretary of the University Court, The University, Glasgow (April 1)

Chair of Geophysics—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (April 1)

Chairs (2) in Biological Sciences—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (April 1)

Staff Tutor (with a good honours degree in one of the biological sciences) in Science in the Department of Adult Education—The Registrar, The University, Hull (April 1)

Senior Lecturer or Lecturer in Mathematics at the University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (April 3)

Lecturers (2) (preferably with interest or experience in the following fields quantitative methods, climatology, hydrology, cartography and survey) in the Department of Geography, University of Ife, Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (April 8)

Reader (with interests in freshwater ecology, hydrobiology and/or ichthyology) in the Department of Zoology, Makerere University College, Uganda (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, WC1 (April 11)

Senior Lecturer or Lecturer in Science Curriculum Development in the Institute of Education, University of the West Indies, Trinidad—The Inter-University Council, 33 Bedford Place, London, WC1 (April 11)

Lecturer in Theoretical Physics—The Secretary to the University Court, The University, Glasgow (April 12)

Lecturer/Assistant Lecturer (preferably with research interests either in learning or cognitive processes) in Psychology at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office

Marlborough House, Pall Mall, London, SW1 (Hong Kong and London, April 15)

OFFICIAL FELLOW IN EXPERIMENTAL PHYSICS—The President, Magdalen College, Oxford (April 15)

UNIVERSITY LECTURERS IN EXPERIMENTAL PHYSICS (Pure or Applied) in the CLARENDON LABORATORY and in the DEPARTMENT OF NUCLEAR PHYSICS—The Secretary of Faculties, University Registry, Broad Street, Oxford (April 15)

ASSISTANT LECTURER and a LECTURER or SENIOR LECTURER (with teaching and research experience (at an advanced level for the senior post) in a branch of physiology) in the DEPARTMENT OF PHYSIOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, April 17)

CHAIR OF SOCIOLOGY in the RESEARCH SCHOOL OF SOCIAL SCIENCES of the Institute of Advanced Studies, Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, May 17)

MASTER to take charge of BIOLOGY—The Rector, Edinburgh Academy, Edinburgh 3

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Education and Science Education Survey 1 Units for Partially-hearing Children Pp v+50 (London H M Stationery Office, 1967) 5*s* net [2212

Schools Council Working Paper No 16 Some Further Proposals for Sixth Form Work Pp v+29 (London H M Stationery Office, 1967) 3*s* 6*d* net [2212

The Zoological Record, Vol 101, Section 19, 1964 Mammalia Compiled by Jean Ingles, John Fitzgibbon, Hazel Bartlett and Michael Dadd Pp 303 (London The Zoological Society of London, 1967) 50*s*, $7 10 [2912

Ministry of Technology Hydraulics Research 1966 The Report of the Hydraulics Research Station Steering Committee with the Report of the Director of Hydraulics Research Pp vi+101+8 plates (London H M Stationery Office, 1967) 11*s* net [2712

Hill Farming Research Organization Fourth Report, 1964–67 Pp 105 (Edinburgh Hill Farming Research Organization, 1967) 5*s* [2712

English Electric Valves—Duplexer Devices Pp 12 (Chelmsford English Electric Valve Company, Ltd, 1967) [2812

Social Science Research Council Research supported by the SSRC, 1967 Pp 96 (London Social Science Research Council, 1967) [2912

Philosophical Transactions of the Royal Society of London Series B Biological Sciences No 783, Vol 253 (21 December, 1967) *Ficus* in the Solomon Islands and Its Bearing on the Post-Jurassic History of Melanesia By E J H Corner Pp 23–159 51*s*, $6 65 No 784, Vol 253 (21 December, 1967) Marine Molluscan Assemblages in the Early Pleistocene of Sidestrand, Bramerton and the Royal Society Borehole at Ludham, Norfolk By P E P Norton Pp 161–200 15*s*, $1 95 (London The Royal Society, 1967) [2912

Department of Education and Science Building Bulletin 39 Designing for Science—Oxford School Development Project Pp v+98 (London H M Stationery Office, 1967) 13*s* 6*d* net [11

The Life of Robert Dick By J A Williamson (Caithness Notebook No 5) Pp 43 4*s* The Fossil Fishes of Caithness By J Saxon (Caithness Notebook No. 6) Pp 31 3*s* 6*d* (Thurso John Humphries, 1, Bank Street, 1967) [21

Chelsea College of Science and Technology (University of London) Annual Report 1966–1967 Pp 51 (London Chelsea College of Science and Technology, 1967) [21

Taxes in the EEC and Britain The Problem of Harmonization. By Douglas Dosser and S S Han Pp 46 (London Political and Economic Planning, 1967) 7*s* 6*d* [31

Ministry of Technology Forest Products Research 1966 Report of the Forest Products Research Laboratory Steering Committee with the Report of the Director of Forest Products Research Pp vi+61+8 plates (London H M Stationery Office, 1967) 8*s* 6*d* net [31

Northern Ireland Ministry of Agriculture Leaflet No 146 Growing and Harvesting Ryegrass Seed Pp 10 (Belfast Ministry of Agriculture, 1967) [41

Enjoying the Countryside By Arthur Blenkinsop (Fabian Research Series, No 265) Pp 20 (London Fabian Society, 1968) 2*s* 6*d* [41

The Less Developed Countries in World Trade a Reference Handbook By Michael Z Cutajar and Alison Franks Pp 209 (London The Overseas Development Institute, 1967) 30*s* [41

Department of Education and Science Building Bulletin 41 Rosebery County School for Girls, Epsom, Surrey Sixth-Form Centre Pp 59 (London H M Stationery Office, 1967) 6*s* net [41

Other Countries

United States Naval Observatory Circular No 116 Total Solar Eclipse of 22 September 1968 By Julena S Duncombe Pp 15 Circular No 117 Ephemeris of the Radio Longitude of the Central Meridian of Jupiter System III (1957 0) By Berenice L Morrison Pp 4 (Washington, D C U S Naval Observatory, 1967) [2712

World Health Organization Specifications for Pesticides Used in Public Health Insecticides—Rodenticides—Molluscicides—Repellents—Methods. Third edition Pp 300 (Geneva World Health Organization, London H M Stationery Office, 1967) 20 Sw francs, 35*s*, $6 75 [2712

Council for Cultural Co-operation of the Council of Europe Education in Europe Section I Higher Education and Research—No 4 How to Qualify as a "Biologist" in the Universities of Europe By Jean Leclercq Pp 240 (Strasbourg Council for Cultural Co-operation of the Council of Europe, London H M Stationery Office, 1967) [2712

Anuario del Observatoria Astronómica de Madrid para 1968 Pp 485 (Madrid Observatorio Astronómico, 1967) [2712

Organization for Economic Co operation and Development, European Nuclear Energy Agency Ninth Report on the Activities of the Agency, December 1967 Pp 71 (Paris Organization for Economic Co-operation and Development, European Nuclear Energy Agency, 1967) [2712

Bulletin of the California Institute of Technology, Vol 76, No 4 The President's Report and Reports of other Officers, 1966–1967 Pp 135 (Pasadena, California California Institute of Technology, 1967) [2812

Mitteilungen aus der Biologischen Bundesenstalt fur Land- und Forstwirtschaft, Berlin-Dahlem, Heft 125 (November, 1967) Neues uber Bacillus Thuringiensis und seine Anwendung Von Dr A Krieg Pp 106 (Berlin-Dahlem Biologische Bundesanstalt für Land- und Forstwirtschaft, 1967) 22 DM [2812

Library of Congress Science and Technology Division Weather Modification in the Soviet Union, 1946–1966—a Selected Annotated Bibliography, By Nikolay T Zikeev and George A Doumani Pp x+78 (Washington, D C Library of Congress, 1967 Obtainable from U S Government Printing Office) $0 55 [2812

United States Department of the Interior Geological Survey Water-Supply Paper 1840–B Floods of June 1964 in Northwestern Montana By F C Boner and Frank Stermitz Pp viii+242+plate 1 (Washington, D C Government Printing Office, 1967) $1 50 [2912

Annals of the South African Museum Vol 50, Part 5 (November 1967) Some Bathypelagic Cephalopods from South African Waters By Gilbert L Voss Pp 61–88+plates 1–9 (Cape Town South African Museum, 1967) 85 c [2912

Annals of the Transvaal Museum Vol 25, No 8 Recent Hyracoidea (Mammalia) of Southern Africa By J du P Bothma Pp 117–152 (Pretoria Transvaal Museum, 1967) [2912

Annales de l'Institut de Physique du Globe de l'Université de Paris Bureau Central de Magnétisme Terrestre Fondees par Ch Maurain, et publiees par les soins de E Thellier, avec le concours du Centre National de la Recherche Scientifique Tome 34 Pp 147 (Paris Institut de Physique du Globe de l'Université de Paris, 1966) [2912

Canada Department of Energy, Mines and Resources Geological Survey of Canada Paper 66–25 Bella Coola and Laredo Sound Map-Areas, British Columbia By A J Baer Pp v+13+maps $1 50 Paper 67–30 Conodont Zonation, Waterways Formation (Upper Devonian), Northeastern and Central Alberta By T T Uyeno Pp v+21 (2 plates) $1 50 Bulletin 153 Lower and Middle Devonian Trilobites of the Canadian Arctic Islands By A R Ormiston Pp 148+17 plates $5 50 Bulletin 156 A Standard for Triassic Time By E T Tozer Pp 103+10 plates $4. (Ottawa Queen's Printer, 1967) [11

United States Department of the Interior Geological Survey Bulletin 1229 Geology of the Circle Cliffs Area, Garfield and Kane Counties, Utah By E S Davidson Pp vi+140+plates 1 and 2 Bulletin 1231 Geology and Ore Deposits of the Lawson-Dumon-Fall River District, Clear Creek County, Colorado By C C Hawley and Frank Baker Moore Pp vi+92+plates 1 and 2 Bulletin 1248 Selected X-ray Crystallographic Data, Molar Volumes, and Densities of Minerals and Related Substances By Richard A Robie, Philip M Bethke, and Keith M Beardsley Pp iv+87 $0 35 Bulletin 1254-B Cretaceous and Lower Tertiary Stratigraphy in West-Central Puerto Rico By Peter H Mattson Pp iii+35 $0 20 Water-Supply Paper 1576-H Ground-Water Resources of the Southern Part of Jicarilla Apache Indian Reservation and Adjacent Areas, New Mexico By Elmer H Baltz and S W West Pp iv+89+plates 1–5 Water-Supply Paper 1824 Ground-Water Levels in the United States, 1960–64, South-Central States Prepared under the direction of C L McGuinness Pp v+152 $0 45 Water-Supply Paper 1839-A Reconnaissance of the Chemical Quality of Surface Water of the Neches River Basin, Texas By Leon S Hughes and Donald K Leifeste Pp iv+63+plates 1–4 Water-Supply Paper 1839-N Hydrologic Data for the Oak Ridge Area, Tennessee By William M McMaster Pp v+60. $0 25 (Washington, D C Government Printing Office, 1967) [11

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Meteorological Physics, 1966–67 Pp 36 (Melbourne Commonwealth Scientific and Industrial Research Organization, 1967) [21

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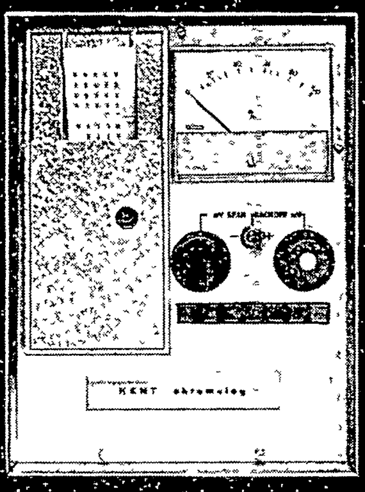

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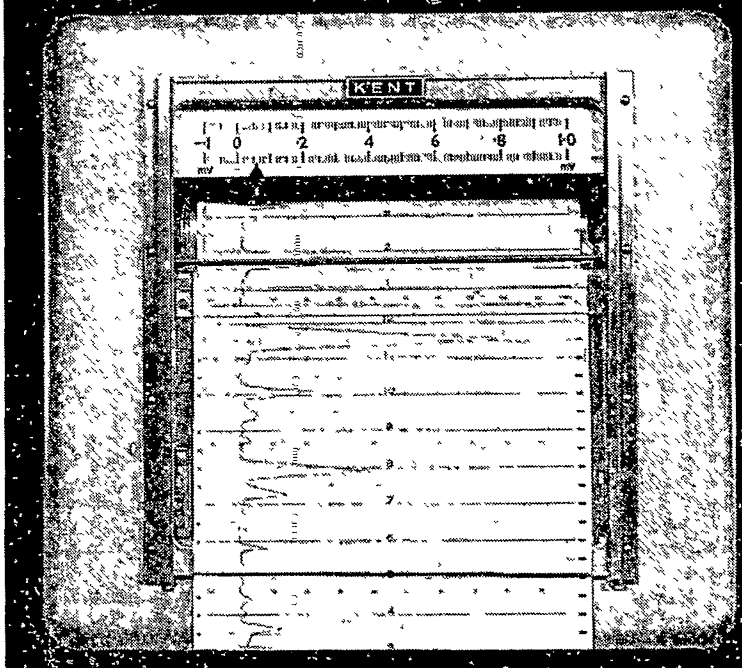

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Application forms and further details of the post can be obtained from the undersigned (S2) at 40 Eastbourne Terrace, London, W 2 (or telephone 01-262-8011 Ext 166) Completed applications to be returned by March 20

E G BRAITHWAITE,
Secretary
(975)

UNIVERSITY OF BRISTOL

TECHNICIAN or SENIOR TECHNICIAN required for Tsetse Research Laboratory Veterinary School, Langford (12 miles south of City) Interest in insect colonies climatic control and biological techniques desirable Salary Technician £653 to £938, Senior Technician £912 to £1,150 plus £30/£80 supplementation for recognised qualifications

Applications, giving full details and the names of two referees, should be addressed to The Director, Tsetse Research Laboratory, Langford House, Langford, Bristol (999)

UNIVERSITY OF MALAYA

CHAIR IN THE DEPARTMENT OF MATHEMATICS

Applications are invited for appointment to the Chair in the Department of Mathematics in the Faculty of Science The post will become vacant when the present incumbent leaves at the end of February, 1968, on resignation Candidates should have high academic qualifications and wide experience in teaching and research Administrative experience in running a Department is essential The successful candidate is expected to assume duty as soon as possible

EMOLUMENTS (approximate sterling equivalents at current exchange rates) are

(a) Salary There is a range of basic salaries, to a point on which a Professor is appointed depending on his qualifications and experience These are £2,882, £2,980, £3,077, £3,175, £3,273 £3,370, £3 468

In addition, the following allowances are payable

(b) Variable Allowance 35 per cent of basic salary, subject to certain maxima depending on marital status Minimum £244 per annum, maximum £651 per annum

(c) Inducement Allowance (if awarded), varies up to £1,628 per annum (In exceptional cases an inducement allowance exceeding this amount may be granted)

HOUSING will be provided if available, for which a rent of £163 to £204 per annum will be charged

PROVIDENT FUND Staff member contributes 5 per cent and University 15 per cent of basic salary

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House Pall Mall, London, S W 1

The closing date for the receipt of applications in London and Kuala Lumpur is April 8, 1968
(982)

UNIVERSITY OF MALAYA

FACULTY OF ENGINEERING

Applications are invited for appointment to the following Chairs in the Faculty of Engineering

CHAIR OF ELECTRICAL ENGINEERING
CHAIR OF MECHANICAL ENGINEERING

Candidates offering themselves for the above Chairs should be corporate members of the Institution of Electrical Engineers and the Institution of Mechanical Engineers respectively and have experience in teaching and research at university level Administrative experience will be an advantage

EMOLUMENTS (approximate sterling equivalents at current exchange rates) are

(a) Salary There is a range of basic salaries to a point of which a Professor is appointed, depending on his qualifications and experience. These are £2,882, £2,980, £3,077, £3,175, £3,273 £3,370, £3,468

In addition, the following allowances are payable

(b) Variable Allowance 35 per cent of basic salary, subject to certain maxima depending on marital status Minimum £244 per annum, maximum £651 per annum

(c) Inducement Allowance (If awarded), varies up to £1 628 per annum (In exceptional cases an inducement allowance exceeding this amount may be granted)

HOUSING will be provided, if available, for which a rent of £163 to £204 per annum will be charged

PROVIDENT FUND Staff member contributes 5 per cent and University 15 per cent of basic salary

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1

The closing date for the receipt of applications in Malaya and London is April 8, 1968 (981)

POSITION OPEN

CURATORIAL ASSISTANT To act as Museum Guide for school groups and to assist in organization care and display of rock mineral and fossil collections

QUALIFICATIONS B Sc or M Sc in Earth Sciences , artistic talent desirable

SALARY Commensurate with experience and potential

WRITE INQUIRIES TO Chairman, Museum Committee, Department of Geology, University of Alberta, Edmonton Alberta, Canada
(572)

NATURE Volume 217
MARCH 16, 1968

Cutting Cakes Blindfold

THE increasing self-consciousness of science policy is, of course, entirely to be welcomed even if, now and again, those responsible for making policy decisions that affect science are a little too inclined to congratulate themselves about it It would be wrong, however, to believe that the potentially good effects of this self-consciousness have already been reflected in the pattern of expenditure on science in countries such as Britain People say, with every appearance of solemnity, that their immediate concern is to establish priorities for expenditure in science and then to see that expenditure follows the appropriate rules, but there are still plenty of examples of how expenditure in crucial fields of science is still more determined by historical than by rational considerations This week, which has seen the regular meeting of the planning group of the European Space Research Organization in Paris and mounting excitement among astronomers about the pulsating radio stars discovered at the Mullard Radio Astronomy Observatory, is a splendid occasion for reflecting that the British pattern of expenditure on space, astronomy and related activities is, to say the least of it, illogical

In Britain, the Science Research Council is the chief source of funds for the support of fundamental research and, quite properly, the council has delegated much of its responsibility to the Astronomy, Space and Radio Board (For practical purposes, the substantial sums of money spent on space research by the Ministry of Technology do not impinge directly on the work of the universities, although much of the work carried out in government establishments is, as it should be, of long-term importance to basic research) In round numbers, the Astronomy, Space and Radio Board of the Science Research Council is responsible for giving advice about the allocation of £10 million a year to various kinds of activities, but it is exceedingly hard to be assured that the pattern of expenditure in this field is one that a body of rational and sensible people would deliberately have chosen if it was really free to choose

For one thing, the board is formally responsible for the expenditure of rather more than £4 million a year (in 1967–68) on ESRO—some 12 per cent of the budget of the Science Research Council as a whole During the same period, the Science Research Council is likely to have paid the Ministry of Technology £1 2 million for engineering work on satellites and for the launching of sounding rockets, and rather more than half a million pounds for the development of upper atmosphere and satellite research in the universities At the same time, the Radio and Space Research Station at Slough will have cost just over a million pounds to run, mostly on recurring expenditure Pure astronomy, as it may be called, is a comparatively poor relation In 1967–68, the cost of running three observatories—Greenwich, the Cape of Good Hope and Edinburgh—was estimated a year ago to be just over a million pounds, and during the same period universities were expected to receive from the Science Research Council some £600,000—more than three-quarters of it for capital equipment such as new kinds of radio telescopes In other words, university astronomy, conventional astronomy and radio astronomy combined cost rather less than 10 per cent of what the Astronomy, Space and Radio Board has to spend, and rather less than 8 per cent of what the Science Research Council spends each year on the support of research in the universities

Although it is easy enough to see how this pattern of expenditure has grown up, and easy to be sympathetic with those who take the line that there is now very little to be done to change it, it is hard to imagine that even the most cursory cost–benefit analysis would justify the expenditure of so much on space research, which has so far been useful but not spectacularly productive so far as Britain is concerned, and comparatively so little on astronomy It is not, after all, as if there were no opportunities for constructive expenditure in astronomy It is hard, for example, to believe that one 98-inch telescope in Sussex and the promise of a larger instrument in Australia will satisfy the needs of British astronomers in the decade ahead There is also good reason to fear that not enough is being spent on the development of instruments which can increase the productivity of optical telescopes and on the research and development from which new techniques must come And what, in any case, about radio telescopes ? The Science Research Council has been extraordinarily far-sighted in the past decade in its support for radio astronomy, but the financial uncertainties of the past few months may yet be reflected in an awkward hiatus unless somebody is resolute

But space research, like astronomy, sustains a demand for new expenditure The space research groups in the universities would no doubt be able to spend quite comfortably twice as much as they have been getting from the Science Research Council The question which they and others should ask, however, is whether, in circumstances in which no amount of spending in Britain can hope to yield a harvest of scientific information comparable with that being gathered in the United States, there would be any good purpose in a substantial increase of expenditure on space research

But this, of course, is not the real question The reason why the pattern of expenditure on astronomy, space and radio is lopsided is that the Science Research Council is bound—or considers itself bound—to chariot wheels created in the past The expenditure on ESRO must, in this spirit, be reckoned more as a political commitment than a deliberate expenditure (which is not to say that Professor Herman Bondi may not yet salvage something of importance from ESRO) And is it possible, anyway, that the Science Research Council should at this stage ask some of the subversive questions about the institutions which it administers which might lead to a radical recasting of the lopsided pattern ? If, for example, a large part of the work of the Radio and Space Research Station and the Greenwich Observatory is no longer determined by the need to provide public services—time signals and the like—is there not a case for transferring these establishments to the universities ? It is true that in the past few months the Greenwich Observatory has taken important steps to make the Isaac Newton telescope accessible to university astronomers, but there is probably a long way to go before the observatory will play its full part in stimulating and sustaining university research in astronomy It would also be good to know just precisely how the work of the Radio and Space Research Station at Slough, costing more than 10 per cent of what is spent in this whole field, contributes to the greater good

To ask all these questions is in the first place merely to suggest that they should be asked and answered If the new broom of self-conscious planning in research and development is to be taken seriously, the Science Research Council must make a determined effort to ensure, first, that it is getting value for the money it spends on organizations like ESRO and, second, that it is spending all that the circumstances require on activities where there are now immediate and promising opportunities for generosity Nobody at this stage would be surprised to learn that only half or even less than half of what is at present being spent on astronomy, space and radio is likely to yield immediate and tangible benefits in science The alarming thing, of course, is that there is every likelihood that expenditures in other fields of science have equally unbalanced distributions The problem of translating the will resolutely to pursue rational priorities in expenditure in science into action is uncommonly difficult Everybody would benefit, however, if the coincidence of a straitened budget and the recent surge of excitement in astronomy served somehow to draw attention to the need that expenditure should be more rational

Are Students Subversive?

From Berkeley to Prague, students in universities are drawing attention to themselves and to their relationship with university authorities in ways which are, it is true, characteristically different but which nevertheless have more than a little in common In Berkeley, the explosions of the past two years have now been reasonably battened down and the report of the Faculty Student Commission on University Governance which has just appeared (see page 1005) promises to provide at least a basis on which the development of the Berkeley campus can continue without too much serious interruption In Eastern Europe, by contrast students appear to be less concerned about their own relationship with the universities than with more general questions of politics and society In Britain, in this respect more like the Californian universities, students are fighting or pretending to fight on two fronts—they are protesting at what seems to be their impotence in the making of university policy and, at the same time, are embarking on foolish and sometimes wild attacks on those rash enough to visit them—Cabinet ministers and officials from foreign embassies It remains to be seen whether the relationship between British students and the rest of British society will be further inflamed by the statement by Mr Patrick Gordon Walker, Secretary of State for Education and Science, a week ago that the taxpayers who provide students with grants cannot be expected to approve the way in which things are now being managed Given his long record for choosing the wrong words in public, however, Mr Gordon Walker himself may now agree that it might have been better to say nothing

The truth is, of course, that it is foolish to behave as if the problems which are arising in a host of universities, not just in Britain, can be chased away with a few prim admonitions There are three quite separate causes of tension between students and the society in which they are embedded, and the chances are that these will become more and not less preoccupying as time goes on First, as developmental biologists must by now be tired of saying, people are maturing earlier and, perhaps more important, the increasing complexity of industrialized societies is continually requiring still more mature behaviour from partly mature people In the circumstances, nobody should be surprised that students waste a lot of time and energy on questions about the use of drugs or the rate at which they should be paid for living in what their critics consider to be an unwarranted condition of idleness and luxury Second, the increasing population of the universities and the increasing diversity of those who attend them have created among students a greater will to be concerned in the planning of universities and in the making of academic policy So long as universities were monkish places, intended to make academics, it was comparatively easy for students to accept the established pattern of the university curriculum Now that students at universities are quite properly concerned with other matters, however, it is understandable that they should want some say in the organization of the curriculum and even in the conduct of the teaching programme The question for university faculties to answer is how much they should yield to the students or, better, how they should devise new means of winning the collaboration and not just the acquiescence of students in forward planning. The third class of problems is probably not a problem at all but something that universities will have to live with Indeed, the most surprising feature of university politics is that universities have usually been much less concerned with the problems of the societies in which they are embedded than they could or even should be Outsiders often find them irritatingly indifferent to the real world and it will be no bad thing if the reawakening of political interest among students turns out to be a brash foretaste of a more solid concern for contemporary problems in the universities as a whole In this respect, British universities are more backward than those in the United States (where faculty members commute to Washington very often) and Eastern Europe

So what is to be done ? The most immediate need for change is in the relationship between students and the university machine The strength of the report of the Faculty Student Commission at Berkeley is that it suggests ways in which students can be fitted into the apparatus for making decisions at all levels on the campus The object, the report argues, is not so much to create formal apparatus by which the conflicting interests of students and the faculty can be brought into confrontation, but rather to create machinery which will somehow remind both sets of people of their mutual interest in a smoothly functioning machine for teaching It remains, of course, to be seen how much of the commission's report will eventually be embodied in the constitution of the University of California, but the mere fact that it has been written is historic in itself In Britain, the nearest tangible step in this direction is that taken at the London School of Economics, where students are to be appointed to a number of committees but specifically not the committees concerned with academic policy, and there are some reasons to believe that other universities will follow suit The trouble, of course, is that it will take time for students to appreciate that devices like these can serve as real instruments of power and possibly even longer for teachers to learn by experience that students have helpful as well as interesting things to say And in the long run, of course, it is hard to see how students can be entirely excluded from academic policy It is no longer sensible for the faculty to attempt to buy off students with tea and muffins in the afternoon and sherry before dinner Until all these things are acknowledged, tension and trouble are likely to persist.

Discussions in Paris

THE ministerial meeting organized last week in Paris by the Organization for Economic Cooperation and Development seems to have been the sort of sucess these meetings generally are The meeting, ttended by ministers of science and education from most of the OECD countries, discussed problems of the technological gap, of fundamental research policy and f information retrieval and dissemination Mr Patrick Gordon Walker, the British Secretary of State for Education and Science, agreed after the meeting that some progress had been made In particular, it seems that one of his own suggestions met with some enthusiasm This was a proposal for the establishment of a European Research Council similar in nature to the ones which operate in Britain

The funds for such an agency would be provided by what the background paper to the conference calls a "matching fund" This would presumably involve the contributions of sums of money to a central organization from the countries of OECD The money would then be distributed to existing research groups in OECD countries As Mr Gordon Walker pointed out, "It would involve a central agency I am sure that such an agency must be composed mainly of scientists and have autonomy in the arrangement of its business and the disposal of whatever funds it may have at its command " The beauty of the idea as far as Europe is concerned is that it would not involve the formation of any new institutions, as the background paper points out, it would instead encourage the development of existing research groups of outstanding quality The paper suggests two fields in which experiments might be carried out, computer science and neurobiology and psychobiology The meeting in Paris agreed that a serious study of the setting up of such a research council in Europe should be carried out during the next year

The meeting also spent some time discussing the technological gap between the United States and other OECD countries The background paper makes a determined effort to define the gaps, concluding that the term can be taken to include almost any feature of life which is different on the other side of the Atlantic Educational systems, the conditions under which government support is given, the techniques of management, scientific and technological capability and the size and homogeneity of the market in the United States are all quoted as causes of the gap Given this diversity, it was hardly surprising if the ministers were unable to define easy ways of reducing the gap Mr Anthony Wedgwood Benn, the British Minister of Technology, came back to the idea of a European Technological Institute This time the institute, which has become almost an obsession for British ministers, was described as a means by which Europe could carry out effective long term planning If Europe could carry out this task of long term market forecasting correctly, Mr Benn said, "it would then be possible to compare the existing need against the capability to meet it" At this point firms would look to their own expansion plans, and see the need for cooperation on a European scale But Mr Benn made it clear what the technological institute would not be—it would not be an academic research institute "Such laboratories may be desirable for their own reasons, as at CERN, but they do not close any technological gaps "

Gas Bonanza

THE British Gas Council has at last reached agreement over the price it is prepared to pay for natural gas from the North Sea So far, the agreement affects only one of the companies lucky enough to discover gas, the Phillips Group The price agreed is 2 87*d* a therm, on a 25 year contract affecting supplies of 350 million cubic feet of gas a day. This is much like the figure which has been widely quoted since negotiations started eighteen months ago, with perhaps a slight upward revision because of the effects of devaluation. The original contract signed with British Petroleum at 5*d*. a therm for 100 million cubic feet a day was obviously not going to be repeated—equally, the Gas Council's original offer of 1·8*d*. per therm was probably unrealistically low. Both Phillips and the Gas Council seem now to be satisfied.

This is entirely as one would expect. Despite accepting a price which some would consider low, Phillips is going to make a very handy profit. When the gas is flowing at full speed through the pipes, it will be collecting £1 million about every three and a half weeks. The investment probably cost £25 million, a sum which it will earn within two or three years. After that, all being well, it will be pure profit. But it is only fair to point out that it may not be quite so easy—the field may need further development in order to keep the gas flowing. And Phillips can fairly claim that without a generous return it would never have made the investment in the first place.

With luck, the agreement with Phillips will encourage the other companies involved to come to terms. Arpet, which shares Phillips's field on the Hewett Bank, must surely now settle on the same terms. And the other companies, Shell/Esso and Amoco (which shares its find with the Gas Council), must also now knuckle under. These finds are somewhat farther out in the North Sea, and rather deeper, so that Shell/Esso and Amoco may be able to squeeze a little more out of the Gas Council. But it is now the Gas Council which is the master, principally because the reserves of gas which have been found far outstrip demand. Even the Phillips find will be enough to supply one-third of present British demand for gas. By 1971, demand is expected to double, but even so there is likely to be something of a glut of natural gas for a decade or so. The oil companies negotiating with the Gas Council thus find themselves in a conspicuously weak position which no amount of bluster can conceal.

This should also have the effect of strengthening the case put forward by the Central Electricity Generating Board for a greater share of the gas. If the CEGB wins its case—and in the long run it can hardly fail—the principal loser will again be coal. But nuclear power, just emerging from a long and bitter struggle with the coal lobby, may now find itself embroiled in a similar struggle with gas. For the ordinary gas consumer, the Gas Council can only promise jam tomorrow. In the short term, it says, gas prices are certain to rise, in part because of the crisis in the Middle East. In the long run, Sir Henry Jones of the Gas Council thinks gas prices will fall, but less optimistic observers fear that natural gas will do no more than prevent an upward spiral in prices. To the domestic consumer, paying almost ten times as much as the Gas Council is paying Phillips for each therm of gas (the average price of gas to the consumer is about 25·5*d*. per therm), this seems very hard to accept.

Exploiting Technology

THE role of public research and development services in British technology has been evaluated by the Institution of Professional Civil Servants, and recommendations for improvements are published this week in a report entitled *Exploiting Technology*. The executive committee of the institution decided to look into the way research and development in the public sector are operating, with the belief that it could make proposals for action as well as examining the facts. The 70,000 members of the institution work in all branches of the public sector—government departments, public bodies, and with the research councils.

The report begins with the assumption that research and development are the key to any nation's prosperity, and Britain is not making the best use of its resources in this direction. Left to itself, industry does not undertake the necessary research and development, and although there are various forms of government encouragement—through such bodies as the Industrial Re-organization Corporation, the National Research Development Corporation and research associations and advisory services—there is scope for much more. The report discusses suggestions that more research and development should be taken from the public sector by industry, and concludes that little would be gained from this, as ideas are often badly thought out or too long-term to be of practical value in the near future. Any shake-out of staff from the public sector would in fact have very little effect in industrial research. Buildings, equipment and experienced staff are already in existence in the public sector, but although many of the organizations have been doing a good job, the report suggests that most of them could be effectively streamlined.

In its recommendations, the report calls first of all for a clear statement of priorities from the Government, so that money is not wasted on research that is too expensive or will shortly be abandoned. After this, closer cooperation between public sector research and industry is the main theme. Rationalization of organizations doing similar research work and industries with similar problems is called for, together with closer working of public bodies with industry so that research teams investigating problems in industry and industrialists are brought into research councils and the management of government establishments. A central body, organized by the government, should take charge of the coordination of scientific and technical information and its dissemination, but commercial security must be safeguarded. Exchanges of manpower are desirable, and the report suggests that mobility can be encouraged by workers having transferable pension schemes. Refresher courses are recommended for qualified staff, and it is suggested that firms will benefit if scientists and engineers are trained in management techniques. With the realization that streamlining of the public sector will affect members of the institution, the report states that there must be adequate preparation for the social problems involved in the changes of employment resulting from this reorganization.

Doctorates in Physics

THE Royal Society has just published a report on th training of PhD students in physics. The report form part of a Royal Society survey, started in Novembe 1965, on postgraduate training in science and technology in the United Kingdom. Sub-committees wer set up to investigate the content and quality of trainin for the PhD degree in six groups of subjects—biology, chemistry, engineering, earth sciences, mathematics and physics The report of the chemistry group was published in March last year, and the Royal Society hopes that the reports of the other four groups will appear before the end of 1968

The physics sub-committee, under Professor C C Butler of Imperial College, limited its attention to a study of postgraduate lecture courses attended by physics PhD students and a comparison with courses available in graduate schools in the United States Butler sent a letter to the heads of all university physics departments asking for brief details of postgraduate lecture courses attended by PhD students, and it is on the replies to this letter that the new report is based Rather surprisingly, the sub-committee did not ask the PhD students themselves for views on postgraduate training

The present undergraduate course is no longer sufficient to prepare students for a full professional career, and the report approves of the recent trend towards lecture courses for PhD students. The sub-committee realizes that it is often difficult for experimentalists to complete a PhD within the three years of a Science Research Council maintenance grant, and any extension of course work will increase the difficulties It therefore recommends that provisions for fourth year grants should be made in certain cases

Comparison with graduate schools in the United States—largely based on the opinions of British physicists who have worked in American universities—revealed no evidence that the standards of PhD training are significantly different in the two countries There seems, however, to be some foundation for the widely held view that the American experimentalist has a broader theoretical knowledge than his British counterpart

Some of the value of these six studies of postgraduate training in science and technology is being lost because the organization of the surveys is being left to the discretion of the individual chairmen The chemistry report, for instance, was based on enquiries among university professors and six centres of chemistry in the United States, while the more ambitious sub-committee for engineering has written to 1,900 postgraduates and to 90 companies and institutions This will make comparison of the training afforded to students in different subject groups difficult

Soul-searching for ESRO

On Wednesday and Thursday of this week there was a meeting of Alternates of the European Space Research Organization (ESRO) where the main preoccupation was expected to be the continuing exercise of preparing ESRO's case (see *Nature*, **216**, 696, 1968) for the European Space Conference in Bonn in June It is hoped that here a policy and programme for European space will finally be settled At the last European Space Conference, in Rome last July, a special committee was set up to evaluate the various proposed projects in European terms This Advisory Committee on Programmes was chaired by J P Causse from the French aerospace industry, supported on the technical side by five independent experts, and it produced its report at the turn of the year The ESRO Alternates were to consider it in detail at this week's meeting There seems no reason why the Causse Committee's report should not then be published

The main dilemma facing ESRO is whether its activities should be substantially redirected towards "useful" satellites, such as the communications satellite sponsored by CETS (the European Conference for Telecommunications by Satellite), or retain its original purpose of providing scientific research opportunities beyond the resources of individual countries The immediate clash is between the European communications satellite and the large astronomical satellite as candidates for ESRO's major project for its first decade. It seems out of the question that both projects could be adopted at the present financial level of European space organizations It will be interesting to see what the Causse Committee has had to say about their relative merits

Full Stop on Decimals

Britain's plans to adopt the metric system of units have run into a little local difficulty. A dispute has arisen over the decision by the Decimal Currency Board to use the full stop or point as a decimal marker, instead of the comma which is common in metric countries The British Standards Institution, which is responsible for organizing the change to the decimal system in British industry, favours the comma, combined with a space to indicate thousands The construction industry, the first to embrace metric units, has already begun to issue reports in this form If the Decimal Currency Board is to have its way, both the BSI and the construction industry will have to think again

The traditional system uses a full point for the decimal sign and a comma for indicating thousands, the new system supported by the BSI would use the comma for the decimal sign, and each group of three figures would be separated by a space Thus the Decimal Currency Board would write 5,650·3286 and the BSI would prefer the form 5 650,328 6 It is no secret that the joint metrication committee of the Ministry of Technology, an advisory body under the chairmanship of Mr A H A Wynne, favours the system adopted by the Decimal Currency Board Unlike most aspects of the metric system, there has so far been no international decision over which system to adopt, and this has made the committee's decision a difficult one In reaching it, it took into account a number of factors

First, there was the evidence provided by two questionnaires sent out by the BSI The first, to the general engineering industry, produced inconclusive results The second, which included financiers and merchant bankers, produced, predictably enough, a result in favour of traditional practices The bankers felt that a system using empty spaces was an open temptation to fraud The Decimal Currency Board—a notably conservative body—was swayed by much the same argument in favour of the traditional system The Confederation of British Industry is also said to prefer the traditional system In spite of this, the BSI continues to favour the space/comma system, and has written to the Decimal Currency Board to say so The Ministry of Technology's answer to this is to say that, as long as there is no international system, it would be best to stick to the existing system If the BSI's appeal fails, it is likely that the construction industry will have to go back on its decision to use the space/comma system

When an international conference is called, of course, it is not impossible that yet another system will emerge There is, for instance, some support to be found for a system which would use a colon to indicate the decimal point But this is still highly speculative Meanwhile, the Ministry of Technology and the Decimal Currency Board say that Britain should stick to conventional practices Colonel J S Vickers of the BSI, the keenest supporter of the space/comma system, will find it difficult to change their minds

Physics Exhibition 1968

by Professor S. Tolansky

THE 52nd exhibition arranged by the Institute of Physics and the Physical Society at Alexandra Palace, London, during March 11–14 was, true to an old tradition, essentially a British show Foreign contributors could be counted on one hand Noteworthy was the distribution of exhibitors, for while industry supplied a little over a hundred, there were in addition no fewer than 45 government-sponsored laboratories represented and some 40 separate higher educational establishments (universities, colleges of technology and teaching hospitals) The encouragement of the latter by the offer of free stands is highly praiseworthy By far the dominant single contributor to the exhibition was the United Kingdom Atomic Energy Authority, which controlled 23 separate stands with displays from 7 different stations

In accordance with usual practice, participation was by invitation Because all exhibits are expected to illustrate novel features, and educational displays require to be passed by referees, the overall standard was both original and attractive The items displayed ran to nearly a thousand, with a distinct bias towards electrical and electronic devices Vacuum technology came off reasonably well, although there was some very expensive equipment, which would be too sophisticated for the university laboratories Quite a number of pieces of microwave equipment and several electrical bridges were shown

One notable item was a Mullard infrared microscope, easily adjustable and using a liquid air cooled indium antimonide detector Performance was attractive and there are clearly many uses for this compact device Mullard also demonstrated the modulation of the infrared output from a filament lamp using yttrium iron garnet Engelhard-Hanovia demonstrated a neat sensitive optical observation device for the detection of small amounts of mercury vapour in laboratory atmospheres My distinguished former teacher, F Paschen, always used to insist in the late twenties and thirties that the mercury level in many university laboratories was a dangerous hazard

Particularly notable was the exploitation of gas lasers at light sources These seem to be quite common laboratory objects now Lasers apart, optics was not strongly represented True, there were some compact high speed cameras and a little-modified long-used optical testing interferometer, yet no special lenses, no visible light microscopes, no new optical materials, etc In fact, "classical" physics was weakly represented, although there were a microcalorimeter, a micromanipulator, a small photometer, an acoustic telemeter, a moisture recorder, cyrostats, new ultraviolet spectrometers and some applications of ultrasonics Holography was represented, but here there was nothing startling, and thin films, either their production or measurement, occupied a few displays

The educational displays with their simple "hook-ups" attracted a good stream of visitors, but here too there was a strong bias towards electrical devices It was a relief to see some fine high resolution interference fringes from Belfast, an ultraviolet radiation meter from Charing Cross Hospital, and a new optical instrument from the Royal College of Surgeons which can measure alcohol vapour down to concentrations of 400 parts per million These and numerous other clever original devices, many designed by the young research workers who were themselves enthusiastically demonstrating their own products, were a heartwarming indication that there is little need to worry about the ultimate future of British physics instrumentation The feed-in from below is certainly sound, although regrettably too electrical

The relatively wealthy government establishments laid on sophisticated devices, all beautifully constructed and magnificently illustrated Yet a lot of this quite expensive publicity polish is quite superfluous, especially in a show for professional physicists A certain number of exhibits were frankly educational, but most of these were dull One, however, a well-conceived atomic optical pumping device from Oxford University using rubidium, clearly has wide teaching applications. There were some excellent book displays in the educational section

I came away from the exhibition somewhat bewildered by the overpowering emphasis on electronic circuitry There seem to be fashions in modern physics as well as much sad disregard of classical subjects There was, for instance, only one display devoted to Mossbauer effect, but no less than ten exhibits exploited lasers Indeed, lasers occupied more space than such normally popular subjects as telemeters or cryogenics or even semiconductor devices

Information on Biodeterioration

BIODETERIORATION means the deterioration of materials of economic importance by living organisms The word was coined in America, but it is a centre at Aston University, Birmingham, which is taking the lead in informing research workers and others of developments in this particular field The centre, the Biodeterioration Information Centre, under the direction of Dr Howard Eggins, was set up in 1965 with the help of a grant of £12,000 from the Office for Scientific and Technical Information It was the first of several information centres now functioning to be sponsored by OSTI The centre is now fully operational and a press conference to publicize it was held last week—the OSTI grant runs out this year, and the organizers naturally hope that more money will be forthcoming from other sources

Some examples of biodeterioration are the attack on wood, paper, textiles and grain by fungi and insects, the blocking and corrosion of pipelines by bacteria, the growth of fungi and algae on concrete and painted surfaces, the fouling of ships and marine structures by barnacles and molluscs, the destruction of stored products by rodents, and hazards caused to aircraft by birds It has been estimated that European countries alone lose £400 million annually through biodeterioration of goods such as timber, textiles, jet fuels, paint, metals and plastics The figure must be very much higher than this for the whole world, because biodeterioration is an ever more serious problem in tropical countries The loss of stored food products from spoilage, contamination and deterioration varies from one country to another, being worst in the developing countries, but over the world as a whole it is probably about 30 per cent As the report of the US President's Science Advisory Committee on *The World Food Problem*, published last year, put it, "If only half of the estimated world loss of food grains was prevented, it would represent an additional 55 million tons or enough to make the diet of 500 million people in developing countries adequate in total calories" And the loss of food supplies is only part of the biodeterioration problem—a problem which is made worse by the interdisciplinary nature of the subject which leads to difficulties in communicating information

The Biodeterioration Information Centre hopes to help solve the problem by providing an information service to workers concerned with biodeterioration all over the world The centre collects articles on the subject selected by specialists in more than 30 countries Each item is indexed with appropriate keywords selected from a specially prepared thesaurus and is recorded in a punched card index system All the accessions of the centre, which number 2,500 to 3,000 articles a year, are listed in a classified bibliography (IBBRIS) which is published quarterly A photocopy service for inaccessible literature is operated and a question-answering service provides either IBBRIS accession numbers or, when available, author abstracts of articles in the collection A further service is the publication of original and review papers in the *International Biodeterioration Bulletin* Associated with the centre are two laboratories where research into microbiological deterioration is done Current research projects include studies on the breakdown of cellulose by fungi, the growth of fungi in high temperatures and the detoxification of preservatives by fungi and bacteria

Not for Drinking

WATER from the mains is not as pure as most people would like to think This was clearly emphasized on March 8 during the afternoon technical sessions of the sixteenth annual general meeting of the Society for Water Treatment and Examination In the rather spartan main hall of the College of Preceptors, Bloomsbury Square, the audience was told how living organisms ranging in size from viruses to beetles are contaminating water in Britain Fortunately, most of the animals involved are not known to involve risk to human health, although infectious hepatitis—a faecally excreted virus—is transmitted by water Dr S F B Poynter, senior assistant bacteriologist to the Metropolitan Water Board, suggested that not only are large numbers of potentially pathogenic organisms excreted—for example, the three types of poliovirus, and adenoviruses—but it has been shown that existing methods of sewage treatment are ineffective inactivators of these viruses A typical disinfectant, combined chloride in its various forms, is relatively useless as a virucidal agent As a result, viruses are discharged in the effluents flowing into rivers and are then borne to water storage reservoirs Trickling filters seem to be particularly inadequate, but a number of investigators have found that activated sludge treatment is more effective During the discussion Mr R W Covill of the Lothian River Board, Edinburgh, said that he knew of a case of partially and unpurified sewage being used to irrigate crops of lettuces and strawberries, and suggested that there is a need for more stringent precautions in this connexion

Animals in mains are also creating an increasing problem, which Mr I C Smalls and Mr G F Greaves of the Biology Division at the Water Research Association have investigated. According to a survey which they have undertaken, infesting animals may give rise to complaints by the appearance of individual animals at the tap, by discolouring the water with, for example, faecal pellets, or possibly by causing tastes and odours as a result of the decomposition of dead animals in the mains by bacteria, fungi and actinomycetes Regular tap samplings of a Scottish supply yielded whole animals, such as *Cyclops strenuus abyssorum*, *Chydorus sphaericus*, *Diaptomus* sp and *Rotifera*

Asellus aquaticus grips tenaciously to nodular encrustations in mains, while some *Nais* sp and some blood worms form protective tubes from organic detritus and rust particles attached to pipe walls Other strains are able to produce resting stages in order to survive unfavourable conditions According to Mr Smalls, underground supplies contain fewer species and smaller total numbers of organisms than supplies from the surface because underground water is less productive of organic matter on which the animals feed Systemic flushing has been used as a control measure, either by itself to flush out free-swimming animals, or together with chemical dosing to remove more tenacious species

Degrees by Television

THE planning committee of the Open University (which is the British Government's name for its proposed television university) has now outlined the pattern of the degrees which will form the core of the university's work when it begins in October 1970 or January 1971 Details of the courses will be left to the university staff to work out, but the basic system, which requires six credits for an ordinary degree and one year's work for each credit, has been put forward by the committee appointed six months ago under the chairmanship of Sir Peter Venables, Vice-Chancellor of the University of Birmingham (see *Nature*, **215**, 1322, 1967) No formal academic qualifications will be required for entry and the normal minimum time for a degree will be four years In exceptional circumstances, particular individuals might be allowed to qualify in a shorter time, but part-time students might well take longer than the minimum

Four broad "lines"—science, technology, social science and arts—have been set out for degrees, but the maximum flexibility will be provided so that students can take courses from each line Two credits in the first year foundation courses will normally be required before students can proceed to further study, but, after that, courses offering different depths of specialization can be followed. All credits for successfully completed courses will be of equal value, eight credits being required for an honours degree, two more than for an ordinary degree. There will probably be continuous assessment of student progress as well as examinations, and certificates will be awarded for each credit obtained. The committee considers that it will be essential to have an efficient student guidance service to advise on the limitations and wide possibilities of the courses and to recommend courses suitable to the various aptitudes and interests of individual students.

Correspondence courses will form the basis of every degree, and viewing–listening centres throughout Britain will have to supplement live broadcasting. The four foundation courses for the four degree "lines" will be broadcast in the first year of the university's life, and will need to be repeated every year to start students off. It will not be possible to broadcast all the component courses every year, but it is hoped that students will be able to obtain the broadcast material by some other means. One contact a week between student and tutor is suggested for foundation courses, but the committee stresses that the subject of contacts must be carefully examined.

The committee considers that other courses—professional, technical and refresher—should be instituted at the opening of the university, some of them with no examinations at the end. The committee adds that "shortage of transmission time of the broadcasting media should not be thought of as an impediment to putting on such courses. Other methods of making them available to potential audiences may very well have been developed by 1970 and should be used."

The university will also encourage other bodies such as local education authorities, extra-mural departments of universities and workers' education associations to organize courses as preparation for the university's own degrees. Regional officers will be appointed to coordinate local efforts.

The planning committee meets at present once a month, but working groups for "ways and means", "students and curriculum" and "constitution and organization" meet once a week for the bulk of the preparatory work. A fourth group is to be set up to consider finance and the costs of decisions that have so far been taken by the committee. With the charter of the university worked out, the constitution and organization group is likely to fade out, and further decisions will be taken by the committee as a whole. The relative merits of opening the university in October or January are at present being discussed, for there could be advantages in beginning the academic year in January and being able to continue through the summer when educational facilities are least used. In organizing local centres for study, the university hopes to pay rent for facilities that are under-used, such as television sets in schools which are not used in the evenings. Library facilities are also being discussed with the appropriate authorities. The 1966 White Paper (*A University of the Air*, HMSO, 1*s*.) stated that an "estimate of the potential audience can only be guesswork". As yet there has been little progress towards translating the guesses into hard figures, but a survey will be carried out this spring. It is expected that students will be older people rather than school leavers.

Finance has yet to be fully investigated, so no figure has yet been set for fees that students will pay. Salaries for academic staff and fees to the BBC for broadcasting will obviously take large chunks of the budget. Arrangements with the BBC have to be finally agreed, but transmission will be on BBC-2 which should, by the time the university begins, be covering at least 95 per cent of the country. The first courses are not expected to be broadcast in colour. No academic staff have been appointed yet, but the planning committee is thinking in terms of a core of full-time staff with an additional floating staff to help in the production of courses. The posts of vice-chancellor and secretary are the only two advertised so far and applications are under consideration.

Chairman for NERC

PROFESSOR V. C. WYNNE-EDWARDS, regius professor of natural history in the University of Aberdeen, is to be the new chairman of the Natural Environment Research Council in place of Sir Graham Sutton, who retires on September 1 this year. Professor Wynne-Edwards is well known for his contributions to ecology—particularly for his studies on animal populations. His book, *Animal Dispersion in Relation to Social Behaviour*, was published in 1962. A fellow of the Royal Society of Edinburgh and of the Royal Society of Canada, he has held appointments at the universities of Bristol and McGill. He has been regius professor of natural history in the University of Aberdeen since 1946, and it is understood that he will continue to hold this post.

From 1954 to 1957, Professor Wynne-Edwards was a member of the Nature Conservancy, from 1954 to 1966 he was a member of the conservancy's Committee for Scotland, and since 1964 he has been a member of the conservancy's Scientific Policy Committee. He was appointed a member of the Natural Environment Research Council when the council came into being in 1965. From 1965 to 1967 he was chairman of the council's Oceanography and Fisheries Research Grants and Training Awards Sub-Committee. He is at present a member of the council's Oceanography and Fisheries Committee and chairman of its recently formed Freshwater Research Sub-Committee.

Defence Research

THE House of Commons Select Committee on Science and Technology is now well set into its examination of defence research and development policy in Britain. In fact it has clearly been doing a good deal of work on the quiet, and showed off its progress at the first open meeting this session, when the witnesses came from the Ministry of Technology and the Ministry of Defence. Both ministries had submitted memoranda to the committee and, perhaps revealingly, both were dated October 1967, a time when the select committee had only a notional existence—according to the rules of the House, it has to be re-established at the beginning of each session.

One result of this was that the Ministry of Defence document was slightly out of date—perhaps an occupational hazard in a department which on its own admission is used to working on projects with lead times of seven to ten years. Much of the discussion was on the procedures by which the Ministry of Defence keeps a check on research projects—the so-called Gibbs–Zuckerman procedures Mr G Leitch, Deputy Under-Secretary (Equipment), said that these were designed to give pauses for thought at crucial stages of any project Less charitable observers have suggested that the procedures are laborious and time-wasting, but Mr Leitch denied this With hindsight, he said, one could say, "Well, perhaps we did waste thousands of pounds by waiting at a certain stage—but isn't this better than wasting millions later ?"

The Ministry of Technology also submitted a memorandum, parts of which bore evidence of the minister's personal touch (As well as calling the Ministry of Technology "Mintech", the memorandum goes on to call the old Ministry of Aviation by the awful title "Avmin") The memorandum explains that the majority of research contracts are let on the basis of "a carefully controlled development cost plan" It might therefore have been better if the memorandum had not gone on in the next paragraph to discuss the projects in which the United States Phantom has been refitted with Rolls-Royce Spey engines This has been a very costly project The first thing the ministry witnesses revealed was that the long promised White Paper on research policy is not now expected until "the second half of this year" (It should originally have been published by now) In part the delay was because of changes in defence requirements, and in part because of the discussions the minister was having about the future of the Atomic Energy Authority

Later, Dr G G Macfarlane, one of the three controllers of research in the ministry, produced an interesting argument Defending the role of the ministry establishments, he said that in reality the balance of research work between research establishments and industry was much the same in Britain as it was in the United States The general impression that the United States puts more research work into industry and does less in establishments was wrong, he suggested The National Aeronautics and Space Administration had large laboratories The Lincoln Laboratory at MIT, publicly supported, was almost a replica of the Royal Radar Establishment at Malvern He did say that the tendency in Britain was now to put more into industry, but this did not mean that the establishments were redundant, and he denied the suggestion by Mr B Parkyn, a member of the committee, that the establishments were needed only to test the feasibility of schemes put up by industry There was plenty of basic research to be done, and the establishments were always useful as a fire-brigade when crises arose

Although this first meeting was dull, the investigation as a whole will be a fascinating exercise The committee is likely to call, among others, Professor P M S Blackett, Sir Solly Zuckerman and Minister of Defence Mr Denis Healey To help it in the investigation, the committee has signed on two advisers, Mr Christopher Harlow, who works for Political and Economic Planning, and Mr W R Thomas, chief scientist of Elliott Automation This is a welcome move, although the way the committee set about finding its advisers was a trifle odd Mr Thomas's experience of government service at the Royal Aircraft Establishment at Farnborough may well be of particular value

Parliament in Britain

by our Parliamentary Correspondent

Radio-telescope

MRS SHIRLEY WILLIAMS could give no information about the siting of the new radio-telescope for the Jodrell Bank Observatory But she did agree that Oswestry was one of the sites considered for the telescope The sites were all within 100 miles of the Jodrell Bank radio-telescope (Written answer, March 7)

Concorde

MR HUGH JENKINS, Labour Member of Parliament for Putney, seems to be opposed to the Concorde supersonic airliner He asked the Minister of Technology if he would take steps to ensure that, when the Concorde started to operate, the noise limits at Heathrow Airport would be the same as they are at present For the Ministry of Technology, Mr Gerry Fowler agreed that this was a design aim, but hedged by saying that the noise estimates were being continually revised as development proceeded He refused to say when Concorde would make its first flight (Oral answer, March 11)

Phantom

MR TAM DALYELL tried to establish how much it has cost to fit the Phantom aircraft bought from the United States with British engines, the Rolls-Royce Spey For the Ministry of Technology, Mr Gerry Fowler said that the cost so far had been about £75 million, which included the cost of developing the engine and adapting the airframe About sixteen Phantoms out of the total order for 170 had been treated so far Mr Fowler added that they had been aware that it might be expensive, but that it had improved the performance of the aircraft, which was necessary for naval operations (Oral answer, March 11)

Defence Research

MR TAM DALYELL has also been trying to establish how much civil research work is carried out at defence establishments So far he has asked dozens of questions without receiving any very surprising answers On March 11, for instance, he asked the Minister of Technology what general policy he had for increasing the amount of civil work in progress at the ministry's defence establishments Mr Gerry Fowler said that it was not the Government's policy to build up these establishments as civil research establishments, or to seek civil work merely to keep them at their present size He seemed quite satisfied at the arrangements that had been made for getting the results of military research over to industry Mr Dalyell later had a go at the chemical and microbiological research establishments at Porton First he asked what the toxicity of CS gas, ortho chloro benzyl malano nitrile, was Mr Roy Mason, for the Ministry of Defence, said that the toxic dose for man was about 150,000 milligram minutes per cubic metre, which was 50,000 times the dose which could be tolerated by human beings, and 200,000 times the dose needed to disperse rioters No authenticated report had been received of CS causing death or injury Mr Mason also gave some information on the civil benefits of the work at Porton—protective clothing, and the study of air pollution But he refused to de-classify all future work carried out at the Porton establishments (Written answers, March 11)

NEWS AND VIEWS

Where *Escherichia coli* K12 Came From

THE chances are that one of the many mutant variations of *Escherichia coli K*12 is included in the stock culture collections of research laboratories investigating problems of bacterial genetics Mutant variations of *E coli K*12 form the basis of microbiological teaching studies in many university degree courses Yet few workers, despite their intimate knowledge of the genetical and biochemical properties of the mutant variants, know the origin and date of isolation of the original parent strain of this organism One of the first records of its use appeared in an article by C H Gray and E L Tatum called "X-ray induced Growth Factor Requirements in Bacteria" (*Proc US Nat Acad Sci*, **30**, 404, 1944) Working in the School of Biological Sciences at Stanford University, Gray and Tatum used for their study the bacterial species *E coli K*12 and *Acetobacter melanogenicum* (*M A* 6 1) The object of the experiment was to see whether it is possible to induce the formation of biochemical variants of the strains by exposure to X-rays Two such variants were isolated from the parent strain of *E coli K*12 The authors in their acknowledgments recognized their indebtedness to Dr—now Professor—C E Clifton of the Department of Bacteriology at Stanford University for providing the strain In a later paper, Tatum (*Proc US Nat Acad Sci*, **31**, 215, 1945) described the isolation of further mutant variants by a second X-ray irradiation of the two mutant strains obtained by Gray and himself in 1944 J Lederberg and Tatum, writing in *Nature* (**158**, 558, 1946), indicated that they had obtained triple mutants

The first and apparently only indication of the origin of *E coli K*12 was put forward by Tatum and Lederberg in an article entitled "Gene Recombination in the Bacterium *Escherichia coli*" (*J Bact*, **53**, 673, 1947) There the authors stated that the mutants used were obtained from a single strain of *Escherichia coli K*12 They described this organism as a "typical coliform bacterium originally isolated from human faeces—a Gram-negative rod, motile, lactose-fermenting, producing indole and susceptible to each of the *coli* phages in the series *T*1 to *T*7 of Demerec and Farro (1945)" It is also mentioned that the strain had been used for several years at Stanford University in the students' laboratory In 1960, Ida and Frits Ørskov pointed out (*Acta Path Microbiol Scand*, **48-49**, 47, 1960) that "12" was a strain number and that the letter "*K*" had nothing to do with the *K* antigen of that strain The resolution of the origin of *E coli K*12 depends, however, on Professor Clifton, whose records show that this particular strain had been isolated from the faeces of a diphtheria convalescent in 1922 and added to the stock collection at Stanford in December 1925 The place of isolation is not clear, but Professor Clifton guesses that it may have been Stanford Hospital The "*K*" is a catalogue symbol and "12" indicates that the particular strain was the twelfth culture of *E coli* that was added to the stock collection. Since 1925, the strain has remained on nutrient agar at Stanford as a stock culture for teaching and research purposes

Comparative Zoology at Harvard

THE Museum of Comparative Zoology, the Agassiz Museum at Harvard University, bristles with famous names among its staff—Professor Ernst Mayr, the director, Professor F Carpenter, Professor P J Darlington, jun, Professor B Patterson, Professor G Gaylord Simpson, Dr A Sherwood Romer, Dr W J Clench—to name a few Some of the work of these and of the other staff of the museum during 1966–67 is described in the latest annual report just published

Like many other museums of its type, the Museum of Comparative Zoology has teaching and curatorial responsibilities, expeditions are organized to build up collections, and staff travel to study collections in other museums Research conducted in the museum covers a wide range of topics—evolution, behaviour, ecology, zoogeography, physiology and biochemistry and taxonomy Almost all the research produces results of interest to the evolutionist One interesting find during the year was the discovery of a fossil insect from Cretaceous amber from New Jersey This is the oldest known ant and is apparently virtually a missing link between ants and wasps The presence of worker characteristics in these insects is evidence of the existence of social Hymenoptera as far back as about 100 million years

The discovery of several new specimens has enabled Professor Simpson to start work on the evolution of an extinct family of South American marsupials, the Argyrolagidae Until recently, it was not even certain whether these animals were marsupials Apart from the mystery surrounding their relationship, these fossils are interesting because they represent an ecological specialization unique among marsupials or any other South American mammals, and they convergently resemble the unrelated "kangaroo" rats of North America A method of food gathering unique for the bivalve molluscs and previously unrecorded was discovered by Dr Turner of the museum—she has found that the elaborately branched ciliated incurrent siphon of the shipworm *Neoteredo reynii* acts as a filter-feeding mechanism

Dr R H Chesher has adopted a new approach to his studies of the classification and variation of heart urchins (Spatangoida) As a deep-sea diver, he has taken his investigations to the sea floor itself, and he has found that some of the features hitherto used in the classification of these animals may be subject to non-genetic modification by the environment

An analysis of the ammonite fauna of the world in one subdivision of the geological time scale has been completed by Dr Bernhard Kummel. A study of all collected taxa of ammonoids for the uppermost zone of the Scythian (Lower Triassic) shows that the total fauna consists of 65 genera and 155 species. A comparison of the faunas of the Tethyan, the eastern and western Pacific, and the circum-Arctic regions shows that there is a diversity gradient, most probably reflecting a latitudinal change in climate: the faunas of the Tethyan region are rich in numbers of individuals as well as in species; the Arctic region, on the other hand, is poor both in number of specimens and in species. These results appear to support the thesis of continental and oceanic permanence for this short segment of time.

Work on thermoregulation in the beaver was completed by Dr Coles. He has been particularly concerned with the role of the tail in this regulation. Beavers are unable to lose much heat from their bodies because of their heavy fur cover; when they are exposed to heat, the naked tail is the main region of heat loss. When they are exposed to cold, however, little heat is lost from the tail because of a counter-current heat exchange system among the arteries and veins.

Long Haul

from our Correspondent in Molecular Biology

DESPITE a great deal of information which has accumulated about the function of the ribosome, it remains a largely inscrutable structure. The large number of protein components in particular gives an impression of discouraging complexity. A number of groups of workers have, however, embarked on the heroic task of fractionating the proteins of *E. coli* ribosomes and characterizing them individually. The first studies were from the laboratories of Tissières and of Moller, and the Geneva group have now reported their findings to date (Moore *et al.*, *J. Mol. Biol.*, **31**, 441, 1968).

They have begun by fractionating the extracted proteins of the 30*S* sub-unit. The evidence of gel electrophoresis and ion exchange chromatography indicates that there are twenty separate components. Of these, thirteen have been isolated in states of 80–90 per cent purity. Their molecular weights have been determined by sedimentation equilibrium, and are found to vary between about 5,500 and 28,000. The relative weights of the various components were obtained by radioactivity measurements on the ^{14}C-labelled components, separated in polyacrylamide gels. This gave the important and unambiguous result—confirming earlier surmise—that one molecule only of each ribosomal protein is present in each 30*S* particle. Moore *et al.* suggest that a true ribosomal protein should be defined as one which obeys this stoichiometry.

In order to ascertain whether common features of sequence exist in the thirteen isolated proteins, tryptic fingerprints were examined. By this criterion there are no detectable homologies. It is thus likely that these are structurally unrelated molecules controlled by different genes.

A promising approach to the study of ribosomal proteins *in situ* is described by Ostner and Hultin (*Biochim. Biophys. Acta*, **154**, 376, 1968). They have treated ribosomes (in this case from rat liver) with proteolytic enzymes, and then extracted the protein and examined it on polyacrylamide gels for fragmentation. Only some of the proteins were susceptible to proteolytic attack in the ribosome and these were degraded by all three proteolytic enzymes which were tried. The remaining proteins were highly resistant. By contrast, extracted proteins are rapidly and extensively degraded. It is concluded that the proteins are in large measure inaccessible in the ribosome. Because treatment with the enzymes brings about some dissociation of the ribosomes, it is not certain to what extent it is the separated sub-units which are attacked. It is also, of course, difficult to be certain that the enzymes contain no nuclease contaminants, though the activity of the ribosomes in respect of incorporation after appreciable exposure to the proteases gives the hope that this may not be serious.

The function of the different ribosomal proteins is another issue, and one on which some progress has been made. The identification of a new protein factor, associated with the ribosome but washed off in ammonium chloride solution, has now been reported by Brown and Doty (*Biochem. Biophys. Res. Commun.*, **30**, 284, 1968). This protein (given the arresting name of Factor 3) is required for the binding of synthetic messenger and probably also viral RNA, and its discovery is therefore an important advance. Apart from direct binding studies it was also shown that only in the presence of Factor 3 were ribosomes able to enhance RNA synthesized by DNA-dependent RNA polymerase: the freshly synthesized RNA causes product inhibition of the enzyme, and the ribosomes can act as a trapping agent for the inhibitor. Brown and Doty also quote as yet unpublished work along these lines by Gros and his collaborators.

Polarity of RNA Phages

from our Cell Biology Correspondent

AN amber mutation at the position of the codon specifying the sixth amino-acid (glutamine) in the coat protein cistron of the virtually identical RNA bacteriophages *R*17 and *f*2 has a polar effect. The mutation not only causes the termination of synthesis of the coat protein but also reduces the level of expression of the cistron which specifies the enzyme RNA synthetase. As a consequence, during single infection with the polar coat mutant there is a marked depression in the synthesis of phage double and single stranded RNA molecules and the polar coat mutant fails to complement amber mutants in the RNA synthetase cistron during mixed infections. This polarity is, however, suppressed by hosts containing strong amber suppressor genes.

The question then is what is the mechanism of polarity in RNA phages. One possibility—a purely translational model—is that the polar amber mutation in the coat cistron prevents the translation of the infecting parental RNA molecule (parental plus strand). This model predicts that, as little or no RNA synthetase is made, very few of the parental RNA molecules should be incorporated into a double stranded replicative form *in vivo* (see *Nature*, **214**, 1287, 1967). In the absence of RNA synthetase the synthesis of a complementary minus strand and then progeny plus strands is obviously blocked from the outset of infection. Alternatively, if, as Stent originally proposed, translation and transcription are coupled, the infecting RNA molecule might be translated, RNA synthetase made and a minus strand and replicative form made

Further transcription, however, of progeny plus strands from the replicative form would be blocked because translation of the nascent plus strands would be terminated by the polar mutation This less likely model requires that the infecting plus strand can be translated whereas nascent progeny plus strands cannot

Recent work by Roberts and Gussin (*J Mol Biol*, **30**, 565, 1967) and latterly Lodish (*J Mol Biol*, **32**, 47, 1968) indicates that the first, purely translational model is correct Roberts and Gussin using the polar coat mutant of R17 showed conclusively that, as predicted by the translational model, infecting RNA molecules are only poorly incorporated into a double stranded replicative form during infection of a host that lacks amber suppressor genes Lodish has shown the same for the polar coat mutant of *f*2 The level of incorporation of parental RNA molecules into a double stranded molecule is only 15–25 per cent of the wild type levels

Furthermore, Lodish has shown that the few double stranded replicative form molecules that are formed with the polar coat mutant RNA function normally as templates for synthesis of progeny plus strand RNA molecules The amber mutation does not therefore block the transcription of progeny RNA molecules from a replicative form (Gussin's 1966 delayed complementation experiments also indicate this)

To prove this, Lodish used a double mutant which has the polar coat mutation and in addition a temperature-sensitive mutation in the RNA synthetase cistron This temperature-sensitive mutation affects the RNA synthetase enzyme so that at 43° C it can synthesize plus strands but not minus strands Thus at 43° C a parental plus strand displaced from the double stranded template by the synthesis of a progeny plus strand does not serve as template for the synthesis of another minus strand At 43° C, therefore, displacement of parental plus strands which are not reincorporated into double stranded molecules becomes a sensitive measure of plus strand synthesis What Lodish found is that both wild type and double mutant RNA molecules are displaced from a double stranded template at the same rate at 43° C The polar mutation therefore has no effect on transcription of double stranded RNA The only effect of the polar mutation on RNA replication is that it depresses the incorporation of parental plus strands into a replicative form

These results, together with the demonstration of polarity in *in vitro* protein synthesizing systems programmed with polar coat mutant RNA (see *Nature*, **216**, 1070, 1967), conclusively show that at least with RNA phages polarity is a translational phenomenon

The Centriole and the Spindle

from our Cytogenetics Correspondent

TECHNIQUES for synchronizing the phases of the cell division cycle have allowed the timing of events in the cycle to be analysed in some detail Most work with synchronized cells has been concerned with events in the nucleus, but now Robbins, Jentzsch and Micali (*J Cell Biol*, **36**, 329, 1968) have studied the timing of centriole replication in a synchronized population of HeLa cells Electron microscope observations of the centriole complex at telophase and early G_1 interphase show that it consists of two orthogonally paired bodies Later in G_1 the members of the pair move apart, and as the cell enters S, each centriolar body appears to bud a replica By the end of S there are two complete centriolar complexes This coincidence of centriole replication and nuclear DNA replication suggests there may be a common control of replication because centrioles may contain DNA

A different view of centriole replication is held by Batisse (*C R Acad Sci*, **266**, 130, 1968) Strictly speaking, he observed kinetosomes (in the Suctorian *Discophrya*), but these structures are probably homologous with centrioles Batisse believes the parental kinetosome does not divide but is able to cause the condensation of precursors for a new kinetosome

In the HeLa cells, signs of centriole activity are first seen in G_2 and as the cell enters prophase In these phases the centrioles are the focus for the aggregation of electron-opaque bodies and less opaque vesicles Moreover, the cytoplasm around just one of the centrioles becomes electron-dense These changes probably represent early stages in the assembly of the mitotic spindle Later in prophase the centriolar complexes migrate to opposite ends of the cell where, at metaphase, they define the apices of the spindle

Hoskins (*Nature*, **217**, 748, 1968) recently suggested that chromosomal DNA at the kinetochore may play a part in ordering the structure of the spindle, it may be equally plausible that the DNA contained by the centriole may play a similar part in spindle organization Hoskins's diagram of kinetochore structure is very similar to electron micrographs of the kinetochore at meiotic metaphase in *Tradescantia* and *Lilium* pollen mother cells, published respectively by Wilson (*Planta*, **78**, 379, 1968) and Dietrich (*C R Acad Sci*, **266**, 579, 1968) In both cases the kinetochore is large, less electron-opaque than the rest of the chromosome and serves as an attachment point for spindle fibres These observations may imply that DNA has another function besides genetic coding, namely, serving as a site for the assembly of protein sub-units for the spindle

Improving Crops

AGRICULTURE is going to have to play an even more important part in the economy of most countries, and plant breeding has a great deal to offer, as it has done in the past twenty years This was the starting point taken by Dr G D H Bell, director of the Plant Breeding Institute in Cambridge, when he gave a review lecture at the Royal Society on March 7 His topic was "Plant Breeding for Crop Improvement in Britain Methods, Achievements, and Objectives"

Among the cereals, since 1946–47 the yield of wheat has increased from 19 to 31 2 cwt/acre, that of barley has increased from 17 8 to 28·7 cwt/acre and that of oats has increased from 16 3 to 24 4 cwt/acre During this time, many new varieties have been developed for commercial use, incorporating higher yields, better straw, and earlier ripening Improvements in barley were made possible by hybridization of British varieties with Scandinavian varieties A spring barley that proved particularly successful was Proctor, which shows a good yield, field resistance to some diseases, and high malting quality Winter malting barleys such as Pioneer and more recently Maris Otter have been developed by hybridization Higher yielding varieties than Proctor are now available, but the chief problem is combating leaf disease, particularly mildew Some of the new varieties have resistance to one or two races of the fungus, but non-specific resistance must be exploited if stability is to be attained.

Resistance to disease in wheat, especially to yellow rust, is also a major concern, while raising the yield and maintaining the baking quality are also important. A useful breeding variety called Compair has recently been produced by introducing a chromosome into hexaploid wheat by crossing with two species of *Aegilops*. This shows resistance to all known strains of yellow rust. The yield of both winter and spring wheat has increased considerably and there are new varieties, such as Maris Ranger, which give 8 per cent higher yield than the standard varieties of the past fifteen years. Maris Widgeon, a winter wheat, and Troll, a spring wheat, are two with a high baking quality. The yield potential of wheat is undoubtedly increasing, and new hybrids are indicating even higher yields, although not combined with good baking quality.

Continued improvement will depend on a more intensive use of more refined techniques and a closer integration of genetics, physiology and pathology. New models are being formulated both morphologically and physiologically and wider genetic variation is being used. Dwarf wheats and barleys are being developed with higher ratios of grain to straw and good yields, while huskless oats with good field characters are also an important objective. Physiological research in the field is showing differences between varieties on which breeding work can be based.

Dr Bell also showed how breeding work has made its contribution with potatoes and sugar beet. The biggest breakthrough with potatoes has been the recent increase in higher yielding main crop varieties such as Maris Piper and Pentland Dell. Maris Piper also shows resistance to one form of eel worm. Quality for processing—chips, crisps and so on—is also of increasing importance. In the case of sugar beet it is less easy to distinguish the advances in yield due to breeding from those due to cultivation. The development of monogerm and bolting-resistant varieties, however, is most significant for efficient husbandry, while polyploidy should make possible improvements in sugar production with better combinations of root yield and sugar percentage. For the greatest expression of yield, diseases such as virus yellows must be controlled, if possible by breeding. The production of Maris Vanguard, a virus-tolerant variety, is important in this respect.

Climate Control

RUSSIAN scientists have made significant advances in climate control, judging by the bibliography *Weather Modification in the Soviet Union, 1946–66*, published by the US Library of Congress in Washington. The Russians seem to have become particularly adept at cloud dispersion—in November 1953 they cleared clouds from an area of 2,500 square kilometres for a parade in Moscow, and in February 1961 a continuous cloud cover over the Crimea was broken in certain places to permit observations of a total eclipse of the Sun. The new bibliography provides an almost complete coverage of Soviet publications on weather modification received by the Library of Congress between the years 1961 and 1966.

The Russians are having some success with fog dispersal. In the winters of 1964 and 1965 fog dispersal methods were applied at 12 airports, making regular flights possible. Noticeable economic effects are claimed for this work. Hail suppression also receives considerable attention in the Soviet Union, especially in meteorological institutions in the Transcaucasus where hailstorms frequently cause serious damage.

The bibliography lists entries under seven headings, and includes tables summarizing Soviet experiments in weather modification. It is available from the US Government Printing Office, Washington, at 55 cents.

Penta-Coordination

from a Correspondent

CHEMISTRY will lose much of its interest if the time arrives when the shapes of molecules and relative—or even absolute—bond lengths can be predicted. A discussion on penta-coordination, arranged by the Chemical Crystallography Group of the Chemical Society on March 5, showed that we are a long way from reaching this state for compounds in which one atom is surrounded by five entities.

In the opening lecture, Professor H M Powell reviewed the problems of defining, for example, whether a lone pair of electrons constitutes a coordinating entity. Many compounds with empirical formulae consistent with penta-coordination crystallize so that the actual coordination is six or more. Where penta-coordination is established, two regular geometries are found, the trigonal bipyramid, favoured by non-transition elements, and the tetragonal pyramid. The latter geometry is found for later transition metals where it is favoured by ligand field stabilization energy, for example, a tetradentate tertiary arsine ligand (TTS) adopts a sterically strained configuration round Pd in Pd TTS Cl to give this geometry, although a trigonal bipyramid would require less strain.

A careful study of Cu(II) complexes with α-hydroxy carboxylate anions by Drs J R Carruthers, C K. Prout and F J C Rossotti established that the structure determining feature in the solid state was the formation of strong hydrogen bonds between the hydroxyl groups of one anion and the keto oxygen of another. This might result in five or six coordination for the copper, with or without the presence of coordinated molecules of water. For this ion, at least, ligand field effects are apparently weaker than hydrogen bonding and other inter-molecular interactions in the crystals. Factors affecting choice of coordination number were also considered by Dr J M Pratt. Stability constant measurements on the replacement or removal of water from the "sixth" position of metal-corrin-X-H_2O systems showed that this was favoured as the electron donor properties of the ligand X increased, six, five and four coordination were preferred by Fe(II), Co(II) and Ni(II) respectively.

Early transition elements form trigonal bipyramidal complexes. Dr J S Wood discussed three of these, correlating the magnetic properties with distortions from 120° in bond angles in terms of ligand field effects. Professor J W Linnett considered possible electronic structures of penta-coordinate non-transition elements, with particular emphasis on the need to explain relative bond lengths, for example, the short axial bond in BrF_5.

There was a wide ranging discussion opened by

Professor G W A Fowles, both he and Dr F G Mann gave examples of apparently inexplicable differences in behaviour on dissolution of similar complexes Dr P Pauling told a cautionary tale about a Ni(II) complex which crystal structure analysis has established as penta-coordinated by one chelate and one tridentate tertiary arsine

Use of Blood Grouping

ON March 6 the second public lecture was held at the Natural History Museum in memory of the late Sir Ronald Fisher, who made many important contributions to both genetics and statistics The lecture—"Blood Groups in Human Genetics"—was delivered to the large audience by Dr R. R Race of the Medical Research Council Blood Group Research Unit, who is well known for his work on blood groups

Sir Ronald realized that blood groups could be a useful tool for the improvement of knowledge in human genetics, especially for the mapping of genes on the chromosome From the bewildering reactions of the various Rh antisera, he saw the simple pattern of a controlling system of three very closely linked loci each with alleles. These loci were assumed to be so close together that crossing over between them could be only an extremely rare event The only way in which this concept has been modified in more recent times is that the three loci are regarded as being in one cistron, with a number of mutational sites for each of the loci *C*, *D* and *E* As postulated by Sir Ronald, the order of these sites is *DCE*.

Sir Ronald also applied mathematical tests to autosomal linkage groups In the early days of blood groups, few marker characters were available, but since then at least eight have been found, and the estimated distances between the pairs of loci range from about 1 to 22 cross-over units (centimorgans) The next step will be to find on which autosomes the loci are located, the most hopeful development in this direction so far has come from Paris, where a boy was found to have an abnormal Hageman factor or factor XII—responsible for a prolonged blood coagulation time It seems that the locus for this factor is on the upper part of the short arm of autosome No. 6

Chromosome mapping may also contribute to an understanding of testicular feminization, a rare and interesting condition People with this condition not only pass as females but tend to be particularly attractive ones, even though their chromosome counts show that they are cytological males It was hoped that the sex chromosome-linked blood group Xg might throw some light on this problem, but these hopes have not yet been realized

Blood grouping has also contributed to the study of twin chimerism, a rare condition in which dissimilar twins have been reported to have two types of blood each It may also be used to study dispermy, which results from the penetration of one ovum by two sperms Human examples are mosaics, often for sex, usually for blood groups and perhaps for eye and skin colour It is thought that one sperm probably fertilizes the egg nucleus, and the other may perhaps fertilize the nucleus of the second polar body, but this is not known for certain The results of testing many father–mother dispermy sets for the blood groups and other markers may in the future allow some estimate to be made of the identity of the two female nuclei which have been fertilized

Antioxidant Metabolism

from our Medical Biochemistry Correspondent

DRUGS and food additives are all tested in laboratory animals before they are ever administered to human beings It is often assumed that enzyme reactions are universal, and species differences are negligible to the biochemist A recent paper on the metabolism of 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) in the rat and in man illustrates how great interspecies differences can be (*Biochem J*, **106**, 783, 1968)

BHT is widely used in food as an antioxidant in fats Very different rates of excretion have been found in man and in the rat In man, half of any dose appears in the urine within the first twenty-four hours, but in the rat there is evidence suggesting that metabolites are retained in a circulation between the intestine and liver by excretion in the bile, reabsorption from the small intestine and transportation to the liver ^{14}C-labelled BHT was given orally in olive oil to laboratory rats, and in gelatine capsules to two male human subjects Urine, faeces and bile were collected from the rats, and urine from the two humans The radioactive metabolites were separated by extraction followed by chromatography, and identified by ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy.

In the rat, most of the activity in the urine was in 3,5-di-*tert*-butyl-4-hydrobenzoic acid (BHT acid), a glucuronic acid conjugate of BHT acid and a mercapturic acid S-3,5-di-*tert*-butyl-4-hydroxybenzyl-N-acetyl cysteine There was some evidence suggesting that the mercapturic acid was formed by a non-enzymic reaction between cysteine and BHT free radical and not by any of the known enzyme systems By contrast, the human urines contained one major labelled product This was an ether insoluble glucuronide in which the BHT residue had carboxyl groups replacing the ring methyl and one tertiary butyl methyl group, while another tertiary butyl methyl group appeared to be oxidized to an aldehyde group BHT acid and its glucuronide were found in only small quantities in human urine, and there was practically none of the mercapturic acid found in large quantities in rat urine.

The mercapturic acid and BHT glucuronide were found in large quantities in rat bile, and must have been the compounds circulating between the small intestine and liver in the rat Since these compounds were only formed in very small amounts in man, this could account for the absence of an enterohepatic circulation in man. The main component in rat faeces was BHT acid, which was presumably excreted in the bile and not reabsorbed in the intestine

The authors point out that these results do not show whether the pharmacological effects of BHT in the rat and man are likely to be similar or different. Where such large differences exist in the detoxification reactions undergone by a compound in two different species, however, it is unsafe to assume that effects observed in the rat will be repeatable in man. With any substance which is likely to be used in considerable quantities by human beings, results like these indicate that there is no substitute for careful studies in man before a compound is released for mass consumption, however reassuring animal studies may have been

All Change at Cal?

by

NICHOLAS WADE

The University of California has had a longer history of crises than most universities, and it has now produced an attempt to resolve its problems which merits the attention and admiration of all concerned with the politics of academic life. The student strikes of November 1966 prompted the Berkeley division of the university's Academic Senate and the Student Senate to set up a joint study commission, composed equally of faculty members and students, to examine the university's problems and propose solutions for them. In ninety-two pages of lapidary prose in which high ideals and legal niceties are given equal attention, the study commission has now produced its report (*The Culture of the University: Governance and Education*). The report is in three parts—a diagnosis of the need for reform, the changes in practice that are required, and the steps that should be taken to effect these changes.

The commission begins its diagnosis of the university's malaise by pointing to the high rate of disaffection and disinterest among students. Only half those who enter Berkeley as freshmen receive degrees, and the drop-outs include many of the most promising students. One reason for this is that "some of our most thoughtful students have come to repudiate many of the social goals and values they are asked to serve in the university and on graduation. . . . The new world emerging seems to exact greater conformity, more routinized lives, more formalized relationships among individuals, and a deeper sense of helplessness amidst an increasingly abstract world devoid of human values". It is little wonder, the commission considers, that students no longer wish to be groomed in the "knowledge industry" to take their place in such a society, especially when the university's atmosphere has come to reproduce the bureaucratic and dehumanized characteristics of that society.

How has this come to be? The demands of external interests—economic lures, the rewards of prestige and now a hostile state administration—have increasingly dominated the ethos of the university and shaped the direction of its educational activities. In the face of these events the university has made two crucial failures—"its failure to develop a student body which respects the value of the intellect itself, and its failure to order its activities according to a conscious conception of its unique purpose of nurturing that intellect".

In diagnosing the consequence of these failures, the commission deplores the incongruity that an institution devoted to the ideals of free enquiry and discussion can be content to manage its own affairs so differently. By doing so, it loses an important educational opportunity. "Campus governance is not simply a method for arriving at decisions about educational policies, it is itself a method of educating those who participate in it." The commission rejects the assumption, implicit in its own terms of reference, that separate spheres of interest should be defined for the administration, faculty and students. It considers it pernicious to suppose that campus society is composed of three such status-bound interest groups whose conflicts can or should be settled by procedures appropriate to national politics. Most issues of university policy require qualitative judgments rooted in values and principles, and should not be decided on the basis of which group has the most bargaining power. The belief that such procedures can solve the university's problems will merely perpetuate the continuous crises of the past, its inevitable result has been "an endless process in which both sides lose, a true minus–minus game in which the game itself becomes the focus while the main purpose of the community, namely education, suffers".

The commission declines to blame any particular group in the university for past troubles, because such a diagnosis would be purely superficial. "The sources of our conflicts are very deep. They lie . . . in the numerous functions, over and above teaching, which the university has acquired or had thrust on it, in the failure of the State of California to give adequate support to education, which has weighted the university in the direction of an impersonal educational environment of large classes and anonymous relationships among and between students, and faculty, in the extreme vulnerability of the campus to outside political pressures, and in the concept of education which—tailored as it is to the demands of a society devoted to economic growth, technological advance, and increased international influence and dominion—has ceased to nourish the best aspirations of the contemporary student and faculty member."

The problem as the commission sees it is to find ways of avoiding the "blunt, non-educational methods of agonistic politics" and to set up new institutions which will resolve conflicts "in accordance with our fundamental educational objectives". A major failing of the present institutions is that they are too large, their dimensions "run counter to genuine and direct participation, discussion and resolution of issues". Dealt with at centralized levels, problems appear abstract and remote and participants lack the experience necessary for genuine discussion. The consequence of centralized authority has been that "only a few can play an active role, while the many are passive spectators whose lethargy is periodically disturbed by a superficial form of participation, namely voting". The remedy for this state of affairs is the delegation of authority to smaller units which are "commensurable with human possibilities" and in which "student–faculty–administration consultation and participation are natural forms of activity rather than contrived formalities".

To what extent is decentralization desirable and possible? The commission points out that the Byrne Report of 1965, appointed by the Board of Regents itself, recommended "drastic devolution and greater local autonomy, but its proposals have been allowed to languish". Too much power has been allowed to reside with the administration, which in consequence has been "badly skewed by repeated confrontations with dissident students and near-confrontations with the faculty". The Academic Senate also has suffered from centralization, the paucity of administrative expertise among its members has led to "a marked tendency for a relatively small number of faculty members to monopolize the membership of the most powerful committees. . . . This situation has contributed significantly to the fact that over the years the Senate has been relatively ineffectual as a vehicle for promoting educational change or stimulating discussion of the fundamental purposes of the university". As to the role of the students, the commission considers that to present them with the trappings of government has not only caused resentment and disillusion, but has "aggravated the tendency to cast students and administration in the roles of contending interest groups". A separate student government is a "non-concept", wholly inapplicable to the university situation. The right solution is to devise "a variety of institutional means through which students can be incorporated into the decision-making process, rather than confined to haggling over the extent of peripheral powers delegated to a separate organization".

From its survey of the present state of affairs the commission draws three main conclusions. The first is that "highly centralized administrative authority has ceased to command much enthusiasm on this or any other comparable campus". The second conclusion is that voluntary compliance with university rules and policy can only be secured by "actions which command respect by the standards appropriate to a university". Third, the accumulated legacy of disloyalty and mistrust "must be dispelled by building new habits of co-operation between students and administrators, as well as between faculty and administrators, so that the administration does not remain politically isolated from the campus community".

In the second part of its report the commission turns its attentions to the ways in which new patterns of co-operation can be built up. The principal requirements for such change are these, that open discussion should precede policy decisions, that there should be substantial decentralization both at state and at campus levels, and that the students' role in governance should be significantly enlarged. The case for open discussion is so strong as to verge on the platitudinous. No group, the commission opines, has a monopoly of wisdom. On several occasions in the past, administrative decisions have been announced without prior consultation and been revoked after student protests. Discussion of an issue after the event occurs in a less cooperative atmosphere than discussion before it. The commission therefore recommends that the processes of policy making should be opened up by instituting public hearings, the public questioning of officials and improved access to university documents.

The case for decentralization is equally strong. In reference to the Regents' decision in December 1966 to suspend any member of the university who participated in a strike, the commission comments that "problems which are extremely complex when seen in their specific context give the illusion of being easily solvable when seen from a Regental distance". The proper function for the Board of Regents is to allow the individual campuses of the university to manage their budgets and their affairs as they think best, and for its own part to resist any attempts by the state government or legislature to interfere. Not only should authority be delegated, but the university should be divided into smaller units. It is difficult to decide at what point a department becomes so large that it is impossible to "speak any longer of its common culture", but the commission suggests a maximum strength of 20 to 30 faculty members, 200 undergraduates and 75 graduate students.

Turning to the question of student participation, the commission rejects any analogy to natural democracy or the right of each member of the university society to have an equal vote in its affairs. But it also rejects the university's claim to stand *in loco parentis* and that of "the minority of parents who wish it to perpetuate its own supervisory role". Pettifogging rules are inconsistent with students' dignity and increasing maturity, nor is it immediately obvious why young people at university should be treated differently than their contemporaries elsewhere. "Such paternalism is anachronistic and should be forthrightly disavowed."

The commission submits definite proposals for increasing student participation, the chief of which is the appointment of students by a student board to the various committees and boards which govern the university. It concludes its report with a list of the basic regulations which it considers should govern the enactment and enforcement of campus rules.

One of the major themes of the commission's argument is perhaps summarized by a paragraph in the final appendix to the report.

"The more opportunities that exist—at all levels of university governance—for students to take active roles, the easier it will be to attract, recognize and utilize the services of promising students. The greater the range of significant and challenging policy questions which engage the student government, the higher the calibre of students likely to volunteer their services becomes."

Human Influence upon Vegetational History in North Cardiganshire

by
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Pollen analytical investigations at Borth Bog and upon the upland blanket peats of Cardiganshire have elucidated the role of man as a factor influencing the development and destruction of the vegetation of the area. The times of the most severe forest destruction appear to have been the Iron Age/Roman period and the fourteenth to eighteenth centuries.

The application of the technique of pollen analysis to the elucidation of problems relating to the influence of man on vegetation is now fairly well established[1,2], but, despite this, detailed analyses coupled with a correlation of pollen and archaeological data for an area are still few. In this article the results of some investigations in the northern part of Cardiganshire will be described and a tentative correlation between these data and local archaeological and historical evidence will be suggested.

For the sake of brevity, only two sites will be considered. Several other sites were studied and the results obtained from these will be published in greater detail elsewhere.

The first site to be considered here is Borth Bog. This is an extensive raised bog on the southern side of the Dovey estuary, which has recently been acquired by the Nature Conservancy as a Nature Reserve. Pollen analysis has been carried out at this site by Godwin[3], but only the figures for the tree pollen have been published. The site selected for the present work was in the north-eastern part of the bog, the results of pollen analysis are shown in Fig. 1 and Fig. 2.

The stratigraphy at this point shows that the bog developed on a stiff blue clay. *Phragmites* reed swamp was the first vegetation, followed by trees, first alder, then birch and finally pine. At other sites on the bog the tree layer is more pronounced. It is this layer which extends beneath the shingle beach at Ynyslas and is there exposed by tidal action[4]. Above this there is a fibrous peat containing the remains of *Eriophorum vaginatum* and above this is a considerable depth of *Sphagnum imbricatum* peat.

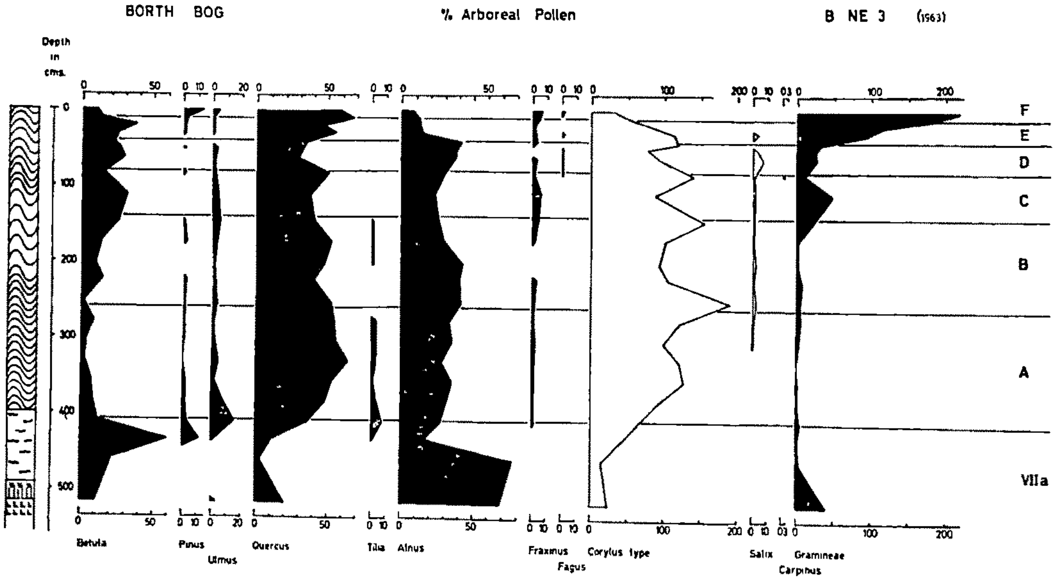

Fig. 1.

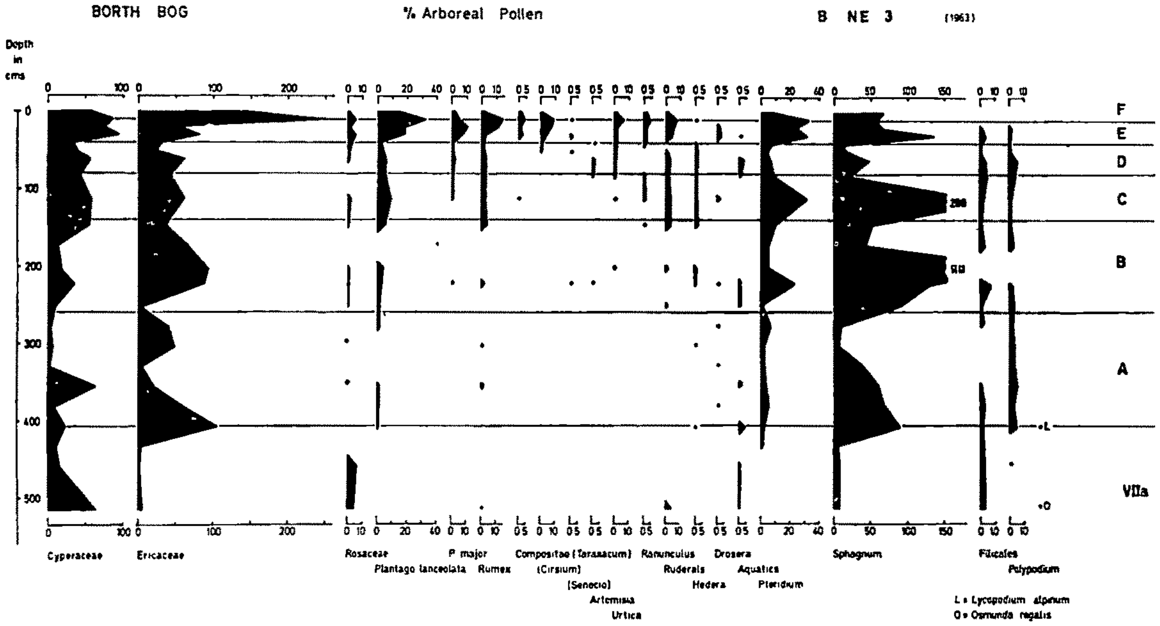

Fig 2.

Figs 1 and 2 Pollen diagrams from Borth Bog, Cardiganshire The zones *A–F* are considered to be of local significance only and represent stages in the development of the vegetation of the area under varying degrees of interference by man

The tree pollen curves at the base of the profile appear to reflect the local succession of trees on the site, but, with the disappearance of woodland in the immediate locality as a result of ombrogenous bog formation, the pollen curves may be regarded as representing more widespread changes in vegetation

For the sake of convenience, the upper part of the diagram has been divided up into zones *A–F* These are considered to be of local significance only They are characterized by the following features

A A decline occurs in the pollen of *Ulmus*, elm, together with the first appearance in quantity of *Plantago lanceolata*, ribwort plantain, and *Pteridium aquilinum*, bracken These are species which are frequently associated with woodland clearance and grazing by domesticated animals, and the fall in elm pollen suggests that this was a species particularly affected by such clearance Human activity, however, may have been of limited extent, for weed pollen does not attain very large proportions in this period

B Increases occur in *Plantago* and in *Pteridium* during this period, particularly at a depth of 200–220 cm. This implies that the influence of man on surrounding vegetation was becoming more severe Pollen of *Betula*, birch, increases during this period, possibly as a result of the invasion by birch of abandoned clearings

C During this period there is yet further evidence of increased human activity Not only do the weed species already mentioned increase, but also numerous other weeds, including *Rumex*, sorrel, become more abundant At the same time both *Alnus*, alder, and *Quercus*, oak, pollen curves suffer a decline, while *Betula* and *Fraxinus*,

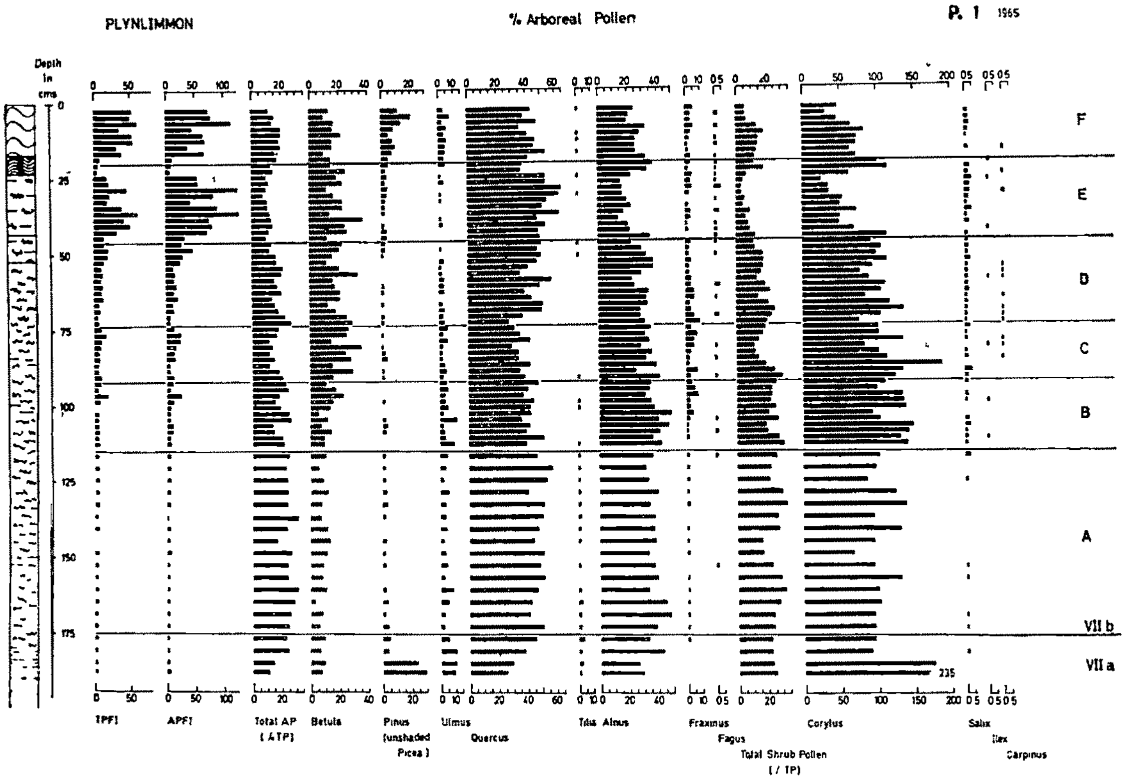

Fig 3

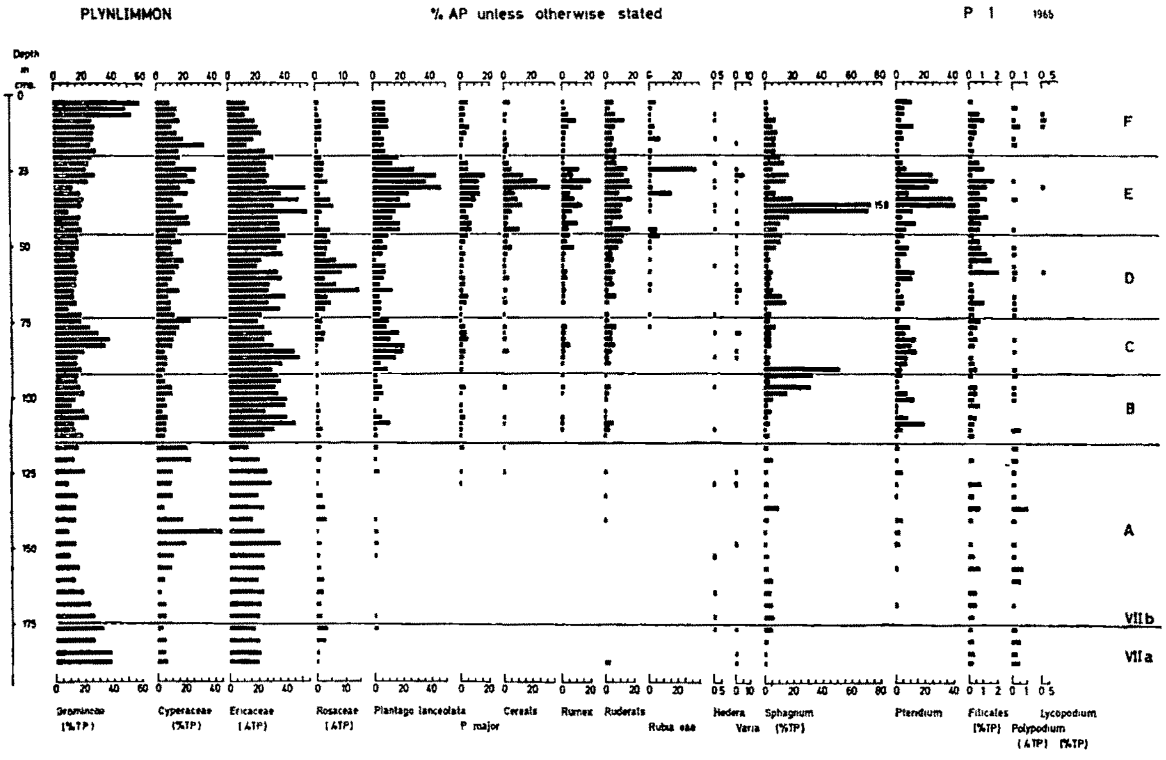

Fig 4

Figs 3 and 4 Pollen diagrams from Plynlimmon, Cardiganshire The zones A–F are thought to correspond to the equivalent zones of the Borth Bog diagrams ash, both trees preferring relatively unshaded habitats, increase Grass pollen becomes more abundant during this period, which, together with *Plantago lanceolata* could indicate a pastoral economy among the local peoples

D. Following the period of intensive clearance there is a period during which some recovery of trees appears to have taken place Alder in particular increases, while many of the weeds and *Pteridium* decrease Evidently the activity of man lessened during this period

E In this period, all weed species increase very rapidly to attain their highest values At the same time there is a marked drop in alder pollen There is some evidence from the *Plantago lanceolata* and *Pteridium* curves to suggest that this intensification of forest clearance and agricultural activity occurred in two phases, separated by a temporary lull It was during the second of these phases that weeds typical of arable farming reached their greatest prominence

F. Following the period of activity there comes an equally sudden decline in many weed species Grass pollen, however, continues to increase in abundance *Pinus* pollen (there are included here some grains of the genus *Picea*, spruce) also increases in period *F* up to the surface of the bog

In many of the upland sites which I examined a very similar pattern emerged, although there was some local variation in detail By means of closer sampling techniques a more detailed account of this pattern was built up from a blanket peat site near the summit of Plynlimmon, at an altitude of 1,850 ft (607 m) The zonation system is the same as that used for the Borth Bog diagram

A few of the more obvious differences are worthy of note At Plynlimmon alder appears to suffer less in relation to oak during periods *C* and *E* It is likely that woodland on the coastal plain and in the foothill valleys was richer in alder than the upland woods Clearance of these lowland woods could result in a proportionately greater decrease in alder pollen, which would be most noticeable in the lowlands, as at Borth Bog The curve for Gramineae at Plynlimmon behaves in quite a different way from the one at Borth, because species of this family were present in the peat-forming vegetation at the upland site Cereal pollen grains are plotted separately from Gramineae at the Plynlimmon site, and reach their greatest abundance in the latter part of period *E*

In order to interpret these data in the context of changing human culture and agricultural activity, it is helpful to have a means of dating certain horizons Radiocarbon dating was not available for this study, and I had to use datable archaeological artefacts For this purpose two peat sites were chosen in which such artefacts occurred In the first, Blaen yr Esgair, a Roman road of A D 78–80 interrupted peat deposition and, in the other, at Llyn Gynon, an early medieval trackway occurred Peat from above and below these structures was analysed At Blaen yr Esgair it was found that the entire period *C* was absent from the diagram, being replaced by the Roman road At Llyn Gynon period *C* and at least some of period *E* were present

With the aid of these data, certain tentative conclusions can be drawn regarding the correlation of vegetational change with archaeological and historical records

A Initial colonization of the region was probably by Neolithic people, whose population was probably very small (no megalithic tombs are known from the immediate area[5]) Their influence on the vegetation was likely to have been small, as is evidenced by the pollen data The one exception is that of elm, which suffered a decline at this time That this was an anthropogenic phenomenon is by no means certain[6], but in all the Cardiganshire sites it was found in association with weed pollen

B The more intensive forest clearance in this zone may be a consequence of the activity of Bronze Age peoples who occupied the uplands of Cardiganshire The Plynlimmon diagram shows evidence of minor clearance episodes, followed by periods of regeneration. This type of shifting culture is in agreement with what is known of the habits of these people, who were probably nomadic pastoralists[7].

C With the coming of Iron Age culture to this region, it is likely that there was a movement of population from the uplands to the foothills and the coastal plain[8] These people, together with the Romans, who invaded Wales between A D 43 and 78, were probably responsible for the considerable destruction of forest which evidently occurred during this period Some forest destruction could have resulted from the fighting which ensued and from the building of roads, but it is likely that an increase in pastoral farming under the *pax Romana* was the most important factor resulting in forest clearance.

D There follows a period of less intense activity and some forest regeneration The withdrawal of Roman legions, probably completed by A D 400, led to times of tribal warfare, which continued throughout much of the Dark Ages As a result, agriculture became once again a rather unstable activity Not until the Monastic period, beginning in Cardiganshire with the founding of Strata Florida Abbey in the south, did this state of affairs change

E The Strata Florida estate covered a large proportion of the uplands of Cardiganshire and supported large numbers of sheep This organized pastoral farming may well have resulted in the renewed forest destruction which occurred during period *E* The direct intervention of Edward I in 1280 could have been of considerable importance for Welsh vegetation, for he sent orders to various officers in Cardiganshire commanding that all woodland should be cleared so that it might not harbour thieves and rebels, with whom he was experiencing considerable difficulties It is quite possible that much of the lowland and valley woods, rich in alder, were cleared at this time, resulting in the marked decrease in alder pollen during period *E*

As has been mentioned, there seems to have been a pause in the destruction of forest during this period, which could have resulted from the reduction of the population of Cardiganshire in the fifteenth century following the Black Death and rebellion against Henry IV

Subsequently forest destruction continued as the cattle breeding industry grew in importance and as the uplands were enclosed The process reached its climax during the Napoleonic Wars, when cereals were grown even at high altitudes, hence the abundance of cereal pollen at Plynlimmon in this period The coming of peace in 1815 resulted in depression and mass emigration, which in turn led to the abandonment of upland holdings

F Depopulation continued throughout the nineteenth century and there is some evidence for the recovery of woodland, particularly in the uplands Added to this there was an increased interest in afforestation by some of the local inhabitants These interests were finally taken up by the Forestry Commission, resulting in the increase in *Pinus* and *Picea* pollen found in period *F*

I thank Mr E H Chater for his supervision of this work and also Dr D D Bartley for help with the techniques of pollen analysis This work was carried out at the Department of Botany, University College of Wales, Aberystwyth, during the tenure of a Science Research Council studentship

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[1] Iversen, J , *Danm Geol Unders* , II R, No 66 (1941)

[2] Turner, J , *New Phytol* , **63**, 73 (1964)

[3] Godwin, H , *The History of the British Flora* (Cambridge University Press 1956)

[4] Godwin, H , and Newton, L , *New Phytol* , **37**, 333 (1938)

[5] Grimes, W F , in *Prehistoric and Early Wales* (Routledge and Kegan Paul, 1965)

[6] Troels-Smith, J , *Danm Geol Unders* , IV R, No 4 (1960)

[7] Savory H N , in *Prehistoric and Early Wales* (Routledge and Kegan Paul, 1965)

[8] Hogg, A H A , in *Prehistoric and Early Wales* (Routledge and Kegan Paul, 1965)

Geochemical Reconnaisance and Hypocuprosis

by

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Regional geochemical reconnaissance in the Southern Pennines can aid the detecting and mapping of the incidence of hypocuprosis—a trace element disorder of agricultural importance.

GEOCHEMICAL reconnaissance by stream sediment sampling was first developed systematically for purposes of mineral exploration in 1952 Subsequently, it has been shown that by using appropriate methods of analysis and interpretation, trace element patterns detected in the active sediments reflect in varying degree the distribution of the elements in the rocks and soils as a whole[1,2] Preliminary studies of the application of this technique to agricultural problems in the Irish Republic and the United Kingdom showed an encouraging degree of correlation between the regional distribution of molybdenum, selenium and cobalt in stream sediments and the incidence in livestock of molybdenosis, selenosis and cobalt "pine", respectively[3,4]. Subsequent geochemical and agricultural reconnaissance of some 3,000 square miles in parts of Devon, North Wales and the southern Pennines has demonstrated a number of similar correlations between the trace element patterns and agricultural disorders The following description is concerned with one aspect of these current studies, namely, the distribution of molybdenum and associated elements in relation to hypocuprosis in cattle in parts of Derbyshire and North Staffordshire

The Study Area

The reconnaissance covered 900 square miles in the rectangle between Stockport, Sheffield, Derby and Stoke-on-Trent (Fig 1) Grossly simplified, the geological structure is that of a dome elongated along an N-S axis and slightly tilted to the east The outcrop of the Lower Carboniferous Limestone dominates the centre of the area and is surrounded by successively younger Carboniferous rocks of Namurian and Westphalian age In the Namurian, a series of basal marine shales is overlain successively by the main Namurian sandstones and the interbedded sandstones and shales of the Westphalian sequence In places, a shale facies of the Carboniferous Limestone separates the Namurian shales from the limestone proper The Carboniferous Limestone shales are lithologically indistinguishable from the Namurian shales with which they have been grouped in Fig 1 In the south, the Carboniferous is overlain unconformably by Permo-Triassic sandstones, isolated outliers of which indicate their former extensive cover

Thick Newer Drift of the Weichsel glaciation covers the Cheshire Plain to the west but thins eastwards to terminate along the 1,250 ft contour on the western slopes of the Staffordshire hills[5] East of this line there are sporadic relics of older Pennine glaciations and periglacial deposits, but, apart from these and loessial deposits on the limestone[6], the soils are essentially residual South of the limestone, however, patches of Pennine drift are mixed with surficial remnants of Permo-Trias, though many of the valleys are drift-free

The limestone outcrop forms a well developed undulating plateau with steep marginal slopes To the north and east, valleys cut in the flanking shales lie between the limestone plateau and the scarps of Millstone Grit West and south, the Namurian rocks give rise to hilly country overlooking the undulating lowland on the Trias

Drainage is predominantly south and east into the Trent basin With the exception of the limestone plateau, the density of tributary streams is sufficient for stream sediment reconnaissance

Stream Sediment Reconnaissance

More than 600 samples of active sediment were collected from tributary streams at a mean density of one sample per square mile, except on the limestone where there is virtually no surface drainage system The unground minus 80-mesh fraction was analysed spectrographically for fifteen elements including molybdenum[7] Arsenic and zinc were determined colorimetrically[8,9]

Anomalous concentrations of 4–40 p p m. of molybdenum were detected in streams draining parts of the lower Namurian shales compared with < 4 p p m elsewhere (Fig 2) North and east of the limestone the anomalies are confined to a narrow zone corresponding to the outcrop of these shales Widening of the shale outcrop to the south and west is accompanied by correspondingly more extensive patterns in the stream sediments, referred to as the Ashbourne and Onecote anomalies, respectively The fact that streams draining comparable shales west and north-west of the Onecote area do not show anomalous contents of molybdenum is almost certainly a result of the increasing persistence of barren drift cover in these directions and the proportion of arenaceous horizons, which are also more prevalent in the shale formation south of Onecote

Similar stream sediment patterns also associated with the Namurian shales were detected for arsenic, nickel, vanadium, chromium, cobalt, titanium and iron Copper and zinc also follow molybdenum, but the pattern is complicated by contamination from old base metal mines around the periphery of the limestone Apart, however, from arsenic, none of these metals gives the high contrast between anomaly and background shown by molybdenum A selection of molybdeniferous samples was also found to carry abnormal concentrations of selenium[10], but here, too, the contrast is usually low

Geochemical Significance of Patterns

Analysis of rock samples shows conclusively that marine facies of the Namurian shales with up to 50 p p m of molybdenum are the predominant source of this element in the area Elsewhere in the succession, shales usually contain < 5 p p m of molybdenum as do the more important arenaceous and calcareous facies (Table 1)

Reconnaissance soil traverses across the two main anomalous areas disclosed zones rich in molybdenum in the overburden above parts of the Lower Namurian succession In the Onecote area, the predominantly gleyed soils are mostly developed on residual parent material Over the shale the topsoil contains 4–35 p p m of molybdenum compared with less than 4 p p m over other rocks (Table 2) Apart from areas of moorland peaty soils where leaching may have been intense, the molybdenum content of the topsoil is largely dependent on the nature of the bedrock

In the Ashbourne area, on the other hand, the higher

Table 1 RANGE AND MEAN METAL CONTENT OF ROCKS FROM THE VARIOUS FORMATIONS

Rock-type and formation	Metal content (p p m) Molybdenum		Copper		No of samples
Marine shale (Namurian and Visean)	11	< 2–50	110	15–500	73
Non-marine shale (Namurian and Westphalian)	< 2	< 2–8	110	15–200	11
Sandstone (Namurian and Westphalian)	< 2	< 2–5	15	6–59	12
Carboniferous limestone	< 2		—		5
Triassic sandstone	< 2		9		4

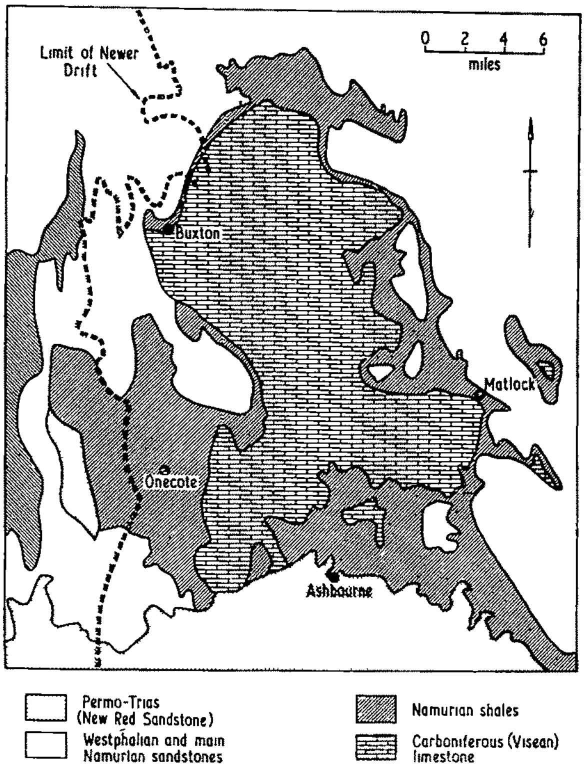
Limit of Newer Drift
0 2 4 6
miles
Buxton
Matlock
Onecote
Ashbourne
Permo-Trias (New Red Sandstone)
Westphalian and main Namurian sandstones
Namurian shales
Carboniferous (Visean) limestone

Fig 1 Simplified geology of the area covered by the regional geochemical survey (After Institute of Geological Sciences and B K Holdsworth, personal communication)

ground between streams is commonly covered by barren drift In these circumstances, the Namurian shales are associated with molybdeniferous soils only in drift-free areas mostly in the valleys and on the lower slopes of the hills

In both areas, soils developed on alluvium flanking anomalous streams are also molybdeniferous Considerable tracts of such soils occur in the valleys of the major rivers.

Reconnaissance soil traverses over molybdeniferous Namurian shale west of the Onecote stream sediment anomaly confirm that the overburden here is mostly barren drift which accounts for the background values recorded in the local stream sediments.

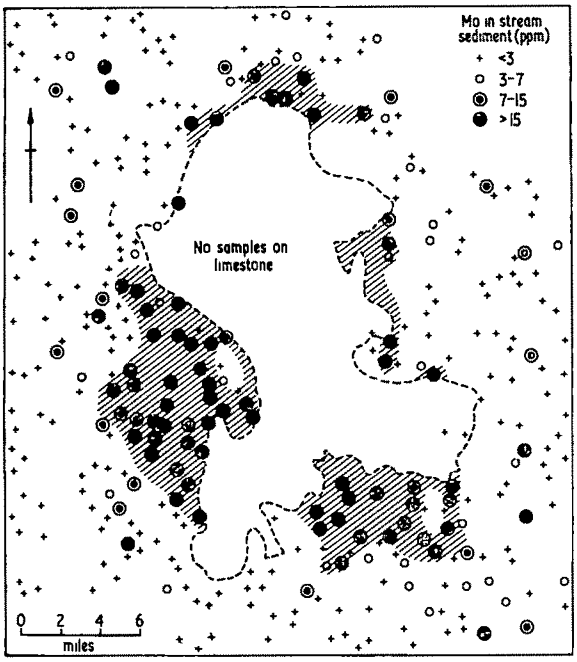

Fig 2 Distribution of molybdenum in the stream sediment of tributary drainage

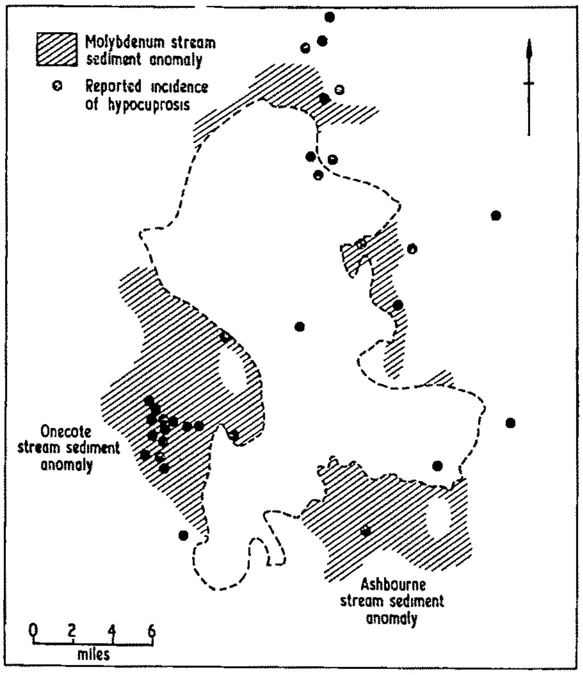
Molybdenum stream sediment anomaly
Reported incidence of hypocuprosis
Onecote stream sediment anomaly
Ashbourne stream sediment anomaly
0 2 4 6
miles

Fig 3 Incidence of hypocuprosis as reported before the regional geochemical survey (Information from National Agricultural Advisory Service, the Veterinary Investigation Service and private veterinary surgeons)

Analysis of soil and pasture herbage from selected plots in anomalous and background stream sediment areas showed a significant degree of correlation between the molybdenum content of the grass and clover and the supporting soil (Table 2) Though mean values for molybdenum in herbage are in each case higher on anomalous than on control soils, the contrast between anomalous and control vegetation is considerably less than that between the corresponding soils With regard to copper, no significant trend is apparent in the mean herbage values which range from 7–16 p p m , although soils from the anomalous plots contain 20–160 p p m of copper compared with 8–50 p p m in the controls

Agricultural Significance of Patterns

Land in the Onecote area is largely poorly or very poorly drained permanent pasture Farms are small and the land is difficult to improve The growing season is short because of the cold wet climate above 1,000 ft , mixed dairy farming is practised, but milk yields are low and stock is frequently in poor condition Farming in the Ashbourne area is again centred around dairying, with a small acreage of corn, but, being at a lower elevation and with a kinder climate than at Onecote, the farms are larger, methods more intensive and animals generally in better condition

The incidence of hypocuprosis in cattle, as reported before the geochemical reconnaissance, is shown in Fig 3 The greatest concentration was in two valleys in the Onecote area where the presence of molybdeniferous soil and herbage had suggested that the copper deficiency was molybdenum-induced, though the source of the molybdenum was not known with certainty (personal communication from D J C Jones) Comparison of the

Table 2 RANGE AND MEAN MOLYBDENUM CONTENT OF TOPSOIL AND HERBAGE FROM PLOTS WITHIN THE STREAM SEDIMENT ANOMALY AND ADJOINING BACKGROUND (CONTROL) AREAS

	Mo (p p m)			
Sample type	Anomalous plots		Control plots	
Topsoil*	16	4–35 (14)	2	<2–3 5 (12)
Grass (May)	3 7	1 0–8 0 (13)	2 1	1 6–3 0 (5)
Grass (July)	3 1	1 4–7 0 (14)	2 0	0 6–5 0 (12)
Clover (July)	7 2	1 8–30 0 (11)	3 6	0 8–8 6 (10)

* Data refer to minus 80-mesh fraction Number of samples in parentheses

reported incidence with the stream sediment pattern for molybdenum indicates an appreciable extension of the suspect area, particularly to the north of Onecote and also around Ashbourne where previous records showed only one herd-incidence of bovine infertility possibly due to copper deficiency (personal communication from C. M. Ford).

To test the agricultural significance of the results of the geochemical reconnaissance in terms of animal health, twenty-six herds comprising 350 cattle were selected for blood copper analysis. The selection was based solely on the distribution of molybdenum in stream sediment: nineteen of the farms were situated within the molybdenum anomaly and seven were in the adjoining background area. Farms where hypocuprosis had previously been reported were specifically excluded.

The results of these tests are summarized in Table 3 in terms of the numbers of deficient animals defined as those with less than the accepted lower normal limit of 0.08 μg of copper per 100 ml. of blood. Although the drawbacks of blood copper analysis as a means of diagnosing clinical hypocuprosis are realized, the principal conclusions, which are similar to those that may be drawn from the actual blood copper values, are as follows.

(1) There is a consistent trend towards a higher incidence of copper deficiency evident in animals of all age groups in both the Onecote and Ashbourne molybdenum-anomalous area as compared with the corresponding controls. This trend is particularly evident (*a*) in young stock (group II) from the Ashbourne area, where the blood copper data show mean values of 0.041 ± 0.004 and 0.095 ± 0.003 mg of copper/100 ml. of blood for anomalous and control groups, respectively, and (*b*) in adults receiving no supplementary feed (group III) in the Onecote area, where the corresponding blood copper values are 0.049 ± 0.004 and 0.090 ± 0.003. It is not known to what extent the limited incidence of copper deficiency observed in the control herds may be a result of (i) farm management, (ii) absolute copper deficiency on parts of the control farms and/or (iii) the local occurrence of patches of molybdeniferous soil too small in extent to influence the molybdenum content of the stream sediments predominantly derived from normal soils. The latter is not unlikely, as the majority of the controls lie within 3 miles of the anomalous stream sediment area.

(2) The effective improvement obtained in the molybdenum-anomalous area by feeding minerals is shown by the significantly smaller percentage of copper deficient adult stock of group IV compared with those receiving no mineral supplement in groups II and III ($P < 0.001$).

(3) Considering the combined data for both areas, the greater incidence of copper deficiency in animals from the anomalous areas compared with the controls is statistically significant in groups II and III ($P < 0.001$). Although it is not valid to compare the bulked data owing to unequal numbers of animals in the various groups, it is none the less impressive that of the total stock sampled, 64 per cent of the animals in the molybdeniferous areas were copper deficient compared with 35 per cent in the controls; excluding animals fed supplementary minerals, the corresponding percentages are 77 per cent and 37 per cent. In either case, the percentage incidence of copper deficiency within the molybdenum stream sediment pattern is approximately double that noted outside the anomaly.

Table 3. INCIDENCE OF COPPER DEFICIENCY (AS INDICATED BY BLOOD COPPER ANALYSIS) IN CATTLE, GROUPED ACCORDING TO AGE AND THE MOLYBDENUM CONTENT OF THE LOCAL STREAM SEDIMENT

District and relative molybdenum content of stream sediment	Percentage of copper-deficient animals (No. tested in parentheses)				
	No mineral supplement given			Minerals given	No minerals
	I < 11 months	II 11 months –2.5 yr	III Adult	IV Adult	I–III Combined
Onecote					
Anomalous	40 (30)	89 (73)	79 (43)	18 (38)	76 (146)
Normal	0 (4)	78 (27)	12 (24)	—	44 (55)
Ashbourne					
Anomalous	—	80 (41)	80 (5)	38 (26)	80 (46)
Normal	—	0 (13)	43 (21)	0 (5)	26 (34)
Combined areas					
Anomalous	40 (30)	86 (114)	79 (48)	27 (64)	77 (192)
Normal	0 (4)	52 (40)	27 (45)	0 (5)	37 (89)

Analysis was undertaken by the Central Veterinary Laboratory, Weybridge.

In several herds, the outward symptoms of copper deficiency were absent despite low blood copper values. Consequently, as a further test of the agricultural significance of the geochemical reconnaissance with particular reference to sub-clinical copper deficiency, supplementation trials were conducted on ten farms within the area of the molybdenum stream sediment anomaly. These experiments, which are being conducted in collaboration with advisers of the National Agricultural Advisory Service and the Veterinary Investigation Service, will be reported in full later. Preliminary results obtained for April–October show a mean increase of 5 per cent in the live weight gain of young cattle receiving injected copper compared with comparable untreated control animals. In some cases, the increased gain in weight was accompanied by a marked improvement in the physical appearance of the animals. Quite apart from the economic significance of these results, it is equally important that in many cases they were obtained on animals showing no clinical evidence of hypocuprosis.

Importance of Geochemical Reconnaissance

Geochemical reconnaissance by stream sediment sampling disclosed molybdenum anomalies covering a total area of 60 square miles in parts of Derbyshire and North Staffordshire. The anomalies are related to molybdeniferous rocks and soils. In places the pasture herbage is also molybdeniferous.

Copper deficiency induced by molybdenum was known to occur in parts of the anomalous stream sediment pattern. The results of blood copper tests carried out elsewhere within the anomaly indicate that all the anomalous area is suspect, the incidence of hypocuprosis being twice that observed outside the anomaly. Subsequent trials carried out within the anomalous area have shown that copper injections can increase the live weight gain of affected herds by more than 5 per cent. It is important that many of the animals which were found to be copper deficient and which responded to copper supplementation showed no outward symptoms of disorder.

In our view, the results fully confirm the potential value of regional geochemical reconnaissance as an aid in delineating suspect agricultural problem areas, wherein to concentrate detailed investigation using conventional methods which, though more precise, are far too costly and time consuming to be applied on the regional scale. The results also stress the unique advantage of the method in focusing attention on areas where clinical symptoms are absent yet where sub-clinical problems may be limiting agricultural productivity or where latent problems may adversely affect intensified farming in the future.

This work was assisted by the Natural Environment Research Council and the Institute of Geological Sciences. We thank the National Agricultural Advisory Service and the Veterinary Investigation Service for their co-operation, M. G. Davies for assistance in the Onecote area, and Miss C. N. Hebert for statistical advice. R. F. Horsnail took part in the reconnaissance sampling which was supervised by I. Nichol. Considerable help was also given by our analytical colleagues, under the direction of E. J. Newman and R. E. Stanton.

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[1] Webb, J. S., Fortescue, J., Nichol, I., and Tooms, J. S., *Tech. Commun. Geochem. Prospect. Res. Cent.*, No. 47 (1964).

[2] Nichol, I., James, L. D., and Viewing, K. A., *Trans. Inst. Min. Metall.*, 75, B146 (1966).

[3] Webb, J. S., *New Sci.*, 23, 504 (1964).

[4] Webb, J. S., and Atkinson, W. J., *Nature*, 208, 1056 (1965).

[5] Jowett, A., and Charlesworth, J. K., *Quart. J. Geol. Soc.*, 85, 307 (1929).

[6] Pigott, C. D., *J. Ecol.*, 50, 145 (1962).

[7] Nichol, I., and Henderson-Hamilton, J. C., *Trans. Inst. Min. Metall.*, 74, 955 (1965).

[8] Stanton, R. E., *Econ. Geol.*, 59, 1599 (1964).

[9] Stanton, R. E., *Tech. Commun. Geochem. Prospect. Res. Cent.*, No. 19 (1962).

[10] Webb, J. S., Thornton, I., and Fletcher, K., *Nature*, 211, 327 (1966).

Binding of Formylmethionyl-*t*RNA to 30*S* Ribosomal Sub-units

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Formylmethionyl-*t*RNA$_F$ seems to bind initially at the site used as a decoding site by all aminoacyl-*t*RNAs. A large part of this entry site must be located on the 30S ribosome particle, but some of it may extend to the 50S particle

THE requirements for correct initiation of protein synthesis in the *Escherichia coli* cell-free system include the following components ribosomes, formylmethionyl-transfer RNA$_F$ (F-met-*t*RNA$_F$), an initiator codon (ApUpG or GpUpG), two or three initiation factors, and GTP (refs. 1–6) Most of the details of the mechanism by which these components interact to form the first peptide bond are unknown The importance of the state of the ribosomes in this reaction has been emphasized recently[7–11]. It is clear that in the usual conditions (8–10 mM Mg^{++}) 70*S* ribosomes display little activity in initiation, whereas 30*S* particles, either alone or mixed with 50*S* particles, may be quite active[8,10]. In contrast, 70*S* particles are very active at about 5 mM Mg^{++} (ref 10). It seems likely that this lower Mg^{++} concentration optimum for 70*S* particles is a result of their ability to dissociate in these conditions into 50*S* and 30*S* sub-units. These facts are consistent with results[7] that suggest that *in vivo* the first steps of the initiation process do not occur on the 70*S* ribosomes, but rather involve the "native" 50*S* and 30*S* particles Nomura and Lowry[8] have proposed an initiation mechanism in which the interaction of *m*RNA, F-met-*t*RNA$_F$ and initiation factor(s) occurs on the 30*S* ribosome, this complex being subsequently joined by a 50*S* particle to form the active 70*S* ribosome

This article describes an investigation of the requirement for the formation of this initial complex involving the 30*S* particle Both native 30*S* particles and those derived from 70*S* ribosomes have been studied In both cases it has been found that GTP and initiation factors are required for the binding of F-met-*t*RNA$_F$ stimulated by ApUpG The bound product is not reactive with puromycin, whereas the addition of derived 50*S* particles to this complex renders the F-met-*t*RNA$_F$ sensitive to puromycin These results confirm the report of Hille *et al* [12] GMP-PCP can substitute for GTP in stimulating binding, but the complex formed does not react with puromycin in the presence of added 50*S* particles Both initiation factors (f_1 and f_2) are involved in the formation of the 30*S* complex

For purposes of comparison, we have made a preliminary investigation of the poly U-directed binding of phe-*t*RNA to 30*S* particles It has been found that this reaction is analogous to that involving F-met-*t*RNA$_F$ inasmuch as GTP (or GMP-PCP) and an enzyme are required. This enzyme does not seem to be an initiation factor, for it is found in the supernatant (*S*-100) fraction. A study of the requirement for GTP in this reaction has revealed that AMP-PCP (the adenosine analogue of GMP-PCP) can serve as a substitute, at about the same level of activity as GMP-PCP This surprising result suggests that the requirement for GTP in protein synthesis may not be as specific as previously supposed

Derived 30*S* and 50*S* particles were prepared by dissociating 70*S* ribosomes, which had been washed with NH$_4$Cl (ref 10), by overnight dialysis against buffer B [0 01 M *tris* HCl, *p*H 7 4, 0 1 mM Mg(OAc)$_2$, 0 05 M NH$_4$Cl, and 1 mM dithiothreitol], followed by sedimentation in a 5–20 per cent sucrose gradient containing buffer B. Fractions from this gradient containing 50*S* and 30*S* particles were pooled and the Mg^{++} concentration was increased to 10 mM The particles were pelleted by centrifugation at 105,000*g*, resuspended in buffer C [0 01 M *tris* HCl, *p*H 7 4, 0 01 M Mg(OAc)$_2$, 0 06 M KCl, 1 mM DTT] and stored at 0° C 30*S* particles prepared in this way were essentially free of contamination by 50*S* particles Conversely, 50*S* particles were considered to be essentially free of contamination by 30*S* particles, for they did not support the binding of detectable amounts of F-met-*t*RNA$_F$

Native ribosomes were prepared as follows. *S*-30 preparations from *E. coli* strain 1113 or *K*12, containing buffer A [0 01 M *tris* HCl, *p*H 7 4, 0 10 M NH$_4$Cl, 0 01 M Mg(OAc)$_2$ and 1 mM DTT] were centrifuged for 1 h at 105,000*g*, to remove polysomes, and the bottom fifth of the supernatant was diluted four-fold and layered on 28 ml of a 10–20 per cent convex sucrose gradient, and then centrifuged at 25,000 r p m. for 10 h at 5° C in a Spinco *SW*-25 rotor. Peaks containing 70*S*, 50*S* and 30*S* components were pooled, and the particles pelleted by centrifugation at 105,000*g* Each component was further purified by one or two more cycles of sucrose gradient sedimentation

Binding assays were conducted essentially as previously described[10], using *t*RNA fully charged with either ^{14}C-met (110 or 187 μCi/μm) or ^{3}H-phe (2 5 μCi/μm), nineteen cold amino-acids and cold formate In addition to the various macromolecular components, the amounts of which are given in the figure legends, binding reactions generally contained 10 mM Mg(OAc)$_2$, 0 1 M NH$_4$Cl, 0 05 M *tris* HCl, *p*H 7 4, and 1 mM dithiothreitol.

Initiation factors were prepared from *E. coli* strain 1113 or *K*12 essentially by the method of Lucas-Lenard and Lipmann[13]. Several preparations of f_1 were further purified by rechromatography on a column of DEAE-cellulose (in 0 02 M *tris* HCl, *p*H 7 4, 1 mM EDTA, 1 mM DTT), to remove any traces of contaminating f_2 These repurified f_1 preparations gave results indistinguishable from the starting material, suggesting that the standard purification procedures give f_1 free from f_2 contamination

In a previous communication[10] it was reported that the optimum Mg^{++} concentration for the binding of F-met-*t*RNA$_F$ to ribosomes depended strikingly on the manner of preparing the ribosomes undissociated 70*S* ribosomes were most active at 4–5 mM Mg^{++}, and virtually inactive at 10 mM, whereas a mixture of 50*S* and 30*S* particles (derived by previous dissociation of 70*S* ribosomes) displayed a broad optimum from 8 to 11 mM Mg^{++}. Inasmuch as these ribosomes were prepared by washing with a high concentration of salt [1 0 M NH$_4$Cl and 40 mM Mg(OAc)$_2$], it was interesting to determine whether the various classes of native ribosomes, which had not been exposed to such a high concentration of salt, would show similar Mg^{++} concentration profiles. The binding of F-met-*t*RNA$_F$ to native 70*S*, 30*S* and an equimolar mixture of 50*S* and 30*S* ribosomes, as a function of Mg^{++} concentration, is shown in Fig 1. The profiles are virtually identical to those obtained with 70*S* ribosomes prepared by washing with a high concentration of salt, on the one hand, and with a mixture of derived 30*S* and 50*S* particles,

Table 1 BINDING OF F-MET-*t*RNA TO DERIVED AND NATIVE 30*S* PARTICLES

	μμMoles of F-met-*t*RNA bound Plus ApUpG	Minus ApUpG
Experiment 1* (derived 30*S*)		
Complete	4 50	0 32
Minus GTP	0 26	0 31
Minus factors	0 25	0 33
Minus factors, minus GTP	0 30	0 31
Experiment 2† (native 30*S*)		
Complete	2 94	0 65
Minus GTP	0 99	—
Minus factors	0 82	—

* The complete reaction mixture contains 15 μμmoles of derived 30*S* particles, 25 μg of charged *t*RNA, 20 μg of initiation factors, 0 08 μmoles of GTP, 9 mμmoles of ApUpG and Mg^{++}, NH$_4^+$, *tris* buffer and DTT as described in the text, in a final volume of 0 1 ml

† The complete reaction mixture contains 19 μμmoles of native 30*S* particles, 7 μg of charged *t*RNA, 32 μg of initiation factors, 20 mμmoles of GTP, 10 mμmoles ApUpG, *tris* buffer, Mg^{++}, NH$^+_4$ and DTT as described in the text, in a final volume of 0 02 ml

Table 2 COMPARISON OF THE BINDING OF F-MET-*t*RNA TO NATIVE AND DERIVED 30*S* PARTICLES GTP REQUIREMENT

	μμMoles of F-met-*t*RNA bound Plus ApUpG	Minus ApUpG
Native 30*S*		
Complete*	14 1	1 9
Minus GTP	3 2	1 6
Minus GTP, plus GMP-PCP	11 9	1 6
Derived 30*S*		
Complete*	10 7	1 3
Minus GTP	1 2	1 2
Minus GTP, plus GMP-PCP	8 7	1 3

* The complete reaction mixtures contain 16 μμmoles of native 30*S* or 12 μμmoles of derived 30*S* particles 20 μg of charged *t*RNA, 33 μg of crude initiation factors, 0 1 μmoles of GTP or 0 01 μmoles of GMP-PCP, as indicated, 9 mμmoles of ApUpG, and NH$_4^+$, *tris* buffer and DTT as described in the text, in a final volume of 0 09 ml The final Mg^{++} concentration was 4 mM in this experiment, the same results were obtained at the usual 10 mM Mg^{++}

on the other[10] In addition, it is evident that native 30*S* particles alone (Fig 1*a*) display a profile similar to that of the mixture of native 50*S* and 30*S* particles (Fig 1*b*), and quite distinct from that of the 70*S* ribosomes (Fig 1*c*) In the case of the latter, it is again apparent that very little binding occurs at or above 10 mM Mg^{++}, whereas at 5 mM Mg^{++} these ribosomes are extremely active As suggested before[10], it seems likely that this very low Mg^{++} concentration optimum may result from the fact that 70*S* ribosomes, which as such may be incapable of binding F-met-*t*RNA$_F$, in these conditions begin to dissociate into actively binding sub-units*

Having established that mixtures of native ribosomal sub-units have an Mg^{++} concentration dependence similar to that of the derived sub-units, and that both preparations are markedly different from undissociated 70*S* ribosomes, we concentrated attention on the binding of F-met-*t*RNA$_F$ to 30*S* particles alone A study of the requirements for this reaction revealed that both GTP and initiation factors were needed for optimum binding This is true both for derived and native 30*S* particles (Table 1) This result shows that the sites of action of GTP and initiation factors (as is shown below, both f_1 and f_2 are involved in binding to 30*S*) are on the 30*S* particle That the complex is indeed specific for F-met-*t*RNA$_F$, as opposed to met-*t*RNA$_M$, is indicated by the fact that all the radioactivity bound in the 30*S* complex reacts with puromycin in the presence of 50*S* particles (see below), to give formylmethionyl-puromycin (F-met-puro) Furthermore, little or no binding of purified met-*t*RNA$_M$ to 30*S* particles could be detected in conditions optimal for F-met-*t*RNA$_F$ binding These results support the view that in the natural initiation process the 30*S* complex (containing F-met-*t*RNA$_F$, *m*RNA, initiation factors, GTP and a native 30*S* particle) is first formed and then subsequently is joined by a 50*S* particle[7,8] If this sequence of events is correct, it implies that the 50*S* particle may have little or nothing to do with the specificity of initiation [that is, the selection of F-met-*t*RNA$_F$ and the initiator codon(s) in the *m*RNA]

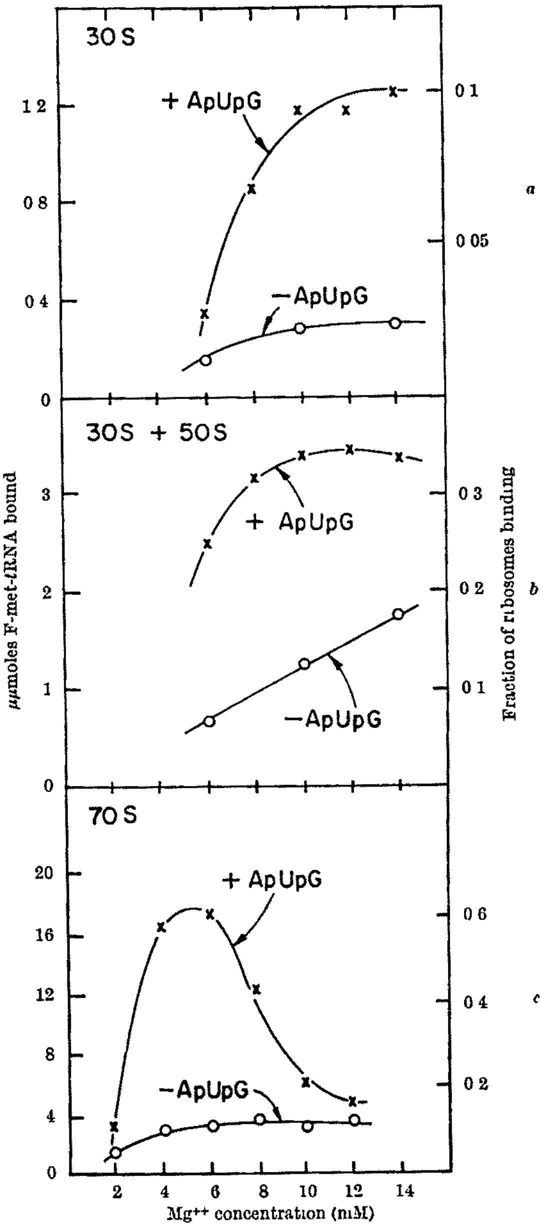

Fig 1 F-met-*t*RNA binding at various concentrations of Mg^{++} The final concentrations of ingredients are 0 05 M *tris* HCl, *p*H 7 4, 0 10 M NH$_4$Cl, Mg(OAc)$_2$ as indicated, 1 mM DTT, 0 40 mg/ml crude initiation factors, 0 6 mg/ml charged *t*RNA 0 5 mM ApUpG, and 1 0 mM GTP The amounts of ribosomes per aliquot are 12 5 μμmoles of native 30*S*, 10 μμmoles of each of native 30*S* and native 50*S*, 28 μμmoles of 70*S* Each binding mixture was filtered and washed with buffer containing the corresponding concentration of salts and Mg^{++}

An investigation of the specificity of the binding reaction has revealed that both GMP-PCP and AMP-PCP can replace GTP, whereas UTP and CTP are completely inactive (Tables 2 and 3) These results are quite consistent with those previously obtained when 70*S* ribosomes were employed[6,10]

Monro[14] has shown that the reaction between puromycin and F-met-*t*RNA$_F$ occurs on the 50*S* particle On the basis of this result one might anticipate that the F-met-*t*RNA$_F$ bound to 30*S* particles would not react with puromycin unless 50*S* particles were added The reactivity of F-met-*t*RNA$_F$ pre-bound to 30*S* particles was measured in the presence or absence of added 50*S* particles, and the results are shown in Table 4 It is clear that the F-met-*t*RNA$_F$ bound to 30*S* particles alone does not react with

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* It is important to note that the Mg^{++} concentrations employed in the wash solutions for these experiments were also varied as shown in Fig 1 When, however the Mg^{++} concentration of the wash is maintained at 10 mM, considerable increase in the level of binding to 30*S* particles is observed when the Mg^{++} concentration in the reaction mixture is low (3–6 mM) This phenomenon accounts for the high activity at low Mg^{++} concentration reported in Tables 2 and 6

Table 3 NUCLEOSIDE TRIPHOSPHATE SPECIFICITY IN BINDING OF F-MET-*t*RNA TO 30*S* PARTICLES

Nucleoside triphosphate Added to complete system*	$\mu\mu$Moles F-met-*t*RNA bound
None	0 8
GTP	4 5
GMP-PCP	4 2
AMP-PCP	3 9
UTP	0 8
CTP	0 8

* The complete reaction mixture contains 6 6 $\mu\mu$moles of derived 30*S* particles, 38 μg of charged *t*RNA, 26 μg of partially purified factor (f_2), 0 05 μmoles each of GTP, UTP, CTP and AMP-PCP, 0 001 μmole of GMP-PCP, 7 mμmoles of ApUpG, and Mg^{++}, NH$_4^+$, *tris* buffer and DTT as described in the text, in a final volume of 0 04 ml

puromycin, whereas the subsequent addition of 50*S* particles renders it reactive Furthermore, when GMP-PCP is substituted for GTP, the bound F-met-*t*RNA$_F$ does not react in any circumstances These results are consistent with the fact that peptide bond formation occurs on the 50*S* particle[12,14], and that the role of GTP in initiation involves two or more sequential functions, only the first of which can be fulfilled by GMP-PCP[10] Furthermore, it is clear that the first of these functions involves the binding of F-met-*t*RNA$_F$ to the 30*S* particle, a reaction in which the GTP molecule is not hydrolysed

Table 4 REACTION OF PUROMYCIN WITH BOUND F-MET-*t*RNA

	$\mu\mu$Moles F-met-*t*RNA bound or F-met-puro formed Plus ApUpG	$\mu\mu$Moles F-met-*t*RNA bound or F-met-puro formed Minus ApUpG
Binding*		
Complete (GTP)	3 5	0 3
Complete (GMP-PCP)	3 2	0 3
F-met-puro synthesis †		
Complete (GTP)	1 3	0 94
Plus 50*S*	5 1	1 00
Complete (GMP-PCP)	1 0	0 98
Plus 50*S*	1 0	1 02

* Reaction mixtures contained 15 $\mu\mu$moles of derived 30*S* particles, 25 μg of charged *t*RNA, 20 μg of initiation factors, 9 mμmoles of ApUpG, 0 08 μmoles of GTP or 0 11 μmoles GMP-PCP as indicated, and Mg^{++}, NH$_4^+$, *tris* buffer and DTT as described in the text, in a final volume of 0 085 ml Incubation was at room temperature for 20 min

† Reaction mixtures were prepared and incubated as in the binding reactions, after which 11 $\mu\mu$moles of derived 50*S* particles was added and the incubation was continued for 20 min at room temperature The reaction mixture was then kept at 0° C, treated with 50 μg of puromycin dihydrochloride and incubated for 3 min F-met-puro synthesis was assayed as previously described[10]

Having found that a crude preparation containing initiation factors stimulated the binding of F-met-*t*RNA$_F$ to 30*S* particles, it seemed important to determine which of the two factors, f_1 and f_2, was responsible for this activity These factors were partially purified as described already, and assayed both individually and together for their ability to stimulate binding to derived 30*S* particles The results of these experiments are shown in Fig 2 It is clear that each factor is active by itself, although maximum binding is obtained only in the presence of both This result is qualitatively similar to those obtained by others using uncharacterized ribosome preparations[3,13], although the relative activity of f_1 reported here is higher than has been observed before Similar results were obtained with f_1 prepared from *E coli* strains *K*12 and 1113, and with samples further purified by rechromatography on DEAE-cellulose to remove possible traces of contaminating f_2 Native 30*S* particles show a pattern of response to f_1 and f_2 which is similar to that observed with derived 30*S*, although the results are less conclusive because of the higher blank values obtained with these preparations Thus there seems to be little basic difference between native and derived 30*S* particles While it is likely that native 30*S* particles are loosely associated with initiation factors *in vivo*[9], the purification procedure used here apparently removes them

The surprising lack of any obvious functional difference between f_1 and f_2 is puzzling on several accounts First, it is difficult to understand why both are present when either one alone displays such high activity It seems possible that one of the factors is contaminated with the other, perhaps in the form of a specific complex between the two (this would account for their clear physical separability on columns of DEAE-cellulose) Second, the activity observed with f_1 alone is considerably higher than that previously reported[3,13] It seems possible that both these anomalies stem from the same source, namely, that the system employed here is quite different in many respects from those used elsewhere In particular, in our system the fraction of 30*S* particles which bind F-met-*t*RNA$_F$ (that is, the efficiency) is between 10 and 400 times higher than that reported by others[8,12] Furthermore, in our hands f_1 activity is extremely unstable, whereas f_2 seems to be more stable As a result of this instability, the level of binding observed with f_1 alone varies from 20 per cent to 70 per cent of the maximum obtainable with both factors present, depending on the age and manner of storage of the preparation

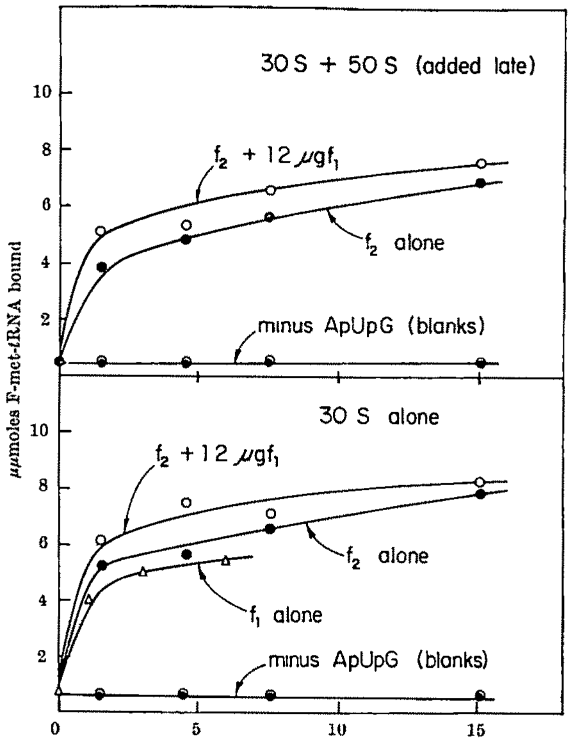

Fig 2 Effect of purified initiation factors, f_1 and f_2, on binding of F-met-*t*RNA to derived 30*S* particles Reaction mixtures contained 11 $\mu\mu$moles of 30*S* particles, 15 $\mu\mu$moles of 50*S* particles (top figure only), 25 μg of charged *t*RNA, factors as indicated, 9 mμmoles of ApUpG where indicated, 0 08 μmoles of GTP, and Mg^{++}, NH$_4^+$, *tris* buffer and DTT as described in the text, in a final volume of 0 1 ml

It was interesting to determine whether the F-met-*t*RNA$_F$ bound in the presence of only one of the initiation factors could react with puromycin, after the addition of 50*S* particles Experiments to investigate this possibility were conducted essentially as described in Table 4, and the results are shown in Table 5 It is clear that either of the factors alone will support F-met-puro synthesis,

Table 5 EFFECT OF f_1 AND f_2 ON F-MET-PURO SYNTHESIS

	$\mu\mu$Moles of F-met-puro Plus ApUpG	$\mu\mu$Moles of F-met-puro Minus ApUpG
Experiment 1*		
30*S*	0 90	0 94
30*S* plus 50*S*	0 89	1 02
30*S* plus f_1	0 83	1 10
30*S* plus 50*S* plus f_1	7 90	1 10
30*S* plus f_1 plus f_2	0 92	0 98
30*S* plus 50*S* plus f_1 plus f_2	9 05	1 10
Experiment 2†		
30*S* plus 50*S* plus f_2 (15 μg)	5 30	—
30*S* plus 50*S* plus f_2 (150 μg)	6 30	—
30*S* plus 50*S* plus f_1 (12 μg) plus f_2 (15 μg)	5 10	—
30*S* plus 50*S* plus f_1 (12 μg) plus f_2 (150 μg)	7 82	—

Reaction mixtures were prepared, incubated and analysed essentially as described in Table 4, except that f_1 and f_2 were used in place of crude factor

* Contains 11 $\mu\mu$moles of 30*S*, and 15 $\mu\mu$moles of 50*S* particles as indicated

† Contains 11 $\mu\mu$moles of 30*S* and 15 $\mu\mu$moles of 50*S* particles

Table 6 BINDING OF PHE-*t*RNA TO 30*S* PARTICLES

	$\mu\mu$Moles of phe-*t*RNA bound
Experiment 1	
Complete*	3 3
Minus GTP	0 3
Minus *S*-100	0 1
Minus GTP minus *S*-100	0 3
Experiment 2	
Complete†	3 4
Minus GTP	0 1
Minus GTP, plus GMP-PCP	0 9
Experiment 3	
Complete†	3 8
Minus GTP	0 1
Minus GTP, plus AMP-PCP	1 9

* The complete reaction mixture in experiment 1 contains 8 0 $\mu\mu$moles of derived 30*S* particles, 25 μg of charged *t*RNA, 50 μg of supernatant enzymes, 0 1 μmole of GTP, 15 μg of poly U, 5 mM of Mg $(OAc)_2$, and NH_4^+, *tris* buffer and DTT as described in the text, in a final volume of 0 09 ml

† The complete reaction mixtures in experiments 2 and 3 contain 6 6 $\mu\mu$moles of 30*S* particles, 10 μg of charged *t*RNA, 25 μg of supernatant enzymes, 0 05 μmoles of GTP, 0 001 μmole of GMP-PCP, 0 05 μmole of AMP-PCP, as indicated, 7 5 μg of poly U, and Mg^{++}, *tris* buffer and DTT as indicated in the text

although the maximum level is obtained only when both factors are present.

Having investigated the requirements for the binding of F-met-*t*RNA$_F$ to 30*S* particles, we next examined the binding of an aminoacyl-*t*RNA, phe-*t*RNA, for the purpose of comparison. It was hoped that such a comparison might shed light on the question of whether F-met-*t*RNA$_F$ initially binds at the same site as other *t*RNAs, that is, the "*A* site" or "single entry site"[10] A preliminary study of the requirements for the binding of phe-*t*RNA to 30*S* particles, directed by poly U, is shown in Table 6 It is clear that both a supernatant enzyme or enzymes present in the *S*-100 fraction and GTP are required for binding, and that AMP-PCP and GMP-PCP can partially replace GTP (recent experiments with GMP-PCP have indicated a greater activity than is evident in Table 6, that is, about half the activity obtained with GTP) That the requirement for nucleoside-triphosphate is specific for the binding step, and that no polypeptide synthesis occurs, is indicated by the following facts (*a*) none of the bound radioactivity is precipitable in hot TCA, and (*b*) neither GMP-PCP nor AMP-PCP support polypeptide synthesis.

Other preliminary experiments suggest that the Mg^{++} concentration dependence for phe-*t*RNA binding is similar to that observed with F-met-*t*RNA$_F$, and that both reactions are inhibited by tetracycline, a drug thought to be specific for the *A* site (ref 15 and unpublished results of S Sarker)

These results show that the requirements for the binding of phe-*t*RNA to 30*S* particles are very similar to those observed for F-met-*t*RNA$_F$ binding The chief difference between the two reactions lies in the nature of the enzymes involved Whereas the supernatant enzyme required for the binding of phe-*t*RNA is probably *T*-factor[16,17], it is not clear whether f_1 or f_2 performs a strictly analogous function in the case of F-met-*t*RNA$_F$ Indeed, it seems possible that one of the initiation factors is actually a modified *T*-factor that is specific for F-met-*t*RNA$_F$, this is suggested by the similarity in the nucleoside triphosphate requirements for the two binding reactions Such a mechanism would, of course, be consistent with the view that F-met-*t*RNA$_F$ initially binds at the same site usually used as a decoding or entry site by all aminoacyl-*t*RNAs It is clear that much of this entry site must be located on the 30*S* particle, however, it also seems possible that this site actually extends to the 50*S* particle to include the site specific for the aminoacyl moiety of aminoacyl-*t*RNA (ref 14) This overlapping site would be equivalent to the *A* site as previously envisaged[5]

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[1] Marcker, K A , Clark, B F C , and Anderson, J S , *Cold Spring Harbor Symp Quant Biol* , **31**, 279 (1966)
[2] Thach, R E , Sundararajan, T A , Dewey, K F , Brown, J C , and Doty, P , *Cold Spring Harbor Symp Quant Biol* , **31**, 85 (1966)
[3] Salas, M , Hille, M B , Last, J A , Wahba, A J , and Ochoa, S , *Proc US Nat Acad Sci* , **57**, 387 (1967)
[4] Revel, M , and Gros, F , *Biochem Biophys Res Commun* , **27**, 12 (1967)
[5] Hershey, J W B , and Thach, R E , *Proc US Nat Acad Sci* , **57**, 759 (1967)
[6] Anderson, J S , Bretscher, M S , Clark, B F C , and Marcker, K A *Nature*, **215**, 490 (1967)
[7] Mangiarotti, G , and Schlessinger, D , *J Mol Biol* , **20**, 123 (1966)
[8] Nomura, M , and Lowry, *Proc US Nat Acad Sci* , **58**, 946 (1967)
[9] Eisenstadt, J M , and Brawerman, G , *Proc US Nat Acad Sci* , **58**, 1560 (1967)
[10] Ohta, T , Sarkar, S , and Thach, R E , *Proc US Nat Acad Sci* , **58**, 1638 (1967)
[11] Kaempfer, R O R , Meselson, M and Raskas, H J , *J Mol Biol* (in the press)
[12] Hille, M B , Miller, M J , Iwasaki, K , and Wahba, A J , *Proc US Nat Acad Sci* , **58**, 1652 (1967)
[13] Lucas-Lenard, J , and Lipmann, F , *Proc US Nat Acad Sci* , **57**, 1050 (1967)
[14] Monro, R E , *J Mol Biol* , **25**, 347 (1967)
[15] Gottesman, M , *Fed Proc Abstracts*, **26**, 612 (1967)
[16] Ravel, J M , *Proc US Nat Acad Sci* , **57**, 1811 (1967)
[17] Gordon, J , *Proc US Nat Acad Sci* , **58**, 1574 (1967)

Haemoglobin Torino – α43 (CD1) Phenylalanine→Valine

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An unstable haemoglobin causing inclusion-body anaemia is described Its amino-acid substitution is in the haem pocket of the α chain, homologous in position to that in Haemoglobin Hammersmith (β CD1 Phe→Ser) The two disease states differ and an explanation is attempted in terms of molecular structure

A CARPENTER from Treviso, Italy, was repeatedly admitted to hospital in Torino from the age of 36 because of haemolytic crises One occurred after treatment with a sulphonamide derivative, when 60 per cent of the circulating red cells contained Heinz bodies After splenectomy he worked for 7 years without requiring any blood transfusions In 1967, nearly 80 per cent of the red cells contained Heinz bodies, the half life of the red cells was measured by tagging with ^{51}Cr, and $T\frac{1}{2}{}^{51}$Cr was found to be only 16 days The fresh blood contained 4 7 per cent of methaemoglobin, and this level increased considerably

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Table 1 AMINO-ACID SEQUENCE OF αTpVI OF HUMAN HAEMOGLOBIN WHICH COMPRISES RESIDUES 41–56 OF THE 141 RESIDUES OF THE α CHAIN[9]

Sequential No	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Helical notation	C6	7	CD1	2	3	4	5	7	8	D6	7	E1	2	3	4	5
Residue	Thr	Tyr	Phe	Pro	His	Phe	Asp	Leu	Ser	His	Gly	Ser	Ala	Gln	Val	Lys
		⇡				↑										

The arrows show where chymotryptic cleavage was demonstrated, and the dotted arrow indicates that hydrolysis at this point is only partial

on incubation for 48 h, even when glucose was added The haemolysate formed a copious precipitate on mild heating[1] The patient has three children, supposedly healthy

The presence of Heinz bodies, the increased proportion of methaemoglobin and the tendency to convert to methaemoglobin on incubation, together with the fact that the haemoglobin could be precipitated by mild heating, suggested that the cause of the anaemia was an unstable haemoglobin These haemoglobins become more easily converted to methaemoglobin, which precipitates and forms Heinz bodies[2,3]

The severe haemolytic crisis following treatment with the sulphonamide derivative could also have been associated with unstable haemoglobin In normal haemoglobin, as in myoglobin, protection of the haem against oxidation of its ferrous ion is due to its being buried in a tightly fitting fold within the globin This fold is held together by the hydrophobic bonding (van der Waals forces) exercised by the internal amino-acid residues lining the pocket[4] In one group of unstable haemoglobins one of these internal residues has been replaced by another of different shape[5] This causes a change in the configuration of the haem pocket, water can enter it, and in the presence of oxygen and water iron is oxidized, that is, methaemoglobin is formed[2] Methaemoglobin loses its haem ten times more easily than oxy or deoxy haemoglobin[6] Mild oxidizing agents, such as sulphonamide at dosages which cause no such oxidation to methaemoglobin in normal red cells, will oxidize the haem in haemoglobin when the haem pocket is disarranged For example, Haemoglobin Zurich (β63 His→Arg) was discovered because sulphonamide had caused haemolysis[7]

Investigation of the Haemoglobin

Electrophoresis of the haemolysate yielded no abnormal fraction On warming to 50° C at *p*H 7 4 (ref. 1) some of the haemoglobin precipitated Haemoglobin solutions were submitted to paper electrophoresis at *p*H 8 9, until the haemoglobin A fraction had moved well from the origin It had thus been separated from any non-haemoglobin fractions which contain heat-labile material The haemoglobin A fraction was eluted from paper and the eluate was concentrated by vacuum dialysis It was then incubated at 50° C (phosphate buffer *p*H 7 4) for 20 min A precipitate appeared which was washed three times with the same buffer which had been brought to a temperature of 50° C The washed haemoglobin precipitate was dissolved in 0 1 N HCl, and precipitated in acetone to obtain the globin After washing three times with acetone the precipitated globin was dried and digested with trypsin for the preparation of fingerprints or peptide chromatograms The techniques used in this laboratory for the preparation of fingerprints and for the specific colour reactions to identify peptides containing histidine, tyrosine, tryptophan and methionine have recently been summarized[8]

Fig 1 shows a fingerprint of the precipitate All the soluble peptides expected are found in the normal position Below the tryptic peptide of the α chain which contains residues 41–56 (see Table 1) of the 141 α chain residues (αTpVI) another peptide can, however, be noted

As expected, colour reactions showed the presence of tyrosine and histidine in αTpVI and these were positive also for the peptide below αTpVI which had the same electrophoretic mobility but was less hydrophobic on chromatography

It has been pointed out[5] that there is a general pattern for unstable haemoglobins which do not separate from haemoglobin A on electrophoresis If present together with haemoglobin A the fingerprint shows, in addition to one of the peptides in the normal position, another with the same electrophoretic but a lower chromatographic mobility This is caused by the replacement of one amino-acid by another less hydrophobic amino-acid carrying the same charge

It was concluded that the purification of the unstable haemoglobin had been only partial and that the precipitate which had been used for fingerprinting contained both normal and unstable αTpVI By more gentle heat precipitation—15 min at 45° C—we have obtained a haemoglobin preparation in which only the abnormal peptide was present

Table 2 shows the results of amino-acid analysis of the two peptides and it will be seen that their composition differs in one amino-acid residue In the abnormal peptide one of the two phenylalanine residues is missing, and two residues of valine are present instead of one To find which of the two phenylalanine residues had been replaced, α43 or α46, both peptides were submitted to chymotryptic digestion In the normal peptide Hill and Konigsberg[9] had described cleavage at Tyr 42 and at Phe α46 (CD4), but not at Phe α43 and Leu 48 Phe α43

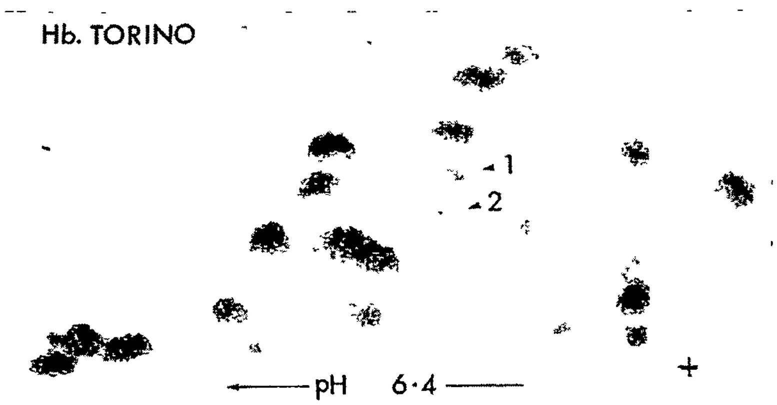

Fig 1 Fingerprint of partially purified haemoglobin Torino (1) α^{A}TpVI, (2) α^{Torino}TpVI In position 43 (CD1) α^{A}TpVI carries a phenylalanine residue and α^{Torino}TpVI one of valine

Table 2 AMINO-ACID COMPOSITION OF NORMAL AND ABNORMAL αTpVI

Amino-acid	μmoles A	Torino	No of residues A	Torino
Aspartic acid	0 017	0 018	1 0	0 9
Threonine	0 016	0 022	1 0	1 1
Serine	0 035	0 036	2 2	1 9
Glutamic acid	0 016	0 022	1 0	1 1
Proline	0 016	0 017	1 0	0 9
Glycine	0 020	0 023	1 3	1 2
Alanine	0 018	0 019	1 1	1 0
Valine	0 018	0 037	1 1	1 9
Leucine	0 017	0 022	1 0	1 1
Tyrosine	0 014	0 019	0 9	1 0
Phenylalanine	0 027	0 020	1 7	1 0
Histidine	0 065	0 031	2 3	2 3
Lysine	0 028	0 038	1 0	1 0

Glutamine appears as glutamic acid after the acid hydrolysis of the peptide which precedes amino-acid analysis Histidine and lysine were estimated separately, hence there are different yields per residue for these basic amino-acids from those of neutral and acidic amino-acids

is protected against chymotrypsin because it is bonded to a proline residue, chymotryptic hydrolysis at Leu α48 is presumably counteracted by the acidic residue Asp α47 Chymotryptic digestion of the normal αTpVI resulted in the expected four peptides 41–46, 41–42, 43–46 and 47–56 The same procedure was applied to the abnormal peptide and gave exactly the same results, indicating that cleavage took place at Phe α46 (CD4) There was, however, a slightly lower chromatographic mobility of peptide 41–46 and 43–46 A valine residue at position α46 (CD4) would not be subject to tryptic hydrolysis, and so the result was interpreted as showing that the residue at α46 (CD4) was phenylalanyl, and that the Phe→Val substitution had occurred at α43 A replacement of phenylalanyl by valyl at this position would be expected to decrease slightly the chromatographic mobility of the chymotryptic peptides 41–46 and 43–46 To verify the exact location of the Phe→Val substitution in the abnormal peptide, it was treated by a combined Dansyl-Edman sequencing method[10] All procedures were carried out also on normal αTpVI as a control

Two preparative fingerprints were prepared each with 9 mg of globin and from both the two peptides were eluted with 0 5 M ammonia The ammonia was removed in a desiccator and the dried material was re-dissolved and submitted to stepwise degradation of the N terminal residues according to Edman After removal of two N terminals each, the new N terminal was dansylated and after hydrolysis identified by polyamide layer chromatography[11]

In this way it was shown that the third residue of normal αTpVI yielded dansyl-phenylalanine and that of the abnormal peptide dansyl-valine The haemoglobin under investigation was therefore a new and so far undescribed variant, an unstable haemoglobin with a substitution in the α chain haemoglobin Torino α43 (CD1) Phe→Val

By combining mild heat denaturation with fingerprinting of the precipitate it could be established that haemoglobin Torino amounted to 8 per cent of the total haemolysate This figure would not include any haemoglobin Torino present as Heinz bodies in the red cells, for these would be removed when the haemolysate is prepared

Haemoglobin Torino is the first unstable haemoglobin to be observed with an amino-acid substitution in the haem pocket of the α chain It is notable that it conforms with the pattern observed in unstable haemoglobins with abnormality of the β chain haem pocket, that is, there is a replacement of an internal amino-acid residue by another with the same charge but less efficient in hydrophobic bonding[5] A previously described unstable haemoglobin—Hammersmith—has its substitution at the homologous site of the β chain CD1 Phe→Ser (ref 12)

Haemoglobin Hammersmith disease is more severe than haemoglobin Torino disease The first causes considerable anaemia in early childhood, and survival to adult age without blood transfusions, as has been the case in the patient with haemoglobin Torino, might have been unlikely in patients with haemoglobin Hammersmith (ref 13 and personal communication from N K Shinton)

There are a number of possible explanations for the greater severity of haemoglobin Hammersmith disease (β CD1 Phe→Ser) as compared with haemoglobin Torino disease (α CD1 Phe→Val), of which four may be mentioned here

(1) The phenylalanyl at CD1 of normal haemoglobins is an invariant residue and is found in this position in all known globins[4] It stabilizes the haem by direct contact and one would expect replacement by an amino-acid with a shorter side chain to cause instability Of the two replacements, serine resembles phenylalanine less than valine does It has a shorter side chain, and, whereas valine and phenylalanine are both non-polar, serine is polar and can form hydrogen bonds either internally or with the surrounding water[14] Thus a replacement of phenylalanine by serine might be expected to cause more instability than a replacement by valine

(2) It is noticeable that the soluble haemoglobin in haemoglobin Hammersmith disease includes 30 per cent of the abnormal pigment, but in haemoglobin Torino disease only 8 per cent of the soluble haemoglobin is abnormal This lower proportion of haemoglobin A in heterozygotes possessing haemoglobin A plus an α chain variant of A has usually been observed For example, in haemoglobins α A13 and β A13 Glu→Asp (haemoglobins J Oxford and J Baltimore) the proportion of haemoglobin A in the heterozygote is higher in the case of haemoglobin J Baltimore than in that of J Oxford[15] The normal α chain gene can if necessary make considerably more than 50 per cent of the total α chains Presumably the pathological risk for an erythrocyte is greater when the level of unstable haemoglobins is higher, and thus the red cells in haemoglobin Hammersmith disease would be more vulnerable than those in haemoglobin Torino disease

(3) There are indications that the α chain haem is less easily removed than the β chain haem[16] The interaction of haem with globin stabilizes the molecule as a whole[17] and the mere loss of haem from myoglobin results in a considerable change of molecular conformation[18] Thus a better hold on the haem by the α chains *per se* might contribute to make haemoglobin Torino disease less severe than haemoglobin Hammersmith disease

(4) Oxidation of the α and β chains occurs stepwise—at first the β93 cysteine residue is oxidized, and the oxidation of the second cysteine residue of the β chain and of the only cysteine residue of the α chain, β112 and α104, follow later[19] Thus a normal β chain may extend some protection even to unstable α chains

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[1] Dacie, J V, Grimes, A J, Meisler, A, Steingold, L, Hemsted E H, Beaven, G H, and White, J C, *Brit J Haematol*, **10**, 388 (1964)

[2] Vaughan Jones, R, Grimes, A J, Carrell, R W, and Lehmann H, *Brit J Haematol*, **13**, 394 (1967)

[3] Hutchison H E, Pinkerton, P H, Waters, P Douglas, A S, Lehmann H, and Beale, D, *Brit Med J*, ii, 1099 (1964)

[4] Perutz, M F, Kendrew, J C, and Watson H C, *J Mol Biol*, **13**, 669 (1965)

[5] Carrell, R W, Lehmann, H, Lorkin, P A Raik, E, and Hunter, E, *Nature*, **215**, 626 (1967)

[6] Bunn, H F, and Jandl, J H, *Proc US Nat Acad Sci*, **56**, 974 (1966)

[7] Hitzig, W H, Frick, P G, Betke, K, and Huisman, T H J, *Helv Paediat Acta*, **15**, 499 (1960)

[8] Sick, K, Beale, D, Irvine, D, Lehmann, H, Goodall, P T, and MacDougall, S, *Biochim Biophys Acta*, **140**, 231 (1967)

[9] Hill, R J, and Konigsberg, W, *J Biol Chem*, **237**, 3151 (1962)

[10] Gray, W R, and Hartley, B S, *Biochem J*, **89**, 379 (1963)

[11] Woods, K R, and Wang, K T, *Biochim Biophys Acta*, **133**, 369 (1967)

[12] Dacie, J V, Shinton, N K, Gaffney, P J, Carrell, R W, and Lehmann H, *Nature*, **216**, 663 (1967)

[13] Grimes, A J, Meisler, A, and Dacie, J V, *Brit J Haematol* **10**, 281 (1964)

[14] Epstein, C J, *Nature*, **215**, 355 (1967)

[15] Liddell J Brown, D, Beale, D, Lehmann, H, and Huntsman, R G, *Nature*, **204**, 269 (1964)

[16] Cassoly, R, Bucci, E, Iwatsubo, M, and Banerjee, R, *Biochem Biophys Acta*, **133**, 557 (1967)

[17] Watson, H C, in *Heme and Hemoproteins* (edit by Chance, B, Estabrooke, R W, and Yonetani, T), 63 (Academic Press, New York, 1966)

[18] Breslow, E, *J Biol Chem*, **237**, PC 3308 (1962)

[19] Rosemeyer, M A, and Huehns, E R, *J Mol Biol*, **25**, 253 (1967)

Amber Suppression: a Nucleotide Change in the Anticodon of a Tyrosine Transfer RNA

by

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In certain mutants a single base change alters the meaning of a messenger codon in such a way that, instead of spelling out an amino-acid, it spells out chain termination. Mutants in a quite different gene, called a suppression gene, allow the chain-terminating triplet to be read as an amino-acid. Experiments have shown that this is caused by a mutated tRNA which carries a single base change in its anticodon. This allows it to read the chain-terminating codon as if it spelt tyrosine.

In *Escherichia coli* the amber codon UAG is one of the three "nonsense" triplets (reviewed in ref. 1) that code as signals for termination of messenger RNA translation. Strains of *E. coli*, which allow translation of UAG as an amino-acid and subsequent propagation of the polypeptide chain, result from mutation in one of several suppressor genes. There are two classes of UAG suppressors: first, ochre suppressors which permit both UAG and the related chain terminating triplet UAA to be read with low efficiency, and, second, the relatively efficient amber suppressors which are specific for UAG. It has been shown for three amber suppressors that suppression is caused by the presence of an altered *t*RNA in the su^+ cell[2-5]. Although there was no doubt that the suppressor itself was a *t*RNA molecule, these experiments did not show how the suppressor gene acts. It could, for example, control the synthesis of a *t*RNA not present in su^- cells, or of an enzyme which modifies *t*RNA. More simply it could specify the sequence of a *t*RNA. We will show that the amber suppressor su_{III} gene is the structural gene for one of the tyrosine *t*RNAs of *E. coli* and that the mutation su^-_{III} to su^+_{III} results in a base change in the anticodon of this *t*RNA.

The nature of the mutational change is shown by comparison of the nucleotide sequences of the su^+_{III} tyrosine *t*RNA and the corresponding *t*RNA specified by the su^-_{III} allele. The su_{III} suppressor *t*RNA is a small part of the total tyrosine *t*RNA in the cell. The rest of the tyrosine *t*RNA consists of two species (I and II). To overcome this difficulty we used a technique designed greatly to increase the amount of su_{III} gene product in the cell by increasing the number of copies of the gene. The su_{III} gene, which maps very near to the attachment site of phage φ80, was placed on a defective transducing φ80. Infection of *E. coli* with the transducing phage resulted in a phage DNA pool containing many copies of the su_{III} gene. In this way, the amount/cell of the su_{III} gene product, su_{III} tyrosine *t*RNA, was greatly increased. This technique also allows selective isotopic labelling of the *t*RNA for sequence determination.

Transducing Phage φ80 carrying the su_{III} Gene

In the experiments described here, a particular defective transducing phage φ80 dsu^+_{III} (V) was used[4]. The su^+_{III} gene was isolated as a spontaneous mutant from an su^-_{III} strain. The homologous phage, transducing the wild type allele, φ80 dsu^-_{III}, was derived as a recombinant from heterozygous strains lysogenic for φ80 dsu^+_{III}. Thus φ80 dsu^-_{III} and φ80 dsu^+_{III} are identical except for the single spontaneous mutation which produced the su^+_{III} allele.

Synthesis of su_{III} Tyrosine tRNA after Infection by φ80 dsu_{III}

Infection of *E. coli CA*274 (su^- lac^- amber) with φ80 dsu^+_{III} resulted in the appearance of suppressor activity as measured by the suppression of an amber mutation in the β galactosidase gene. This was closely paralleled by an increase in the amount of tyrosine *t*RNA in the cell (Fig. 1). No increase occurs in the relative proportion of any other *t*RNA.

Ribosome binding experiments with the *t*RNA synthesized after infection with φ80 dsu^+_{III} showed that the su^+_{III} strains contain a tyrosine *t*RNA which recognizes the amber codon. The corresponding *t*RNA in cells infected with φ80 dsu^-_{III} recognizes the normal tyrosine codons UAU and UAC[4]. Although these experiments do not prove that the suppressor gene specifies a tyrosine *t*RNA, this, however, is likely because φ80 dsu^+_{III} does contain a structural gene for tyrosine *t*RNA as shown by specific hybridization of tyrosine *t*RNA with the DNA from this phage[6].

Nucleotide Sequences of su^+_{III} and su^-_{III} Tyrosine tRNAs

If the su_{III} gene is the structural gene for a tyrosine *t*RNA, we expect that the mutation from the su^-_{III} to su^+_{III} allele will have produced a nucleotide change in the su_{III} *t*RNA. To test this idea we have determined the nucleotide sequence of the su^+_{III} tyrosine *t*RNA and compared it with that of the su^-_{III} *t*RNA.

The su^+_{III} *t*RNA labelled with phosphorus-32 was prepared as previously described[6] or by a slightly modified procedure (see legend of Fig. 1). The selective labelling of su_{III} tyrosine *t*RNA after infection was evident from the high proportion of *t*RNA labelled with phosphorus-32 eluting with tyrosine *t*RNA during purification on the DEAE 'Sephadex' column. Pooled fractions from this region often contained about 70 per cent radiochemically pure tyrosine *t*RNA and were sufficiently homogeneous

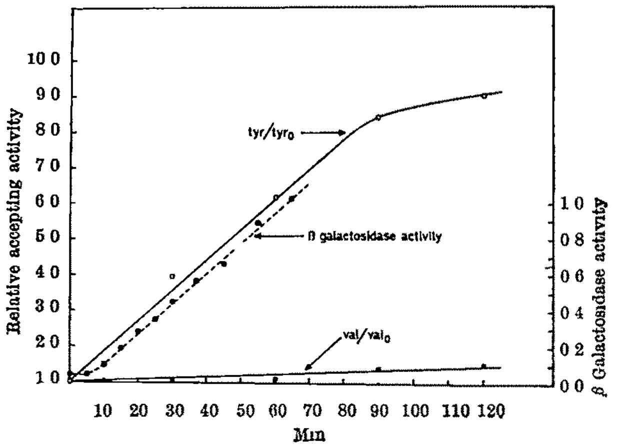


Fig 1 Increase of tyrosine *t*RNA and suppressor activity in *E coli CA274 su⁻ lac⁻* amber following infection by φ80 *dsu*$_{\mathrm{III}}^{+}$ *E coli CA274* was grown to 2×10^8 cells/ml in LP medium[6] The cells were collected by centrifugation and concentrated ten-fold in preconditioned LP medium $MgSO_4$ was added to 0 02 M and the cell suspension was irradiated with ultraviolet light for 90 s This dose kills 70 per cent of the cells The culture was then infected with 30 φ80 *dsu*$_{\mathrm{III}}^{+}$ particles/bacterium After adsorption for 7 min the cell suspension was diluted ten-fold in preconditioned media Two separate experiments are shown In one experiment, the suppression of an amber mutation in the β-galactosidase gene was measured by assaying enzyme activity at various times after dilution into the growth medium One mM of isopropylthiogalactoside was added to induce the β-galactosidase In the second experiment, aliquots of the infected culture were removed, *t*RNA was extracted, and tested for its ability to be acylated with several different amino-acids Fifty μg/ml of chloramphenicol added at 25 min prevented lysis which normally occurs at 60 min after dilution In experiments where the *t*RNA is labelled with ^{32}P, 0 05 m Ci/ml is added 5 min after dilution of the infected cells and the culture is collected after 2 h

for some of the nucleotide sequence work In other cases, these fractions were further purified by either of two chemical methods which depend on the ability of the *t*RNA to be acylated with tyrosine[7,8]

The sequence of the su_{III}^{+} tyrosine *t*RNA labelled with phosphorus-32 was determined using the methods of Sanger *et al* [9,10] The end-products of complete digestion with T_1 ribonuclease or pancreatic ribonuclease were separated on a two-dimensional electrophoretic system and their sequences determined by further enzymatic digestion. These are listed in Table 1 The combination of products from the two enzyme digests gave only two significant overlaps—GAAGGTψCG and CCAAAGGGAGC

To overlap the remaining end-products, larger fragments were obtained by partial enzyme digestion and separated as described before[11] The sequences of these partial fragments were determined from the complete products of their digestion with T_1 ribonuclease and pancreatic ribonuclease About one hundred large fragments were obtained by partial digestion with various enzymes and their sequences were determined. Fig 2 shows a selected set of fragments from which the complete sequence was deducted Fig 3*a* shows the su_{III}^{+} tyrosine *t*RNA sequence arranged in the "clover leaf"

Table 1. END-PRODUCTS OF RIBONUCLEASE DIGESTION OF su_{III}^{+} TYROSINE-*t*RNA

T_1 ribonuclease	Pancreatic ribonuclease
7 G	20 C
2 AG	8 U
UG	ψ
pGp	AoH
CCG	2 AC
CAG	GC
AAG	AU
CG$_{2'\mathrm{OMe}}$G	GAC
CCAAAG	AGAC
TψCG	G$_{2'\mathrm{OMe}}$GC
U*UCCG	AA*Aψ
UCAUCG	GAGC
ACUUCG	GU
AAUCCUUCCCCCACCACCA–OH	GAAU
ACUCUAA*AψCUG	AAAGGGAGC
	GAAGGT
	GGGGU*
	pGGU

pattern characteristic of the yeast *t*RNA structures For comparison, the sequence of yeast tyrosine *t*RNA as determined by Madison, Everett and Kung[12] is shown in Fig 3*b*. Note that in su_{III}^{+} tyrosine *t*RNA, CUA is in the position expected for the anticodon

There is only a single nucleotide difference between the su_{III}^{+} and su_{III}^{-} tyrosine *t*RNAs and this is best illustrated by comparison of their T_1 ribonuclease digestion products Fig. 4 compares the fingerprints of the T_1 ribonuclease digestion products of the two *t*RNAs

Comparison of the T_1 products shows that oligonucleotide 17, ACUCUAA*AψCUG, in the su_{III}^{+} *t*RNA is replaced in the su_{III}^{-} *t*RNA by the two oligonucleotides ACUG and UAA*AψCUG For the moment we will ignore the presence in the su_{III}^{+} fingerprint of oligonucleotide 17a (which is related to 17); its significance will be discussed later There are no differences in any other T_1 ribonuclease digestion products In the su_{III}^{-} *t*RNA the sequence corresponding to oligonucleotide 17 (su_{III}^{+}) must therefore be ACUGUAA*AψCUG which is split by T_1 ribonuclease to give ACUG and UAA*AψCUG The sequence GUA in su_{III}^{-} *t*RNA is thus changed to CUA in the su_{III}^{+} *t*RNA GUA is the expected anticodon for the dual recognition of the tyrosine codons UAU and UAC (ref 13) and in fact GψA is found in the expected anticodon position of yeast tyrosine *t*RNA (Fig 3*b*) which recognizes both these codons[14] CUA is the anticodon expected for the unique recognition of the amber codon UAG. In the complete sequence (Fig 3*a*) of su_{III}^{+} *t*RNA, CUA occupies the anticodon position These results show that the mutational event giving rise to the su_{III}^{+} allele can be accounted for by a G to C change in the structural gene for a tyrosine *t*RNA.

G in the Anticodon is Normally Modified

In these experiments, the su_{III}^{-} *t*RNA had an unmodified G in the first position of the anticodon Normally, however, the G in the anticodon of the su_{III}^{-} tyrosine *t*RNA is

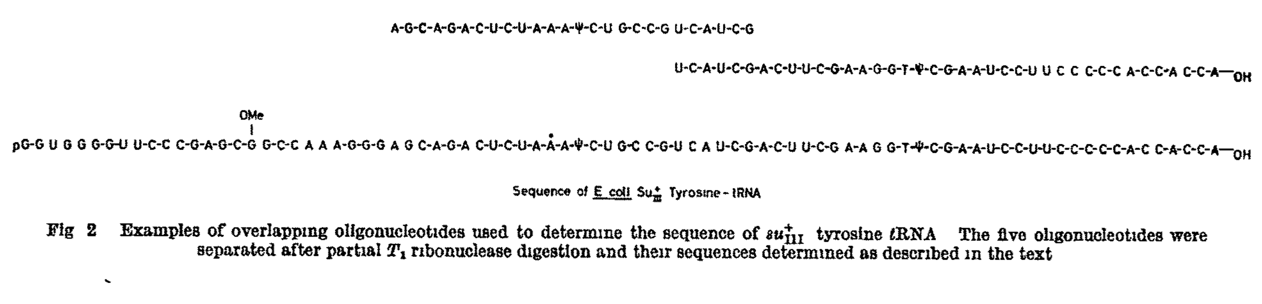
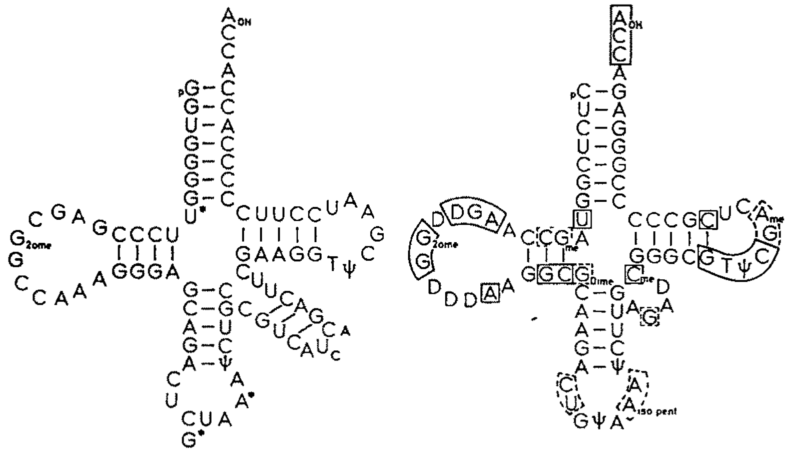

pG-G-U G-G-G-G-U̇ U-C-C-C-G
OMe
U-G-G-G-G-U U-C-C C-G-A-G-C-G-G-C-C-A-A-A-G
C-C A-A-A-G-G G-A-G C-A-G
A-G-C-A-G-A-C-U-C-U-A-A-A-Ψ-C-U G-C-C-G U-C-A-U-C-G
U-C-A-U-C-G-A-C-U-U-C-G-A-A-G-G-T-Ψ-C-G-A-A-U-C-C-U U C C C-C-C A-C-C-A C-C-A—OH
OMe
pG-G U G G G-G-U U-C-C C-G-A-G-C-G G-C-C A A A-G-G-G A G C-A-G-A C-U-C-U-A-Ȧ-A-Ψ-C-U G-C C-G-U C A U-C-G-A-C-U U-C-G A-A G G-T-Ψ-C-G-A-A-U-C-C-U-U-C-C-C-C-C-A-C C-A-C-C-A—OH
Sequence of E coli Su$_{\mathrm{III}}^{+}$ Tyrosine-tRNA

Fig 2 Examples of overlapping oligonucleotides used to determine the sequence of su_{III}^{+} tyrosine *t*RNA The five oligonucleotides were separated after partial T_1 ribonuclease digestion and their sequences determined as described in the text



Su+ Tyrosine tRNA E coli

(*a*)

Tyrosine tRNA Yeast

(*b*)

Fig 3 Clover leaf models of *E coli* and yeast tyrosine *t*RNAs The sequence of the su^+_{III} tyrosine *t*RNA is shown with the differences that occur in su^-_{III}, and species I and II (*a*) For identification of the minor bases see Fig 4 and the text The su^-_{III} and species I tyrosine *t*RNAs have identical sequences and differ from su^+_{III} in having a G* in the anticodon Species II *tyr* *t*RNA has the same anticodon sequence containing G* but is different in two positions where the T_1 ribonuclease product UCAUCG is replaced by UCACAG The latter oligonucleotide has been independently identified by B P Doctor and S Nishimura The sequence of the yeast tyrosine *t*RNA is taken from Madison *et al*[12] (*b*) The sequences enclosed in solid boxes are common to all the five yeast *t*RNA structures so far determined (neglecting differences in modification), while those enclosed in dotted boxes are common to most of the sequences

a modified guanine derivative (G*) In su_{III} tyrosine *t*RNA synthesized in conditions where the cells were starved before infection[6] we have observed that A^+ and $G_{2'OMe}$ remain largely unmodified The same is true of the G residue in the anticodon In su^-_{III} *t*RNA prepared from cells not starved before infection (legend to Fig 1), the G residue in the anticodon is partly or completely modified to G* This is not split by T_1 ribonuclease so that the T_1 ribonuclease product containing the anticodon is $\text{ACU}\underline{\text{G*UAA}}\text{*A}\psi\text{CUG}$ The same anticodon G*UA also occurs in the two principal tyrosine *t*RNA components of *E. coli* (Fig 3*a*, I and II), and therefore it was possible to isolate small quantities of non-radioactive G^*_p to study its properties G^*_p has an ultraviolet absorption spectrum very similar to that of G_p It differs from G_p in having an additional basic group with a pK_a between 5 and 6 Neither T_1 nor pancreatic ribonuclease splits the anticodon at the position of G^*_p and hence in a pancreatic ribonuclease digest it is obtained as the dinucleotide $G^*_pU_p$ From ribosome binding studies on *E coli* tyrosine *t*RNAs it is clear that G* has the same codon recognition properties as G The nature of the modification (which we suspect is on the imidazole part of the purine ring) and its biological significance remain unknown

Heterogeneity of su^+_{III} Tyrosine *t*RNA

In the su^+_{III} *t*RNA T_1 ribonuclease fingerprint we noted the presence of an additional oligonucleotide 17a (Fig 4) This has the sequence $\text{ACU}\underline{\text{G*UAA}}\text{*A}\psi\text{CUG}$ and is present in amounts varying between 20 per cent and 40 per cent of the su^+_{III} anticodon-containing T_1 product $\text{ACU}\underline{\text{CUAA}}\text{*A}\psi\text{CUG}$ The sum of the two is in molar yield in the *t*RNA Thus the tyrosine *t*RNA synthesized after infection by φ80 dsu^+_{III} contains in addition to su^+_{III} *t*RNA a smaller proportion of a tyrosine *t*RNA with the su^- anticodon sequence As will be shown later, the tyrosine *t*RNA in *E coli* can be separated into two kinds of molecules One of these, tyrosine *t*RNA I, has the same sequence as the su^- *t*RNA The other tyrosine *t*RNA II, differs by two nucleotides Fingerprints of the su^+_{III} tyrosine *t*RNA exclude the presence of tyrosine *t*RNA II as the source of the contaminating su^- anticodon sequence. We have considered four possible explanations for this. (i) The φ80 dsu^+_{III} carries an additional tyrosine *t*RNA gene determining the structure of tyrosine *t*RNA species I This is not supported by hybridization experiments between φ80 dsu^+_{III} DNA and tyrosine *t*RNA, which show that the transducing phage carries a single *t*RNA gene[6] (ii) The φ80 dsu^+_{III} phage preparations always contain a proportion of φ80 dsu^-_{III} transducing phage Such phages could be produced by abnormal excisions occurring in the induction of the heterogenote We have no good method of assessing the frequency of such events although the general phenomenon is known to occur[15] (iii) The transcription of genes near the φ80 attachment site is specifically promoted on infection by the φ80 transducing phage (iv) The presence of many copies of the structural gene for the su_{III} tyrosine *t*RNA specifically induces the expression of one class of chromosomal tyrosine *t*RNA genes

At present we cannot say which of these mechanisms produces the effect we have observed Experiments are in progress which should distinguish between them

Tyrosine *t*RNAs in Uninfected *E coli*

There are two principal tyrosine *t*RNAs in *E coli* which can be separated by column chromatography on DEAE 'Sephadex'[16] These are referred to as I and II in the order they elute from the column They are present in proportions of approximately 40 per cent (I) and 60 per cent (II) We have determined the sequences of I and II from *CA*265 su^+_{III} uniformly labelled with phosphorus-32 The labelled tyrosine *t*RNAs were isolated by a combination of chromatography on DEAE 'Sephadex', and either reversed phase partition chromatography[17] or chromatography on benzoylated DEAE cellulose[18] The sequences were derived in the same way as for su^+_{III} *t*RNA Certain overlaps of the complete T_1 and pancreatic digestion

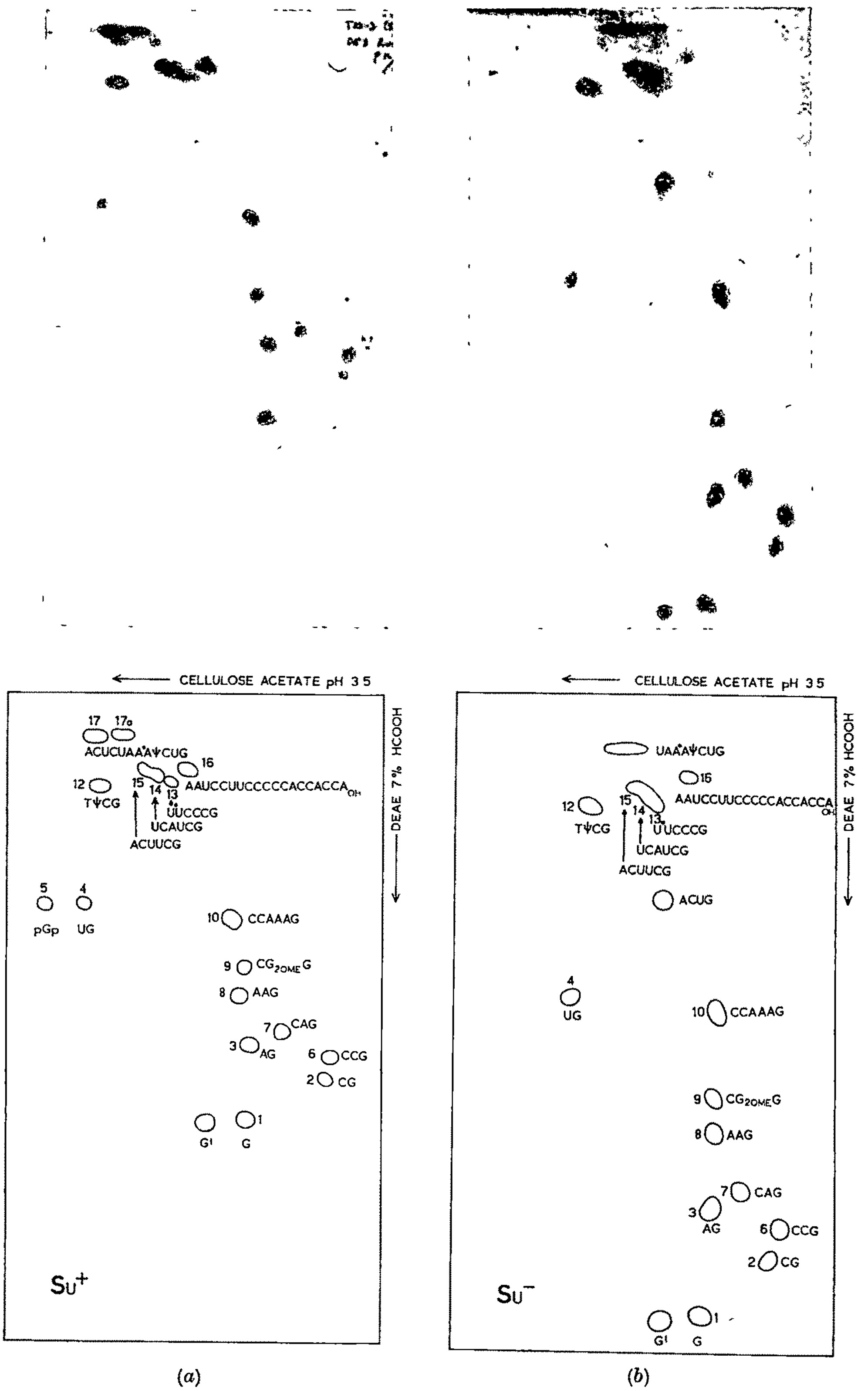


Fig 4 Two dimensional electrophoretic separation of a ribonuclease T_1 digest of su^+_{III} tyrosine *t*RNA labelled with ^{32}P. *a*, The end-products of ribonuclease T_1 digestion were separated by two dimensional electrophoresis first on cellulose acetate in pyridine–acetate buffer, *p*H 3.5, and then on DEAE–cellulose paper in 7 per cent formic acid (v/v). The lower diagram shows the identification of the nucleotides. The three hexanucleotides 13, 14 and 15 can be resolved after removal of their terminal 3′-phosphates with phosphomonoesterase. The appearance of CG is a consequence of incomplete 2′-*O*-methylation of $CG_{2'OMe}G$ giving rise to CG plus G after digestion with ribonuclease T_1. The hexanucleotide, U*UCCCG, always occurs in low yield, a fact that has not yet been adequately explained. pGp streaks badly and has partially migrated off the paper, making it difficult to detect. The two spots to the right and slightly above UG are minor contaminants which disappear on further purification of the *t*RNA. U* is a sulphur-containing nucleotide, tentatively identified as 4-thiouridylic acid. A* is derived from adenylic acid and, because it can be labelled with (^{35}S) sulphate and (^{14}C)-methyl methionine, it presumably contains both sulphur and a methyl group. *b*, Two dimensional electrophoretic separation of a T_1 ribonuclease digest of su^-_{III} tyrosine *t*RNA labelled with ^{32}P produced after infection with φ80 *dsu*$^-_{III}$. In this separation spot 16 has trailed but is normally present in molar yield. The time of electrophoresis on DEAE paper was longer than in *a*.

products in the first sixteen residues have not yet been obtained, and the sequence in this region is assigned by analogy with that of the su^{+}_{III} *t*RNA.

Both tyrosine *t*RNAs I and II contain the anticodon G*UA and differ only in a sequence of two nucleotides in the loop between the anticodon and TψCG arms (Fig. 3*a*). Tyrosine I has a sequence apparently identical with that of su^{-}_{III} tyrosine *t*RNA so that in *E. coli* there are only two basic types of tyrosine *t*RNA sequences.

Thus it is clear that the two different sequences (I and II) must be specified by separate genes. If tyrosine *t*RNA I and su^{-}_{III} were determined by the same gene, then we would expect that in an su^{+}_{III} strain all of tyrosine *t*RNA I would be converted to su^{+}_{III} *t*RNA. When tyrosine *t*RNA from *E. coli CA*275 su^{+}_{III} is fractionated on a DEAE 'Sephadex' column, assays for su^{+}_{III} *t*RNA show that it is located between the peaks of tyrosine *t*RNAs I and II (Fig. 5). This is confirmed by fingerprinting. We conclude that there is a separate gene for su^{-}_{III} *t*RNA even though it has the same sequence as tyrosine I.

Comparison between Yeast and *E. coli* Tyrosine tRNAs

It is interesting to compare the sequences of the yeast and *E. coli* tyrosine *t*RNAs. The shape and hydrogen bonding pattern of the clover leaf model are common to the two organisms. As indicated on the diagram, most of the specific sequence similarities between the two tyrosine *t*RNAs are also shared by all the other yeast *t*RNA structures[19–22], so that when these are taken into account the sequence differences between the two tyrosine *t*RNAs are very striking. An interesting feature is the complete absence of dihydro-uridylic acid (D) from the *E. coli* tyrosine *t*RNA. The greatest similarity is in the anticodon loop and the lower three base pairs of the anticodon arm, which, except for modified bases, are identical in the two *t*RNAs.

At this stage it is not useful to speculate which part of the structures serve as the amino-acyl synthetase recognition site. Neither yeast nor *E. coli* tyrosine *t*RNA can be acylated by the heterologous synthetase[23], so the two transfer RNAs may have quite different recognition sites.

Discussion

The mutation giving rise to the su^{+}_{III} gene changes the anticodon of a minor tyrosine *t*RNA species from GUA to CUA. This change enables the *t*RNA to recognize the amber codon UAG instead of the tyrosine codons UAU and UAC. It seems likely that the mutations leading to the su^{+}_{I} and su^{+}_{II} amber suppressor genes have similarly affected structural genes for serine and glutamine *t*RNA. These *t*RNAs could have anticodons related to the amber anticodon by a single base change.

The ochre suppressor gene su_{c} maps in the same position as the su_{III} gene[24] and also causes the insertion of tyrosine[25]. The su_{c} gene may therefore be another allele of the su_{III} gene. Amber suppressor genes may only arise where there are multiple genes for a particular *t*RNA species. In the case of tyrosine *t*RNA we have provided evidence that there are at least three structural genes. If there are no more than three genes our results suggest that the genes are expressed at different rates. We estimate that tyrosine *t*RNAs I, II and *su* are present in the approximate ratios 25:60:15. The need for this differential control is obscure because the three *t*RNAs have nearly identical sequences.

One might predict that multiple amber suppressor genes could arise corresponding to the multiple tyrosine *t*RNA genes. Perhaps not all of these could be obtained as the large amount of tyrosine II *t*RNA in the cell might make an amber suppressor mutant of this gene lethal. We have examined three independently isolated amber suppressor strains which insert tyrosine. All these map very close to the original su^{+}_{III}. In addition, Garen's *su*3 (ref. 26) and Yanofsky's *suYmel* (personal communication from G. Dennert and U. Henning) insert tyrosine and map in this region. These results suggest either that only one of the tyrosine *t*RNA genes can mutate to su^{+}_{III} or that at least two of the genes are located close to each other.

The G to C change in the nucleotide sequence of the *su* tyrosine *t*RNA specifically changes its codon recognition but does not affect any of the other functional properties of the *t*RNA. This formally designates this position as part of the anticodon and also shows that at least the first position of the anticodon is not necessary for recogni-

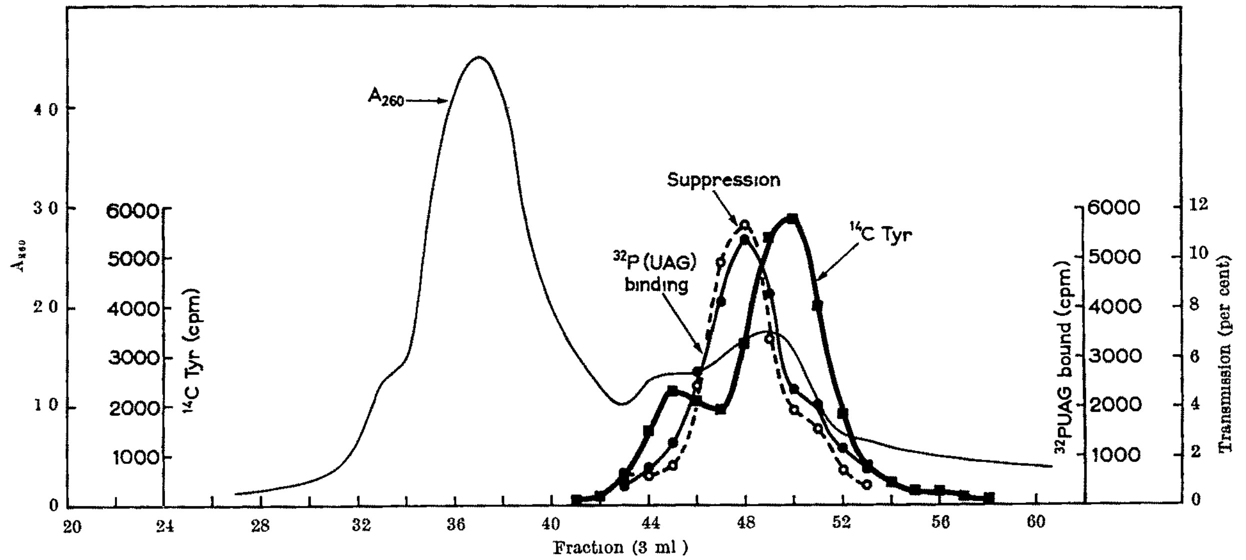


Fig. 5. Separation of the tyrosine *t*RNA components from *E. coli CA*275 su^{+}_{III} and identification of the su^{+}_{III} tyrosine *t*RNA. Tyrosine *t*RNA (from *CA*265 su^{+}_{III}) was purified approximately five-fold by chromatography on benzoylated DEAE cellulose. The figure shows the separation of this material by chromatography on a DEAE 'Sephadex' column (0.5 × 200 cm). Tyrosine *t*RNA elutes in two peaks, I and II (in order of elution). The suppressor *t*RNA was located by two methods. (i) fractions were tested for their ability to bind (^{32}P)pUpApG to ribosomes at 30 mM Mg^{++} using the Nirenberg and Leder assay[27]. (ii) Fractions were also tested for their ability to suppress *in vitro* the *sus* 3 amber mutation of the coat protein of the phage *f*2. This assay was performed by Dr Harvey Lodish using a modification[28] of the method of Engelhardt *et al.*[3]. The measure of suppression is the percentage of transmission, defined as the ratio of completed coat protein molecules/the total number of polypeptide chains initiated, at a concentration of 8 μg/ml. of suppressor *t*RNA added.

tion of amino-acyl synthetase To identify the function of other parts of the molecule, different mutants of this gene must be studied These can be obtained by isolating *su^-* revertants of the *su^+* gene Any mutation which gives rise to a non-functional *t*RNA will have the *su^-* phenotype Garen, Garen and Wilhelm[26] have isolated *su^-* revertants of the *su_{I}^+* gene These map in five closely linked sites indicating that different parts of the *su_{I}^+* gene may be mutated to give the *su^-* phenotype

With L Barnett we have isolated a number of *su^-* revertants of the *su_{III}^+* gene We are in the process of characterizing the structural and functional changes in the *su^-* tyrosine *t*RNA caused by these mutations This should provide insight into the structural basis for function in *t*RNA molecules

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[1] Weigert, M G , Galluci, E , Lanka, E , and Garen, A , *Cold Spring Harbor Symp Quant Biol* , **31**, 145 (1966), Stretton, A O W , Kaplan, S , and Brenner, S ibid , **31**, 173 (1966)
[2] Capecchi, M , and Gussin, G , *Science*, **149**, 417 (1965)
[3] Engelhardt, D L , Webster, R E Wilhelm, R C , and Zinder, N D , *Proc US Nat Acad Sci* , **54**, 1791 (1965)
[4] Smith, J D , Abelson, J N , Clark, B F C , Goodman, H M , and Brenner, S , *Cold Spring Harbor Symp Quant Biol* , **31**, 479 (1966)
[5] Gesteland, R F , Salser, W , and Bolle, A , *Proc US Nat Acad Sci* , **58**, 2036 (1967)
[6] Landy, A , Abelson, J , Goodman, H M , and Smith, J D , *J Mol Biol* , **29**, 457 (1967)
[7] von Portatius, H , Doty, P , and Stephenson, M L , *J Amer Chem Soc* , **83** 3351 (1961)
[8] Katchalsky E , Yankofsky, S , Novogradsky, A , Galenter, Y , and Littauer, U Z , *Biochim Biophys Acta*, **123**, 641 (1966)
[9] Sanger, F , Brownlee, G G , and Barrell, B G , *J Mol Biol* , **13**, 373 (1965)
[10] Brownlee, G G , and Sanger, F , *J Mol Biol* , **23**, 337 (1967)
[11] Brownlee, G G , Sanger, F , and Barrell, B G , *J Mol Biol* (in the press)
[12] Madison, J T , Everett, G A , and Kung, H K , *Science*, **153**, 531 (1966)
[13] Crick, F H C , *J Mol Biol* , **19**, 548 (1966)
[14] Doctor, B P , Loebel, J E and Kellogg, D A , *Cold Spring Harbor Symp Quant Biol* , **31**, 543 (1966)
[15] Appleyard, R K , *Genetics*, **39**, 440 (1954)
[16] Nishimura, S , Harada, F , Narushima, U , and Seno, T , *Biochim Biophys Acta*, **142**, 133 (1967)
[17] Kelmers, A D , Novelli, G D , and Stulberg, M P , *J Biol Chem* , **240**, 3979 (1965)
[18] Gillam, I , Millward, S , Blew, D , von Tigerstrom, M , Wimmer, E , and Tener, G M , *Biochemistry*, **6**, 3043 (1967)
[19] Holley, R W , Apgar, J , Everett, G A , Madison, J T , Marquisee, M , Merrill, S H , Penswick, J R , and Zamir, A , *Science*, **147**, 1462 (1965)
[20] Zachau, H G , Dutting, D , and Feldman, H , *Ang Chemie*, **5**, 122 (1966)
[21] Bayer, A A , Venkstern, T V , Mirsabekov, A D , Krutilina, A I , Li, L , and Axelrod, V D , *Molecular Biology* (*USSR*), **1**, 754 (1967)
[22] Raj Bhandary, U L , Chang, S H , Stuart, A , Faulkner, R D , Hoskinson, R M , and Khorana, H G , *Proc US Nat Acad Sci* **57**, 751 (1967)
[23] Doctor, B P , and Mudd, J , *J Biol Chem* , **238**, 3677 (1963)
[24] Signer, E R , Beckwith, J R , and Brenner, S , *J Mol Biol* , **14**, 153 (1965)
[25] Weigert, M G , Lanka, E , and Garen, A , *J Mol Biol* , **23**, 401 (1967)
[26] Garen, A , Garen, S , and Wilhelm, R C , *J Mol Biol* , **14**, 167 (1965)
[27] Nirenberg, M , and Leder, P *Science*, **145**, 1399 (1964)
[28] Lodish, H F , Abelson, J , and Clark, B F C , *Abst of Fed Europ Biochem Soc Fourth Meeting, Oslo* (1967)

Erythropoietin and the Development of Erythrocytes

During the differentiation of erythrocytes some cells develop which contain a high concentration of haemoglobin and no cytoplasmic basophilia Erythropoietin has the effect of increasing the proportion of these cells in preparations of marrow Two of the communications which follow discuss the effect on these cells of erythropoietin and of ageing and haemorrhage The third communication describes a factor in the serum of a polycythaemic calf which enhances the activity of erythropoietin

Effect of Erythropoietin *in vitro* which Simulates that of a Massive Dose *in vivo*

IN smears of normal rabbit marrow a few erythroblasts can be seen which are of the same size as the haemocytoblasts, pronormoblasts and basophilic erythroblasts, with almost the same large ratio of nucleus to cytoplasm They differ, however, in that they have no cytoplasmic basophilia. The concentration of haemoglobin seems to be greater in these than in their basophilic counterparts The high ratio of nucleus to cytoplasm as well as the larger size distinguishes them from polychromatic and orthochromatic erythroblasts For convenience they are referred to as line 2 cells and the typical early basophilic erythroblasts are referred to as line 1 cells

It is well known that in the marrow of a severely anaemic animal there is, with the erythroid hyperplasia, a shift of the distribution towards the less mature erythroblasts In such animals the number of early line 1 cells and all line 2 cells are increased In the rabbit made severely anaemic by injections of phenylhydrazine, the ratio of line 2 line 1 is increased several times. Early line 2 cells are seen more often in some stage of cell division than any other class of erythroblasts Often their nuclei became pyknotic, indicating their early conversion to large non-nucleated erythroid cells—reticulocytes and erythrocytes

There are three possibilities regarding the origin of line 2 cells first, that they arise directly from the stem cell compartment, second, from early line 1 cells; or third, by both routes In the search for ways of obtaining direct evidence it was found that rabbit erythroblasts kept overnight in normal rabbit plasma at 5°–15° C seemed from their staining characteristics to be well preserved At higher temperatures many had degenerated When 10 U/ml of erythropoietin was added to the plasma, there was a significant increase in the number of early line 2 cells on the following day. Without the erythropoietin there was no increase

The following procedure was adopted to investigate the effect of erythropoietin in these conditions Rabbit marrow erythroblasts were fractionated in a buoyant density gradient of bovine serum albumin (unpublished work of Borsook, Gunderson and Teigler) Cell fractions, washed free of bovine serum albumin, were suspended in normal rabbit plasma which contained 50 mg per cent of sodium heparin and which had been heated at 56° C for 30 min and filtered Penicillin *G*, streptomycin sulphate, and tetracycline were added in the respective quantities, 0 2, 0 2 and 0 05 mg/ml Aliquots of 1 ml containing about 10^7 cells were transferred to 15 ml plastic beakers, covered with aluminium foil, and set on one side at 37 5° C for 1 h to prevent the growth of micro-organisms The materials the effects of which were to be tested were added after incubation at 37 5° C The cell suspensions in the covered beakers were set on one side at 10° C Better yields seemed to be obtained by keeping the suspensions still in an atmosphere of oxygen or air + 5 per cent carbon dioxide After 16–20 h the cells were removed and centrifuged, and smears were made of the pellet These were stained with benzidine and counterstained with Giemsa solution After 20 h at 10° C in these conditions the cells incorporated ^{14}C-2-glycine into haem and protein at 80 and 100 per cent of the respective initial rates

The phenomenon under investigation was the disappearance of cytoplasmic basophilia from the erythroblasts To describe the changes the following scoring system was used, based on the yellow colour that benzidine confers on haemoglobin clear yellow, 5, yellow with a few greenish areas, 4, greenish yellow, 3, one-quarter of the circumference of the cell deep green or blue, 2, one-half to three-quarters blue, 1, more than three-quarters blue, 0 The long diameter of the cell was measured with a micrometer in the eyepiece

Table 1 shows a representative effect of erythropoietin (EP) and calf serum erythropoietin enhancing factor (EH) (see communication by D Van Dyke, M L Nohr and B Tennant) on an erythroblast fraction from normal rabbit The distribution of scores of the different sized cells allowed them to be grouped into those with diameters 9μ or smaller and 10μ or larger, corresponding to those of normal polychromatic–orthochromatic erythroblasts and of the early haemocytoblast, pronormoblast, basophil group According to conventional classification, the cytoplasm of the one is acidophilic, the other basophilic. The cells scoring 0 in the 9μ or smaller group would be designated as small basophils, those scoring 3 or higher in the 10μ or larger group are the early line 2 cells Table 1 shows that EP+EH accelerated the disappearance of the cytoplasmic basophilia in both groups of cells, irrespective of whether the scores are expressed as an increase in average cell score, as a percentage of the total scoring 3 or higher, or as a decrease in the percentage scoring 0 In plasma alone the scores differed little from the initial values

Tables 2 and 3 summarize results from five experiments with similar erythroblast fractions from normal rabbits as used in Table 1. They show the range of variation observed in the response to EP+EH

The percentage of cells scoring 3 or higher in the experiments of Tables 1, 2 and 3 was greater than in the whole erythroblast population of the marrow because a selected fraction was used

Table 1 EFFECT OF ERYTHROPOIETIN *in vitro* AT 10° C ON THE DISAPPEARANCE OF CYTOPLASMIC BASOPHILIA IN ERYTHROBLASTS FROM NORMAL RABBITS

Treatment and additive	Diameter 9μ or less Average cell score	Percentage of the total scoring 0	3 or higher	Diameter 10μ or larger Average cell score	Percentage of the total scoring 0	3 or higher
Initially	1 63	35	30	0 62	72	0
Normal plasma 16 h at 10° C	1 60	35	30	0 80	66	11
Normal plasma + EP + EH 16 h* at 10° C	2 58	16	59	2 18	31	50

* EP, 10 u/ml , EH, 0 09 ml /ml

Table 2 SUMMARY OF RESPONSES OBSERVED BY ERYTHROBLAST FRACTION OF INTERMEDIATE MATURITY FROM NORMAL RABBITS (DIAMETERS 9μ OR LESS)

Experiment No	Average cell score		Percentage of total cells with score of 0		3 or higher	
	Plasma alone	EP+EH	Plasma alone	EP+EH	Plasma alone	EP+EH
1	1 60	2 58	35	16	30	59
2	1 32	2 43	42	18	26	53
3	0 92	2 18	60	18	14	43
4	1 26	2 18	44	31	29	50
5	1 25	2 14	52	25	21	53

EP and EH as in Table 1

Table 3 EFFECT OF ERYTHROPOIETIN *in vitro* AT 10° C ON THE DISAPPEARANCE OF CYTOPLASMIC BASOPHILIA IN ERYTHROBLASTS FROM NORMAL RABBITS (DIAMETER 10μ OR LARGER)

Experiment No	Average cell score		Percentage of total cells scoring 0		3 or higher	
	Plasma alone	EP+EH	Plasma alone	EP+EH	Plasma alone	EP+EH
1	0 80	2 18	66	31	11	50
2	0 49	2 00	73	30	6	48
3	0 61	1 34	68	38	8	18
4	0 39	0 93	86	61	9	21
5	0 61	1 35	74	32	10	53

EP and EH as in Table 1

All the different gradient fractions of erythroblasts were examined from both normal and severely anaemic animals (by injection of phenylhydrazine), to find the group which would show the effect of EP+EH most clearly An accelerated disappearance of cytoplasmic basophilia in the presence of EP+EH was observed in all the cell fractions and in all cell size groups with diameters varying from 7μ to 17μ, whether from normal or severely anaemic rabbits

It is reasonable to surmise that the accelerated disappearance of cytoplasmic basophilia under the influence of erythropoietin is several times faster *in vivo* than at 10° C *in vitro* Because basophilic erythroblasts of all sizes respond, the *in vivo* response must be of considerable magnitude

Tables 4 and 5 summarize observations on the effects of actinomycin *D* on the accelerated loss of cytoplasmic basophilia Only the results in the size group 10μ or larger are given, but they are similar to those found in the smaller cells It can be seen that actinomycin *D* can completely nullify the effects of EH + EP The effects of EH and EP, separately and together, are similar to those observed by Van Dyke *et al* (see p 1027) on uptake of ^{59}Fe after injection into mice The competitive counteraction of erythropoietin and actinomycin *D* has also been observed *in vivo*[1]

Some exploratory experiments were carried out on the effect of EP+EH on the ability to synthesize haem and protein Cells exposed to EP+EH incorporated 20–25 per cent more ^{14}C-2-glycine into the haem and total protein

These observations show that erythropoietin also acts on cells peripheral to the stem cell compartment They explain the quick response *in vivo* to a large dose of erythropoietin that is, the appearance in the blood of abnormally large cells more quickly than can be accounted for as coming from the stem cell compartment They also offer an explanation for the possible mechanism involved in the earlier observations of Gallagher *et al* [2] on the effect of erythropoietin on nucleated red cells

The loss of cytoplasmic basophilia is the terminal differentiation in erythropoiesis[3,4] and is concomitant with a considerable biochemical change Haemoglobin synthesis becomes 85–90 per cent instead of 20 per cent of the total protein synthesis, and it is the principal, whereas earlier it is an acidic minor, haemoglobin, that is chiefly synthesized[4,5] This terminal differentiation is inhibited by actinomycin *D* For example, when 100 μg/kg of actinomycin *D* is injected into a rabbit, in 4 days all acidophilic erythroblasts and reticulocytes disappear from the marrow Haemocytoblasts, pronormoblasts and baso-

Table 4 EFFECTS OF EH ON EP AND OF ACTINOMYCIN *D* ON EH+EP IN ERYTHROBLASTS FROM NORMAL ANIMALS

Additive to normal plasma	Average cell score	Percentage of total cells scoring 3 or higher
None	0 61	7 8
EH alone	0 65	6 0
EP alone	0 85	11 0
EP+EH	1 34	22 0
EP+EH+actinomycin *D*, 3 5 μg/ml	0 47	8 6

EP and EH as in Table 1

Table 5 EFFECTS OF EH ON EP AND OF ACTINOMYCIN *D* ON EH+EP IN ERYTHROBLASTS FROM ANAEMIC ANIMALS (BLOOD RETICULOCYTOSIS 56 PER CENT)

Additive to normal plasma	Average cell score	Percentage of total cells scoring 3 or higher
None	1 41	24
EH alone	1 40	22
EP alone	2 00	35
EP+EH	2 60	57
EP+EH+actinomycin *D* (0 7 μg/ml)	2 12	46
EP+EH+actinomycin *D* (3 5 μg/ml)	1 40	24

EP and EH as in Table 1

philic erythroblasts, however, are present in normal amounts (results to be published) Tables 4 and 5 show the same effect *in vitro*

The phenomenon described causes a quick increase in circulating erythrocytes by accelerated maturation and possibly by skipping some divisions[6] Because erythropoietin certainly causes differentiation of stem cells into erythroid cells[7], there seem to be two "sites" at which it can act on an undifferentiated stem cell population in which it can induce differentiation, and on already differentiated basophilic erythroblasts increasing their rate of maturation

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[1] Reissmann, K R , and Ito, K , *Blood*, **28**, 201 (1966)

[2] Gallagher, N I , and Lange, R D , *Proc Soc Exp Biol and Med* , **110**, 422 (1962) Gallagher, N I , Seifert, G L , Callinan, J L , Maes, A A , and Lange, R D , *J Lab Clin Med* , **61**, 258 (1963)

[3] Borsook, H , *Biol Rev* , **41**, 259 (1966)

[4] Borsook, H , Teigler, D , and Gunderson, A , *Arch Biochem Biophys* (in the press)

[5] Borsook, H , *Ann NY Acad Sci* (in the press).

[6] Lajtha, L G , and Oliver, R , in *Ciba Foundation Symposium on Haemopoiesis*, 289 (Churchill, London, 1960) Stohlman, F , Howard, D , and Beland, A , *Proc Soc Exp Biol and Med* , **113**, 986 (1963)

[7] Erslev, A J , *Blood*, **14**, 386 (1959) Alpen, E L , and Cranmore, D , in *The Kinetics of Cellular Proliferation*, 290 (Grune and Stratton, NY, 1959) Filmanowicz, E , and Gurney, C W , *J Lab Clin Med* , **57**, 65 (1961)

Emergence of Two Erythroblast Lines in Erythropoiesis during Growth and Haemorrhage in the Rat

DURING studies of erythropoiesis in rats of different age groups and in rats which had been heavily bled, line 1 and line 2 normoblast cells, as described in the preceding communication, appeared in varying numbers Consequently, the proportion of line 1 and line 2 cells present in the bone marrows and spleens of these animals has been examined

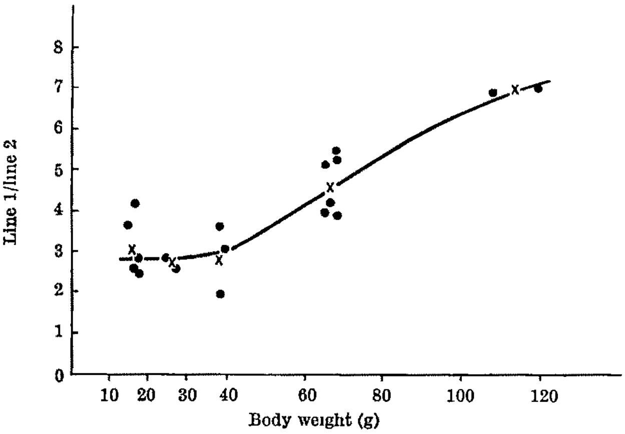

Fig 1 Variation in the ratio of line 1 to line 2 erythroid cells in the bone marrow of rats during the first 8 weeks of life

Using groups of August rats weighing approximately 15 g (7–10 days old), 25, 40, 65 and 110 g (6–8 weeks old), smears of spleen and femoral marrow were made and differential counts of early and intermediate normoblasts with the usual degrees of cytoplasmic basophilia or with the high haemoglobin content—line 1 and line 2 normoblasts—were obtained Omitting the late normoblasts which, by definition, have a high degree of haemoglobinization and therefore cannot with confidence be described as line 1 or line 2, the ratio of the numbers of line 1 to line 2 normoblasts was calculated The results for the bone marrow are shown in Fig 1

Up to 40 g (3 weeks old) the ratio is about 3 1, after which the proportion of line 2 normoblasts gradually declines until in the adult animal the ratio is about 7 1 Thus it would seem that the line 2 normoblasts are associated with the high rate of production of erythrocytes necessary during the early growth period when the blood volume is expanding rapidly The curve also probably corresponds to earlier work[1] in which it was shown that a high degree of cell proliferation in the bone marrow remains constant up to about 40 g body weight, after which it declines

In the second series of experiments, adult rats were bled approximately 10 per cent of their blood volume by cardiac puncture and were killed after 48 h Spleen and marrow smears were made and differential counts of normoblasts were made Table 1 summarizes the ratios of line 1 to line 2 normoblasts, again omitting the late normoblasts

Table 1 shows that bleeding the animal causes an increase in the proportion of line 2 cells in the marrow with the result that the picture becomes similar to that in the 1 week old rats In the spleen, also, the ratio of line 2 to line 1 normoblasts is very similar in the bled adult rats and the 1 week old rats In the case of this organ, however, comparisons are difficult because of the almost complete absence of normoblasts in the adult rat spleen Consequently, this increase in line 2 cells is a real increase in the absolute number of line 2 cells, probably resulting from stimulation of the stem cells

In the marrow of the bled rats, the origin of the line 2 cells was not so clear Identification of many of the line 2

Table 1 RATIO OF LINE 1 TO LINE 2 ERYTHROID CELLS IN THE BONE MARROW AND SPLEEN OF RATS OF DIFFERENT AGES AND FOLLOWING HAEMORRHAGE IN THE ADULT RAT

Ratio of $\frac{\text{line 1}}{\text{line 2}}$	Bone marrow	Spleen
1 week old	3	1 2
7 weeks old	7	—
Bled adult	2 2	1 3

cells was not so positive and a number of cells classified as line 2 should, on morphological grounds, more properly have been put in a group between the two lines The appearance was therefore that the line 1 cells were passing through a transitional stage into the line 2 cells This type of change would agree with the observation, described in the preceding communication, that transformation from line 1 to line 2 cells was stimulated *in vitro* with erythropoietin

In summary, in the 1 week old rat there is a high proportion of line 2 normoblasts present in the nucleated erythroid cell populations of both bone marrow and spleen During growth, the proportion of line 2 cells gradually decreases When the adult animal experiences a severe haemorrhage, line 2 cells appear in large numbers at both sites In the marrow, part of the increase may be caused by stimulation of the stem cell compartment, but part also seems to be the result of a transition from the early line 1 to early line 2 cells In the spleen, the appearance of large numbers of line 2 cells is probably a result only of a stimulation of the stem cell compartment Thus the differentiation step to major haemoglobin production can take place at either the stem cell or the committed erythroid cell level

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[1] Lord, B I, *Brit J Haematol*, 11, 525 (1965)

Erythropoietin Enhancing Factor in Serum of a Calf with Primary Familial Polycythaemia

PRIMARY familial polycythaemia occurs occasionally in calves of an inbred Jersey herd maintained at the University of California, Davis The genetic pattern of this disease suggests a simple autosomal recessive mode of inheritance[1] During investigation of the aetiology of this disease, it was discovered that serum from the one currently surviving calf with polycythaemia, although itself devoid of measurable erythropoietic activity, when incubated with erythropoietin resulted in a four-fold enhancement of activity Incubation with normal calf serum did not enhance the activity of erythropoietin in the assay system employed in these studies

Gardner *et al*[2] have reported enhancement of purified erythropoietin by serum from normal human beings, normal rabbits and normal mice Although in these studies serum from normal calves consistently failed to enhance erythropoietin activity, incubation of erythropoietin with sera from other species, dog, monkey and man, was found to increase erythropoietin activity 1 5–2 3-fold

Serum was obtained from the 6 month old female calf with familial primary polycythaemia and from four normal Jersey female calves of the same age in the herd All sera were frozen immediately after separation and stored until use The haematocrit of the polycythaemic calf was 65 per cent, haemoglobin was 21 g/100 ml The haematocrit of the normal calves was 31, 34, 39 5 and 39 per cent, haemoglobin was 11 7 and 10 8 g/100 ml (calves *C* and *D* were not measured)

Erythropoietic activity was assayed in hypertransfused female *BALB/c* mice, according to the method of DeGowin *et al*[3] Erythropoietin was obtained from the urine of a patient with aplastic anaemia[4] and concentrated by collodion adsorption[5] This erythropoietin was standardized by comparison with the International Reference Preparation[6], using either saline or serum as diluent

All sera were incubated with erythropoietin for 2 days in the cold (8° C) Erythropoietin alone and serum alone were also incubated in the same conditions

Table 1 shows the enhancement of erythropoietin after incubation with various amounts of polycythaemic calf serum Serum obtained before the calf was 8 5 months old produced a consistent and significant enhancement of activity of erythropoietin even with as little as 0 1 ml of polycythaemic calf serum No enhancement of activity occurred in the case of incubation with serum from normal calves When this experiment was repeated with serum collected a fortnight later, identical results were obtained, 1 ml of serum gave a four-fold enhancement of erythropoietin activity Table 1 also shows the results of simultaneous subcutaneous injections of erythropoietin and polycythaemic calf serum at different sites, significant enhancement was not observed.

Table 1 ERYTHROPOIETIN ENHANCING FACTOR IN SERUM OF A POLYCYTHAEMIC CALF

Group (No of mice)	Activity (× ESF control)	Significance of difference*
Polycythaemic calf		
1 ml of serum + ESF (6)	4 0	0 001
0 5 ml of serum + ESF (10)	3 0	0 001
0 1 ml of serum + ESF (10)	2 4	0 001
1 ml of serum and ESF at two sites (7)	1 8	0 1
Normal calves 1 ml of serum + ESF		
Calf *A* (6)	0 7	0 02
Calf *B* (9)	0 7	0 001
Calf *C* (8)	1 0	—
Calf *D* (9)	1 4	0 3

ESF, erythropoietic serum factor

* Fisher's *t* test

These findings indicate that the serum of this polycythaemic calf contained something not present in norma calf serum which in itself had no erythropoietic effect but which on incubation with erythropoietin resulted in four-fold enhancement of the activity Spontaneous recovery has been observed in affected calves which survive for more than one year, and in an attempt to ensure survival of this calf until remission occurred, phlebotomies were carried out at approximately monthly intervals Erythropoietin enhancing activity was assayed in serum from successive bleedings and a decrease in activity at 8 5 months with complete disappearance of enhancing activity by 10 months was revealed (Table 2) Although it seemed reasonable to assume that this factor was causally related to the polycythaemia in this calf and to the severe and often fatal polycythaemia occurring in other calves of this herd, we found no remission in polycythaemia corresponding to, or within 4 months of, the disappearance of the detectable level in the serum

Table 2 DECREASE IN ERYTHROPOIETIN ENHANCING FACTOR WITH AGE OF POLYCYTHAEMIC CALF

Age (months)	Haematocrit (%)	Activity (× ESF control) 1 ml	0 5 ml	0 1 ml
5 3	66	2 9	2 4	2 4
6 4	64 5	4 0	3 0	2 4
7 2	66	4 2	3 7	3 9
8 5	69	1 6	1 9*	
9 5	67	1 0	1 0	
10 0	70	0 6		

* Average of ten assays, one of which was negative

Enhancement of erythropoietin by incubation with renal erythropoietic factor (REF)[7] suggested that the enhancing factor present in this calf might be REF Further investigation of the physiological and chemical characteristics of the enhancing factor in the intact mouse assay system being employed, which requires relatively large amounts of material, was not possible The small remaining supply of active serum (from bleedings at 6 and 7 months) was reserved for study in the *in vitro* system described in the first of these three communications Further investigation of this subject must await birth of another calf afflicted with primary familial polycythaemia

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[1] Tennant, B , Astbury, A C , Laben, R C , Richards, W P C , Kaneko, J J , and Cupps, P T , *J Amer Vet Med Assoc* ,**150**, 1493 (1967)

[2] Gardner, jun , E , Wright, C -S , Lewis, J P , and Moores, R R , *Brit. J Haematol* ,**13**, 317 (1967)

[3] DeGowin, R L , Hofstra, D , and Gurney, C W , *Proc Soc. Exp Biol and Med* , **110**, 48 (1962)

[4] Van Dyke, D , Layrisse, M , Lawrence, J H , Garcia, J F , and Pollycove M., *Blood*, **18**, 187 (1961)

[5] Van Dyke, D C , *Haemopoiesis*, 397 (J and A Churchill, London, 1960)

[6] Cotes, P M , and Bangham, D R , *Bull WHO*, **35**, 751 (1966)

[7] Contrera, J F , Gordon, A S , and Weintraub, A H , *Blood* **28**, 330 (1966)

Polymorphism of Lipids

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The structures of two new phases of divalent cation soaps have been solved The polar groups (cations and carboxyl groups) form planar two-dimensional networks, either square or hexagonal, embedded in a continuous matrix formed by the disordered paraffin chains. These and earlier results make possible a unified picture of a novel class of structures in all of which the polar groups are clustered in rod-like regions, but which differ in the way the rods are arranged in one, two or three-dimensional networks.

THE work described here arose from the casual observation of striking structural analogies between the high-lipid, high-temperature phases of lecithin–water systems and the anhydrous high-temperature phases of several divalent cation soaps This observation has led to an analysis of the literature, to the reinterpretation of some of the published data, and in some cases to new experiments The result has been the discovery of a novel family of structures, formed by identical rod-like elements, which are ordered into either two or three-dimensional lattices The "structure elements", to which we often refer in this article, are by definition the locus of the polar groups, those elements are embedded in a paraffin matrix in which the electron density is supposed to be uniform throughout The structures observed with soaps are described here the lecithins will be discussed later

Spegt and Skoulios studied many anhydrous soaps of the divalent cations of group II as a function of temperature, using X-ray diffraction techniques[1-4]. Many of the data used here come from that work when new experiments were carried out, the techniques were those described by Spegt and Skoulios. A few examples of the phase diagrams established by these authors are given in Table 1 The low-temperature phases C_1 and C_2 are lamellar, with stiff paraffin chains; the conformation of the chains in all the other phases is disordered, as shown[5] by the presence of a diffuse band near $s=(2 \sin \theta/\lambda)=(4\ 5\ \text{Å})^{-1}$ Phases H, T, R, Q are each characterized by a number of sharp small-angle reflexions Phases H, H_1 and H_2 are two-dimensional hexagonal arrays of rigid rods, the rods containing the polar groups of the soaps Spegt and Skoulios have shown that the number n of cations per unit length of rod, a parameter easily determined for this type of structure, is constant for all the soaps of the same cation[1-4]. When two hexagonal phases (H_1 and H_2) are observed in one soap, each phase is characterized by a different value of n (refs 1 and 4) Phase Q is body-centred cubic[3]; its structure[6] consists of rods of finite length, linked three by three, and forming two interwoven three-dimensional networks (ref 6, Fig 2) The distribution of the cations seems to be uniform along the rods, with the exception of a short gap (approximately 1·5 Å long), devoid of cations, near the ends In this structure the number n is the same for all the strontium soaps; furthermore, n is almost identical in phases Q and H (ref. 6 and Table 2)

Here we are mainly concerned with the structure of phases T and R Spegt and Skoulios have indexed the small-angle reflexions of both phases on two equatorial planes, assuming arbitrarily that the two planes are at right angles they propose a face-centred orthorhombic lattice for phase T (ref 2), and a face-centred monoclinic lattice for phase R (ref 3) In fact, an inspection of the data shows that for all the soaps and at all temperatures the spacings of the reflexions are in perfect agreement with a body-centred tetragonal lattice for phase T ($a/c=\sqrt{2}$ in Tables 3 and 4 of ref 2), and with a rhombohedral lattice

Table 1 PHASE DIAGRAMS OF THE ANHYDROUS STEARATES OF THE CATIONS OF GROUP II

Mg^1	C_1 or C_2 (109°) H_1 (195°) H_2 (210°) melt
Ca^2	C_1 (100°) C_2 (123°) T (152°) (179°) H (>350°)
Zn^1	C_1 (130°) melt
Sr^3	C_1 (130°) (170°) R (197°) Q (246°) H (>400°)
Cd^4	C_1 (99°) H_1 (211°) H_2 (~230°) melt
Ba^1	C_1 (150°) (220°) Q (>400°)

Transition temperatures are given in parentheses The dotted lines show regions in which the phases have not been fully characterized The phase diagrams of the other saturated soaps of the same cation are quite similar to stearate (see refs), although the phase T was observed recently in strontium arachidate, near 170° C (unpublished results) The correspondence of the nomenclature adopted here with that of Spegt and Skoulios is the following for the calcium soaps T and H replace D and F, for the strontium soaps R replaces Δ, for the cadmium soaps H_1 and H_2 replace B and C

Table 2 SOME DATA ON THE STRONTIUM SOAPS

	Phase R						Phase Q			Phase H	
	t °C	δ g cm^{-3}	a Å	c Å	N	n	t °C	N	n	t °C	n
Laurate	180	$0\ 91_0$	29 0	78 0	21 4	$0\ 52_4$	235	215	$0\ 51_1$	265	$0\ 49_8$
Myristate	180	$0\ 89_3$	31 0	84 8	23 3	$0\ 52_5$	235	233	$0\ 51_0$	265	
Palmitate							235	248	$0\ 50_9$	265	
Stearate	181	$0\ 87_2$	36 0	93 3	28 0	$0\ 52_8$	235	261	$0\ 50_7$	265	
Arachidate*	200	$0\ 85_0$	37 9	97 5	29 3	$0\ 52_1$	235	278	$0\ 50_6$	265	
Behenate							235	285	$0\ 50_0$	265	

The structures of phases R and Q are shown in Fig 1 and in ref 6, Fig 2 The parameters a and c of the hexagonal cell were deduced from the parameters c', b' and γ' of the face-centred monoclinic cell of ref 3 ($a=2c'$, $c=3/2\, b' \sin \gamma'$), and were verified with new experiments (The density δ is determined as in ref 6, Table 2) $N=0\ 602\ V\delta/M$ is the number of cations associated with one lattice point (V, volume of the elementary cell, M, molecular weight of the soap) The symbol n is the number of cations per unit length of rod $n=N/3(D-d)$, where $D=a/\sqrt{3}$ is the length of one side of the hexagon, $d/2=1\ 56$ Å is the length of the gap devoid of cations, at each end of the rods The dimensions of phases Q and H are taken from refs 3 and 6

* See comment in Table 1

Table 3 SOME DATA ON THE CALCIUM SOAPS

	Phase T						Phase H	
	t °C	δ g cm^{-3}	a Å	c Å	N	n	t	n
Laurate							240	0 52$_9$
Myristate	165	0 89$_0$	31 7	55 0	30 1	0 58$_5$	240	0 52$_9$
Palmitate	159	0 88$_5$	34 1	59 1	33 2	0 59$_6$	240	0 53$_4$
Stearate	152	0 88$_0$	35 9	62 2	35 1	0 58$_7$	240	0 53$_4$
Arachidate	152	0 87$_5$	38 3	66 4	38 7	0 59$_5$	240	0 53$_4$

The structure of phase T is shown in Fig 2 The parameters a and c are taken from ref 2, Table 4 (c here is b in ref 2), and were verified with new experiments N is determined as in Table 2, with δ given in ref 2 $n=\frac{N}{2(D-d)}$, $D=a$, $d/2=3$ Å The lattices are pseudo-hexagonal ($c=\sqrt{3}\ a$) at the temperature of Table 3 The data of ref 2 permit calculation of N and n at different temperature, for calcium myristate the results are the same as in the table The value n of phase H is taken from ref 2

for phase R ($a/c=\sqrt{3}$ and $\cos\gamma\simeq a/3b$ in Table 3 of ref 3), these lattices will be adopted here, on the ground that the number of independent parameters is minimal (two for the tetragonal and rhombohedral, instead of three for the orthorhombic and four for the monoclinic) The agreement of the observed and calculated spacings is shown in Tables 4 and 5

By analogy with phases H and Q we propose that phases T and R are ordered arrangements of identical rod-like elements of finite length The structures are represented in Figs 1 and 2 the rods are linked together four by four in phase T, three by three in phase R, and form planar two-dimensional networks, either square or hexagonal These structures explain the observations in a far more satisfactory manner than the structures formed by disk-shaped structure elements, as suggested before (ref 2, Fig 5)

Observed and calculated intensities agree very well for the rod-like structures of Figs 1 and 2 (Tables 4 and 5), if, as in phase Q of the strontium soaps[6], the distribution of the cations is uniform along the rods*, with the exception of a short gap at the ends, devoid of cations It should be noted, nevertheless, that for disk-shaped structure elements the agreement would not be much worse, as long as the size and shape of the disks are properly adjusted, different soaps of the same cation should be compared if the intensity distribution were to provide a discrimination between rods and disks (this problem will be discussed elsewhere)

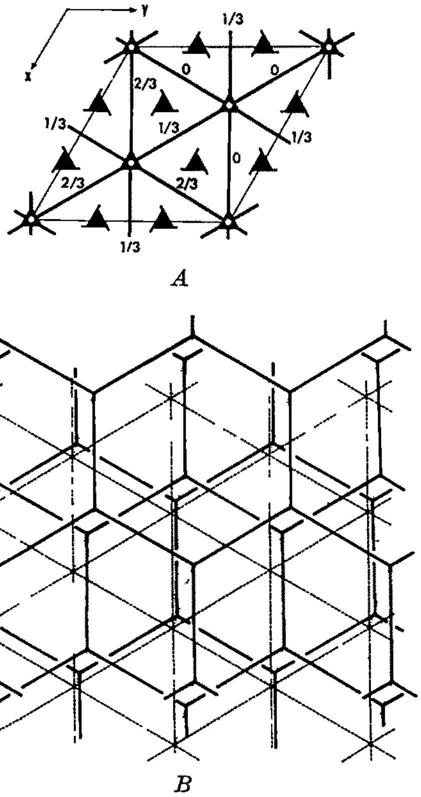

Fig 1 Phase R space group $R\bar{3}m$ The loci of the polar groups, represented by heavy lines, are rods of finite length that sit on mirror planes The rods are linked three by three, forming planar two-dimensional networks A, Projection of one unit cell along the hexagonal axis, with the position of some of the symmetry elements The rods are perpendicular to the c axis the fractional figures are the z co-ordinates of the rods B, Perspective view Note the two-dimensional hexagonal networks stacked and mutually unconnected

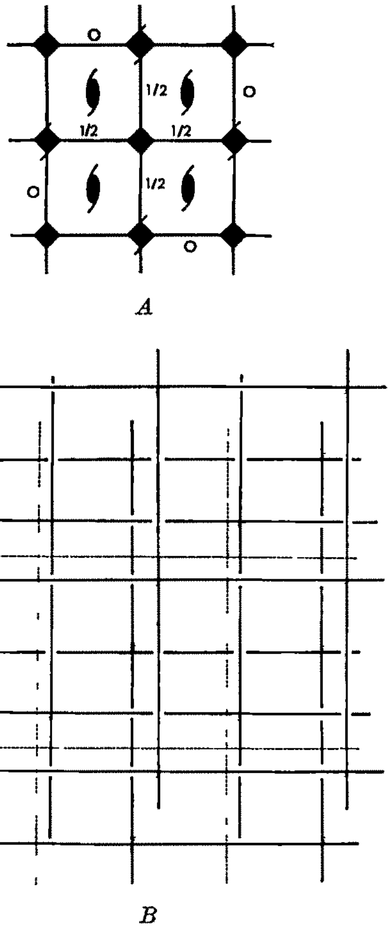

Fig 2 Phase T space group $I422$ (see legend of Fig 1) The polar groups sit on two-fold axes A, Projection of one unit cell along the tetragonal axis c The rods are parallel to the plane a, b, the fractional figures are the z co-ordinates of the rods B, Perspective view Note that the structure is formed by identical rod-like elements, linked four by four, forming two dimensional planar square networks The networks are stacked, otherwise unconnected

More direct arguments in favour of the rod-like structures can be obtained from analysis of the dimensions of the structure elements Tables 2 and 3 show the following (a) In each phase (T, R, Q, H) n is constant for all the soaps of the same cation (b) In the strontium soaps n has almost the same value in the three phases R, Q and H, the small differences between the phases T and H of the calcium soaps are of the same order as those observed between the phases H_1 and H_2 of the magnesium and cadmium soaps[1,4], and can be similarly explained, as discussed here (c) At high temperature, the lattices of phase T of all the calcium soaps become pseudo-hexagonal[2] (Table 3), a consequence of the fact that each of the bundles of rods parallel either to the a or to the b axis becomes a two-dimensional hexagonal array

Clearly all these numerical coincidences would be meaningless if the structure elements were assumed to

---

* The uniform distribution of the cations must be taken as a simplification justified by the low resolution of the X-ray data ($s^{-1}>8$ Å, see Tables 4 and 5

Table 4 X-RAY DIFFRACTION DATA OF PHASE *R* STRONTIUM MYRISTATE, 185° C

hkl	s_{obs} $(10^3$ Å$)^{-1}$	s_{calc} $(10^3$ Å$)^{-1}$	I_{obs}	I_{calc}	F
000					+44 0
003	35 8	36 0	1,500	1,313	+44 0
101	39 5	39 4	459	397	−17 7
012	44 7	44 6	261	341	−17 7
104	60 7	60 9	55	172	−17 7
110	65 4	65 0	21	14	+8 5
015	69 9	70 7	27	104	−17 7
006	71 7	72 0	321	331	+44 0
113	74 1	74 2	20	17	+8 5
021	75 9	75 9	10	17	+15 9
202	79 9	78 9	9	15	+15 9
024	—	89 1	<8	7	+15 9
107	91 9	92 0	26	28	−17 7
205	96 3	96 0	18	5	+15 9
116		97 0		4	+8 5
211	102 5	99 6	25	1	−10 9
122		101 8		1	−10 9
108		103 2		12	−17 7
009	107 9	108 2	72	35	+44 0
214	—	110 0	<10	<1	−10 9
300	—	112 5	<10	<1	−9 5
027	—	112 5	<10	<1	+15 9

The X-ray pictures were recorded on a Guinier camera The values s_{obs} and s_{cal} are the reciprocal spacing ($s=2 \sin \theta/\lambda$) of the observed reflexions, and calculated for the hexagonal lattice (space group $R\bar{3}m$), with $a=30\ 8$ Å, $c=83\ 3$ Å Values of I_{obs} are the observed intensities, strong intensities were measured on densitometer tracings, and weak ones were estimated visually by recording the X-ray pictures on packs of films and comparing different films (the absorption coefficient of which is known) The intensities were corrected for the proper geometric factor The calculated intensities can be expressed as follows

$$I_{\text{calc}} = m\ F^2 \exp\left[-\alpha s_l^2 - \beta\,(s^2 - s_l^2)\right]$$

m is the multiplicity factor, F is the structure factor, normalized to $F_{000}=3(D-d)$ (that is, the total length of rods in one elementary cell), the gaussian function (s_l is the l component of s) takes into account several effects, such as the anisotropic thermal motion, the spread of the electron density around the axis of the rods, the scattering of the length of the rods (in Tables 2 and 3 n is not an integer) The structure factors are those of space group $R\bar{3}m$, special positions (x, $x/2$, $2/3$, and so on), integrated over the length of the rods (ref 10, page 472), the gap at the end of the rods is taken into account in the form of a delta function, at (0, 0, 2/3)

$$F_{hkl} = D/4 \int_0^{1/3} A_{hkl}\,(x,\ x/2,\ 2/3)\,\mathrm{d}x - 3d$$

The result is $F_{00l} = 3\,(D-d)$, $F_{hkl}=D-d$, $F_{hkl}=3D/\pi\,[(2h+k)^{-1} + (k-h)^{-1} - (h+2k)^{-1}] \sin 2\pi l/3 - 3d \cos 2\pi l/3$ The parameters used in the calculations are $D=17\ 8$ Å, $d=3\ 12$ Å, $\alpha=310$ Å^2, $\beta=680$ Å^2

Table 5 X-RAY DIFFRACTION DATA OF PHASE *T* CALCIUM STEARATE, 140° C

hkl	s_{obs} $(10^3$ Å$)^{-1}$	s_{calc} $(10^3$ Å$)^{-1}$	I_{obs}	I_{calc}	F
000					−58 4
002	30 9	30 9	3,650	4,240	+58 4
101	32 4	32 3	4,730	3,370	+23 2
110	40 3	40 2	385	151	−12 0
112	50 5	50 6	150	187	−12 0
103	54 2	54 3	742	1,310	+23 2
200	56 8	56 8	161	163	+23 2
004	61 8	61 8	666	1,060	+58 4
202	64 9	64 6	205	202	+23 2
211		65 3		77	−12 0
114	—	73 6	<60	47	−12 0
213	—	78 6	<60	33	−12 0
220	—	80 3	<60	3	−12 0
105	82 2	82 3	204	205	+23 2
204	—	83 9	<60	51	+23 2
222	—	86 0	<60	4	−12 0
301	—	86 6	<60	11	+23 2
130	—	89 8	<60	2	−12 0
006	92 6	92 7	163	100	+58 4
303	—	96 9	<70	45	+23 2

Experiments and calculations were carried out according to the legend of Table 4 Space group $I422$, $a=35\ 2$ Å, $c=64\ 7$ Å The structure factors take the form $F_{00l}=2(D-d)$, $F_{h0l}=D-2d$, $F_{hkl}=\ -2d$ The parameters are $D=35\ 2$ Å, $d=6$ Å, $\alpha=490$ Å^2, $\beta=810$ Å^2

be disk-shaped, furthermore, the number of cations per disk (N in Tables 2 and 3) would vary with the length of the paraffin chain, and the high temperature form of phase T would be an odd pseudo-hexagonal organization of flat disks parallel to the "six-fold" axis

Other properties are consistent with the rod-like structures Fairly large crystals are observed in phase Q much more frequently than in any other phase this property should be correlated with the presence of the three-dimensional network Phases R and T can be visualized as lamellar structures, with stronger cohesion in the plane of the lamellae than between lamellae, and can be expected to display order–disorder phenomena whenever the interlamellar correlations become sufficiently loose Spegt and Skoulios[1–3] mention phenomena of that nature, characterized by the presence of both sharp and diffuse small-angle reflexions (these phenomena will be discussed elsewhere)

The most satisfying result emerging from the variety of structures described here is perhaps that one can construct a unified picture embodying them all Indeed, it is now clear that the high temperature phases of the anhydrous soaps of the divalent cations of group II are all constructed according to the same principle The polar groups are clustered in rod-like regions, and the rods, all identical and crystallographically equivalent, are organized in either a two or three-dimensional lattice Structure H consists of infinitely long isolated rods (one-dimensional network) arranged in a hexagonal two-dimensional lattice, structures R and T consist of rods of finite length, linked three by three or four by four, respectively, forming two-dimensional hexagonal or rectangular networks, which are stacked regularly into three-dimensional lattices Structure Q consists of rods joining three by three, and forming two interwoven three-dimensional networks This class of structures has been discussed by Wells[7], all the structures discussed in this article were considered by him

It is appropriate, in the context of this work, to mention another class of structures, originally described by Skoulios and Luzzati[8], that displays interesting analogies with the rod-like class In this class of structures the elements are lamellar the lamellae may be in the form of infinite planar sheets packed in one-dimensional (smectic) lattice[8], infinitely long ribbons organized in two-dimensional centred-rectangular[8] or oblique[9] lattices or, finally, disks organized in three-dimensional lattices[8] It is remarkable that all the high temperature phases of each of the anhydrous soaps studied so far belong to one or other of the two classes according to the nature of the cation rod-like for the cations of group II, lamellar for sodium[8], potassium[9], lithium[9] The highly developed organization of all these phases is achieved in spite of a great disorder of the paraffin chains, it is thus clear that the correlation between the chemical composition of the lipid and the class of the structures must be sought in the organization of the polar groups Although little direct evidence is available on this question, various indirect arguments suggest that the degree of order is quite high the fact that for different soaps of the same cation the number of molecules per unit length of rod (this work) or of ribbon[5] is constant clearly points to an ordered organization of the polar regions, the occurrence of two hexagonal phases in the magnesium and cadmium soaps is interpreted by Spegt and Skoulios[4] as a consequence of a polymorphic transition between two ordered arrangements of the polar groups The finite length of the rods, and the finite width of the ribbons and of the disks, can best be explained by the interplay of some ordered arrangement of the polar groups and of a disordered conformation of the paraffin chains (see discussion in refs 6 and 8)

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[1] Spegt, P A , thesis, Univ Strasbourg (1964)

[2] Spegt, P A., and Skoulios, A E , *Acta Cryst* ,17, 198 (1964)

[3] Spegt, P A , and Skoulios, A E , *Acta Cryst* ,21, 892 (1966)

[4] Spegt, P A , and Skoulios, A E , *Acta Cryst* ,16, 301 (1963)

[5] Luzzati, V , in *Biological Membranes* (edit by Chapman, D) (Academic Press, in the press)

[6] Luzzati, V , and Spegt, P A , *Nature*,215, 701 (1967)

[7] Wells, A F , *Acta Cryst* ,7, 535, 545, 842, 849 (1954)

[8] Skoulios, A E , and Luzzati, V , *Acta Cryst* ,14, 278 (1961)

[9] Gallot, B , and Skoulios, A E , *Acta Cryst* ,15, 826 (1962)

[10] *International Tables for X-ray Crystallography*, 1 (The Kynoch Press, Birmingham, 1952)

LETTERS TO THE EDITOR

PLANETARY SCIENCE

Holocene Earth Movements and Eustatic Sea Level Change in the Peloponnese

TWENTY-THREE archaeological sites on the east and south-east coast of the Peloponnese have been studied to determine the relative change of sea level at each site and the date of the latest occupation One site was uplifted, one undisplaced, seventeen submerged and four unproved (Fig 1 and Table 1) The displaced sites were at widely differing depths, and there was no simple correlation between age and depth, or location and depth It has been widely supposed, though on no certain evidence, that the Peloponnese is tilting downwards as a semi-rigid block to the south, about a hinge-line at the Gulf of Corinth, but the evidence does not support this

The displacement, approximate age to the nearest half millennium, rate of displacement and other data for each site are shown in Table 1 Displacement and age data are available for the western Mediterranean[1] based on fifty-four proved sites, and the distribution curves for the two areas are plotted in Fig 2

The curve for the western Mediterranean is markedly skew, because the data are effectively drawn from two populations Most sites are on stable lengths of coast such as that from Gibraltar to Toulon, while others are grouped in volcanic areas such as the Bay of Naples and round Etna where there is high seismic activity[2] The data for the Peloponnese come from an area which is uniformly seismically active[2] and show a random distribution of sites vertically

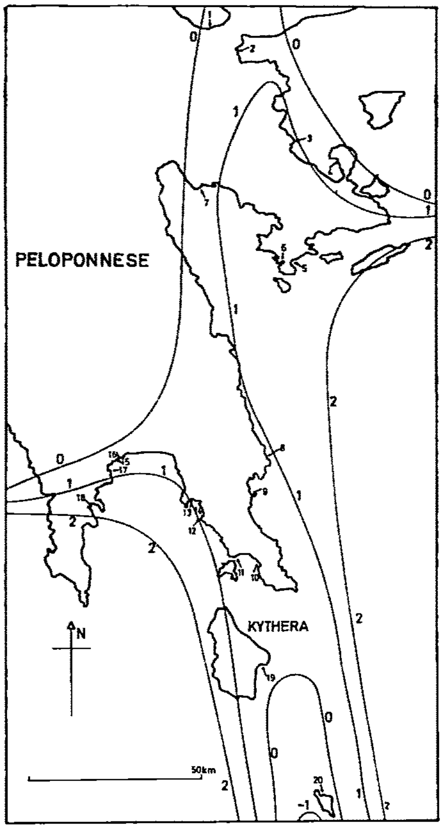

Fig 1 The coast of the eastern Peloponnese, southern Greece, showing the sites which produced definite data The contours indicate the best fit third order surface for the rate of displacement of each site in m/millennium downwards relative to present sea level

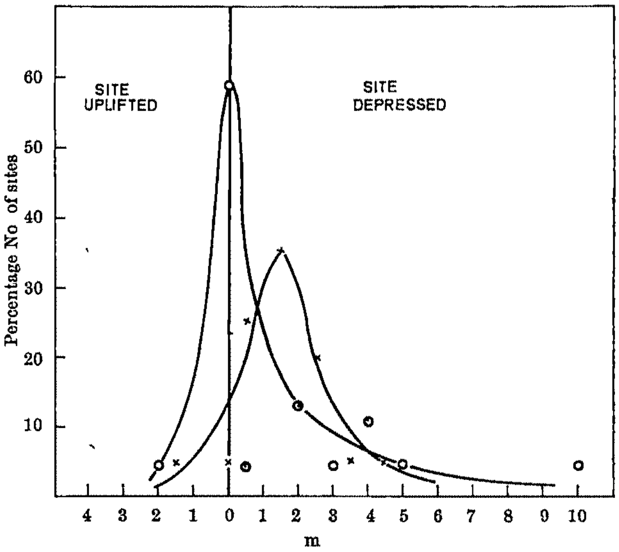

Fig 2 Percentage frequency of occurrence of archaeological sites plotted against magnitude of displacement during 2,000 yr, relative to present sea level O, Western Mediterranean, ×, Peloponnese

The Peloponnese data alone suggest a eustatic change of sea level equivalent to the mean displacement of sites, superimposed on a random tectonic displacement which was equally likely to be up or down at each site Other authors working from less complete field data have either stressed the tectonic factor alone[3] or the eustatic factor alone[4]

Evidence from the Atlantic coasts of Europe and America indicates that net eustatic change of sea level during the past 2 millennia has been less than 2 m (refs 5 and 6) and possibly less than 1 m (ref 7) The evidence from the western Mediterranean[1] shows that undisplaced sites occur in non-seismic areas, and that, to within an accuracy of observation of ± 0 5 m, the net eustatic change over 2,000 years has been zero, though sea level may have varied by 0 5–1 0 m in the interval Blackman (personal communication) has archaeological data showing that several sites on the Aegean coast of Turkey are also undisplaced

The Peloponnese data were tested statistically to look for a eustatic factor No correlation could be found with any of the proposed eustatic curves for the last 3,000 yr (refs 5–9) It was considered that rate of displacement might be related to geographical location, seismicity or isostatic gravity anomaly The number of epicentres published[2] and the accuracy of location of epicentres are

Table 1

Site No	Name	Age (millennia)	Depth (m)	Depth/age (m/millennium)	Bouger (mgal)	Gravity gradient (mgals/km)
1	Lechaeum	2 0	0 7	0 3	04	1 0
2	Kenchreai	2 5	2 0	0 8	15	1 0
3	P Epidaurus	2 0	2 7	1 3	35	1 0
4	Methane	2 0	1 0	0 5	60	1 3
5	Lorenzon	1 0	2 0	2 0	60	4 0
6	Halieis	2 4	2 7	1 1	60	4 0
7	Asine	3 0	2 0	0 7	20	1 0
8	Xarax	2 5	3 0	1 2	60	1 0
9	Monemvasia	2 0	1 0	0 5	60	1 0
10	Neapolis	1 5	0 3	0 2	55	2 5
11	Elaphonisos	3 5	3 0	0 9	40	4 0
12	Arkangelos	2 0	0 2	0 1	05	4 0
13	Plitra (1)	3 0	3 0	1 0	−05	4 0
14	Plitra (2)	1 5	2 0	1 3	−05	4 0
15	Trinisi (1)	1 0	1 0	1 0	−70	1 0
16	Trinisi (2)	1 0	1 0	1 0	−70	1 0
17	Gythion	1 5	2 5	1 5	−70	1 0
18	Skutari	1 5	3 5	2 2	−70	1 0
19	Kythera	4 0	0 0	0 0	10	2 5
20	Antikythera	5 0	−3 0	−0 6	−30	0 0

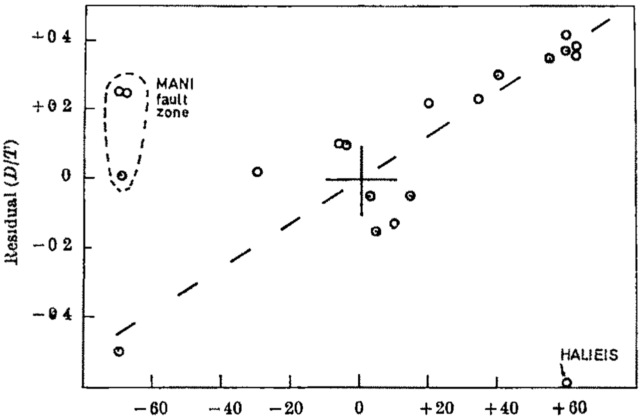

Fig. 3. The difference between the actual rate of displacement at each site and the predicted value from the computed surface is plotted against local Bouger gravity anomaly. Data from the areas of maximum faulting plot off the main trend. There are more points above the indicated mean than below because three values were originally minimum estimates and have been weighted towards the most probable value

not adequate to obtain a correlation. Gravity data were obtained from unpublished gravity maps of the eastern Mediterranean from the NATO SACLANT laboratory at La Spezia. There is no Bouger correction to apply at the shoreline, and the isogams of isostatic anomaly published by De Bruyn[10] follow the same general trend as the Bouger isogams available. It is the relative difference of value between sites which is significant, and there are insufficient data to construct more accurate isostatic maps, so that the Bouger data were used in the following calculations.

In order to isolate a possible eustatic factor the depth of each site was correlated with its age and local gravity anomaly, and then with the age and gravity gradient. Correlation in both cases was low (mean square residual (δ) = 0.365 and 0.503, respectively), and there was no time-dependent term which could be attributed to eustatic change.

If tectonic movements have been random in time, infrequent and of relatively large amplitude at time of movement, there would be no correlation between age and total displacement of a site. And if the period of study is sufficiently long for most sites to be displaced once, then there would be a correlation between magnitude of displacement and tectonic factors independent of time. This was tested by correlating depth with geographical co-ordinates, then with gravity anomaly and then with gravity gradient. All tests yielded very low correlation (δ = 0.495, 2.03, 1.72, respectively). This suggests that tectonic processes are continuous or at shortly spaced intervals compared with the period of the study. Correlation of the rate of displacement, however, in metres per millennium with the local gravity anomaly also produced low correlation (δ = 0.450).

Rate of displacement was correlated with geographical co-ordinates, and a third order equation produced a good fit (δ = 0.082, maximum residual = 0.59). The contours of this surface are plotted in Fig. 1. The close spaced contours to the south-west, indicating maximum variation in rate of displacement, coincide with a heavily faulted zone intersecting the coast between Skutari and Scala[11]. The close spaced contours to the north-east are probably an artificial effect caused by the absence of data further to the north-east, and the fact that the value for Methane is an open-ended minimum estimate, added to the real difference in values obtained at Halieis and Lorenzon.

The most notable feature of the contour pattern is the trend of the zero-displacement line to the north-west which indicates a doming of the Peloponnese with depression of the coastline about a hinge-line set back from the mean coastline. An uplifted ridge stretches south-east from the Elos peninsula through Kythera and Antikythera towards Crete, while a trough of depression extends into the Gulf of Argolis.

Fig. 3 shows a plot of the residual displacement—that is, the difference between the actual measured rate of displacement and that predicted by the equation—against local Bouger gravity anomaly. Allowing for the sites from the areas of maximum variation in rate of displacement caused by faulting, there is a positive correlation between high gravity anomaly and excessive displacement downwards. Thus, if the contours represent the mean rate of displacement of a group of sites in an area, the departure of sites from this mean is proportional to gravity anomaly.

The subsidence of the South Aegean basin since the Tertiary[12] coincides with an area of high positive isostatic anomaly[10], while the oceanic trench to the south of the Peloponnese is an area of negative anomaly, as is usual with trenches[13]. The South Aegean consists of subsiding continental material and so these findings are compatible with an association between high gravity anomaly and high rate of subsidence of continental material in this area. The recent sediments in the trench dip northwards (personal communication from W. B. F. Ryan) indicating a relative depression of the northern margin by approximately 1.0 m/millennium, comparable with the average rate of 0.87 m/millennium for the data discussed here.

Displacement of the coastline due to faulting would be expected to be erratic and of relatively high spatial frequency, while displacements resulting from small variations in isostatic anomaly would be of a broader nature with less local variation, due to the rigidity of the Earth's crust. This seems to be the case. The departures from the broad dome-like warping which are attributed to gravity in this study have a magnitude of 0.2–0.5 m/millennium and a spacing of the order of 50 km, while the variation attributed to faulting is of the order of 1.0 m/millennium over 25 km. This interpretation is tentative until there are more data on the seismicity of the area and on rates of displacement in other parts of the Aegean.

In view of the manifest tectonic differences between the Peloponnese and the average conditions in the western Mediterranean, the simplest explanation of the difference between the two curves in Fig. 2 is that the net eustatic change in the last 2,000 yr has been only of the order of 0.5 m, that the spread of data for the western Mediterranean is a result of local areas of vulcanism and seismicity, and that the spread of data for the Peloponnese is a result of a general depression of the margins of the Peloponnese, with areas of faulting in the Mani, and an uplifted ridge through Antikythera separating the trench to the south and the Aegean to the north, with a possible correlation between local variations in displacement and isostatic anomalies.

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[1] Flemming, N. C., *Geol. Soc. Amer., Special Paper* (in the press).
[2] Gutenberg, B., and Richter, C. F., *Seismicity of the Earth* (Princeton, 1954).
[3] Negris, P., *Athenischer Mitteilungen*, 29, 340 (1904).
[4] Hafemann, D., *Die Umschau*, 60, 7, 193 (1960).
[5] Jelgersma, S., *Mededlinger van de Geologische Stichting*, Maastricht, Series C (1961).
[6] Coleman, J. M., and Smith, W. G., *Geol. Soc. Amer. Bul.*, 75, 833 (1964).
[7] Scholl, D. W., and Stuiver, M., *Geol. Soc. Amer. Bul.*, 78, 437 (1967).
[8] Fairbridge, R. W., *Physics and Chemistry of the Earth*, 4, 99 (Pergamon Press, 1961).
[9] Sheppard, F. P., *Recent Sediments in NW Gulf of Mexico*, 338 (Amer. Assoc. Petrol. Geol., Oklahoma, 1960).
[10] De Bruyn, J. W., *Geophysical Prospecting*, 3, 1 (1955).
[11] Renz, C., and Liatsikas, N., *Geological Map of Greece*, 1 (1954).
[12] Bourcart, J., *Géographie du Fond des Mers*, 1 (Payot, Paris, 1949).
[13] Worzel, J. M., *Continental Margins and Island Arcs*, 357 (Geological Survey of Canada, 1966).

Extension of the Mid-Labrador Sea Ridge

EVIDENCE obtained during geophysical investigations aboard the USCGC Mariposa in the summer of 1967 strongly supports the hypothesis[1] that an inactive branch of the Mid-Oceanic Ridge lies buried beneath the axis of the Labrador Sea

Between 61° and 62° N the crest of the ridge is overlain by 1 km of sediment (Fig 1, profile 4, ref 1, profile *C–D*). To the south, where sediment accumulation rates are perhaps somewhat less rapid, the crest outcrops as isolated knolls on the sea floor North of 62° the ridge crest is covered by a thinner veneer of sediment (profiles 2–3) The Mariposa data apparently extend the known portion of the Mid-Labrador Sea Ridge to at least 64° The basement high strikes N N × W near the 55° W meridian as an apparent continuation from profile *C–D* (ref 1) The axis of the buried ridge lies slightly to the east of a line bisecting that part of the ocean basin which lies between the continental shelves In profile 3 the structural high has apparently acted as a partial dam for detritus Near 64° (profile 1) the basement rock has shoaled sufficiently to form Davis Strait and its rugged relief is replaced by a seemingly monolithic swell The absence of detectable sediment over this feature is probably attributable to current scour

The crest of the Mid-Oceanic Ridge is normally characterized by a unique magnetic signature consisting of a high amplitude anomaly and earthquake epicentres[2] Correlation of the postulated Mid-Labrador Sea Ridge with magnetic anomalies has been presented by Godby *et al*[3]. These investigators found two parallel zones with anomalously high magnetic signatures flanking a central swath of low anomalies and earthquake epicentres The crest of the prominent sub-bottom reflector which has been interpreted as basement (5 5 km/s layer of ref 1) correlates with the eastern magnetic anomaly zone The earthquake belt and the western magnetic zone, however, seem anomalous The frequency of earthquakes in the

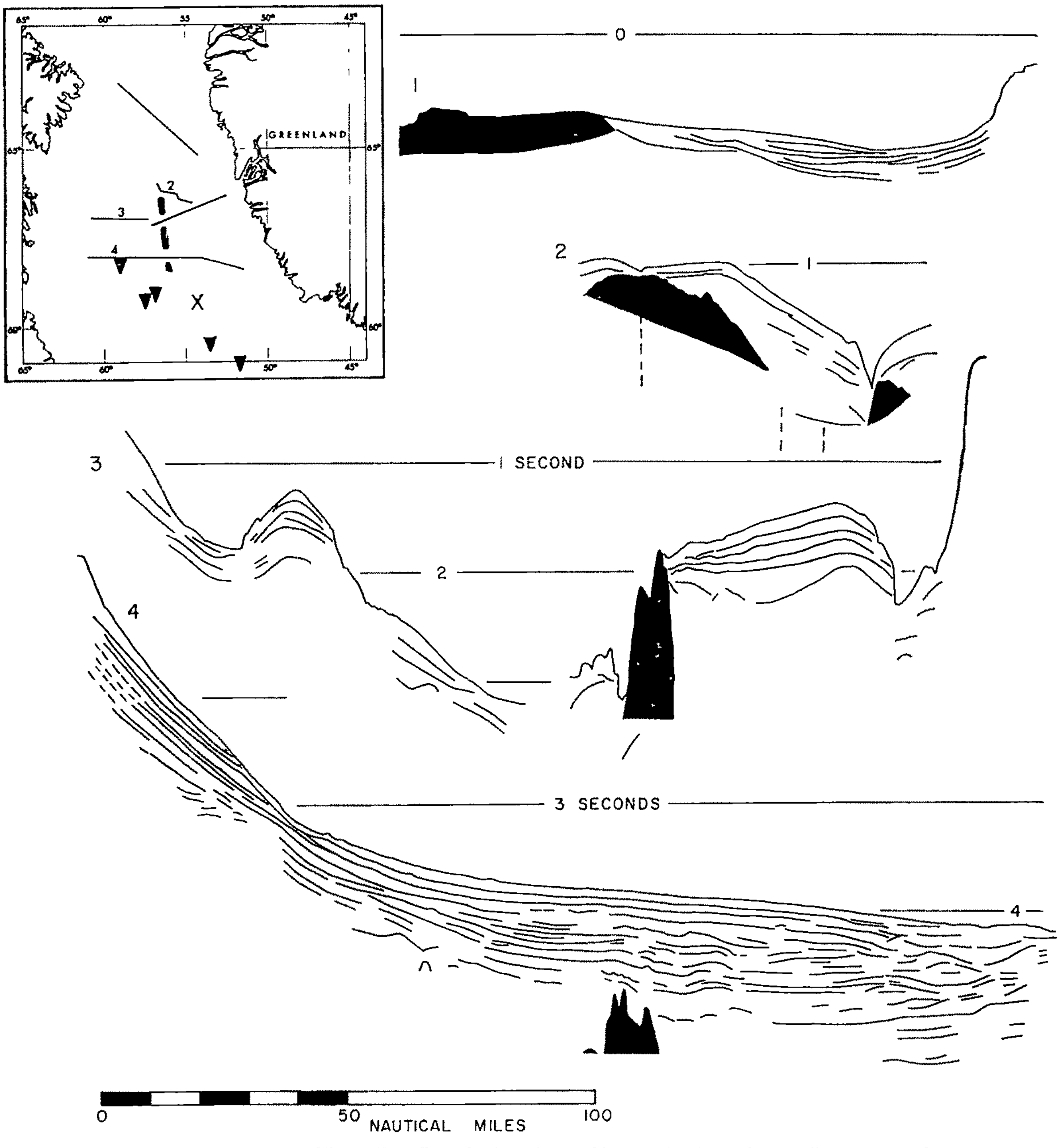

Fig 1 Four seismic reflexion traverses of the northern Labrador Sea Assumed basement is dark coloured Inverted triangles on index chart are earthquake epicentres, dotted lines indicate axes of high value magnetic anomalies[2], × marks axis of Drake *et al* profile of *C–D*[1] basement high, heavy dashed line indicates axis of basement high Travel time is two way—1 s is approximately equal to 1 km

northern Labrador Sea is about 10 per cent that of normal crestal regions of the Mid-Oceanic Ridge (personal communication from L Sykes) It seems plausible that these few earthquakes are associated with the graben structure of Cumberland Sound[4] The western magnetic anomaly belt which terminates abruptly near 60° N perhaps by a fracture zone or depth of burial[3] is anomalous and at the present is difficult to explain An unlikely possibility is that the prominent basement high may represent the eastern belt of rift mountains with the slightly seismically active centre swath, the ancient "rift valley" The western rift mountains would then be deeply buried beneath the continental rise The lack of an axial magnetic anomaly could be interpreted as a genesis during a reversed period of the Earth's polarity[5] This would, however, create an anomalously wide ridge–rift system within a narrow basin The more likely explanation that the basement high is the crestal region is favoured The relatively small amplitude (±400 gammas) of the axial magnetic anomaly suggests the origin of the feature during a reversed epoch, or perhaps the blanketing effect of sediments[6]

The seismic reflexion data do not indicate any support for a proposed submerged continental remnant in the Labrador Sea[4,7], however, the axial position of the crest would suggest the creation of Baffin Bay by ridge growth[6] with, as already suggested[8-10], the Nares Strait north of Greenland acting as a transform fault

Dementskaya and Dibner[11] suggested that a north-west–south-east geotectonic linear feature extends from Davis Strait through the Bay of Biscay Magnetic anomalies and topographic trends in the eastern Atlantic[12] may rather be a consequence of the north-west–south-east fracture pattern[13] The magnetics and buried relief of the Labrador Sea region (unpublished work of Johnson, Closuit and Pew) tend to support this hypothesis, however, we believe that the inactive ridge branch originated just to the west of the crestal region of the Mid-Atlantic Ridge near 53° A former axis of the Mid-Oceanic Ridge, now subsided, and in a north-west–south-east trend diagonally across the North Atlantic, not unlike Darwin Rise in the Pacific[14], is a possibility warranting further investigation Rates of subsidence for the Mid-Oceanic Ridge have recently been presented[15] and appear adequate to have suppressed this feature, assuming an early Tertiary or Mesozoic age

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[1] Drake, C L , Campbell, N J , Sander, G , and Nafe, J E , *Nature*, **200**, 1085 (1963)
[2] Heezen, B C , Tharp, M , and Ewing, M , *The Floors of the Oceans*, I *The North Atlantic* (Geol Soc Amer , 1959)
[3] Godby, E A , Baker, R C , Bower, M E , and Hood P J , *J Geophys Res* , **71**, 511 (1966)
[4] Kerr, J W , *Earth Planet Sci Lett* , **2**, 283 (1967)
[5] Vine, F J , and Matthews, D H , *Nature*, **199**, 947 (1963)
[6] Johnson, G L , and Heezen, B C , *Nature*, **215**, 724 (1967)
[7] Rvachev, V D , *Okeanologiia, Akad Nauk, SSSR*, **7**, 477 (1967)
[8] Wilson, J T , *Nature*, **198**, 925 (1963)
[9] Wilson, J T , *Nature*, **207**, 342 (1965)
[10] Wegener, A , *The Origin of Continents and Oceans* (E P Dutton and Co New York, 1924)
[11] Dementskaya, R M , and Dibner, V D , in *Continental Margins and Island Arcs*, 63 (Geol Survey Canada 66–16, 1966)
[12] Laughton, A S , in *Proc Seventeenth Colston Res Soc* , 175 (Butterworths, London, 1965)
[13] Johnson, G L , *Earth Planet Sci Lett* , **2**, 445 (1967)
[14] Menard, H W , *Marine Geology of the Pacific* (McGraw-Hill, New York, 1964).
[15] Schneider, E D , and Vogt, P R , *Nature* (in the press)

Has the Pacific Basin moved as a Rigid Plate since the Cretaceous ?

McKenzie and Parker[1] have shown from displacements along faults and from seismic first motions that the North Pacific seems to be moving as a rigid plate relative to the surrounding continents This motion is uniquely defined by a rotation axis which they estimated to be located near 50° N , 85° W at the southern end of Hudson Bay The sense is towards the north-west, clockwise as viewed from the pole Palaeomagnetic evidence from North America and the magnetization of Pacific Ocean seamounts suggest that this rotation dates from Cretaceous time (10^8 yr), and that the total angle is perhaps as great as 100°, an average rate of 1° per million years

Grommé, Merrill and Verhoogen[2] critically examined the palaeomagnetic data for the Cretaceous of North America, presenting the results in the conventional manner as virtual geomagnetic poles (a VGP is the pole of that unique axial dipole field which gives the observed magnetization at a given site) Six out of the nine poles that they consider reliable are tightly grouped about a mean position 67° N , 172° W These poles have all been radiometrically dated and the ages range from 84 to 138×10^6 yr So, within the present accuracy of the palaeomagnetic measurements, the North American continent remained fixed relative to the assumed geomagnetic pole for most of the Cretaceous, 50×10^6 yr

Richards, Vacquier and Van Voorhis[3] recently summarized the results on the magnetization of Pacific Ocean seamounts as determined by the method of Vacquier[4] They assume that the total magnetic field anomaly associated with a seamount is primarily a consequence of a uniform magnetization throughout its volume, and with this simple assumption they estimate the direction and intensity of magnetization They can explain about half of the anomaly in this way and "best" fits require that the magnetization induced in the present geomagnetic field is a small fraction of the total magnetization The average virtual geomagnetic poles for six seamounts in the Hawaii development area and four seamounts near Japan are 21° E , 58° N ($\alpha_{95} = 12°$, $k = 19$) and 14° W , 51° N ($\alpha_{95} = 7°$, $k = 10$), respectively Richards *et al* [3] discussed K–Ar age determinations made by others on dredge samples from the two groups of seamounts they are probably mid-Cretaceous (80×10^6 yr)

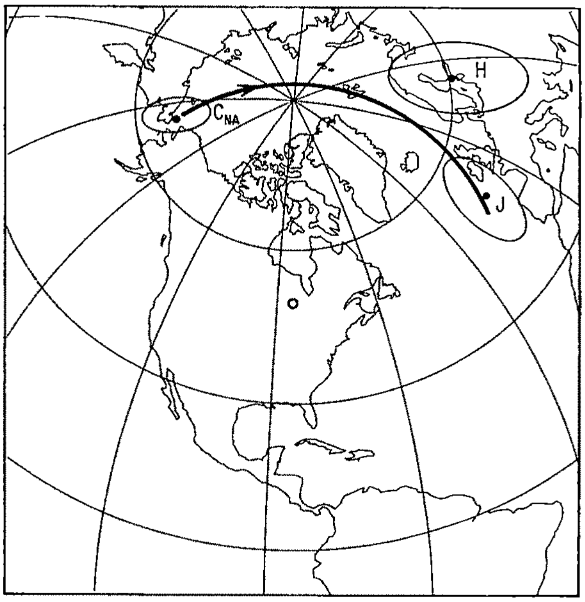

Fig 1 Cretaceous virtual geomagnetic poles for North America, C_{NA}, Hawaiian seamounts, H, and Japanese seamounts, J An equal area projection about the axis of relative rotation determined from seismic first motions

If the Pacific Basin has rotated about the McKenzie-Parker axis since the Cretaceous and if the seamount virtual geomagnetic poles were once coincident with the Cretaceous poles of North America, then the motion would carry the seamount poles along a small circle about the axis of relative rotation The present position along the small circle of the seamount poles, relative to the continental pole, would indicate the total angle by which the Pacific has rotated The geometry is depicted in Fig 1, an equal area projection about the axis of relative rotation, showing the North American Cretaceous, C_{NA}, and oceanic, H, J, virtual geomagnetic poles The angles between the North American pole and the two mean seamount poles, one near Ireland, the other in the Gulf of Finland, are 70° and 90°, respectively, indicating an average rotation rate of the Pacific Basin of 1°/million years

Admittedly, the Hawaiian result alone is not convincing and there is a huge scatter in numerous unpublished seamount poles (personal communication from M Richards) But these preliminary data are certainly consistent with the hypothesis of large scale rotation of the Pacific relative to North America The rotation axis used here comes from the analysis of only ten years of seismic data and is uncertain to perhaps ten degrees Even so, Fig 1 suggests that the Pacific Basin has moved as a rigid plate about an axis, fixed relative to North America, not too different from the McKenzie–Parker axis and that this rotation began at least in the mid-Cretaceous It may be older

A more detailed study of the palaeomagnetic evidence, the tectonic implications and suggestions for corroborative experiments, is in preparation From preliminary results it seems possible that the rotation dates from Lower Cretaceous time (140×10^6 yr) and that the Pacific Basin has been a rigid plate moving about an axis constant in time and fixed with respect to the surrounding continents

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[1] McKenzie, D P, and Parker, R L, *Nature*, 216, 1276 (1967)

[2] Grommé, C S, Merrill, R T, and Verhoogen, J, *J Geophys Res*, 72, 5661 (1967)

[3] Richards, M L, Vacquier, V, and Van Voorhis, G D, *Geophysics*, 32, 687 (1967)

[4] Vacquier, V, Proceedings, *Benedum Earth Magnetism Symposium*, 123 (1962)

Nature of Glaciated Landforms

GLACIAL erosion has been accepted for more than a hundred years, yet there are many loose assumptions about the real nature of glaciated landforms This lack of knowledge makes it difficult to explain the erosion processes concerned If more precise information were available, these processes and landforms could be applied to test several theories in ice physics

Descriptions of glaciated landforms are surprisingly vague Much has been made of the textbook U-shaped valley or the schrundline corrie, but there is little information to suggest how typical these landforms really are More needs to be known about their exact form, the frequency of different types and about their relationships to the surrounding relief, geological structures and to the processes which have shaped them

Any study has to be exploratory, and for this reason the corrie (or cirque) seems a very suitable choice of landform Using this helps to minimize the difficulty that most of the glaciated areas of northern Europe were covered during the Ice Age by a complex sequence of several different types of ice cover Corries have also obviously been submerged by ice sheets for long periods, but it seems from their distinctiveness as landforms that most of them have been relatively little affected by this phase in their history This is really what would be expected, for observations on the Antarctic ice sheet today[1] show that it is relatively slowly moving and presumably fairly protective in its effects away from the principal lines of ice flow

Corries are mostly on mountain flanks and are bypassed by the chief avenues of ice discharge They have been found to possess a great overall similarity from area to area, but where on other grounds there is reason to suppose that they have been overrun by more strongly flowing ice than normal, they have slightly different characteristics or else are rendered almost unrecognizable by dissection In addition, really good examples of corries tend to be absent from densely dissected areas and presumably, therefore, from areas of rapid ice streaming—such as various south-westerly parts of the Scottish Highlands

Second, corries are suitable for a preliminary study of glaciated landforms because a much better estimate of the thickness of the ice assumed to be responsible for the hollow can be made than for a valley glacier or for the ice covering a roche moutonnée Finally, after the well integrated work of Lewis[2] and his associates on Veslskautbreen—a small corrie glacier in Norway—we know much more about the characteristics and detailed three-dimensional flow pattern of corrie glaciers than of any other glacial type

The most suitable way of tackling the problem of the origin of a variable natural occurrence such as a corrie is to analyse various aspects of the shape of large numbers of examples A sample containing more than one hundred well formed chiefly Scottish corries as well as, for comparison, other similar mountain hollows of much more disputed origin was taken and their similarities and differences were analysed Various aspects of corrie shape were measured, one of the most important being the longitudinal profile The shape of this is obviously very pertinent to the question of corrie formation, because it is a profile in the direction of ice movement The most satisfactory way to define similarity of longitudinal profiles between different corries was thought to be to find a best-fit curve with as simple a formula as possible, to fit as much of the sample as possible Various types of curve were tried, and the most suitable was found to be a logarithmic curve of the type $y=(1-x)e^{-x}$ A term k was also introduced into the equation $[y=k(1-x)e^{-x}]$ to allow for the fact that corrie steepness varies, while still retaining the same type of curve for all examples

This curve has many advantages (1) It can be fitted to 81 per cent of the corries An arc of a circle has previously been used to characterize the bed of a corrie glacier (for example, by McCall[3]), but this was found to be inappropriate in the present study—it bore no resemblance to almost half of the corrie profiles

(2) The k curve could also be used to distinguish corries from non-corries, few of which it fitted well In contrast, no distinction could be made between the two groups of mountain hollows by using an arc of a circle With the latter the proportion of hollows fitting was precisely the same for both groups (53 per cent fit)

(3) The formula of the k-curve is simple and it is an easy curve to fit Obviously more complex curves could have been found to fit each individual corrie exactly, but this would defeat one of the chief purposes of this method of landform analysis

(4) The value of the term k can be used to define several types within the corrie group of landforms itself It can also be used as a single parameter to plot against the various independent variables which may influence corrie shape—rock type, aspect, climatic factors, environmental relief, the stage reached in erosion and so on

This is an obvious advantage over the arc of a circle. With the latter, in order to describe a corrie accurately it is necessary to know the radius of curvature, the gradient of the hollow and the angle subtended at the centre of the circle by the length of the corrie profile The *k*-number on the other hand, within one term, describes both the shape and steepness of the corrie

Corries may look very different, but it seems that many of these apparent differences are partially caused by the surrounding relief and are not reflected in the longitudinal profiles of the corries themselves Relevant features are the difference between a peak towering above a corrie, as there is in the case of Glaslyn on Snowdon, or a surrounding plateau as in the Cairngorms Despite the different superficial appearances of different corries their profiles are astonishingly similar—81 per cent of those studied fitted the curves very closely Most of the remaining 19 per cent were more angular caricatures of the rest, caused by specialized lithological situations In fact, the corrie profiles which have been studied can be arranged in a more or less continuous series, with the bulk of the corries—those fitted by the *k*-curves—in the middle At one end of the series they gradually become more angular until the classic schrundline corrie is reached Oddly enough, at the other end of the series is the exaggerated L-shaped corrie, which has also attracted attention in the past It is perhaps symptomatic of the contrast between classical geomorphology and the present day more multivariate and quantitative approach that the two corrie types which had previously been regarded as typical textbook forms are, in fact, quite rare and are exaggerated caricatures of the bulk of the forms

The similarity which has been discovered between corries in very different situations must be the result of common factors The most obvious one is the influence of glacial erosion processes, in particular the characteristic "rotational" flow pattern of the corrie glacier

With the similarities defined it is possible to pinpoint and analyse the differences between the hollows These differences are of three types (1) Large divergences from the *k*-curves, which define mountain hollows not caused by corrie glacier erosion (2) Smaller random divergences caused by occurrences like ice sheet overflow, lithological changes and so on (3) Systematic variations which give rise to the different values of *k* Corries with different values of *k* are found all over Highland Britain, and many areas contain all types, which suggests that the controlling factors are very local —principally structural rather than climatic The schrundline corrie, for example, is especially associated with granitic rocks on account of particular jointing characteristics often found in these

One general conclusion which can be drawn from the whole study can be mentioned It is quite clear that both erosion processes (related to the glacial flow pattern) and rock structure are very important in the production of glaciated landforms They act together and neither can really be said to dominate the other

The research described here was carried out while I held a DSIR research studentship in the Department of Geography, University of Cambridge, from 1963–66 I thank all those who gave their support

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[1] Swithinbank, C W M, *Glaciology*, I, 1949 52, *Sci Res*, III (1959)
[2] *R G S Res Ser*, 4 (edit by Lewis, W V) (1960)
[3] McCall, J G, thesis Univ Cambridge (1953)

Petrogenetic Significance of Alkali Feldspar Megacrysts and their Inclusions in Cornubian Granites

THE problems peculiar to the Land's End granites are, by virtue of their chemical mineralogical and geophysical affinities[1], common to other exposed granites in Cornubia Findings concerning the Land's End granites can therefore be extended to all granites in south-west England and possibly to similar granites throughout the world

The porphyritic biotite granites contain large alkali feldspar crystals of variable length (up to 18 cm) These big crystals (megacrysts) form a coarse microperthite with albite and may contain small crystals of all the other minerals comprising the granite The most common inclusion is biotite which may be centrally or zonally arranged or irregularly distributed

This preliminary communication describes the nature of the zonal distribution of minerals within the megacrysts and discusses their petrogenetic significance

Alkali feldspar megacrysts are primarily a feature of the coarse porphyritic granites of Cornubia At Land's End they are an average of 6 cm long and constitute about a quarter of the total rock volume (excluding groundmass perthite) The megacrysts are white when fresh and tabular in habit, they are flattened parallel to (010) and generally elongated about a line bisecting the acute angle between the *a* and *c* crystallographic axes Twinning is usually on the Carlsbad law and the composition plane (010) may be stepped or curved The crystals are set in a coarsely granular groundmass of anidiomorphic quartz and perthite, plagioclase (albite–oligoclase), biotite, subsidiary muscovite and frequent tourmaline The margins of the megacrysts often show implicate texture, especially where the crystals are proximal to quartz, although straight contacts are not unusual Contacts with plagioclase, which is invariably replaced, are serrate or lobate, while mica/megacryst contacts are straight or zigzag depending on the orientation of the mica Secondary muscovite frequently penetrates the megacrysts along cleavage cracks or follows the direction of perthite strings, primary nucleation would be expected at sites where the anorthite molecule was breaking down

In hand specimens both biotite and quartz are obvious inclusions; the biotite is often arranged in well defined zones which are frequently parallel to the boundary of the megacryst The number of zones varies, with at least one principal inner zone and occasionally one or more peripheral zones 1–2 mm from the margin Quartz inclusions are similarly arranged in peripheral zones as

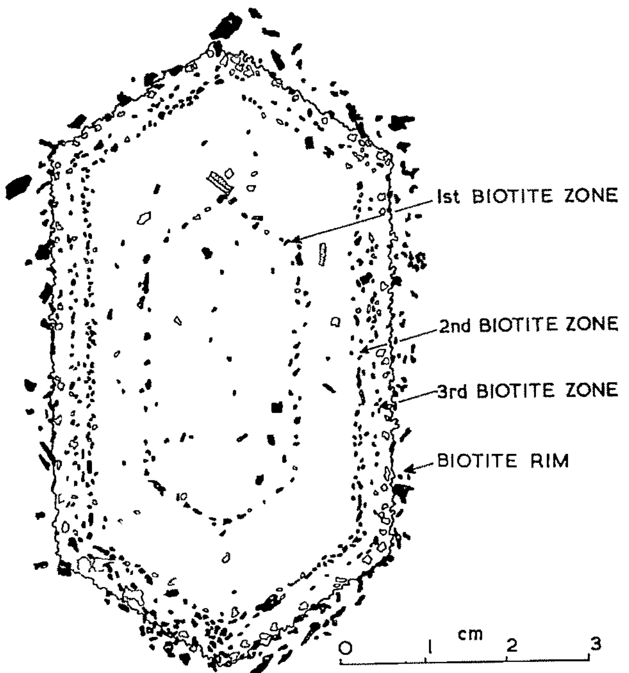

Fig 1 Perthite megacryst showing zones of inclusions Black, biotite, clear, quartz, stippled, plagioclase (Specimen from Carn-a-langa quarry, Land's End)

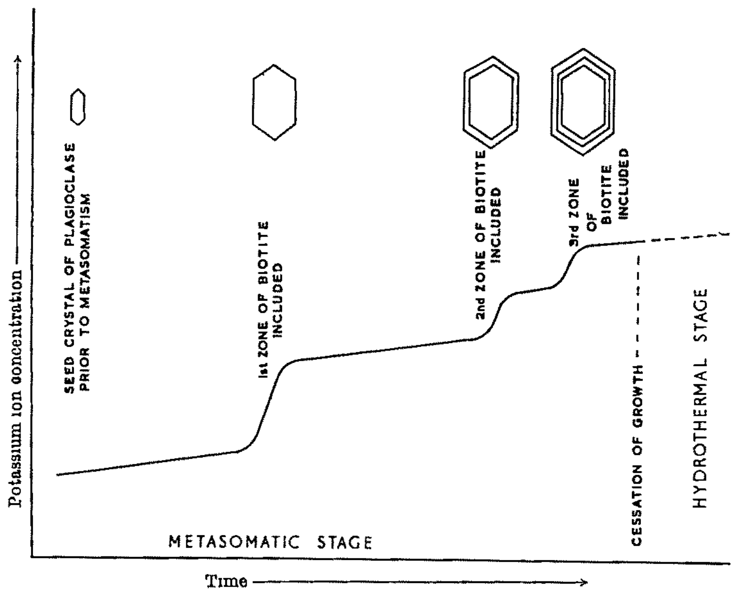

Fig 2 Time versus concentration of potassium ions, illustrating the stages at which the three inner biotite zones of Fig 1 feldspar become included

discrete, elongated, clear patches Plagioclase occurs as small (0 6 mm) subhedral to euhedral crystal inclusions which vary considerably in shape and size and, although the centres are often preferentially altered to secondary micas or clay minerals (a reflexion of the breakdown of the more calcic core), the crystals are frequently mantled with a thin pellicle of clear albite Tourmaline inclusions usually show a replacement texture towards the feldspar and are therefore secondary in origin[1]

In thin section the inclusions reveal significant features The quartz grains which occur as rounded water-clear blebs, 0 3–1 mm long, rarely exhibit strain effects, while the metasomatic quartz "pools" or composite masses in the groundmass frequently show strain shadows and indicate late stage replacement textures; likewise, the interfeldspathic biotites are not, as a rule, as large (< 2 mm) or as chloritized as the intrafeldspathic varieties These two facts suggest that the included biotite and quartz are derived from the initial fine grained crystal "mush" early in the pluton's history This pre-dated the extensive metasomatic and contamination effects which were superimposed on this aplogranitic material[1,2] as crystallization proceeded, and the important silico-potassic aqueous phase separated from the "magma", inclusions suggest a grain size of 0 3–2 mm for the aplogranite

Fig 1 illustrates a typical megacryst with several zones of included biotite and quartz, these zones of oikocrysts within the chadocryst can be expressed in diagrammatic—graphic form (Fig 2), which illustrates the relationship between time and the concentration of potassium ions in the perthite megacrysts The correlation between the biotite zones, megacryst growth and accelerated periods of potassium metasomatism is evident, and it can often be shown that the obtuse angles in the biotite zones lie on common axes These represent uniform directions of growth, and by tracing the axes back to their origin some estimate of the original size of the seed crystal can be obtained The distribution and orientation of these seed crystals within the peripheral zones of the pluton, and their subsequent metasomatic history, can be shown to have some bearing on the distribution of the coarse porphyritic and non-porphyritic granites of south-west England[1]

The granites of the Cornubian peninsula are composite, serokinematic to postkinematic rocks representing the upper protrusions of a high-level pluton elongated from W S W to E N E , the possibility that the granite outcrops of Devon and Cornwall could belong to the same mass was realized by de la Beche in 1839 and referred to in his classic report[3] Furthermore, it has been shown that the Land's End granite is a composite body consisting of a coarse grained porphyritic outer phase, and a medium to fine grained (non-porphyritic) core[1], in several localities this core has extensively veined or broken through the porphyritic envelope

Fig 3 is a cross-section of the Cornubian pluton In it a correlation is made between the granites of the Scillies, Land's End, Carnmenellis, St Austell, Bodmin and Dartmoor, and illustrates the composite nature of the pluton. In the west, very little non-porphyritic granite is exposed, this being overlain by the coarse porphyritic envelope, in the east, the non-porphyritic granite is often the rule, with isolated outcrops of the coarse porphyritic envelope exposed on hills and tors (for example, Hay Tor, Dartmoor), and nearer the margin These facts indicate a lower level of erosion in the Dartmoor granite and the sloping nature of the roof zone from east to west This is confirmed by geophysical evidence which shows that the pluton and its roof become thinner from east to west[4]

Although field evidence at Land's End suggests partial remobilization of the finer core[1], the composite nature of the granites is not attributed to separate intrusions, but rather to the concentration of the metasomatizing agent, potassium, beneath the granite roof or within a differentiation "front". The potassium ions are believed to have been concentrated around the cooling margin of the granite by migration along a thermal and/or chemical gradient[1,5] The action of potassium metasomatism outside the main body of the granite is demonstrated by the

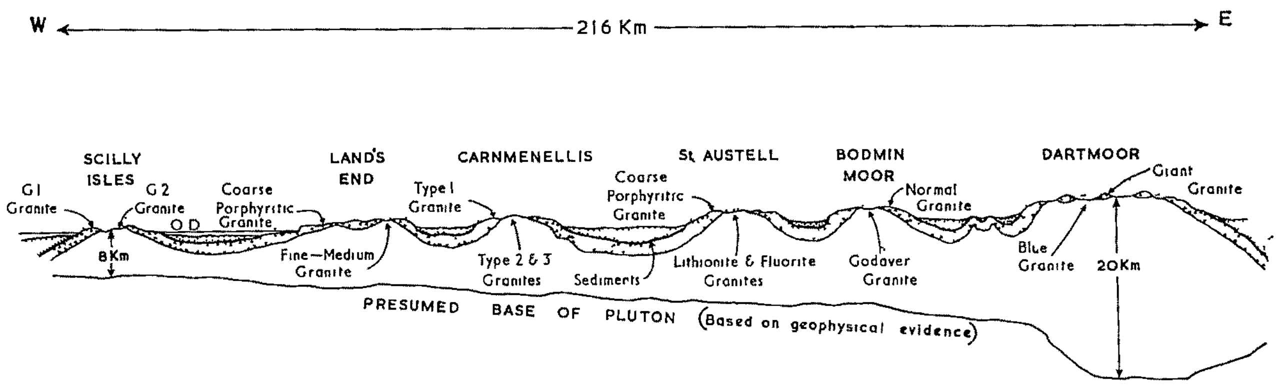

Fig 3 Correlation diagram of Cornubian granites Fine stipple, country rock, coarse stipple, coarse porphyritic granite envelope, white, medium to fine grained granite (texture of the granite below 2,000 ft is unknown) (Thickness of pluton based on geophysical evidence[6])

development of biotite-rich hornfelses formed by the biotization of hornblende–plagioclase hornfelses[5,6]. The effect of late-stage potassium metasomatism within the granite (autometasomatism) is illustrated by the development of double-enclaves, and the metasomatic replacement of early formed plagioclases which then continued to grow across granite/hornfels and granite/aplite contacts. The development of these secondary alkali feldspar megacrysts across aplite/pegmatite and aplite/granite contacts in the Carnmenellis granite has previously been cited as evidence for the postconsolidation development of these crystals[7].

Field evidence permits the interpretation that the alkali feldspars grew under the influence of potassium metasomatism within a crystal "mush" or near solid environment. It is, however, the primary origin of these feldspars which is being questioned, and I conclude that the cores of many of the alkali feldspar megacrysts were originally magmatic in origin and albitic (An 0–An 10) in composition; a quartz–orthoclase–plagioclase–biotite matrix would be a natural presumption for anatectic granites[8,9]. The crystals were orientated into parallel alignment and occasional convection swirls by viscous flow; this took place during the progressive emplacement of the crystal mush while under the influence of a regional stress-gradient field. During periods of ensuing potassium metasomatism, replacement of these "seed crystals" was accompanied by irregular endoblastic growth, the groundmass minerals immediately adjacent to the seed crystals probably constituted an "aplitic" matrix of quartz, plagioclase and biotite, lubricated by a hydrous phase[2], and were enveloped during the increased rate of growth of the seed crystal which later slowed down because of impoverishment of suitable adjacent material. During periods of slow growth, it seems that the surrounding minerals were pushed aside, only to become enveloped when crystal growth accelerated in some way. These zones or periods of accelerated growth are correlated with advancing waves of potassium metasomatism, or increases in potassium concentration within the granite which, by virtue of an established concentration and temperature gradient, tended to migrate towards the periphery and give rise to a highly metasomatized marginal granite. Alternations of highly porphyritic granite within the coarse non-porphyritic granite are attributed to metasomatic fronts the activity of which was curtailed by the attenuation of the metasomatic physicochemical conditions.

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[1] Booth, B., thesis, Univ. Keele (1966).
[2] Exley, C. S., and Stone, M., *Roy. Geol. Soc. Cornwall*, 131 (1964).
[3] De la Beche, H. T., *Mem. Geol. Surv. Eng. Wales* (1839).
[4] Bott, M. H. P., Day, A. A., and Masson-Smith, D., *Phil. Trans. Roy. Soc.*, A, 251, 161 (1958).
[5] Booth, B., *Proc. Ussher Soc.*, 1, 212 (1966).
[6] Floyd, P. A., *J. Petrol.*, 6, 223 (1965).
[7] Stone, M., and Austin, W. G. C., *J. Geol.*, 69, 464 (1961).
[8] Booth, B., *Nature*, 213, 896 (1967).
[9] Booth, B., *Proc. Ussher Soc.*, 1, 162 (1965).

Mesospheric–Stratospheric Coupling

THIS communication describes an investigation of mesospheric–stratospheric relationships between electron densities and collision frequencies with stratospheric temperature or constant pressure heights as measured during winter periods of 1964 and 1965 by the wave interaction method of Ferraro and Lee[1].

Shapley and Beynon[2] indicated evidence for the correlation of the phenomenon known as stratospheric warmings with the winter anomaly in ionospheric radiowave absorption. They showed that periods of an extraordinarily warm stratosphere coincide with periods of strong radiowave absorption. Beynon and Jones[3] believe there is a common cause behind the similarities in behaviour of the seasonal variations of monthly means of stratospheric temperature and mesospheric neutral density and collision frequency. Regression equations developed by Quiroz and Thompson[4] show correlations between neutral density, pressure and temperature in the stratosphere and mesosphere to be good in general. We have investigated associations of electrical effects and neutral temperature and of mesospheric–stratospheric coupling.

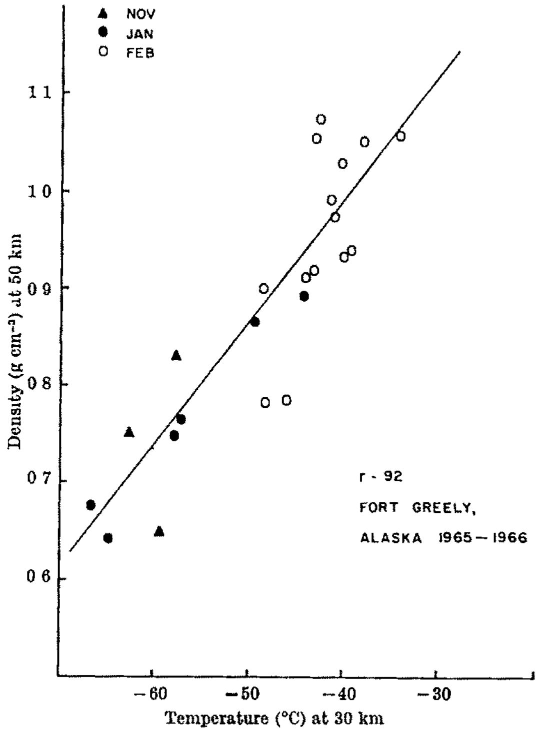

Fig. 1. Fort Greely temperature and density.

In the *D* region, where the electron density generally increases with height, an increase in neutral temperature in and below a layer will cause the layer to lift. Thus the electron density at a fixed height in the layer decreases. Solar influences may, however, interfere with this relationship. An increase in solar activity may increase the electron density in a layer, whereas an increase in zenith angle tends to raise the layer.

Mawdsley[5] suggests that enhanced electron density is related to winter high-latitude anomalies of temperature through a photochemical process involving nitric oxide. Motions responsible for high densities and temperatures in the mesosphere might also be responsible for temporary enhancements of nitric oxide content of the mesosphere, leading to greater electron production by Lyman alpha radiation. J. B. Gregory (private communication) feels that "... the basic mechanism (for such correlations) is the upward development of long planetary waves of the troposphere and stratosphere".

Electron collision frequency is a function of electron temperature and neutral density. Electron temperature in the mesosphere is influenced by solar radiation, heat conduction—particularly from the thermosphere—and heat convection. Convection which would increase neutral density and electron temperature would also increase collision frequency.

Absorption of radiowaves is caused by a loss of energy through electron-neutral collisions, the absorption co-

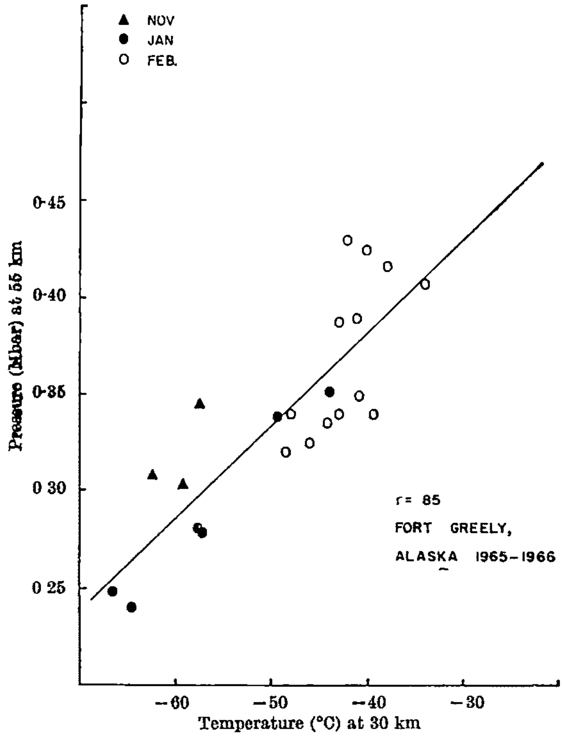

Fig 2. Fort Greely pressure and temperature

efficient is proportional to local electron collision frequency and electron density, and an increase of either or both of these variables might cause the kind of associations reported before[2,3]. The absorbing layer may be raised without change in absorption if, for example, an increase in temperature at lower layers causes an increase in neutral density and collision frequency and a decrease in electron density.

If planetary waves are solely responsible for electrical-neutral coupling, then there should be some associated time lag. This was not evident in Shapley's investigation although it may account for lack of coupling when "quiet" winters are studied.

Combination of the gas law and the hydrostatic approximation gives

$$\mathrm{Ln}(p)-\mathrm{Ln}(p_0)=-\int_{Z_0}^{Z}\frac{g}{R_0T}\,\mathrm{d}Z$$

This equation shows that through hydrostatic coupling, an increase in temperature in a column of air between Z and Z_0 would cause an increase in pressure at the height Z if the pressure at Z_0 did not vary appreciably. At sufficiently high levels, the pressure increase is of such magnitude as to cause a density increase in spite of the temperature increase. This mechanism accounts qualitatively for the results of Quiroz and Thompson.

Perturbations moving vertically in the wind fields may cause similar disturbances at different levels. This could result in the type of linking suggested by Mawdsley. Apparent linking of parameters at different levels may only be the result of similar reactions of the various parameters to outside influences. For example, an increase in solar activity might cause an increase in neutral and electron temperature in the mesosphere and in temperature in the stratospheric ozone layer.

The Meteorological Rocket Net (MRN) data reports at Fort Greely (Alaska), Fort Churchill (Canada) and Cape Kennedy (Florida) were chosen because of their larger number of rocket shots reaching 55 km. These reports were used for comparing stratospheric temperature with density and pressure at 50 and 55 km. *NASA Technical Reports* *R*-211 and *R*-245 were chosen as sources of data for comparison of atmospheric density at 70 km with stratospheric temperature for Wallops Island.

Table 1. SUMMARY OF TEMPERATURE AND DENSITY COUPLING

Site	Correlation coefficient
Fort Greely, Alaska 1965–66	R = 0·92 at 50 km Nov., Jan., and Feb. data
Cape Kennedy, Florida 1965–66	R = 0·58 at 50 km Nov., Jan., and Feb. data
Wallops Island, Virginia 1961–63	R = 0·59 at 70 km Nov., Dec., Jan., Feb., and March data

Fig. 1 shows temperature and density coupling at a northern location, and Table 1 summarizes the results at other locations.

The greater correlation coefficient and more evident seasonal trend of monthly data at Fort Greely indicate the greater degree of covariant changes associated with high latitude wintertime stations. The Wallops Island result indicates that the coupling shown here and previously by Quiroz and Thompson does extend well into the mesosphere. Fig. 2 shows the existence of coupling of pressure at 55 km with stratospheric temperature.

In order to determine "small scale" coupling, changes of temperature and density over 1 to 5 days at different levels were compared. Because observations were not available on consecutive days, changes over the shortest period that the frequency of the soundings permitted were used. Fig. 3 indicates that small scale coupling does exist, although it is not of the magnitude of the larger scale seasonal coupling. Notice that seasonal trends are now absent along the line of regression.

Because of the scarcity of wind data at 40 and 50 km levels, only a small portion of the data was spaced closely enough for a study of vertical coupling.

Fig. 4 shows time series graphs for Fort Greely and White Sands, selected for the years 1962–63 because of the significant change in zonal winds during the "sudden warming" which occurred on January 18, 1963, at Fort Greely. There is no significant coupling, except for some large scale events, such as the slow trend reversing the easterly wind to a westerly wind after the "sudden

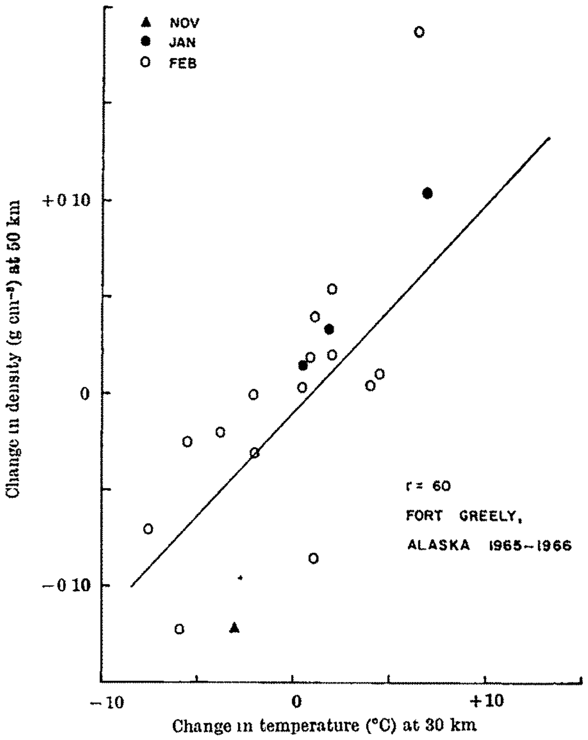

Fig 3. Fort Greely change in density at 50 km and change in temperature

warming" It is difficult to detect coupling of smaller perturbations because of missing data

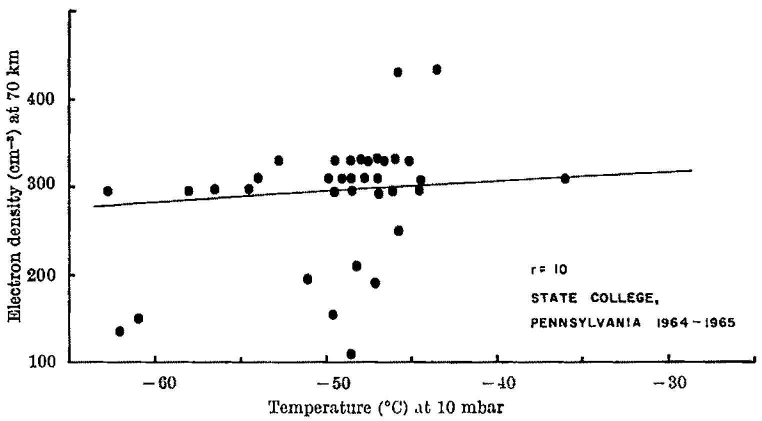

Fig 5 Electron density and temperature at State College, Pennsylvania

Electron density and collision frequency measurements have been provided on a semi-daily basis by phase and amplitude wave interaction experiments conducted by the Ionosphere Research Laboratory at the Pennsylvania State University by Ferraro and Lee The electron collision frequency used is representative of electron-neutral collisions Because the measurements were made in the mesosphere over State College, the heights of upper air constant pressure levels and temperature at 10 mbar at the nearest station, Pittsburgh, Pennsylvania, were chosen for comparison Radiosonde balloon ascents are made here twice daily with special care in order to reach high elevations The measurements at 0700 h and 1900 h were averaged to give a representative 10 mbar height or temperature

Measurements of electron collision frequency were available for 16 days in January and 16 days in February 1964 The measurements were made for heights of 70, 75, 80 and 85 km several times daily, usually from 1000 h to 1500 h EST, and then averaged to give a daily average collision frequency Electron density measurements at 70 km for 39 days in January, February, March and December of 1964 and January 1965 were also available for comparison In this case, an approximate value was chosen for 1200 h EST to be compared against 10 mbar temperature in Fig 5

The coupling between temperature at 30 km and atmospheric pressure and density at higher levels as detected by Quiroz and Thompson has been confirmed and found to extend to higher levels then previously noted The coupling seems to be stronger for high than for low latitudes Correlations are largely the result of covariant seasonal changes, and only partly the result of short period changes This indicates that the coupling of these parameters is strongest in periods of large temperature variations at high latitudes in the winter hemisphere

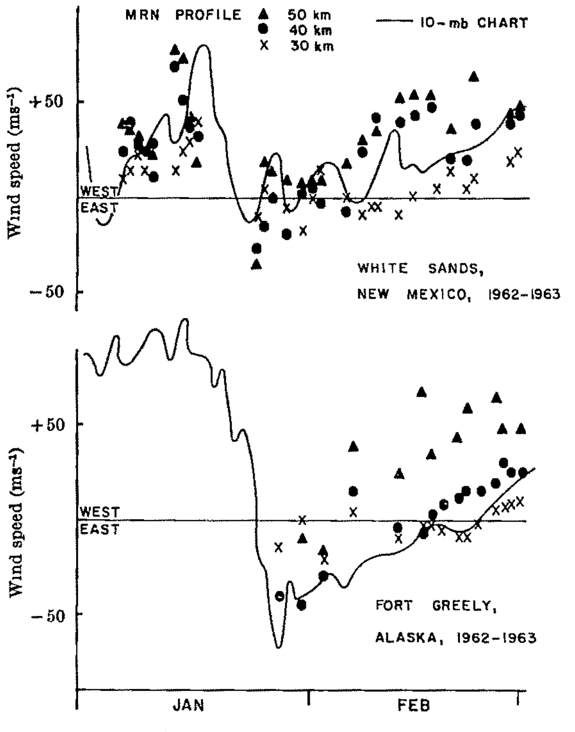

Fig 4 Westerly winds at 10 mbar and at 30, 40 and 50 km

Correlations between 70 and 35 km were smaller, possibly partly because of increased error of measurements and the smaller number of measurements made at these levels In general, some hydrostatic-type coupling exists between middle stratosphere temperature and pressure and neutral density of the upper stratosphere and lower mesosphere The existence of short-period dynamic wind coupling is not evident from the results, but zonal winds of different levels do seem to undergo similar major long-period changes during the winter months

Because of the large variability of temperature, neutral density, electron collision frequency and electron density during the winter months, it was hoped that a coupling of these parameters could be discovered No significant relation was, however, found between temperature in the stratosphere and electron density or collision frequency Significant relationships between these may not exist, or may be obscured by time lags, observational errors or by the influence of other variables Because previous "successful" comparisons were made during periods of "explosive warmings", it is still possible that there is no significant coupling between electric variables in the mesosphere and middle stratosphere temperatures during ordinary warmings.

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[1] Heard, W C, *Scientific Report 297*, Ionosphere Res Lab, Pennsylvania State Univ (1967)

[2] Shapley, A H, and Beynon, W J G, *Nature*, **206**, 1242 (1965)

[3] Beynon, W J G, and Jones, E S O, *Nature*, **206**, 1243 (1965)

[4] Quiroz, R S, and Thompson, G J, *Tech Rep*, *Air Weather Service, USAF*, No 187 (1966)

[5] Mawdsley, J, *J Geophys Res*, **66**, 1298 (1961)

PHYSICS

Effects of Drag-reducing Additives on Vortex Stretching

Giles and Pettit[1] have shown that, in tubes of small diameters, solutions of additives which reduce turbulent drag can apparently maintain laminar flow at Reynolds numbers greatly above the limit of about 2,000, which for water usually marks the onset of turbulent flow The friction factor rises above the Hagen–Poiseuille line, however, and Giles and Pettit attribute this to a shear-thickening of the fluid, and suggest that the viscosity of the additive solution effectively increases at sufficiently high shear rates.

This explanation seems improbable because results for two different tube sizes, one nearly twice the diameter of the other, correlate on the basis of Reynolds number. If shear thickening were involved, the apparent viscosity would be expected to be a function of shear stress, which must be greater in a smaller diameter tube than in a larger one for equal Reynolds numbers. Thus the results should not correlate with Reynolds number. An alternative explanation could be that although dye traces at the centre of the tube indicate that the flow there is laminar, nevertheless non-linear laminar oscillations occur close to the walls of the tube. As Stuart has shown[2], this could give rise to an increased friction factor. (Although Stuart's work refers to plane Poiseuille flow, the probability that similar finite-amplitude disturbances can occur in Poiseuille flow through a pipe has been demonstrated by Dixon and Hellums[3].) If the disturbance flows were geometrically similar for the same Reynolds numbers in different tubes, the friction factor would then be a function of Reynolds number.

Evidence that the flow with additives is not completely steady in quasilaminar conditions was obtained as follows. Dye was injected into the flow through a glass tube (Fig. 1) so that the dye would flow close to the walls of the tube. There were two dye injection holes at 90° spacing. At the top of the tube was a rounded entry slightly smaller in diameter than the bore of the tube: the resulting step should tend to provoke early transition. The speed could be varied by varying the height of the outflow point. Fig. 2*a* shows the dye flow with 92 p.p.m. polyethylene oxide 'Polyox *WSR* 301' at a Reynolds number, based on diameter, of about 4,000. (In Fig. 2*a*, as in Figs. 2*b* and 2*c*, the flow is from top to bottom and the exposure time is about 30 μs.) Although the dye filament does not break up into turbulence it is nevertheless disturbed. Such disturbances could have caused the manometer oscillations reported in ref. 4. For comparison, Figs. 2*b* and 2*c* show dye traces for plain water and for 11 p.p.m. of 'Polyox', respectively, at a Reynolds number of about 8,000. The latter very dilute 'Polyox' flow, although apparently turbulent, shows much less complete mixing of the dye than the water flow.

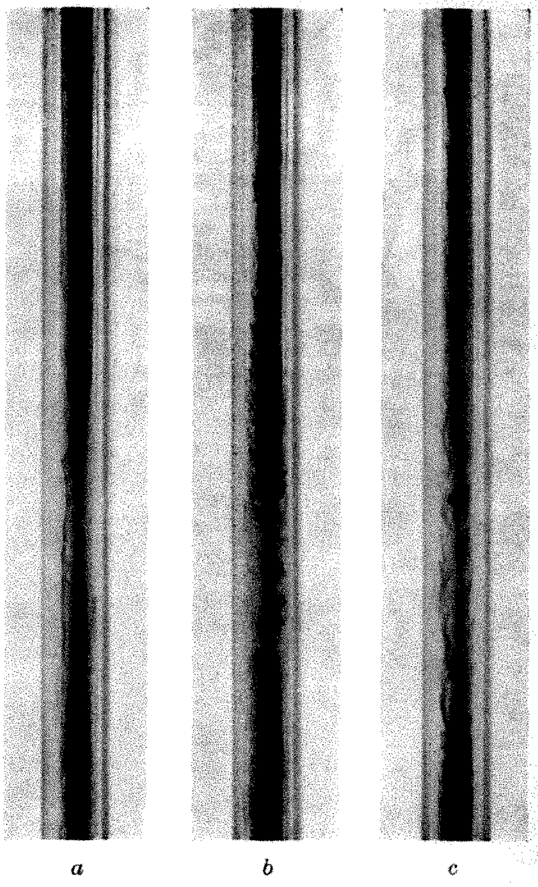

Fig. 2. Dye filament photographs.

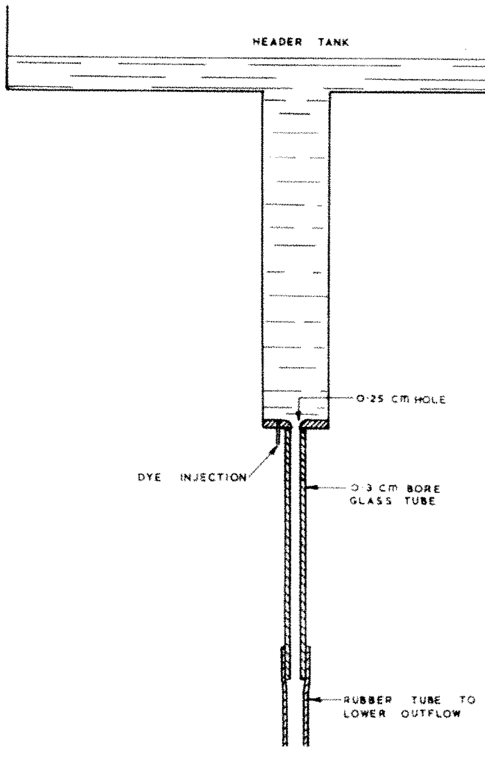

Fig. 1. Flow visualization apparatus.

Pfenninger gave a possible explanation of such effects[5]—additives can inhibit vortex stretching. This could delay the breakdown of non-linear laminar oscillations into turbulence, because the oscillations can be regarded as a pattern of vortex filaments close to the pipe wall, chiefly transverse to the flow direction, and breakdown into turbulence involves a convolution and hence a stretching of these vortex filaments. Similarly less mixing would occur in fully turbulent flows if vortex stretching were reduced. Some support for this hypothesis was provided by the experimental arrangement sketched in Fig. 3. The rod shown was rotated at about 5,000 r.p.m., so generating a thin core of vorticity on the axis of the tube leading to the orifice. As the flow passed through this, the vortex filament was stretched and intensified as in a bath-plug vortex. In Fig. 4 the time for a given quantity of fluid in the header tank to pass through the hole under the action of gravity is plotted against the concentration of dissolved 'Polyox'. It can be seen that the additive increases the flow time, presumably by making it more difficult to stretch the vortex. (When the rod was not rotated the flow time was the same as for water.) Similar effects were obtained with solutions of the polyacrylamide 'Separan *AP* 30', and of cetrimide–alpha naphthol mixtures of a type found[6,7] to reduce turbulent drag. Guar-gum solutions, however, behaved the same as water.

The threshold shear stress for the onset of turbulent drag reduction[6] is higher for guar-gum than for the other substances quoted, so these results might be interpreted as supporting Pfenninger's hypothesis, on the assumption that in the experiments of Fig. 3 the vorticity was simply not intense enough or the stretching violent enough to produce measurable effects with guar-gum. It was

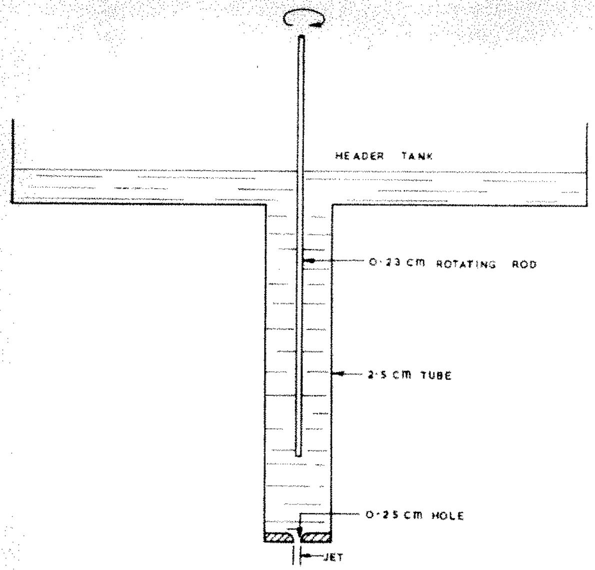

Fig. 3. Vortex stretching apparatus.

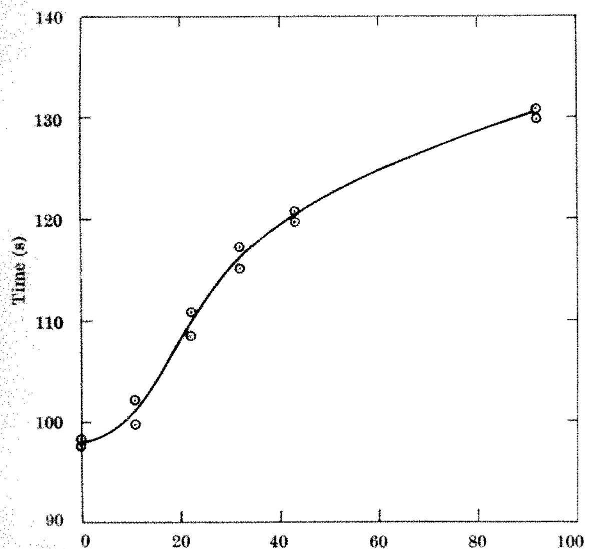

Fig. 4. Effect of 'Polyox' on flow times.

found, however, that 'Polyox' solutions, aged either by storage for about a week or by gentle pumping for a few hours, also lost the ability to slow down the vortex flow of Fig. 3. Moreover, they produced less suppression of turbulent mixing in the dye experiments of Fig. 1 than fresh solutions. (Figs. 2*a* and 2*c* are for fresh solutions.) It had previously been found[8,9] that aged solutions produce as much turbulent drag reduction as fresh ones, so this seems at first sight to show that the inhibition of vortex stretching cannot be relevant to drag reduction.

Further investigations have shown, however, that though the effects of ageing on drag reduction are indeed small at higher concentrations and high levels of the friction stress, there is some loss of effectiveness at lower concentrations around 10 p.p.m., and this loss is very marked at relatively low levels of the friction stress (for example, when the flow speed at the edge of the boundary layer, or at the centre of the pipe in pipe flow, is only of the order of 150 cm s^{-1}). At these lower levels of the friction stress the turbulence structure is coarser, so that the vorticity stretching processes perhaps approach more nearly in scale those of Fig. 3. To modify these, the presence of molecular aggregates may be needed, but when the aggregates are broken up by ageing[8] the fluid may still perhaps be able to affect the smaller, more intense stretching processes found in highly sheared turbulent flows. Thus it may be that Pfenninger's hypothesis it correct, though to establish it firmly it would be desirable to set up a vortex stretching experiment in which the length scales are smaller and the accelerations more intense than those of Fig. 3.

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Received February 7, 1968.

[1] Giles, W. B., and Pettit, W. T., *Nature*, **216**, 470 (1967).
[2] Stuart, J. T., *J. Fluid Mech.*, **4**, 1 (1958).
[3] Dixon, T. N., and Hellums, J. D., *A.I.Ch.E. J.*, **13**, 866 (1967).
[4] Jones, W. M., and Maddock, J. L., *Nature*, **212**, 388 (1966).
[5] Pfenninger, W., *Northrop Corporation, Norair Div. Report BLC-179* (1967).
[6] Gadd, G. E., *Nature*, **212**, 874 (1966).
[7] White, A., *Nature*, **214**, 585 (1967).
[8] Brennen, C., and Gadd, G. E., *Nature*, **215**, 1368 (1967).
[9] White, A., *Nature*, **216**, 994 (1967).

Interference of Sub-millimetre Waves over 450 m Path Difference

SUB-MILLIMETRE waves are attractive for metrology over long distances, because they are not much scattered by fog or cloud[1] and can be specularly reflected from ordinary machined surfaces. A device using interference over a long path difference would give a simple method of measuring small movements of a distant body and could be developed for the precise measurement of distances.

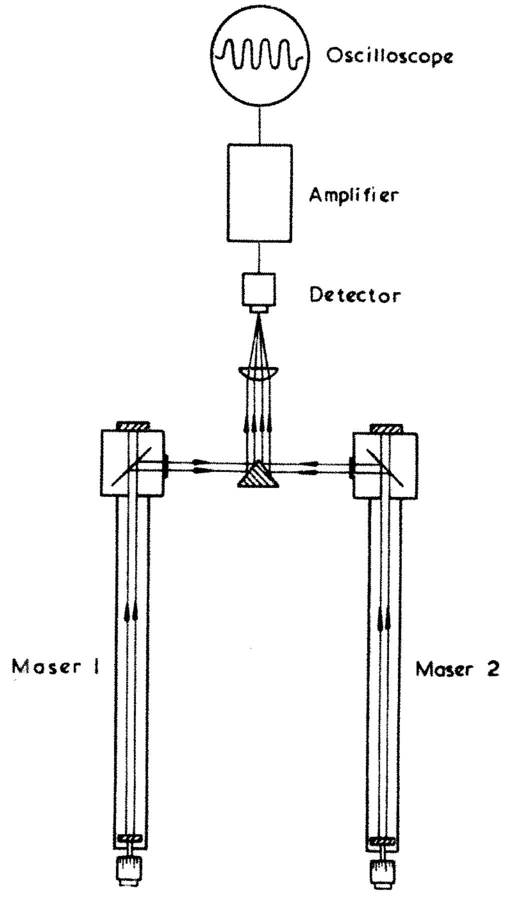

Fig. 1. Diagram of beating experiment.

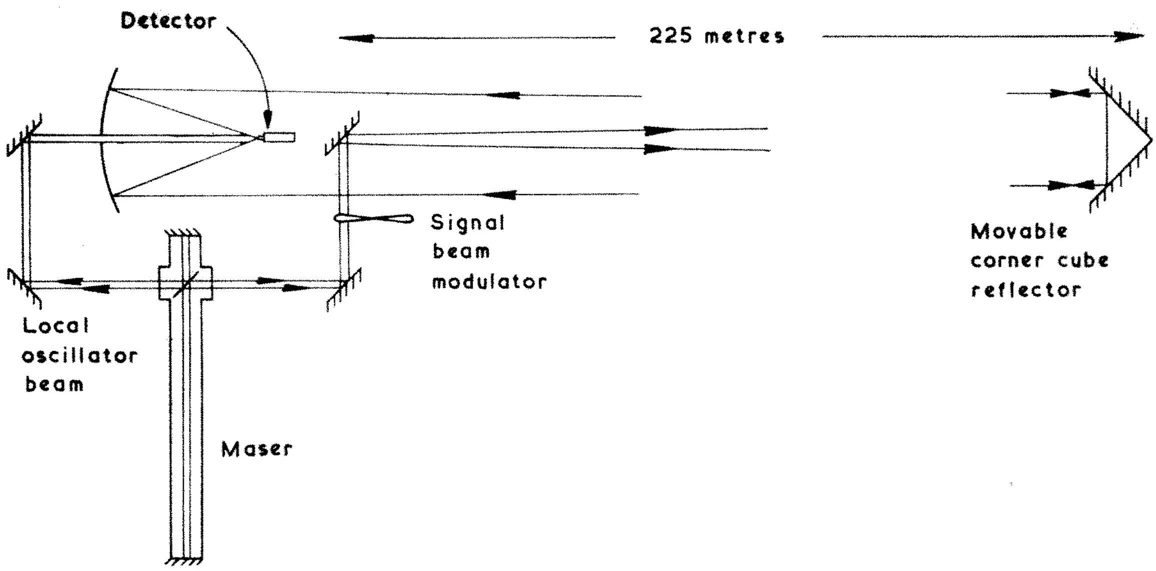

Fig. 2. Apparatus for recording interference over 450 m path difference.

Interference between beams which have traversed a large path difference requires that the radiation be confined to a narrow bandwidth, and to this end we were interested in finding the effective line width of the continuous-wave 337μ maser[2]. A preliminary experiment was carried out by beating two masers together as shown in Fig. 1, using a Putley indium-antimonide detector[3] as the non-linear mixing element.

Using single sweep operation of the oscilloscope, transient beat frequencies ranging between 20 kHz and 3 MHz were recorded, but instability of the masers causes the beat frequency to vary over periods of a few milliseconds. If, however, one of the masers is set to give maximum signal, while the second is tuned through its resonance position, the beat frequency first appears at about 3 MHz, decreases through a minimum of a few kHz and then increases again. This indicates that the maser can radiate over a band-width of a few MHz, but for short periods the emission line is much narrower.

To make a quantitative determination of the coherence length of the radiation, we built the interferometric apparatus shown in Fig. 2. A corner cube (made by Plessey Radar, Ltd.), accurately constructed to preserve phase coherence of the reflected beam, was used as the distant reflector. A maser with a dielectric film in the resonant cavity gives two beams of 337μ radiation. The "signal" beam, which is reflected back along its path by the corner cube, is modulated at 15 Hz and is mixed with the unmodulated short path or "local oscillator" beam at the Golay detector. This heterodyne method of detection as applied to submillimetre waves has been described elsewhere[4]. Interference fringes were recorded as the corner cube was slowly displaced, and have been obtained when the signal beam has travelled a total distance of 450 m. Fig. 3 shows fringes obtained at an intermediate distance.

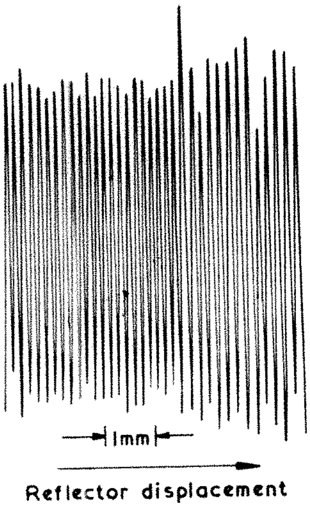

Fig. 3. Fringes recorded over 160 m path difference.

Using the expression

$$L = \frac{c}{\Delta\nu}$$

to define the coherence length L, where $\Delta\nu$ is the frequency bandwidth and c the speed of light, we see from this result that the radiation line width is not greater than 700 kHz and may well be much less. In addition, in the atmospheric conditions prevailing, phase coherence was preserved over the 450 m path.

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Received February 9, 1968.

[1] Burroughs, W. J., Pyatt, E. C., and Gebbie, H. A., *Nature*, **212**, 387 (1966).

[2] Gebbie, H. A., Stone, N. W. B., Slough, W., Chamberlain, J. E., and Sheraton, W. A., *Nature*, **211**, 62 (1966).

[3] Putley, E. H., *Applied Optics*, **4**, 649 (1965).

[4] Gebbie, H. A., Stone, N. W. B., Putley, E. H., and Shaw, N., *Nature*, **214**, 165 (1967).

THE SOLID STATE

Interstratified Structure formed from a Pre-heated Mica by Acid Treatments

In this study we used sericite specimens of the $2M_1$ type. The chemical composition of one of them is shown in Table 1*a*. This sample was pulverized to a fine powder in an agate mortar, heated in a platinum crucible for some time (5 min–4 h), quenched to room temperature and treated with hydrochloric or sulphuric acid. In this way the sample was easily changed, wholly or in part, into an interstratified mineral.

We found that for the sample to change into an interstratified mineral it should be heated to the temperature range of dehydroxylation. In our experiments, the duration of pre-heating did not seriously affect the amount of interstratified mineral produced, but the duration of treatment with the acids was important. The interstratified structure was formed even when the pre-heated sample was boiled for a few minutes with the acids. The longer the treatment with acids, the more interstratified mineral formed.

The product obtained after the treatment with 6 N hydrochloric acid for 1 h is shown in Table 1*b*, which gives the following structural formula

$$\begin{pmatrix}\mathrm{Ca}\ 0{\cdot}01\\ \mathrm{K}\ \ 0{\cdot}53\\ \mathrm{Na}\ 0{\cdot}17\end{pmatrix}\begin{pmatrix}\mathrm{Al} & 1{\cdot}95\\ \mathrm{Fe}^{+++} & 0{\cdot}03\\ \mathrm{Mg} & 0{\cdot}02\end{pmatrix}\begin{pmatrix}\mathrm{Si}\ 3{\cdot}30\\ \mathrm{Al}\ 0{\cdot}70\end{pmatrix}\mathrm{O}_{9\cdot99}(\mathrm{OH})_{2\cdot01}(\mathrm{H_2O})_{0\cdot76}$$

The structural formula of the original sericite was

$$\begin{pmatrix}\mathrm{Ca}\ 0{\cdot}01\\ \mathrm{K}\ \ 0{\cdot}73\\ \mathrm{Na}\ 0{\cdot}17\end{pmatrix}\begin{pmatrix}\mathrm{Al} & 1{\cdot}83\\ \mathrm{Fe}^{+++} & 0{\cdot}03\\ \mathrm{Mg} & 0{\cdot}02\end{pmatrix}\begin{pmatrix}\mathrm{Si}\ 3{\cdot}10\\ \mathrm{Al}\ 0{\cdot}88\\ \mathrm{Ti}\ 0{\cdot}02\end{pmatrix}\ \mathrm{O}_{9\cdot58}(\mathrm{OH})_{2\cdot42}$$

Under the electron microscope, the original shapes and sizes are well preserved.

The pre-heated sample, treated with 0·8 N sulphuric acid for 8 h showed a 22·7 Å reflexion (Table 2*a*), which expanded to a 23·3 Å spacing on treatment with ethylene glycol, and contracted to a 10 Å spacing when heated at 500° C for 1 h and then quenched to room temperature. This suggests that the structure consists of a nearly regular alternation of mica-like layers (*M*) 10 Å thick and hydrous mica-like layers (*H*) 12·7 Å thick, because there is a reflexion with a long spacing which has a repeat distance of 22·7 Å (10 + 12·7) and basal reflexions at submultiples of this distance. The X-ray properties are those of an "allevardite" structure with a single layer of water molecules, as reported by Brindley[1]. 'Allevardite' has been shown to be identical to 'Rectorite'[2].

Table 1. PERCENTAGE CHEMICAL COMPOSITIONS OF THE ORIGINAL SERICITE (*a*) AND THE SYNTHETIC 22·7 Å MINERAL (*b*)

	(*a*)	(*b*)
SiO_2	47·24	48·45
TiO_2	0·38	tr.
Al_2O_3	35·04	33·02
Fe_2O_3 FeO	0·59	0·58
MnO	tr.	tr.
MgO	0·21	0·18
CaO	0·16	0·08
K_2O	8·75	6·10
Na_2O	1·37	1·28
$H_2O(+)$	5·52	7·77
$H_2O(-)$	0·32	2·22
P_2O_5	None	None
Total	99·58	99·68

Table 2. X-RAY POWDER DIFFRACTION DATA

(*a*)		(*b*)	
d (Å)	*I*	*d* (Å)	*I*
22·7	202	25·6	115
11·1	424 (*s*)	12·5	86
7·56	2		
5·64	60	5·07	29 (*s*)
4·55	19 (*s*)		
3·25	244 (*s*)	3·62	2
2·82	8	3·20	1·3
2·52	23 (*s*)	2·823	1
2·25	1·3 (*s*)		
2·036	80 (*s*)		
1·881	7		
1·508	1	1·678	1·5
1·417	7		
1·258	7 (*s*)	1·439	3

a, An interstratified mineral synthesized from sericite; *b*, a sample treated with 1·2 per cent magnesium chloride solution for 1 h. Cu $K\alpha$ radiation was used.

s, Reflexions overlapped by mica reflexions.

When this product was boiled with 1·2 per cent magnesium chloride solution for 6 h, the X-ray behaviour of the sample resembled that of the regular interstratified mineral mica-montmorillonite; that is, a 25·6 Å reflexion was obtained (Table 2*b*), which expanded to a 27·2 Å spacing after treatment with ethylene glycol.

The Fourier transform method of MacEwan *et al.*[3] revealed the following probabilities of stacking: $P_M = 0{\cdot}57$, $P_H = 0{\cdot}43$, $P_{MM} = 0{\cdot}30$, $P_{MH} = 0{\cdot}70$, $P_{HH} = 0{\cdot}07$, indicating that the structure is close to the completely regular type.

It is interesting that the original sample clearly showed an endothermic peak, caused by dehydroxylation, at about 670° C, which disappeared when the sample was heated at 800° C, and reappeared at a lower temperature after treatment with acids (Fig. 1). Such rehydroxylation was also confirmed by infrared absorption spectroscopy.

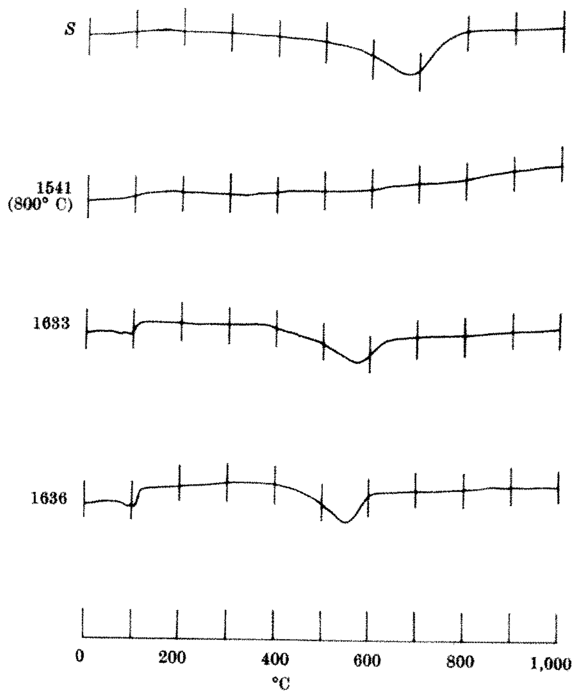

Fig. 1. Differential thermal analysis curves of the original mica sample (*S*), after the sample was heated at 800° C (No. 1541), after the pre-heated sample was treated by sulphuric acid (0·8 N) for 10 min (No. 1633), and after the pre-heated sample was treated with sulphuric acid (0·8 N) for 4 h (No. 1636).

When the physico-chemical methods used to examine the sericite were also applied to macroscopic muscovite we found that an interstratified structure of the same type had been formed in part in the flakes, but we could not confirm this for the phlogopite flakes.

As shown before[4], this is an easy method of forming an interstratified structure, and the result seems to be pertinent to the discussion of the origin of interstratified clay minerals.

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[1] Brindley, G. W., *Amer. Miner.*, **41**, 91 (1956).

[2] Brown, G., and Weir, A. H., *Intern. Clay Conf.*, 1963 (AIPEA, Pergamon Press, 1963).

[3] MacEwan, D. M. C., Amil, A. R., and Brown, G., *The X-ray Identification and Crystal Structure of Clay Minerals* (edit. by Brown, G.), 393 (1961).

[4] Ueda, S., and Sudo, T., *Nature*, **211**, 1393 (1966).

CHEMISTRY

Hydrothermal Synthesis of Sodium–Mordenite in Pebble Form

MORDENITE is one of the more common low-temperature sedimentary zeolites[1]. It can be regarded as a hydration product of volcanic glasses and lavas and is, for example, formed by the crystallization of obsidian glass on exposure to a natural, acid hydrothermal solution at 230° C in a New Zealand borehole[2]. Such observations have led to the industrial development of sodium–mordenite syntheses from naturally occurring materials, particularly volcanic glasses and pumice, in well defined experimental conditions[3]. Thus synthetic mordenites are usually formed as hard or semi-hard aggregates of extremely small crystals ($\leqslant 20\mu$ diameter) after hydrothermal preparation at high *p*H (*c*. 11·5) and temperatures ranging from 100° to 260° C.

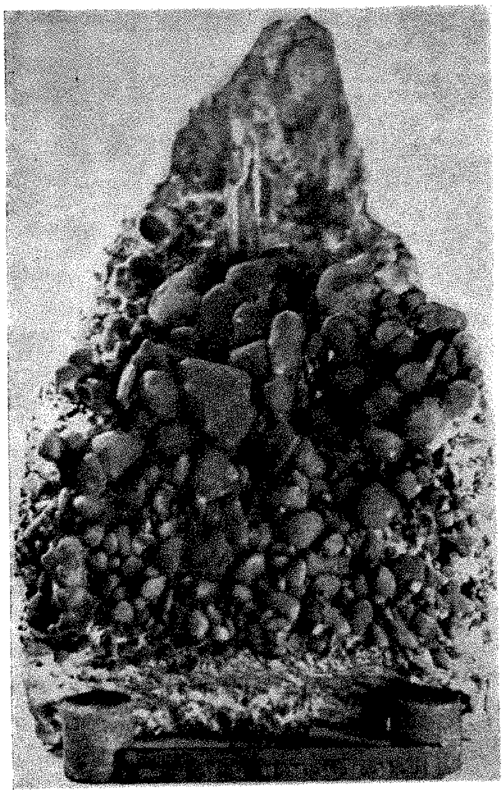

Fig. 1. "Pebbles" containing 75 per cent weight mordenite. General view looking down on mass removed from end of autoclave.

We have recently reinvestigated the hydrothermal synthesis of sodium–mordenite from finely divided pumice and aqueous "water-glasses"[3,4]. The preparative conditions were chosen to give sodium–mordenite as a single crystalline phase[4]. We found it difficult to increase the yield of mordenite above about 75 per cent weight, and the products were invariably contaminated with small amounts of albite, analcite, α-quartz and unreacted pumice. The hard crystalline masses were characterized by stratification, the mordenite being concentrated at the top in contact with the reaction mother-liquor. For this reason, the syntheses were repeated using constant agitation of the reactants at about 250 lb/in.^{2} gauge in a "rocking" autoclave. The hydrothermal products were found to be chiefly "pebbles" (Figs. 1 and 2) of from 70 to 90 per cent weight mordenite, as a single crystalline phase, set in a matrix of powdered mordenite, unreacted pumice and crystallized "water-glass". The size of the "pebbles" and their mordenite content were found to increase with the duration of hydrothermal treatment at the same pressure.

The unexpected appearance of "pebbles" in the synthesis led to speculation on their mode of origin, particularly because they slightly resembled some natural pebbles of mordenite from the type-locality, Morden, King's County, Nova Scotia[5], which had been used in earlier experiments. These pebbles were amygdales weathered from a basalt and sometimes contained needles of just sufficient breadth—about 30μ—for X-ray single crystal structure determinations.

Thin sections of the "pebbles" show a carious, fine-grained mass of overlapping anhedra of mordenite about 0·01 mm in diameter, together with dust (possibly pumice fragments), a few small anhedra of quartz and very rare fragments of pyroxene presumably unreacted portions of the starting material. The small cavities were subcircular or flattened; into them projected rectangular or acicular edges of mordenite.

No sign of a layered, concentric texture could be seen in the "pebbles", so they probably did not form by accretion. The larger ones in particular were, however, slightly moulded on one another, and on the autoclave, so that at some stage of their development they were plastic. Perhaps once nucleation of the silicate slurry started, it proceeded rapidly so that the whole passed

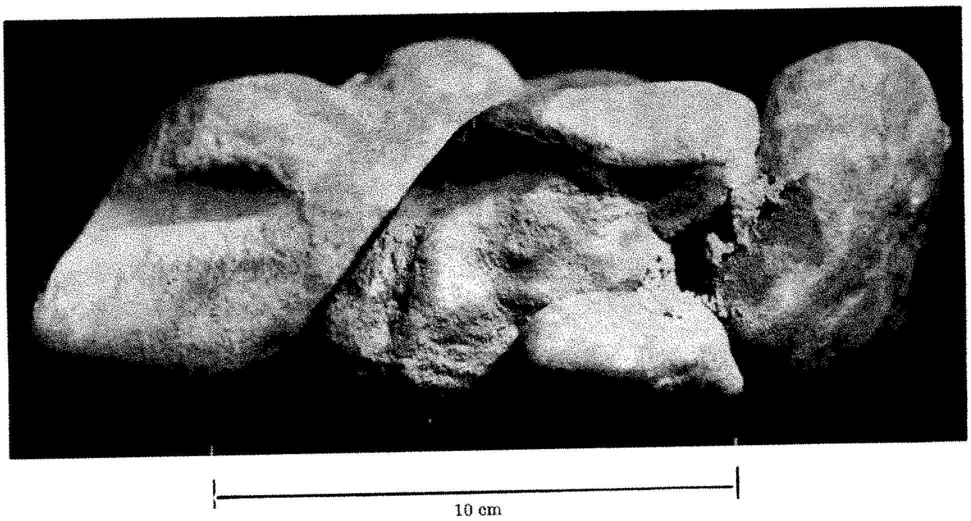

Fig. 2. "Pebbles" containing 90 per cent weight mordenite obtained by extension by 12 h of the hydrothermal treatment giving product 1 above; product moulded to part of autoclave fittings.

into a semi-solid "pasty" condition. The quite violent movement of the autoclave fragmented the mass and the "pebbles" developed by mutual attrition. They remained slightly plastic for part of the agitated cooling period, during which they sagged on to one another and moulded themselves to the autoclave fittings.

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[1] Hay, R. L., *Zeolites and Zeolitic Reactions in Sedimentary Rocks*, 12, Spec. GSA Pap. No. 85 (New York, 1966).
[2] Ellis, A. J., *Geochim. Cosmochim. Acta*, **19**, 145 (1960).
[3] UK patents 983,756, published 17.2.65, and 992,872, published 26.5.65.
[4] Sand, L. B., *Mordenite Synthesis*, paper presented at Molecular Sieve Conf., London (April 1967).
[5] How, H., *Mineralogy of Nova Scotia* (1869).

Rates of Adsorption of Wetting Agents and Detergents

ZETTLEMOYER *et al.*[1] have recently reported considerable differences in the rates of adsorption of some isomeric surfactants, for example, sodium octyl α-sulphopelargonate (I), a wetting agent, and sodium methyl α-sulphopalmitate (II), a detergent, to 'Graphon'. Both I and II have the formula $R\text{CH}(\text{SO}_3^-\text{Na}^+)\text{COO}R'$, but differ in the lengths of the chains R and R'. Type I compounds seem to adsorb three–six times faster than type II compounds. The explanations cited are a higher diffusion coefficient (D) or a faster sticking to the 'Graphon' surface for I (ref. 1).

I suggest that the important factor is the difference in the activities of the actual adsorbing species, the monomers and, perhaps, dimers, of I and II in the experiments performed. Double-chain salts such as I have lower surface activity than their long-chain isomers, presumably because of greater difficulty of interfacial adsorption[2]. Indeed, at equilibrium, II adsorbs more to 'Graphon' than I (ref. 1). Similarly, the packing needed to produce large micelles is more difficult for molecules of type I and therefore micelle formation is energetically less favourable, and the critical micelle concentration (CMC) is higher. The CMC of I, for example, is $2\cdot1 \times 10^{-3}$ M (ref. 3), obtained by the somewhat uncertain dye-spectral change method[4], while that of II is 4×10^{-4} M (ref. 3) as obtained by the dye method and $3\cdot2 \times 10^{-4}$ M from surface tensions[5]. Conductance measurements on many isomeric $R\text{SO}_4^-\text{Na}^+$ salts, with SO_4^- in different chain positions, show similar changes in the CMC (ref. 6). The differences expected between molecules of types I and II were discussed qualitatively by Hartley[2] in 1941, who showed that type I compounds can produce very low interfacial tensions, a factor closely connected with their superior wetting power.

Zettlemoyer *et al.*[1] used an initial concentration of $5\cdot8 \times 10^{-3}$ M, above the CMC for both I and II. Monomer activities change very little above the CMC and micelles are not surface active, so the "effective" activities of the adsorbing species for I and II are in the ratio of roughly 5 at $5\cdot8 \times 10^{-3}$ M. The adsorption of I should therefore be about five times faster even if there are no differences in D and rates of "sticking".

Small but significant differences may, nevertheless, exist, particularly in D. The free energies of transfer from aqueous to hydrocarbon-like media suggest that long-chain compounds in water are not tightly coiled, and probably have extended random-coil type structures[7]. On such grounds, I should have a higher D than II because of greater compactness. Experimental evidence here, however, is conflicting. D can be estimated from conductance and transference data using the Nernst–Hartley relationship[8]. The conductance data of Evans[6] suggest an overall decrease in D as SO_4^- is moved towards the middle of a chain. The data, unfortunately, show a wide scatter. On the other hand, Flockhart[9] noted a higher conductance of $\text{C}_{14}\text{H}_{29}\text{SO}_4^-\text{Na}^+$ as SO_4^- was moved from position 1 to 2 and 4. The calculated D for the 4-sulphate is higher than that for the 1-sulphate by the significant factor of 1·26.

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[1] Zettlemoyer, A. C., Subba Rao, V. V., and Fix, R. J., *Nature*, **216**, 683 (1967); *Chem. Eng. News*, 52 (December 11, 1967).
[2] Hartley, G. S., *Trans. Faraday Soc.*, **37**, 130 (1941).
[3] Stirton, A. J., Bistline, jun., B. G., Weil, J. K., and Ault, W. C., *J. Amer. Oil Chem. Soc.*, **39**, 55 (1962).
[4] Mukerjee, P., and Mysels, K. J., *J. Amer. Chem. Soc.*, **77**, 2937 (1955).
[5] Weil, J. K., and Stirton, A. J., *J. Phys. Chem.*, **60**, 899 (1956).
[6] Evans, H. C., *J. Chem. Soc.*, 579 (1956).
Mukerjee, P., *Advances in Colloid and Interface Science*, **1**, 241 (1967).
[8] Robinson, R. A., and Stokes, R. H., *Electrolyte Solutions*, second ed., 285 (Butterworths, 1959).
[9] Flockhart, B. D., *J. Colloid Sci.*, **17**, 305 (1962).

Standard Potential of the Silver–Silver Bromide Electrode in Formamide

As part of an investigation of the behaviour of electrolytes in formamide, we have now measured the molal standard e.m.f. of the cell $\text{H}_2/\text{HBr}(m)/\text{AgBr}/\text{Ag}$ in formamide at 25° C.

Hydrogen and thermal-electrolytic silver–silver bromide electrodes were prepared according to the methods of Ives and Janz[1]. Only those silver–silver bromide electrodes with bias potentials below 150 μV were used.

Pure formamide was prepared by repeated distillation, and had a freezing point of 2·55° C and a specific conductance in the range $3\text{–}6 \times 10^{-6}$ ohm^{-1} cm^{-1}, which is in good agreement with values given in the literature[2]. Dry hydrogen bromide gas, generated by the action of bromine on tetrahydronaphthalene, was passed through formamide, in the presence of dry nitrogen. It was found possible to analyse the hydrogen bromides olutions by potentiometric titration against aqueous silver nitrate solution. The molal standard potential, E^0, was determined by extrapolating to zero molality the function E', given by

$$E' = E + 2\cdot303 \times 2RT/F\ (\log(m) - A\sqrt{m})$$

where the symbols have their usual meanings. The value of the Debye–Hückel constant A for formamide at 25° C is 0·3286 $(\text{moles/kg})^{-\frac{1}{2}}$. The values of E' are shown plotted against m in Fig. 1. The best straight line, fitted

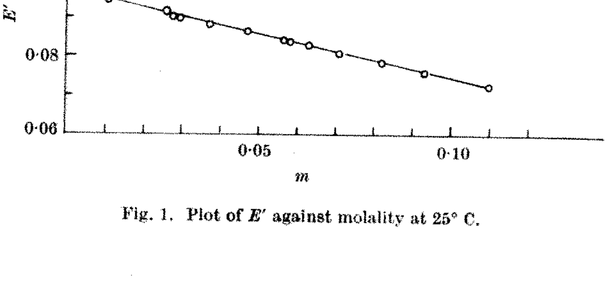

Fig. 1. Plot of E' against molality at 25° C.

using a least squares treatment, gives the intercept, E^0, as 0.0967 V with a root mean square deviation of 0.0007 V.

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[1] Ives, D. J. G., *Reference Electrodes, Theory and Practice*, chaps. 2 and 4 (Academic Press Inc., 1961).
[2] Smith, G. F., *J. Chem. Soc.*, 53, 3257 (1931).

Enhanced Catalytic Activity of Platinum Electrodes resulting from Cathode Pretreatment

A recent report[1,2] describes the effect on Raney platinum electrodes of cathodic pretreatment in 10N H_2SO_4. Current-voltage curves for the oxidation of formic acid in 3N H_2SO_4 before and after pretreatment were compared and the enhanced currents measured for the treated electrodes were attributed to an adsorbed layer of sulphur formed by deposition during the cathodic pretreatment. This is surprising because the inhibiting effect of sulphur adsorbed on platinum on many other surface reactions and electrode reactions is well known and was observed by Faraday[3]. Enhanced catalytic activity was also observed[1,2] by using electrodes pretreated with gaseous hydrogen sulphide at high temperatures and with electrodes cathodically polarized in solutions of selenium oxide in dilute sulphuric acid. Extended cathodic pretreatment in 10 N H_2SO_4 yielded a less active electrode. Here, the sulphur was said to have formed a monolayer.

At our laboratories, measurements have been made on bright platinum electrodes in a half cell arrangement with a hydrogen electrode as a reference electrode in stirred solutions of formic acid in 2 N H_2SO_4. Electrochemical pretreatment steps, similar to those used by Gilman[4], ensured that the electrode was in a reproducible state. The most important part of this treatment consisted of holding the electrode at 1.55 V for 30 s and at 0.05 V for a further 15 s. The steady state current-voltage curve for the oxidation of formic acid was measured (Fig. 1). Cathodic pretreatment in 10 N solutions of three strong acids (H_2SO_4, $HClO_4$ and HNO_3), stirred by bubbling pure nitrogen over the electrode, was then carried out. This treatment consisted of holding the electrode at −0.25 V for an arbitrary time (90 s). After the electrode had been washed its potential was held at +50 mV for a few seconds before measurement of the current-voltage curve for formic acid oxidation (Fig. 1). The enhanced oxidation rate (increased currents at lower potentials) remained stable provided the electrode potential was not increased beyond 600 mV for long periods of time (that is, more than 4 min).

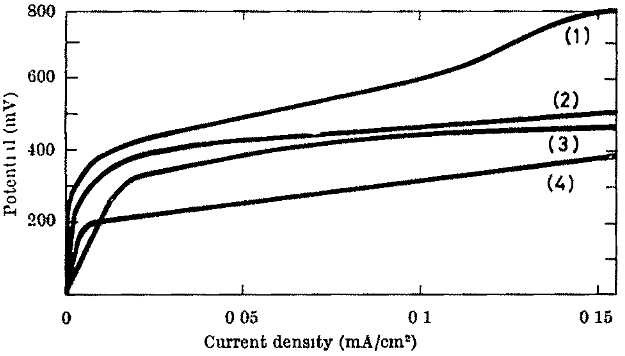

Fig. 1. Anodic oxidation of formic acid in 2 N H_2SO_4 on bright platinum electrodes. Curve 1, results for untreated electrode, curves 2, 3 and 4, results for electrodes "activated" in perchloric acid, sulphuric acid and nitric acid, respectively.

The form of these results agrees with that of those previously obtained by the other workers for activation in sulphuric acid[1,2]. The extension of the measurements to other strong acids, however, leaves some doubts as to the interpretation of these effects.

There are several possible explanations. Cathodic pretreatment might simply reduce any remaining "oxide" on the platinum surface; such a reduction could also be effected by hydrogen sulphide at high temperatures. It may be, however, that some hydrogen remains on the surface of the platinum at potentials where hydrogen is not normally expected to be present[5]. If so, the catalytic activity would be altered, for there would no longer be a true platinum surface. Furthermore, it could be that sorption layers are formed in which the ions NO_3^-, ClO_4^- and SO_4^{2-} are adsorbed on the platinum surface in some partially reduced state in such a way that they catalyse the oxidation of formic acid by a redox process. It is also conceivable that this type of layer could be formed by chemical action of hydrogen sulphide on "platinum oxide". The lower activity of the Raney electrodes which had undergone extended cathodic polarization was probably caused by adsorbed impurities or even by inhibition by sulphur.

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[1] Binder, H., Kohling, A., and Sandstede, G., *Nature*, 214, 268 (1967).
[2] Binder, H., Kohling, A., and Sandstede, G., *Advanced Energy Conversion*, 7, 77 (1967).
[3] Faraday, H. M., *Phil. Trans. Roy. Soc.*, 55, 72 (1834).
[4] Gilman, S., *J. Phys. Chem.*, 68, 70 (1964).
[5] Schuldiner, S., and Warner, T. B., *Electrochimica Acta*, 11, 307 (1966).

Bistability caused by Substrate Inhibition of Peroxidase in an Open Reaction System

Several authors have suggested that more than one stable stationary state exists in an open homogeneous reaction system, and this suggestion has served as a basis for speculative explanations of various biological phenomena[1-4]. Although bistability is well known in open heterogeneous chemical reaction systems, as for example an electrolysis cell with a passivable electrode[6], it seems that bistability has never been observed in an open homogeneous system. This communication describes such a case.

The reaction used in the experiment is the oxidation of NADH by O_2 catalysed by horseradish peroxidase. This reaction was studied in an open system by Yamazaki *et al.*[7,8], who found the reaction to be autocatalytic and that damped oscillations in the oxygen concentration of the solution may occur when oxygen is supplied continuously by bubbling with a mixture of oxygen and nitrogen. Although this is not explicitly stated by Yamazaki *et al.*, it seems from their experimental results that the oxidation of NADH is inhibited by oxygen, because at high oxygen concentrations the enzyme is converted to an inactive form spectrophotometrically identified as complex III. This substance was originally discovered by Keilin and Mann as a reaction product of peroxidase and H_2O_2 (ref. 9).

Substrate inhibition is one of the possible mechanisms which can lead to bistability in an open system, as predicted by Sel'kov[10] and Higgins[11]. Consider, for example, the following formal reaction scheme suggested by Higgins[12]

$$S+E\xrightarrow{k_1}ES$$

$$ES\xrightarrow{k_2}E+P$$

$$S+ES\underset{k_4}{\overset{k_3}{\rightleftharpoons}}ES_2$$

where a reversible reaction between the substrate and the enzyme–substrate complex to form an inactive complex (ES_2) is added to the classical Michaelis–Menten scheme The steady state rate of consumption of the substrate according to this scheme is

$$-\frac{ds}{dt}=\frac{k_1e_0s}{1+\dfrac{k_1}{k_2}s+\dfrac{k_1k_3}{k_2k_4}s^2} \qquad (1)$$

where e_0 is the initial enzyme concentration It is assumed that the reaction occurs in a stirred solution which can exchange substrate by diffusion with a reservoir of constant concentration The rate of transport of substrate into the reaction solution is then given by

$$\frac{ds}{dt}=K(s_x-s) \qquad (2)$$

where K is a constant and s_x is the concentration of the substrate in the reservoir For the open system to be in a stationary state, the rate of consumption of substrate given by (1) must be equal to the rate of transport of substrate into the reaction solution given by (2) For positive rate constants the curve described by (1) must always have a form as shown in Fig 1 Such a curve can have one (*A*) or three (*B*, *C* and *D*) intersections with the straight line given by (2) depending on the slope and position of this line In the case of only one point of intersection this point corresponds to one stable stationary state of the system If there are three intersections, one of them (*C*) corresponds to an unstable stationary state and the two others correspond to stable stationary states

In the present work, the peroxidase catalysed oxidation of NADH by O_2 was studied in an open system slightly different from that of Yamazaki *et al* The experiments were performed in a 1 × 1 × 4 cm cuvette in a double beam spectrophotometer The light absorption was measured at 418 mμ, where complex III has an absorption maximum The wavelength of the reference light was 615 mμ, which is an isosbestic point of the peroxidase-complex III couple The oxygen concentration in the liquid was measured polarographically by a vibrating platinum electrode A mixture of O_2 and N_2 was blown at a constant rate on the surface of the solution The vibration of the electrode caused an efficient stirring and a suitable supply rate of oxygen to the solution could be obtained without bubbling the gas mixture into the solution Oxygen was continuously supplied to the system but NADH was not The initial concentration of NADH was made so high, however, that the decrease of NADH concentration during the experiment was not significant Fig 2 describes the reaction system schematically

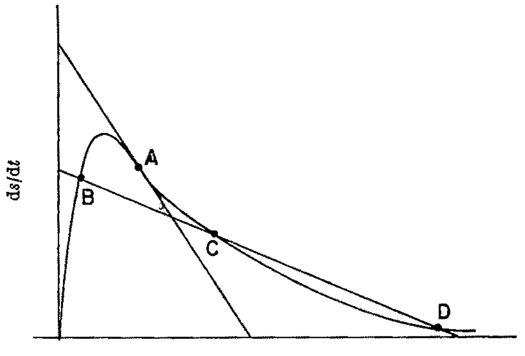

Fig 1 Characteristic curve of substrate inhibited enzyme reaction For explanation see text

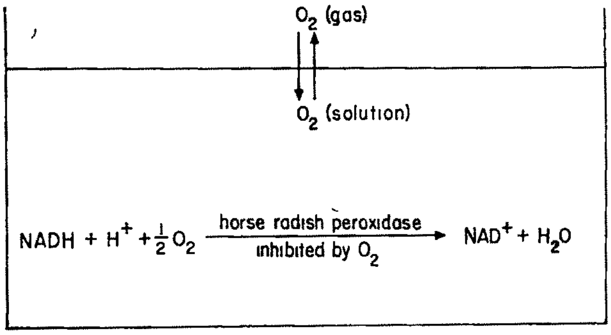

Fig 2 Schematic representation of experimental system yielding results shown in Fig 3

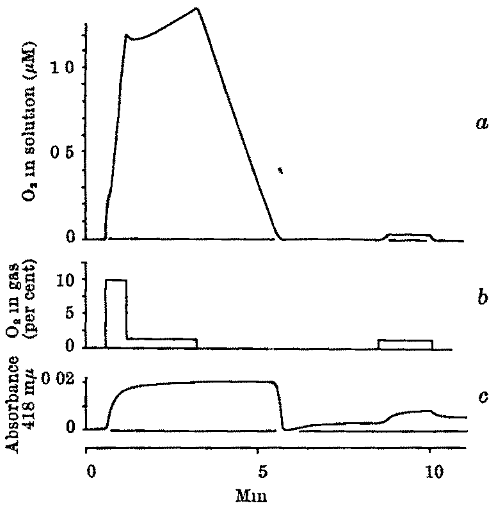

Fig 3 Simultaneous measurement of oxygen concentration (*a*) and light absorption at 418 mμ (*c*) of 0 4 μM horseradish peroxidase (Boehringer und Soehne) and 1 4 mM NADH in 0 07 M phosphate buffer at pH 5 5 Oxygen was supplied to the solution by a mixture of O_2 and N_2 blown on the surface at a rate of 10 ml /s The setting of the oxygen content of the gas mixture is indicated in (*b*)

Fig 3 shows the simultaneous measurements of the oxygen concentration in the solution (*a*) and the light absorption at 418 mμ (*c*) when the oxygen content of the gas mixture (*b*) was increased from zero to a constant value, in one case after an initial pulse and in another case without such a pulse It is seen that in the first case the oxygen concentration in the solution increased rapidly during the pulse and continued to increase more slowly as long as the oxygen content of the gas mixture was kept on the constant value When the oxygen in the gas was turned off, the oxygen concentration of the solution fell slowly to zero In the second case, when the oxygen content of the gas mixture was set at the same constant value as before but without an initial pulse, the oxygen concentration in the solution increased to a very low level where it remained constant until the oxygen in the gas was turned off, after which it fell to zero

In the experiment illustrated in Fig. 3 a stationary concentration of oxygen in the solution was not reached in the first case when an initial pulse of oxygen was used It was found in similar experiments that a high stationary concentration of oxygen could be obtained, but to show this it was necessary to use a scale of measurement on which the low stationary concentration of oxygen could not be recorded The very large difference between the two stationary oxygen concentrations indicates that there is a large difference between the activities of the enzyme in the active and the inhibited states It was anticipated that the distance between the two stationary oxygen concentrations could be decreased by the addition of a second oxygen consuming reaction which is not inhibited by oxygen It was found that the addition of the glucose oxidase catalysed oxidation of glucose to the reaction system as shown in Fig 4 had the anticipated effect The production of H_2O_2 as an end product in the oxidation of glucose did not seem to play any important part in the reaction system

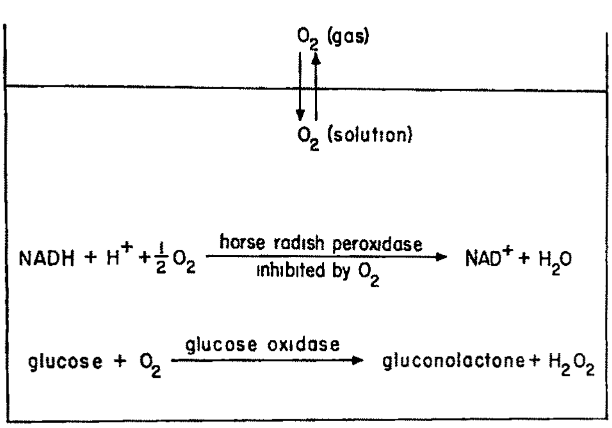
O_2 (gas)
O_2 (solution)
$NADH + H^+ + \frac{1}{2}O_2 \xrightarrow[\text{inhibited by } O_2]{\text{horse radish peroxidase}} NAD^+ + H_2O$
$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconolactone} + H_2O_2$

Fig 4 Schematic representation of experimental system yielding results shown in Fig 5

Fig 5 shows an experiment similar to that shown in Fig 3 except that glucose and glucose oxidase were added to the solution besides peroxidase and NADH It is seen that a high stationary concentration of oxygen in the solution was obtained when the constant oxygen content of the gas mixture was preceded by a pulse The stability of the high stationary oxygen concentration is evident from the fact that it is approachable from both sides The low stationary concentration of oxygen which is obtained at the same constant oxygen content of the gas mixture when an initial pulse is not used is also approachable from both sides When a small oxygen pulse was applied after the low stationary oxygen concentration was reached, the oxygen concentration in the solution increased a little but fell back to the original value When the pulse was a little higher, however, the system was triggered into the high oxygen state

The light absorption measurements in Figs 3 and 5 show that the admission of oxygen to the solution leads to the formation of a substance absorbing at 418 mμ This substance can have two different stationary concentrations at the same oxygen content of the gas mixture A high oxygen concentration in the solution, whether stationary or not, is accompanied by a high stationary concentration of the light absorbing substance At a low stationary oxygen concentration in the solution the light absorbing substance has a low stationary concentration In the low oxygen state the concentration of the absorbing substance varies with the concentration of oxygen in the solution The autocatalytic character of the NADH oxidation reaction is indicated by a lag time between the beginning of the increase of the oxygen concentration in the solution and the beginning of the increase of the light absorption Also it is observed that the rate of increase of oxygen concentration during the initial pulse exhibits a deflexion when the light absorption is increasing These phenomena are more evident at lower DPNH concentrations and higher pH The fact that the reaction is autocatalytic is not essential for the bistability

These findings are consistent with the assumption that the light absorbing substance is an inactive form of the enzyme, presumably complex III, and that at high oxygen concentrations the enzyme is almost completely converted to the inactive form, whereas at low oxygen concentrations a partial conversion, to a degree determined by the oxygen concentration, takes place It should be noted that peroxidase alone does not react with oxygen to form complex III In the present system, the complex is only formed along with the oxidation of NADH

It may be questioned whether the bistability observed in the present experimental system has any physiological relevance Of interest in this connexion is the finding by Harrison and Pirt[13] that the respiration of a continuous culture of *Klebsiella aerogenes* is inhibited by oxygen and that abrupt transitions between high and low oxygen concentrations in the liquid take place at certain values of the oxygen content of the mixture of oxygen and nitrogen used to supply oxygen to the culture Such phenomena in the continuous bacterial culture may be caused by a respiratory enzyme which is inhibited by oxygen, as is the horseradish peroxidase, but a more

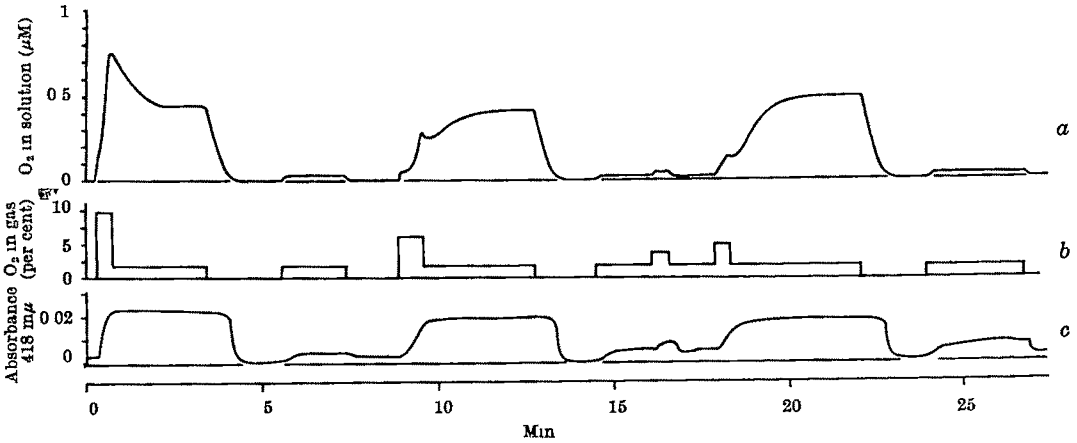

Fig 5 Experiment similar to that in Fig 3 except that 3 5 μg/ml glucose oxidase and 17 mM glucose in addition to 0 4 μM peroxidase and 7 mM NADH are present in the solution

indirect control of the respiration rate by oxygen is also possible

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[1] Denbigh, K G , Hicks, M , and Page, F M , *Trans Faraday Soc* , **44**, 47 (1948)
[2] Szilard, L , *Proc US Nat Acad Sci* , **46**, 277 (1960)
[3] Kacser, H , *Soc Exp Biol Symp* , **14**, 13 (1960)
[4] Anker, H S , *Nature*, **188**, 938 (1960)
[5] Monod, J , and Jacob, F , *Cold Spring Harbor Symp Quant Biol* , **26**, 389 (1961)
[6] Franck, U F , *Elektrochem* , **62**, 649 (1958)
[7] Yamazaki, I , Yokota, K , and Nakajima, R , *Biochem Biophys Res Commun* , **21**, 582 (1965)
[8] Yamazaki, I , and Yokota, K , *Biochim Biophys Acta*, **132**, 310 (1967)
[9] Keilin, D , and Mann, T , *Proc Roy Soc* , B, **122**, 119 (1937)
[10] Sel'kov, E E , in *Some Biocybernetics Problems, Application of Electronics to Biology and Medicine* (edit by Anosov, N M) 1 (Naukova Dumka, Kiev, 1966)
[11] Higgins, J , *Mémoires Acad Roy Med Belgique*, **6**, 235 (1967)
[12] Higgins, J , *Ind Eng Chem* , **59** (5), 19 (1967)
[13] Harrison, D E F , and Pirt, S J *J Gen Microbiol* , **46**, 193 (1967)

BIOCHEMISTRY

Sub-unit Nature of Catalase Compound II

THE absorption spectrum of catalase compound II is well established and provides the principal basis for the characterization of this species The nature of the reactions involved in the conversion of catalase to compound II is not well understood, but it is clear that compound II plays a very important part in the reversible deactivation of catalase by its substrates Although there has been no suggestion in the literature that the formation of compound II involves a major disruption of the catalase molecule, recent kinetic studies in this laboratory[1] suggested that compound II may be a sub-unit species This compound is sufficiently stable to permit a direct test of this idea by sedimentation velocity studies in the analytical ultracentrifuge

The catalase used in our experiments was prepared from *M lysodeikticus* and had an activity[2] of 6 5 × 10^{7} M^{-1} s^{-1} and an RZ value of 0 8 sedimentation velocity experiments showed no lower molecular weight impurities Catalase was partially converted to compound II by the addition to catalase solutions (~ 10^{-5} M, phosphate buffer *p*H 7·0) of either several very small amounts of H_2O_2 (allowing the catalatic reaction to subside after each addition) or, in much milder conditions, by a single addition of dilute aqueous alkyl hydroperoxide solution The results obtained were independent of the mode of formation of compound II The absorption spectra of the solutions were time invariant for several hours and these measurements permitted calculation of the percentage of compound II using the data of Chance and Herbert[3]

Fig 1 shows the sedimentation velocity pattern obtained with the Beckman Model E Ultracentrifuge, from a catalase solution containing 17 5 per cent compound II by spectrophotometric estimation, the faster peak ($S_{20,w}$ = 10 7 *S*) corresponds to catalase (ref 4, S_{20} bacterial catalase = 11 0 *S*) The fraction of slower moving material ($S_{20,w}$ = 2 7 *S*) corresponded well with the percentage of compound II in the solution (for example, 15 per cent slower moving material for the experiment shown in Fig 1, corrected for sectorial dilution) We therefore assign the slower peak to compound II and, if compound II resembles a globular protein, the reduced *S* value implies a much lower molecular weight than that of catalase Using the method of Van Holde[5], we have calculated a diffusion coefficient for compound II of 5 4 × 10^{-7} cm^{2} s^{-1}, which yields a molecular weight of about 5 × 10^{4} (compare ref 4, bacterial catalase 2 3 × 10^{5}) A more detailed quantitative characterization of compound II is in progress and other catalase compounds are also being investigated

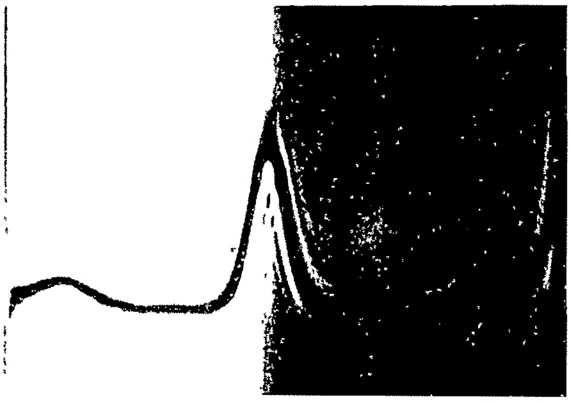

Fig 1 Sedimentation velocity pattern of mixture of catalase and compound II Speed 60,000 r p m , *T* = 22° C, photograph taken 31 min after reaching full speed, bar angle 60° Sedimentation occurs to the right

The occurrence of catalase sub-units has been reported in extreme conditions[6] and after lyophilization[7] Caravaca *et al* [8] have shown that a hepatocatalase sub-unit, which lacks significant catalatic activity, is a powerful peroxidase *in vitro* They have also shown that, *in vivo*, this sub-unit may be of value in the prevention of induced atherosclerosis[9] Our result that compound II is a sub-unit species opens a new chapter in fundamental studies of the catalase system We believe that a variety of sub-unit species play important parts in catalase action and our evidence to support this more general proposition will be published in detail elsewhere

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[1] Suggett, A , thesis, Univ Newcastle upon Tyne (1967)
[2] Bonnichsen, R , Chance, B , and Theorell, H , *Acta Chem Scand* , **1**, 685 (1947)
[3] Chance, B , and Herbert, D , *Biochem J* , **46**, 402 (1950)
[4] Cecil, R , and Ogston, A G , *Biochem J* , **43**, 205 (1948)
[5] Van Holde, K E , *J Phys Chem* , **64**, 1582 (1960)
[6] Samejima, T , *J Biochem* , Tokyo, **46**, 155, 1101 (1959)
[7] Tanford, C , and Lovrien, R , *J Amer Chem Soc* , **84**, 1892 (1962)
[8] Caravaca, J , and May, M D , *Biochem Biophys Res Commun* , **16**, 528 (1964)
[9] Caravaca, J , Dimond, E G , Sommers, S C , and Wenk, R , *Science*, **155**, 1284 (1967)

Evidence that there are Two Forms of Fluoride in Human Serum

IT has been assumed that there is only one form of fluoride in serum, the inorganic F ion It would therefore seem that either the value for serum fluoride which I found (1 μM) (refs 1 and 2) or that found by Singer and Armstrong (7 5 μM) (ref 3) must be in error While the diffusion method of Singer and Armstrong has been shown to produce erroneous values, the same cannot be said for their ashing and distillation procedure The evidence that the serum fluoride is about 1 µM in a fluoridated community does not rule out the possibility that more fluoride could be made available from serum by ashing, it only indicates that the fluoride in serum able to exchange with added radioactive fluoride can be measured and is 1 µM

To test the hypothesis that ashing makes more fluoride available, 10 ml samples of serum were ashed with a fixative and the fluoride was diffused from the ash at room temperature The fixative, 5 ml of a solution of 1 M $MgCl_2$ and 1 mM Na_2CO_3, was shown in experiments using standard solutions or serum to retain 94–99 per cent of radioactive fluoride at an ashing temperature of 600° C for 3 h The fluoride present in the neutralized diffusate was measured by the fluoride electrode[4] and then by the morin–thorium reagent[1] The readings in serum were compared with bracketing standards which had been similarly handled Thus the blanks, standards and samples had identical ionic strengths and pH for measurement by the fluoride electrode

As shown in Table 1, there is excellent agreement between the findings with the fluoride electrode and those employing the morin–thorium reagent The values obtained are about 5 µM, which are considered by Singer and Armstrong to be within normal limits by their ashing procedure, but are much greater than those shown in brackets, found by my diffusion method (25° C), which are all less than 1 µM The blank value from diffusion was usually about 0 15 µM and the blank from the fixative and ashing 0 8–1 0 µM, that is, 0 2 of the amounts of fluoride being analysed

Using radioactive fluoride as a control of completeness of recovery, the results of the fluoride electrode were compared with those of the morin–thorium reagent after direct diffusion of fluoride from 10 ml aliquots of serum Identical values (0 9 µM) were obtained, the details are being prepared for publication elsewhere as part of the validation of the new method Comparisons of measurements of serum fluoride with the fluoride electrode and with the morin–thorium reagent after diffusion at 60° C as recommended by Singer and Armstrong show a marked discrepancy, 1 5 ± 0 5 µM and 10 3 ± 2 2 µM, respectively (eight samples in each, $\pm S\ E$), confirming earlier findings of false positive values generated by diffusion at 60° C

Commercial human gamma globulin was included in the third experiment of Table 1 because it provides a control against the possibility that ashing of protein allows more contamination either from the platinum crucibles or from the air Four ml of the gamma globulin solution was used/sample because the protein was more concentrated (16 5 per cent) than the protein in serum (7 per cent) Small quantities of fluoride (about 1 µM) were found, much less than the increment following the ashing of serum or plasma

Preliminary data on the distribution of the extra fluoride in serum are shown in Table 2 The morin–thorium reagent was used to measure the fluoride after diffusion at 25° C either directly or after ashing Extra fluoride seems to be associated with the albumin and cannot be ultrafiltrated Concentrating the serum proteins concentrated the extra fluoride in proportion

Table 1 COMPARISON OF FLUORIDE CONCENTRATION (µM) AFTER ASHING USING MORIN–THORIUM REAGENT AGAINST FLUORIDE ELECTRODE

	M–T reagent	F electrode
Exp 1 Serum	5 9	5 8
(0 8 µM)*	4 4	4 5
	5 5	5 6
Exp 2 Plasma	4 8	4 0
(0 5 µM)*	4 3	3 7
	4 4	3 7
Exp 3 Serum	4 3	4 2
(0 8 µM)*	3 9	4 2
	4 2	4 5
Gamma globulin	0 8	1 5
16 per cent protein (0 8 µM)*	1 0	1 5

* Average values from non-ashed samples by my method

Table 2 FLUORIDE CONCENTRATION (µM) IN VARIOUS COMPONENTS OF SERUM

		Ashed	Not ashed
Exp 4	Gamma globulin	1 8	1 0
	16 per cent protein	1 0	0 6
	Albumin	8 8	1 0
	25 per cent protein	8 5	0 9
	Plasma	3 8	0 6
	7 per cent protein	3 5	
Exp 5*	Serum ultrafiltrate	1 3	1 1
		1 2	1 1
	Serum, volume reduced to	8 3	0 9
	one half by ultrafiltration	9 5	0 9

* Same serum used for Exp 3 and 5

These results are consistent with the hypothesis that there are two forms of fluoride in serum, exchangeable and non-exchangeable

In 1950, Smith, Gardner and Hodge[5] found normal values of 1 7 µM for serum fluoride in a fluoridated community, implying that they also were measuring only exchangeable fluoride They distilled fluoride from blood with H_2SO_4 at 135° C and then ashed the distillate[6] If in fact there is a non-exchangeable fluoride in serum, it did not break down or diffuse under these conditions, implying a large stable molecule These findings are consistent with the presence of a fluorocarbon molecule

There seems to have been very little consideration of this possibility in any biological work Peters[7] found that fluoroacetate is synthesized by certain toxic plants, but that it is not a general phenomenon His work, however, leaves open the possibility of other forms of organically bound fluorine

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[1] Taves, D R , *Nature*, 211, 192 (1966)
[2] Taves, D R , *Nature*, 215, 1380 (1967)
[3] Singer, L , and Armstrong, W D , *J App Physiol* , 15, 508 (1960)
[4] Frant, M S , and Ross, J W , *Science*, 154, 1553 (1966)
[5] Smith, F A , Gardner, D E , and Hodge, H C , *J Dental Res* , 29, 596 (1950)
[6] Smith, F A , and Gardner, D E , *J Dental Res* , 30, 182 (1951)
[7] Peters, R , and Shorthouse, M , *Nature*, 202, 21 (1964)

New Form of Diphtheria Toxin

ULTRACENTRIFUGAL studies have shown that purified diphtheria toxin is a single protein with a molecular weight of 64,500 and an $S_{20,w}$ of 4 2 (ref 1) In this communication, I report experiments with a sample of toxin that contains the 4 2 S molecule, and an unusual species with a higher sedimentation constant These two toxin molecules have been separated by disc electrophoresis on polyacrylamide gels, by ultracentrifugation and by chromatography on a thin layer of 'Sephadex *G*-100'

Partially purified diphtheria toxin (supplied by Mr Leo Levine of the Massachusetts Department of Public Health, Boston) was further purified by fractionation with ammonium sulphate The fraction obtained between 45 and 60 per cent saturated ammonium sulphate was chromatographed on DEAE-cellulose as previously described[2] The purified toxin thus obtained contained 60 *MLD*/Lf and 2 6 µg of protein/Lf Its specific activity compares favourably with that reported by others[1,3] for highly purified toxin preparations

Electrophoresis on polyacrylamide disc gels was performed at room temperature The 7 5 per cent polyacrylamide gels were run in a *tris* glycine buffer, pH 8 9, at 3 3 mA/tube for 2 h[4] After electrophoresis, the gels were fixed with 10 per cent trichloroacetic acid (TCA) and

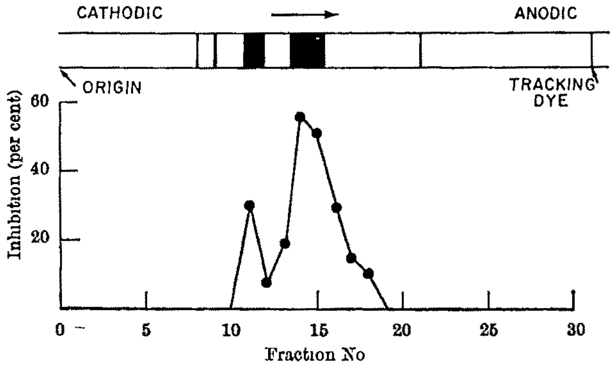

Fig 1 Line drawing of a disc electrophoresis of purified diphtheria toxin run on a 7 5 per cent polyacrylamide gel and stained as described in the text A duplicate, unstained gel was sliced into thirty thin fractions and the protein in each fraction eluted into a phosphate buffer containing BSA An aliquot of each eluate was tested for its ability to inhibit poly-U-directed incorporation of ^{14}C-phenylalanine into TCA precipitable material in a cell-free system derived from rabbit reticulocytes The results are expressed in terms of the percentage inhibition of the level of ^{14}C-phenylalanine incorporation into TCA precipitable material exhibited by the control to which no toxin or eluate was added

stained with Coomassie blue[5] As shown in Fig 1, two principal bands and three lesser bands are visible Eighty per cent of the toxic activity can be recovered from a duplicate, unstained gel after electrophoresis The gel was first chilled to 0° C and sliced into approximately thirty thin sections Toxin was eluted by shaking each slice overnight at 25° C in a 2 mM potassium phosphate buffer, pH 7, containing 100 µg/ml bovine serum albumin (BSA) The eluate from each fraction was tested for its ability to inhibit incorporation of amino-acids in a cell-free rabbit reticulocyte system[2,6] All assays were performed in the presence of 0 03 mM NAD, which is needed for toxic activity in the cell-free system[2,6] As shown in Fig 1, there are two peaks of inhibitory activity (fractions 11 and 14) which correspond to the two heavily stained bands on the gel A comparison of fractions 11 and 14 shows that fraction 14 not only stains more darkly than fraction 11 but also contains more inhibitory activity The eluates from fractions 11 and 14 were also tested for their ability to kill guinea-pigs The results of these routine *MLD* determinations showed that the ratio of *MLD*/Lf of each fraction is approximately that of the mixture

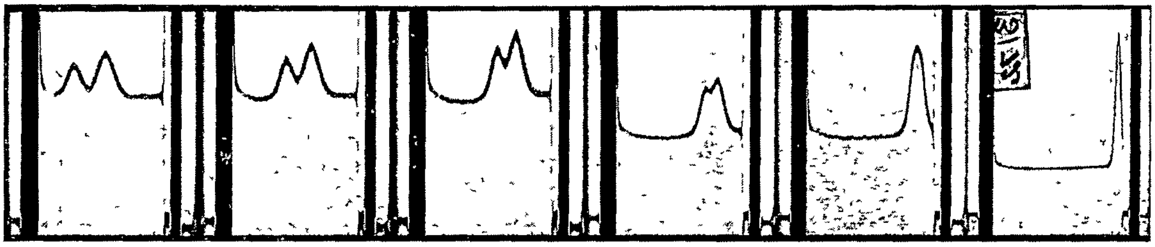

Fig 2 Schlieren patterns from ultracentrifugal sedimentation of purified diphtheria toxin, 8 mg/ml, in 0 01 M potassium phosphate buffer, pH 7 From right to left 19, 35, 51, 67, 83 and 99 min after running at 60,000 r p m

Ultracentrifugal analysis of a solution containing 8 mg/ml diphtheria toxin in 0 01 M potassium phosphate buffer, pH 7, was performed at 60,000 r p m and 25° C As shown in Fig 2, this preparation of toxin exhibits two peaks with uncorrected sedimentation constants of 4 6 and 6 8 These peaks sediment independently of one another The areas under the peaks show that the heavy component is present in lower concentrations than the light one The ultracentrifugal pattern is not altered by the presence of 1 5 mM NAD

Chromatography of this preparation of toxin was performed on a thin layer of 'Sephadex *G*-100' superfine[7] Approximately 40 µg of toxin was spotted on the 'Sephadex' thin layer which was developed with 0 01 M *tris* buffer, pH 7 1, for 6–8 h at 25° C On completing the chromatography, the 'Sephadex' was overlaid with a sheet of dry Whatman 3 *MM* filter paper After 10 min at 25° C, the paper was dried and sprayed with ninhydrin As shown in Fig 3, the toxin was resolved into two ninhydrin-positive components The spot obtained from the slow component was always darker than that from the fast one The fast component in chromatography and in ultracentrifugation thus probably corresponds to the slow one in electrophoresis

A second sample of toxin obtained from the Massachusetts Department of Health and purified in the same way as the preparation described gave identical results on 'Sephadex' On the other hand, a sample of crude toxin supplied by Dr H Devlin of Parke, Davis and Co showed only the usual slow component after chromatography on 'Sephadex *G*-100' (Fig 3)

The unusual, fast component can be completely converted to the slow one by treating the toxin with the sulphydryl reagent, dithiothreitol (DTT) A sample of toxin (250 µg) was incubated overnight at 25° C in 0 01 M *tris* buffer, pH 7 3, containing 0 33 M DTT and 0 08 M EDTA After this treatment, only the slow component was detected following chromatography on 'Sephadex *G*-100' (Fig 3) The fact that the fast component was converted to the slow one by DTT implies that it may be a polymer composed of two or more 4 2 S components held together by disulphide bonds Raynaud *et al*[1] have shown that 4 2 S toxin has no free sulphydryl groups

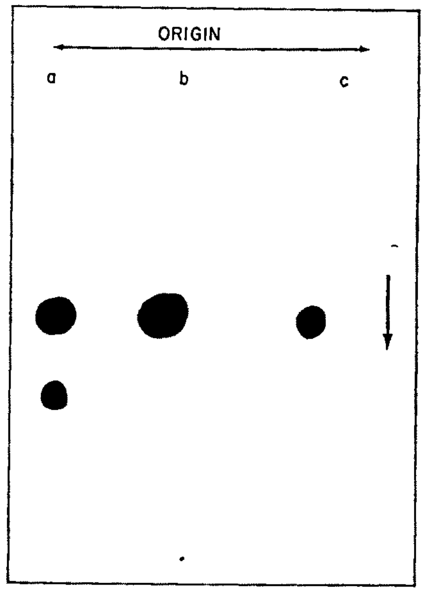

Fig 3 Chromatography of diphtheria toxin on a 12 × 20 cm thin layer of 'Sephadex *G*-100' superfine (*a*) Purified toxin obtained from the Massachusetts Department of Health, (*b*) toxin from (*a*) that has been treated overnight with 0 33 M DTT and 0 08 M EDTA before being chromatographed, (*c*) toxin obtained from Parke, Davis and Co

but does have two disulphide bonds. It is possible that in certain conditions one or both of these disulphides is able to form a disulphide bridge between two 4.2 *S* molecules.

The unusual, heavy form of toxin reported here might have been formed as a result of mutation either in the *Corynebacterium diphtheriae* or in its lysogenic phage, or as a result of unusual growth conditions during toxin synthesis.

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Received January 22, 1968.

[1] Raynaud, M., Bizzini, B., and Relyveld, E., *Bull. Soc. Chim. Biol.*, **47**, 261 (1965).
[2] Goor, R. S., and Pappenheimer, jun., A. M., *J. Exp. Med.*, **126**, 899 (1967).
[3] Pope, C. G., and Stevens, M. F., *Brit. J. Exp. Pathol.*, **39**, 139 (1958).
[4] Reisfeld, R. A., and Small, jun., P. A., *Science*, **152**, 1253 (1966).
[5] Chrambach, A., Reisfeld, R. A., Wyckoff, M., and Zaccari, J., *Anal. Biochem.*, **20**, 150 (1967).
[6] Collier, R. J., and Pappenheimer, jun., A. M., *J. Exp. Med.*, **120**, 1019 (1964).
[7] Morris, C. J. O. R., *J. Chromatog.* **16**, 167 (1964).

Amino-acid Composition of "Tubulin" constituting Microtubules of Sperm Flagella

PHYSICOCHEMICAL characterization of components of flagella and cilia is essential for an understanding of the mechanisms underlying flagellar and ciliary movement. The so-called "9+2" fibres (microtubules) are one of the chief ultrastructures in both flagella and cilia, and can be isolated with minimum contamination of other components[1,2].

Microtubules were obtained from sperm flagella of the sea-urchins *Pseudocentrotus depressus* and *Anthocidaris crassispina*. Semen was collected by injecting 0.5 M KCl into the body cavity, and diluting with artificial sea water free from calcium and magnesium. The suspension of sperm was subjected to mild sonication, and centrifuged at 900*g* for 10 min to separate detached flagella from heads and mid-pieces. The precipitate was washed with this solution, and the combined supernatants were centrifuged first at 900*g* for 10 min to remove any remaining heads and mid-pieces, and then at 15,000*g* for 10 min to collect flagella. At this stage, the preparation showed almost no cytochrome oxidase activity. The isolated flagella were treated with a 0.5 per cent digitonin solution to remove cell membranes, centrifuged at 20,000*g* for 10 min and then washed twice with 30 mM *tris* HCl, *p*H 8.2, and 2.5 mM $MgCl_2$ solution. The microtubules were isolated from the flagellar preparation by two different methods—dialysis of the preparation against 1 mM *tris* HCl, *p*H 8.2, and 0.1 mM EDTA solution for 2 days, followed by centrifuging at 100,000*g* for 15 min and extraction of the preparation with Weber–Edsall solution for 2 days, followed by centrifuging at 20,000*g* for 10 min. The precipitates obtained by these two methods were found under the electron microscope to consist exclusively of microtubules. The isolated microtubules were then suspended in 10 mM *tris* HCl, *p*H 8.2. The suspensions were dialysed against distilled water, lyophilized and kept in a deep freezer until amino-acid analyses were carried out.

Table 1 shows the results of the analyses of the component protein of microtubules. The amount of tryptophan was estimated from the ultraviolet absorption spectra of the protein solutions[3]. Half cystine was determined by measuring the amount of cysteic acid obtained by performic acid oxidation before hydrolysis with 6 N HCl. No significant difference was observed between the results obtained by the two methods, or between the results obtained with the two different species. The amino-acid residues accounted for 75–80 per cent of the total weight of the samples.

If we take the amount of half cystine and of tryptophan as a unit, the minimum molecular weight of the protein is calculated to be about 7,000. Using the protein isolated from the mitotic apparatus of sea-urchin eggs, Sakai[4] found that the dimer (3.5*S* particle) of the protein contained six SH groups and one S–S group. The spindle fibres of the mitotic apparatus are also microtubules, and are similar to the "9+2" micrtubules of flagella and cilia. When the minimum molecular weight is multiplied by eight, the resulting value is about 56,000. The molecular weight of the 3.5*S* particle of the mitotic apparatus was reported to be 68,700, and that of the monomer (2.5*S* particle) to be 34,700. Furthermore, Renaud *et al.*[5] reported a molecular weight of 60,000 for the constituent unit of microtubules isolated from cilia of *Tetrahymena*. Whether the value of about 60,000 is the molecular weight of the monomer or of the dimer of the microtubule protein has yet to be determined.

A comparison of amino-acid composition among structural proteins concerned with cell movement is illustrated in Fig. 1. The distances from the centre of the star to each angle on the outline correspond to the proportions of individual amino-acids. Sixteen amino-acids, excluding tryptophan and half cystine, are arranged from the top of each star clockwise according to the order shown in Table 1. It is clear that the amino-acid composition of the microtubule protein (5) is quite similar to that of invertebrate (insect flight muscle)

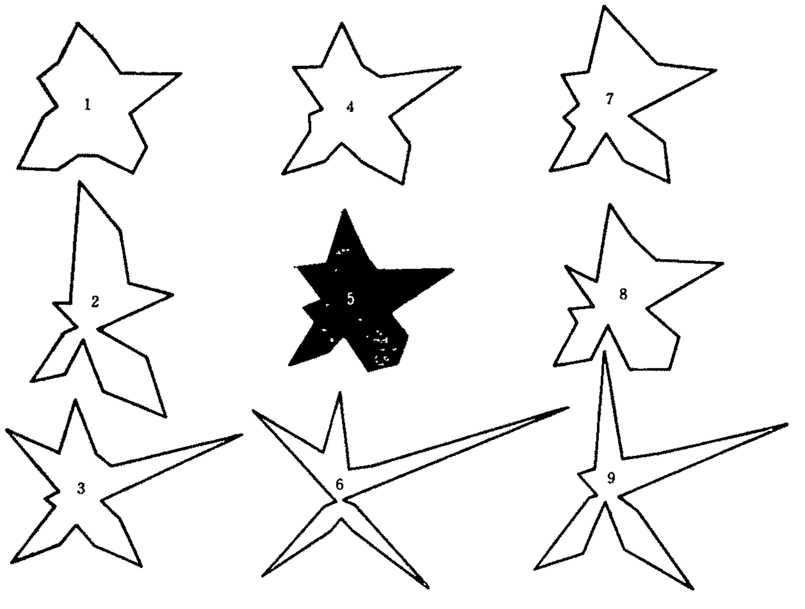

Fig. 1. Comparison of amino-acid composition among several structural proteins. 1, Rabbit actin[6]; 2, bacterial flagellin[7]; 3, rabbit myosin[6]; 4, insect actin[6]; 5, "tubulin"; 6, tropomyosin[6]; 7, acetic acid extract of *Tetrahymena* cilia[5]; 8, ethanol-insoluble fraction from 7[5]; 9, ethanol-soluble fraction from 7[5].

Table 1 AMINO-ACID COMPOSITION OF "TUBULIN" (MOLES PER 1,000 MOLES RECOVERED AMINO-ACIDS)

Amino-acid	*Anthocidaris crassispina**	*Pseudocentrotus depressus**	*Pseudocentrotus depressus*†
Asp	105	102	102
Thr	58	58	58
Ser	50	52	53
Glu	121	120	118
Pro	46	48	46
Gly	69	72	73
Ala	76	70	72
Val	65	59	60
Met	20	37	33
Ileu	44	49	43
Leu	77	77	80
Tyr	31	29	31
Phe	41	39	45
His	22	23	22
Lys	61	65	62
Arg	50	52	49
1/2 Cys	—	29	20
Try	—	17	16

* After dialysis of digitonin treated flagella versus 1 mM *tris* HCl, *p*H 8 2–0 1 mM EDTA

† After extraction of the same material with Weber–Edsall solution

actin (4), except for a few amino-acids On the other hand, the composition is entirely different from that of flagellin which constitutes bacterial flagella (2), even though the microtubules of sperm flagella resemble bacterial flagella, as revealed under the electron microscope It is not strange that the acetic acid extracts of *Tetrahymena* cilia (7), especially its ethanol-insoluble fraction (8) (ref 8), have amino-acid compositions similar to that of the microtubule protein

Although the protein has other resemblances to actin, such as a super-precipitation-like phenomenon induced by ATP if combined with rabbit skeletal muscle myosin in low ionic strength[2], it differs from actin in the following ways The protein does not activate myosin ATPase in low ionic strength, nor is a viscosity drop observed after the addition of ATP to a mixture of myosin and the protein in high ionic strength[2] Furthermore, the protein contains guanosine tri and di-phosphate instead of adenosine tri and di-phosphate[9,10] The appearance of the microtubules is also different from that of actin filaments or thin filaments of muscle From these facts, we believe that the microtubule constituent is a different protein, for which we propose the name "tubulin"[11]

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[1] Gibbons, I R , *Arch Biol* (Liège), **76**, 317 (1965)

[2] Mohri, H , Murakami S , and Maruyama, K , *J Biochem* , **61**, 518 (1967)

[3] Goodwin, T W , and Morton, R A , *Biochem J* , **40**, 628 (1946)

[4] Sakai, H , *Biochim Biophys Acta*, **112**, 132 (1966)

[5] Renaud, F L , Rowe, A J , and Gibbons, I R , *J Cell Biol* , **31**, 92*A* (1966)

[6] Kominz, D R , Maruyama, K Levenbook, L , and Lewis, M S , *Biochim Biophys Acta*, **63**, 106 (1962)

[7] Asakura, S , *J Biochem Tokyo*, **38**, 797 (1966)

[8] Watson, M R , Alexander, J B , and Silvester, N R , *Exp Cell Res* , **33**, 112 (1964)

[9] Mohri, H , and Yanagisawa, T , *Zool Mag* , **76**, 276 (1967)

[10] Stevens, R E , Renaud, F L , and Gibbons, I R , *Science*, **156**, 1606 (1967).

[11] Yanagisawa, T , Hasegawa, S , and Mohri, H , *Exp Cell Res* (in the press)

Nor$_2$Chlorpromazine Sulphoxide and 3,4-Dimethoxyphenethylamine

SEVERAL workers have found a spot isographic with 3,4-dimethoxyphenethylamine (DMPEA) in urine chromatograms of some schizophrenic patients but never or rarely in those of non-schizophrenic persons[1–3] A metabolite of chlorpromazine (CPZ), however, noted[4] to have been identified as nor$_2$chlorpromazine sulphoxide (N$_2$CPSO)[6], causes a spot close to or isographic with DMPEA in the chromatographic system originally used to detect the spots apparently diagnostic of schizophrenia[4,5] (referred to below as system 1) If a phenothiazine metabolite caused the apparently diagnostic spots (the pink spots) the following conditions must be fulfilled (1) The R_F for the metabolite in system 1 should be almost identical to that of DMPEA because fifteen pink spots were originally studied and even a small consistent difference in R_F between these spots and authentic DMPEA would have been detectable (2) The metabolite must be isographic with DMPEA in the two-way system used by Kuehl *et al* [2], for they reported that certain samples found positive by Friedhoff and van Winkle showed a ninhydrin positive spot isographic with DMPEA in their system (3) The metabolite must persist in urine after the parent drug has been stopped because all of the original patients and many of those found subsequently to have pink spots had been withdrawn from drugs for at least 2 weeks (4) The metabolite must give a pink spot with modified Ehrlich's reagent (DABA) following ninhydrin-pyridine and a negative reaction with DABA alone because this test was applied to the original spots[1] Also, an explanation must be found for the fact that material isolated from the original spots was indistinguishable from authentic DMPEA in several sensitive tests of identity[1,11] and for the fact that Takesada *et al* , who were aware of the problem of confounding drug metabolites and particularly N$_2$CPSO, found pink spots in the urine of schizophrenic patients and others as well[5]

(1) The spot caused by administration of CPZ is virtually isographic with DMPEA in system 1 Twenty-four hour urine samples were collected from five patients on CPZ (about 400 mg/day) Samples (500 ml) were extracted and chromatographed in butanol acetic acid water (4 1 1, system 1) according to the procedure of Friedhoff and Van Winkle[1] (omitting the BaCl$_2$ step) Three samples were also collected after substitution of other phenothiazines for CPZ (17 days being the maximum period off CPZ) Extracts equivalent to 20–50 ml of urine (on CPZ) and 150–250 ml (off CPZ) were chromatographed and spots located with ninhydrin[2] or ninhydrin-pyridine[1] Duplicate strips were sprayed with sulphuric acid 50 per cent v/v ethanol 4 1, which turns most non-phenolic CPZ metabolites pink In each case on CPZ dense spots were found which were purple with ninhydrin and pink with 50 per cent sulphuric acid Mean difference in R_F between these spots and DMPEA was 0 01 (3 patients, 10 pairs of spots on CPZ, 4 pairs off CPZ) (2) A sample of 15 μg of DMPEA enlarged or intensified the spot in the system of Kuehl *et al* [2] Also authentic N$_2$CPSO and DMPEA were found to be inseparable in this system (spotting a mixture of 10 μg each) (3) A ninhydrin positive spot isographic with DMPEA in system 1 was found in all specimens obtained after CPZ had been stopped, but the significance of this finding is limited by the fact that other phenothiazines had been substituted for CPZ It was possible to overcome this limitation, however, because a metabolite of CPZ isographic with DMPEA in chromatographic systems other than system 1 had been reported to persist in urine for up to 25 days after the withdrawal of CPZ[6,7] (in the absence of substitute medication) and we were able to show that this metabolite was N$_2$CPSO (15 μg of DMPEA or N$_2$CPSO mixed with the unknown enlarges or intensifies the spot in both of the other two-way systems and DMPEA and N$_2$CPSO spotted as a mixture of 10 μg each are also not separated) There is an unusual chromatographic similarity between DMPEA and N$_2$CPSO because the three two-way thin-layer systems in which we have found them inseparable easily resolve closely related metabolites of CPZ Our studies strongly support the previous identification of the CPZ metabolite isographic with DMPEA in system 1 as N$_2$CPSO because an eluate of the spot produced a streak isographic with N$_2$CPSO in a second

chromatographic system (isopropanol 30 per cent ammonia water 20 1 2—system 2) and an eluate of this twice chromatographed material (unsprayed) gave an ultraviolet spectrum perfectly characteristic of N_2CPSO Also we recover at least 50 per cent of added N_2CPSO which normally accounts for 2 4–5 0 per cent of an administered dose of CPZ[8–10] and there is no other spot on our chromatograms of sufficient density to be N_2CPSO A slight discrepancy between the unknown spot and authentic N_2CPSO in system 1 was reduced if authentic N_2CPSO was added to an extract before chromatography or if the unknown was eluted and re-chromatographed in system 1 We can therefore conclude that the spot studied by us is N_2CPSO and that it persists in urine for at least 25 days after the withdrawal of CPZ (4) In concentrations greater than 5 µg, N_2CPSO gives a pink spot with DABA alone in contrast to DMPEA With 2 5 µg on a spot, however, authentic N_2CPSO gives only a very faint reaction with DABA alone, and if the spot is developed in system 1 before dipping in DABA the reaction with DABA is negative The purple colour with ninhydrin-pyridine, however, is definite with 2 5 µg both on an undeveloped spot or after development in system 1, and the spot turns pink with subsequent treatment with DABA

N_2CPSO, however, is not the material isolated by Friedhoff and Van Winkle Their unknown was indistinguishable from DMPEA in the two systems used by them to study eluates of the spots[1] whereas N_2CPSO streaks in these systems and clearly separates from DMPEA in one of them (system 2) Also the ultraviolet absorption spectrum for N_2CPSO carried through the extraction and chromatography has maxima at 240, 276, 300 and 343 whereas the unknown of Friedhoff and Van Winkle had maxima only at 234 and 279 (ref 1) (maxima for DMPEA were found by us to be 232 and 280) and the melting point of the unknown material of Friedhoff and Van Winkle is 152–154° C[11] (virtually identical to DMPEA) while that of N_2CPSO HCl is 300° C Finally, DMPEA has been identified in human urine by mass spectrometry[12] and the acid metabolite of DMPEA, 3,4-dimethoxyphenylacetic acid (DMPAA), has also been found in human urine using specific methods[7,13] It is therefore likely that DMPEA was present on the spot and isolated by Friedhoff and Van Winkle There is, however, considerable evidence suggesting that DMPEA may not cause a visible pink spot diagnostic of schizophrenia (*a*) Those workers other than Friedhoff and Van Winkle who have found what they thought was DMPEA[2,7,14] have not found it in amounts capable of causing a definitely detectable spot in system 1 (spotting a 100 mg creatinine equivalent as did Friedhoff and Van Winkle) (*b*) DMPAA has been found in equal amounts in schizophrenic and non-schizophrenic persons[7,13] (*c*) Those workers who have studied patients never treated with drugs[15] or who have been aware of confounding spots caused by phenothiazine metabolites[6,16–18] have either not found apparently diagnostic spots or have found the characteristic spots only in very low frequency in schizophrenic urine and have shown them not to be preponderantly DMPEA Our findings and all of those cited are consistent, however, with the possibility that N_2CPSO or a phenothiazine metabolite with similar properties caused spots apparently diagnostic of schizophrenia, but that DMPEA was present on the spot as well in low amounts and was isolated by Friedhoff and Van Winkle, leading them to believe that the apparently diagnostic spots were DMPEA If this occurred, it is not clear why N_2CPSO did not appear in the systems used to study eluates of the original spots N_2CPSO streaks in these systems, however, and if present in low concentrations originally might not have produced a visible spot or if eluates were combined might have caused a streak which looked like "background" The postulated trace amounts of DMPEA may have become more visible in the secondary systems in the absence of other constituents of the extract, or eluates may have been combined, possibly accounting for the presence of visible spots in the secondary systems but not in the chromatograms of non-schizophrenic persons This, of course, is speculation and the circumstantial evidence adduced here does not definitely exclude the possibility that schizophrenic patients excrete DMPEA in greater amounts than normals

Takesada *et al*[5] assumed that the spot which they found to be isographic with DMPEA (rather than N_2CPSO which was nearby) and which they found in a high percentage of normals was the one studied by Friedhoff and Van Winkle But subsequently it was confirmed that the pink spot never, or only rarely, occurred in the urine of non-schizophrenic persons[2,3] and it was shown that the substance studied by Takesada *et al* is only poorly extracted using the methods of Friedhoff and Van Winkle and is not DMPEA[6] It disappears with dietary restriction and may not be more prevalent in the urine of schizophrenic patients than others[19]

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[1] Friedhoff, A J, and Van Winkle, E, *J Nerv Ment Dis*, **135**, 550 (1962)
[2] Kuehl, F A, Hichens, M, Ormond, R E, Meisinger, M A P, Gale, P H, Cirillo, V J, and Brink, N G, *Nature*, **203**, 154 (1964)
[3] Bourdillon, R E, Clarke, C A, Ridges, A P, Sheppard, P M, Harper, P, and Leslie, S A, *Nature*, **208**, 453 (1965)
[4] Closs, K, Wad, N, and Ose, E, *Nature*, **214**, 483 (1967)
[5] Takesada, M, Kakimoto, Y, Sano, I, and Kaneko, A, *Nature*, **199**, 203 (1963)
[6] Pind, K, and Faurbye, A, *Acta Psychiat Scand*, **42**, 246 (1966)
[7] Vogel, W H, Ahlberg, D C, and Horwitt, M K, *Intern J Neuropsychiatry*, **3**, 292 (1967)
[8] Goldenberg, H, and Fishman, V, *Proc Soc Exp Biol and Med*, **108**, 178 (1961)
[9] Posner, H S, Culpan, E, and Levine, J, *J Pharm Exp Ther*, **141**, 377 (1963)
[10] Rose, R M, DiMascio, A, and Klerman, G L, *J Psychiat Res*, **2**, 299 (1964)
[11] Friedhoff, A J, and Van Winkle, E, *Nature*, **202**, 520 (1964)
[12] Creveling, C R, and Daly, J W, *Nature*, **216**, 190 (1967)
[13] Kuehl, F A, Ormond, R E, and Vandenheuvel, W J A, *Nature*, **211**, 606 (1966)
[14] Sen, N P, and McGeer, P L, *Biochem Biophys Res Commun*, **14**, 227 (1964)
[15] Williams, C H, Gibson, J E, and McCormick, W O *Nature*, **211**, 1195 (1966)
[16] Boulton, A A, and Felton, C A, *Nature*, **211**, 1404 (1966)
[17] Bell C E, and Somerville, A R, *Nature*, **211**, 1405 (1966)
[18] Boulton, A A, Pollit, R J, and Majer, J R, *Nature*, **215**, 132 (1967)
[19] Von Studnitz, W, and Nyman, G E, *Acta Psychiat Scand*, **41**, 117 (1965)

Effects of Very Large Doses of Penicillin G given Orally to Rats

Nimni and Bavetta[1] reported a connective tissue disorder in rats fed penicillamine The disorder was reflected by decreased breaking strength of unwounded and wounded skin, decreased collagen synthesis in polyvinyl sponge implants and a diminished proportion of insoluble to soluble collagen in such granulomas We have studied this phenomenon in rats and guinea-pigs[2–4] and have likened it to the lathyritic state induced by feeding β-amino propionitrile, a view also expressed by Nimni[5] Penicillamine can be derived[6] from both the *in vitro* and the *in vivo* hydrolysis of penicillin, so we set out to determine whether the feeding of very large doses of penicillin would cause a collagen defect similar to that caused by penicillamine

Twenty-two Cobbs rats weighing 120–150 g were fed freely on a diet of commercial chow for 10 days, paired and assigned to one of two groups The rats were anaesthetized with pentobarbital and standard paravertebral skin incisions were made and polyvinyl alcohol sponges implanted subcutaneously. The wounds were sutured and the animals were then fed freely on experimental diets. One group received ground chow supplemented with 25 mg of pyridoxine, the other received a similar diet which also contained 50 g of potassium penicillin *G*/kg of dry food. After 2 weeks the rats were killed, clipped and their pelts removed. Each pelt was cut into seven single strips 0.5 cm wide with a multi-blade cutting device[7]. Adjacent strips were measured for breaking strengths immediately or after immersion in 10 per cent buffered formalin[8]. Unwounded ventral abdominal skin was cut into a single strip and its breaking strength measured immediately[7]. Student's *t* test for paired samples was used to calculate the statistical significance of the data.

The unwounded skin of the animals treated with penicillin was weaker breaking strength 1,289±149 g as opposed to 1,600±146 g for the controls ($P<0.1$). The mean breaking strength of the wounds of rats treated with penicillin was 478±40.2 g, however, compared with 603±43 g for the controls, this 21 per cent decrease in breaking strength was significant at the 0.001 level. Fixation of the wounds in formalin resulted in mean breaking strengths of 2,225 g for both groups.

Penicillin produced alterations in the intestinal flora (unpublished results from this laboratory) and it occurred to us that the effects of penicillin on wound strength might be reflexions of the altered bacteriological status. Accordingly, an experiment was conducted using twenty-two Fischer germ-free rats. The conditions were similar to those in the previous experiment with several exceptions (1) the animals were pair fed, (2) whereas the intake of potassium between groups was not controlled in the first experiment, it was regulated in the second experiment by supplemental administration of potassium acetate, bicarbonate and citrate to the group not receiving potassium penicillin *G*, (3) the animals were killed 3 weeks after wounding. The wounds of the animals treated with penicillin were again substantially weaker than the controls (breaking strength 1,508±63 g as opposed to 2,196±111 g, $P<0.001$) as was the unwounded abdominal skin (2,672±174 g as opposed to 3,120±127, $P<0.05$). Fixation in formalin resulted in breaking strengths of 3,892±206 and 4,381±227 ($P<0.2$).

The experiment with germ-free rats implies that the decreased wound strength of rats treated with penicillin is not dependent on the changes in microbial flora.

In both experiments, the oral administration of potassium penicillin *G* resulted in a significant decrease (21 per cent and 30 per cent) in the breaking strength of wounds in rat skin tested immediately after killing. After immersion of adjacent wound strips in 10 per cent formalin, the breaking strength of the wounds was similar in both groups. The tanning effect of formaldehyde on collagenous proteins was described by Gustavson[9] and its effect on the breaking strength of wounds was reported by Levenson *et al.*[8] The "correction" by formaldehyde of the decreased breaking strength of the wound of the rats treated with penicillin is evidence that the total amount of collagen present in the wounds of both groups is similar and that the defect is qualitative rather than quantitative—namely, decreased crosslinking of collagen. This is supported by the similar quantities of collagen (hydroxyproline) in the sponge granulomas of both penicillin treated and control animals, and, as will be reported elsewhere, the similar histological appearances of the wounds and sponge granulomas in both groups.

We think that the collagen maturation defect resulting from orally administered potassium penicillin *G* is mediated through one or more of its naturally occurring metabolites, such as penicillamine. Schwartz and Buckwalter[10] and Dennen and Davis[11] have shown that in conditions of *p*H and temperature close to physiological conditions, penicillin undergoes significant degradation even in the absence of enzymes. From the point of view of the stay of ingested penicillin in the acidic environment of the stomach of rats and the mildly alkaline milieu of the remainder of the intestinal tract, appreciable breakdown of benzyl penicillin to benzyl penicilloic acid (cleavage of the lactam), penicillic acid, penicillenic acid, penaldic acid and some penicillamine would be expected.

It is possible that some of these compounds inhibit the crosslinking of collagen because they interact with the active sites (aldehydes) of collagen which are usually involved in crosslinking. Another possible explanation involves the interaction of penicillin degradation products with copper. Penicilloic acids react with heavy metals to yield mercaptides, penicillamine chelates, and phenacetylaminomalonaldehyde. Thus copper would be sequestered in such a reaction. Copper could also be sequestered by the other breakdown products of penicillin catabolism, where copper itself is not contributing to the hydrolysis. It is known that copper deficiency leads to connective tissue disorders related to lathyrism[12] and that the lathyrism induced by β-amino propionitrile can be antagonized by increased copper supplementation[13].

Extremely high doses of penicillin *G* were used in these experiments. These doses are far greater on a relative basis than those used clinically, even for the treatment of patients with certain life-threatening infections. Our data should not be construed as indicating that penicillin as used for the treatment of patients interferes with their collagen metabolism.

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[1] Nimni, M., and Bavetta, L., *Science*, 150, 905 (1965).
[2] Youssef, S., Geever, E., and Levenson, S. M., *Surg. Forum*, 17, 89 (1966).
[3] Geever, E. F., Youssef, S., Seifter, E., and Levenson, S. M., *J. Surg. Res.*, 7, No. 4, 160 (1967).
[4] Berberian, G., Kuyama, T., Geever, E., and Levenson, S. M., *Surg. Forum*, 15, 50 (1964).
[5] Nimni, M., Gert, N., and Bavetta, L. Z., *Nature*, 213, 921 (1967).
[6] Scheinberg, S. H., *J. Chron. Dis.*, 17, 293 (1964).
[7] Geever, E. F., *Proc. Workshop on Wound Healing*, National Academy of Sciences, Washington, DC, 283 (1966).
[8] Levenson, S. M., Crowley, L. V., Geever, E. F., Rosen, H., and Berard, D. W., *J. Trauma*, 4, 543 (1964).
[9] Gustavson, K. L., *J. Amer. Leather Chem. Assoc.*, 43, 741 (1948).
[10] Schwartz, M. A., and Buckwalter, F. H., *J. Pharm. Sci.*, 51, 1119 (1962).
[11] Dennen, D. W., and Davis, W. W., *Antimicrobial Agents and Chemotherapy*, 53 (Amer. Soc. Microbiol., 1961).
[12] Miller, E. J., and Fullmer, H. M., *J. Exp. Med.*, 123, 1097 (1966).
[13] Duffy, J., and Shield, G. S., *Collagen Currents*, 6, No. 7, 246 (Jan. 1966).

Epidermal Iron Pigments in Red Species

FROM the hair of red mongrel dogs and New Zealand red rabbits we have extracted iron pigments which in most respects are similar to the pigments isolated before from red human hair (trichosiderin)[1-3] and Rhode Island Red chicken feathers (feather siderin)[3,4]. These findings suggest that iron compounds play a central part in certain types of red pigmentation. To facilitate studies in other species, a general description of these pigments is presented.

The amounts of extractable pigment depend on the compactness of the horny material, so it is advisable to use at least 4–5 g of hair for extraction. When boiled with 0.1 N HCl, loose rabbit fur and chicken feathers yield a deep red-brown solution within 10 min. The compact human and canine hair requires boiling at least for 1 h in acid to give pale pink to brown–yellow solutions. From rabbit fur and chicken feathers the pigments can also be obtained in mild conditions (weak acids or alkalis at 40° C) as orange-coloured protopigments[4].

The pigments extracted with boiling acids have the following characteristics: at *p*Hs greater than 2 the red extracts turn yellow and can reverse to red; in other words, they behave like indicators. The pigment is precipitated at *p*H 7. From the supernate at *p*H 4·3 another small fraction frequently precipitates on standing. This fraction contains relatively large amounts of the iron-chromophore responsible for the indicator properties of the siderins.

When redissolved in 0·1 N HCl, the pigment can be split with KSCN (2 per cent final concentration) to brown protein which either yields traces of or no iron, and a red supernate which is rich in iron. The latter retains the indicator properties of the original extract, but is dialysable. When the pigment precipitated from this supernate at *p*H 7 is dissolved in 0·1 N NaOH and passed through a column of 'Sephadex *G*-25' it separates into two bands: a more rapidly moving brown protein and an orange-pink chromophore. By precipitation at *p*H 4·3, and repeated passage through 'Sephadex *G*-25', the chromophore can be further purified until it contains no protein.

In man, rabbit and chicken—the species studied so far—the purified chromophore contains 1·4–1·7 per cent iron in the ferric form, very firmly bound to the molecule. The two chromophores analysed (human and rabbit) contain sulphur[2]. The chromophores are stable at all *p*Hs. Their absorption bands in acid solution (maximum about 535 mμ) become less pronounced and shift to shorter wavelengths on alkalinization. The characteristic vivid purple colour in acids, changing reversibly to orange when alkalinized, permits detection in concentrations up to 10^{-6}.

To these pigments the following general structure can be assigned: (chromophore-Fe^{III}) . . . protein. In the siderins the protein part accounts for about 98 per cent of the molecule.

The iron pigments are the principal pigments in red human hair, rabbit fur and chicken feathers. The limited yield of trichosiderin from compact horny structures is caused by gradual destruction during boiling with acids[3,4]. On prolonged extraction, rabbit fur and chicken feathers turn dark grey.

The consistent presence of these unique substances in some red species suggests synthetic pathways involving iron which differ from the classical black melanogenesis. Our findings do not support the theory implicating tyrosinase in the synthesis of human red hair pigments[5]. The alleged role of tyrosinase in this process is based essentially on two observations: the DOPA-positivity, and the purported tyrosinase activity of red melanocytes in the hair matrix; the latter is derived from autoradiographic evidence. The DOPA-positivity can be accounted for by a non-specific catalytic action of the iron pigment on the autoxidation of DOPA[1,6]; ferric ions have a similar effect. The autoradiographic findings obtained with labelled tyrosine could be caused by incorporation of this amino-acid into the protein part of the iron pigment without any further oxidation.

The discovery of a new pigmentary system of phylogenetic antiquity has implications for anthropology, genetics and biochemical studies of nonhaem iron proteins to which these pigments belong. The mild methods with which the protein may be split off from the chromophore could be useful for obtaining the protein part of a melanoprotein and for studying its mode of attachment to the pigmented portion. In preliminary analyses various protein fractions had a remarkably similar amino-acid composition within the same species.

These iron pigments isolated from red hair and feathers of four species are probably widespread in nature and are unlike any known to us.

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[1] Flesch, P., and Rothman, S., *J. Invest. Dermatol.*, **6**, 257 (1945).

[2] Boldt, P., *Naturwissenschaften*, **51**, 265 (1964).

[3] Flesch, P., Esoda, E. J., and Katz, S. A., *J. Soc. Cosmetic Chemists*, **18**, 777 (1967).

[4] Flesch, P., Esoda, E. J., and Katz, S. A., *J. Invest. Dermatol.*, **47**, 595 (1966).

[5] Fitzpatrick, T. B., Brunet, P., and Kukita, A., *The Biology of Hair Growth* (Academic Press, London, 1958).

[6] Flesch, P., *J. Invest. Dermatol.*,

IMMUNOLOGY

Relation between Rat Slow Alpha$_2$-globulin and Alpha$_2$-macroglobulin of Other Mammals

SLOW alpha$_2$-globulin[1] (SA_2G) of the rat is a peculiar serum protein in that it is absent from the serum of the normal, non-pregnant adult rat[1-4] but appears in a number of conditions, including the foetal and neonatal states[3-5], during pregnancy[2-6], during neoplastic growth[2-4,6-9], after interference with the kidney[10,11] and other conditions[12]. On the other hand, alpha$_2$-macroglobulin[13] (A_2MG) is a protein found in the sera of normal, non-pregnant humans[14-16] and other mammals[17-21] but not in the mouse (ref. 22 and my unpublished work). There are indications in the literature that the two materials may be similar despite the divergent situation in the adult.

The concentration of human A_2MG is increased above the normal adult state in at least four situations similar to some of those in which SA_2G appears in the rat: pregnancy[23-25], youth[16,25,26], during neoplastic growth[15,28] and in the nephrotic syndrome[15]. The molecular weights of porcine and human A_2MG are given as 960,000 and 900,000, respectively[21,28], and I calculate the molecular weight of rat SA_2G to be 900,000 to 1,000,000 on the basis of gel exclusion chromatography on 'Bio-Gel *A*15*m*' (BioRad Laboratories, Richmond, California).

In view of these indications of similarity, I have investigated whether or not the two materials are also similar when examined by starch gel electrophoresis, immunodiffusion and immunoelectrophoresis. Rats used, general procedures and vertical starch gel electrophoresis have already been described[4]. SA_2G was caused to appear in the rats, that is, the rats were made positive, by the administration of the lipopolysaccharide (endotoxin) of *Salmonella abortus equi*[29]. Human sera were obtained from four healthy, non-pregnant adults of both sexes or in lyophilized form (Pentex Incorporated, Kankakee, Illinois). For the purpose of this work, no appreciable differences were noted among the sera from the various sources. Other animal sera were also obtained in lyophilized form from Pentex. Antiserum to human A_2MG, produced in rabbits, was that of the Behringwerke, Marburg-Lahn, Germany (Hoechst Pharmaceuticals, Inc., Kansas City, Missouri). Antisera to rat SA_2G were prepared in rabbits by the injection of an impure preparation of SA_2G and complete Freund's adjuvant. The antisera obtained were absorbed to completion with sera from normal, that is, SA_2G negative, rats. The double immunodiffusion method of Ouchterlony[30] was carried out either in Petri dishes of 45 mm diameter or on microscope slides using 1 per cent agar with 1 per cent sodium chloride and 0·1 per cent sodium azide. The gels were examined in a dark-field arrangement and, in some cases, also after drying and staining. Immunoelectrophoresis was carried out with, and according to the instructions for, the model 6800 *A* of the LKB-Produkter AB (Stockholm, Sweden).

Fig. 1 gives representative examples of the results obtained. On the starch gel electrophoresis plate (Fig. 1*a*), sera from man, rabbit, cow and horse all show a band coincident in position with that of SA_2G in the serum of the positive rat, a position that is empty, of course, in the pattern of the serum from the negative rat. These results are in accord with a similar finding by Jacquot-Armand and Guinand[21] concerning human serum. In view of the close phylogenetic relationship between the Rodentia and the Lagomorpha, it is interesting to note that this band is consistently weaker in patterns of rabbit sera than in those from other, non-rodent, mammals.

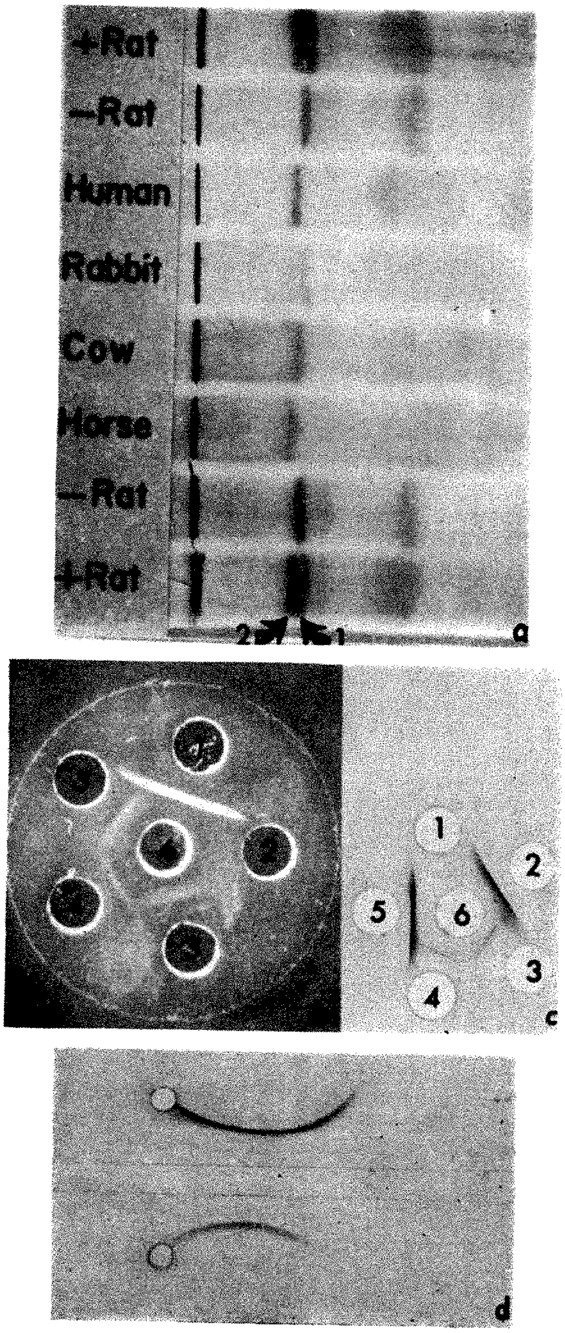

Fig. 1. *a*, Cathodal portion of vertical starch gel electrophoresis plate. Starting wells immediately to the right of the label; migration from left to right. Arrow 1, slow $alpha_1$-globulin; arrow 2, slow $alpha_2$-globulin. *b*, Dark field view of double diffusion plate. Well 1, rat serum containing slow $alpha_2$-globulin; well 2, rat serum not containing slow $alpha_2$-globulin; well 3, bovine serum; well 4, canine serum; well 5, human serum; well 6, antiserum against an impure preparation of rat slow $alpha_2$-globulin absorbed to completion with normal rat serum. *c*, Dried and stained double diffusion plate. Well 1, rat serum not containing slow $alpha_2$-globulin; well 2, human serum; well 3, impure preparation of rat slow $alpha_2$-globulin; well 4, rat serum containing slow $alpha_2$-globulin; well 5, human serum; well 6, antiserum to human $alpha_2$-macroglobulin produced in rabbits. *d*, Dried and stained immunoelectrophoresis plate. Migration from left to right. Top well, human serum; bottom well, rat serum containing slow $alpha_2$-globulin; centre trough, antiserum to human $alpha_2$-macroglobulin produced in rabbits.

Fig. 1*b* demonstrates, first, the specificity of the absorbed antiserum for rat SA_2G in that it will still react with rat serum containing SA_2G but will not do so with serum from a normal, SA_2G negative rat. Second, there is shown the cross-reaction of this specific antiserum with a component in normal human, bovine and canine sera. The spurs formed indicate that the various sera do not contain an immunologically identical but only similar material. This finding is in accordance with similar findings on the A_2MG of other mammals[17,20]. The sera of these three mammals therefore contain a material which is immunologically very similar to rat SA_2G.

These conclusions are supported by the type of result, shown in Fig. 1*c*, obtained when the antiserum in the centre well is commercial anti-human A_2MG. A definite reaction is obtained between this material and the positive rat serum in well 4 while there is no reaction with negative rat serum (well 1). This again demonstrates the specificity of the reaction. That rat SA_2G is immunologically only similar to human A_2MG is again shown by the spur formation at the intersection of the reaction lines with positive rat serum (well 4) and with human serum (wells 2 and 5). An impure preparation of rat SA_2G, used as the injection antigen for the production of the antisera used in Fig. 1*b*, was placed in well 3 and reacted like positive rat serum.

In immunoelectrophoresis also (Fig. 1*d*) positive rat serum and human serum, reacted against commercial anti-human A_2MG, show similar reaction arcs in similar locations.

I conclude from the data in the literature and the experimental evidence described that: (*a*) the SA_2G of the rat is chemically, immunologically and probably functionally similar to the A_2MG of other, non-rodent, mammals and (*b*) the rat, and possibly other rodents, differ from most mammals in that the normal range of concentration of this material in the serum includes or comes very close to zero.

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[1] Beaton, G. H., Selby, A. E., and Wright, A. M., *J. Biol. Chem.*, **236**, 2001 (1961).

[2] Beaton, G. H., Selby, A. E., Veen, M. J., and Wright, A. M., *J. Biol. Chem.*, **236**, 2005 (1961).

[3] Heim, W. G., *Amer. Zool.*, **1**, 359 (1961).

[4] Heim, W. G., *Nature*, **193**, 491 (1962).

[5] Boffa, G. A., Fine, J. M., Jacquot-Armand, Y., Gaudin-Harding, F., and Susbielle, H., *CR Acad. Sci.*, **159**, 1342 (1965).

[6] Weimer, H. E., and Benjamin, D. C., *Amer. J. Physiol.*, **209**, 736 (1965).

[7] Bogden, A. E., *Cancer Res.*, **21**, 1258 (1961).

[8] Boffa, G. A., Fine, J. M., and Zajdela, F., *Cancerologie*, **255**, 803 (1962).

[9] Weimer, H. E., and Benjamin, D. C., *Fed. Proc.*, **23**, 475 (1964).

[10] Heim, W. G., Kerrigan, J. M., and Lane, P. H., *Nature*, **200**, 688 (1963).

[11] Boffa, G. A., Nadal, C., Zajdela, F., and Fine, J. M., *Nature*, **203**, 1182 (1964).

[12] Heim, W. G., and Ellenson, S. R., *Nature*, **208**, 1330 (1965).

[13] Smithies, O., *Biochem. J.*, **61**, 629 (1955).

[14] Schultze, H. E., Gölner, I., Heide, K., Schönenberger, M., and Schwick, G., *Naturforschung.*, **10**b, 643 (1955).

[15] Poulik, M. D., and Smithies, O., *Biochem. J.*, **68**, 636 (1958).

[16] Ganrot, P. O., and Schersten, B., *Clin. Chim. Acta*, **15**, 113 (1967).

[17] Picard, J., Heremans, J., and Vandebroek, G., *Vox Sang.*, **7**, 425 (1962).

[18] Picard, J., Heremans, J., and Vandebroek, G., *Mammalia*, **27**, 285 (1963).

[19] Picard, J., Vandebroek, G., Heremans, J. F., and Defossé, G., *Biochim. Biophys. Acta*, **117**, 111 (1966).

[20] James, K., *Immunology*, **8**, 55 (1965).
[21] Jacquot-Armand, Y., and Guinand, S., *Biochim. Biophys. Acta*, **133**, 289 (1967).
[22] Rowen, R., and Wiest, M. A., *J. Exp. Med.*, **122**, 547 (1965).
[23] Schubert, G., Bettendorf, G., Höhne, G., Kunkel, H. A., Maass, H., and Uhlman, G., *Geburtsh. u. Frauenheilk.*, **22**, 1006 (1962).
[24] Schumacher, G., and Schlumberger, H. D., *Proc. Eighth Cong. Europ. Soc. Haematol.*, article 228 (1962).
[25] Ganrot, P. O., *Clin. Chim. Acta*, **14**, 493 (1966).
[26] Simpson, N. E., Gunson, H. H., and Smithies, O., *Diabetes*, **11**, 329 (1962).
[27] Oliver-Gonzalez, J., and Gadea, D., *Proc. Soc. Exp. Biol. and Med.*, **123**, 802 (1966).
[28] Schultze, H. E., *Dtsch. Med. Wschr.*, **83**, 1742 (1958).
[29] Heim, W. G., and Lane, P. H., *Nature*, **203**, 1077 (1964).
[30] Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, **26**, 507 (1949).

Differences in Localization of *Salmonella* Flagella in Lymph Node Follicles of Germ-free and Conventional Rats

STUDIES on the induction of antibody production have shown that subcutaneously injected immunogens can be processed in two ways by draining lymph nodes[1]. Most of the immunogen retained in the node is actively phagocytosed by the macrophages of the sinuses of the medulla, while a smaller portion becomes attached to the surfaces of dendritic cellular processes in the primary follicles of the cortex[1–5]. This latter phenomenon seems to be important because germinal centres subsequently form in immediate relation to this surface-retained immunogen[1–5]. Because the ability of follicular cells to capture immunogens is decreased in tolerant animals and increased in actively or passively immunized animals[6–8], it may be that opsonins, specific or non-specific, are required for follicular localization of immunogens and thus for the formation of germinal centres in lymph nodes. To test this hypothesis further we have compared the degree of follicular localization of flagella of *Salmonella adelaide* labelled with iodine-125 in germ-free rats and in conventionally raised rats, assuming that germ-free rats would have experienced less exposure to antigens which might have induced cross-reacting or non-specific opsonins.

Germ-free and conventional female Sprague–Dawley rats weighing 75–115 g were used. On arrival, the germ-free rats were transferred into a peracetic acid-sterilized flexible plastic isolator (Labco, Columbus, Ohio) by conventional sterile techniques. Two were kept in each cage. Purina 5010*C* laboratory chow, water, bedding and the necessary glassware and instruments were introduced into the isolator after sterilization in an autoclave. Bedding from the isolator was changed, removed and cultured for bacteria twice weekly. At the time of injection of ^{125}I labelled flagella, two bacteria were found contaminating the isolator, *Clostridium perfringens* and *Cl. capriocum* (identified by the Communicable Disease Center, Atlanta, Georgia). In the experiment testing the immunogenicity of flagella in germ-free rats, *Cl. perfringens* and *Cl. multifermentans* became contaminants 2 weeks after primary immunization. No other bacteria were ever cultured from the bedding, isolator or rats. Conventional rats injected subcutaneously four times at 2 day intervals with $1–1\cdot5 \times 10^8$ organisms of each of these *Clostridium* species killed with formalin did not develop detectable immobilizing antibody to *Salmonella adelaide*.

Flagella from *Salmonella adelaide* were prepared and iodinated with iodine-125 (New England Nuclear Corp., Boston, Mass., lot. No. 2–674) by the chloramine-*T* method described earlier[9]. The radioactive antigen was injected in doses of 0·8 μg of flagella in 0·1 ml./hind foot pad into groups of ten germ-free and ten conventional rats under pentobarbitol anaesthesia. Pentobarbitol solutions and flagella suspensions were sealed in glass ampoules which were exposed for 1 h to a 10 per cent peracetic acid spray before being put in the isolator. Suspensions of flagella injected into the conventional rats were handled in the same way as those injected into the germ-free rats except that they were kept at room temperature for a time equivalent to that required for ampoules to pass into the isolator. One conventional rat died 25 min after injection of antigen and one germ-free rat was killed after 60 min. Two rats from each group were killed at intervals of 4 h and 1 and 7 days after injection of antigen, and single rats were killed after intervals of 8 h and 2 and 3 days. Both popliteal lymph nodes, a para-aortic lymph node and the spleen were removed from each rat, fixed in 10 per cent formol-saline for 24 h, transferred to 70 per cent ethanol, and counted individually on a Packard 'Auto-Gamma' scintillation counter simultaneously with diluted samples of serum from each animal and with a diluted sample of the injected radioactive suspension of flagella. Subsequently, autoradiographs of 5μ sections of the organs were prepared using Kodak *NTB*3 emulsion diluted 1 : 1 with distilled water, and methyl green-pyronin stain.

At times later than 24 h after the injection of labelled flagella the levels of radioactivity in the popliteal nodes and in the spleen, but not in the serum, were slightly lower in the "germ-free" rats than in the conventional rats. After exposure for 33 days, autoradiographs of the popliteal nodes from germ-free and conventional rats had distinctly different patterns of concentration of label in follicular cells (Figs. 1 and 2.) In popliteal nodes from conventional rats, label appeared in follicles within 4 h of injection, was heavily concentrated in follicles by 8 h and persisted here until 7 days after injection. In the germ-free rats, however, no label was found over follicles at 4 h

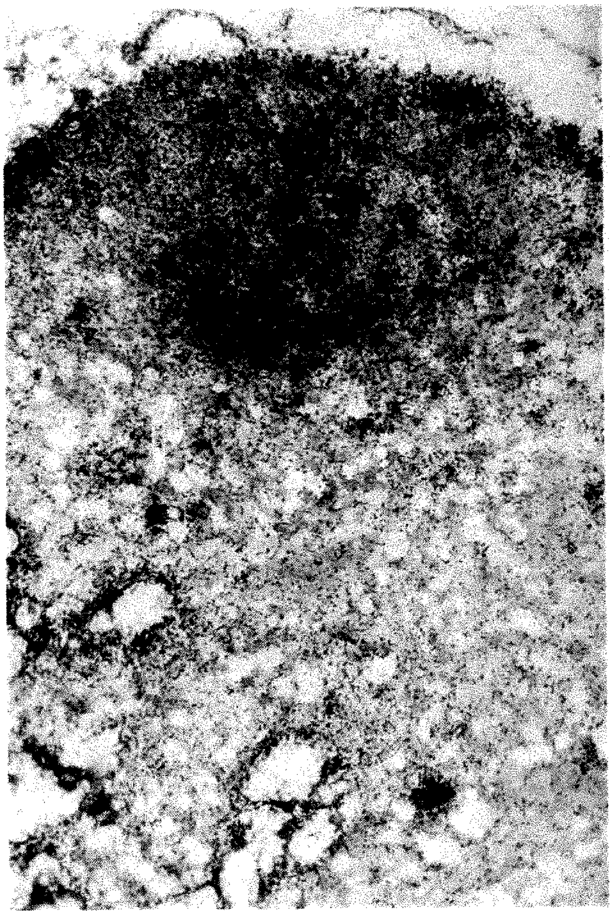

Fig. 1. Photomicrograph of an autoradiograph exposed for 33 days, showing a section of a popliteal node from a conventional rat 8 h after injection of flagella labelled with iodine-125. Labelling is present over the phagocytic cells of the peripheral and medullary sinuses and is concentrated over a primary cortical follicle. Methyl green-pyronin stain, × 60.

Table 1. COMPARISON OF THE CONCENTRATION OF LABELLED FLAGELLA IN THE PRIMARY FOLLICLES OF THE POPLITEAL LYMPH NODES IN THE GERM-FREE AND CONVENTIONAL RATS 24 h AFTER INJECTION

Rats	Nodes examined	Proportion of follicles counted with grains	Statistic	Size of follicular cross-section (μ^2)	Individual* follicular concentration of grains	Grains/follicular cross-section
Conventional	4	14/17	Mean	26,590	147	9,401
			Median	20,800	117	2,995
			Range	(11,200–52,800)	(0–385)	(0–36,960)
Germ-free	4	12/13	Mean	20,280	47	2,660
			Median	17,600	34	979
			Range	(4,480–37,600)	(0–166)	(0–9,030)

* Follicular concentration = grains/500μ^2 in follicle – grains/500μ^2 in adjacent cortex.

and only a trace amount was seen by 8 h. At 24 h and thereafter there was always some label over the follicles, but the concentration of this label was less than that in nodes from comparable conventional rats. In contrast, within 4 h of injection of radioactive flagella equally heavy labelling was found over the medullary areas of the popliteal nodes from both groups of rats.

The difference in degree of concentration of antigen in follicles in the popliteal nodes of the "germ-free" rats compared with those of the conventional rats was more accurately established by grain counting[10]. Autoradiographs (exposed for 10 days) of popliteal nodes of all rats killed 24 h after antigen injection were examined. Follicles containing germinal centres were not counted. The concentration in the follicles was expressed as the excess of this concentration over the concentration in the diffuse cortex, the latter being determined individually for each node. These data are shown in Table 1. The range of values, mean values, and median values of the concentration of labelled flagella in follicles and of grains/cross-section of follicles are all smaller in the germ-free rats than in the conventional rats. The sizes of cross-sections of follicles were comparable. There was little difference in the proportion of follicles which retained antigen between the two groups.

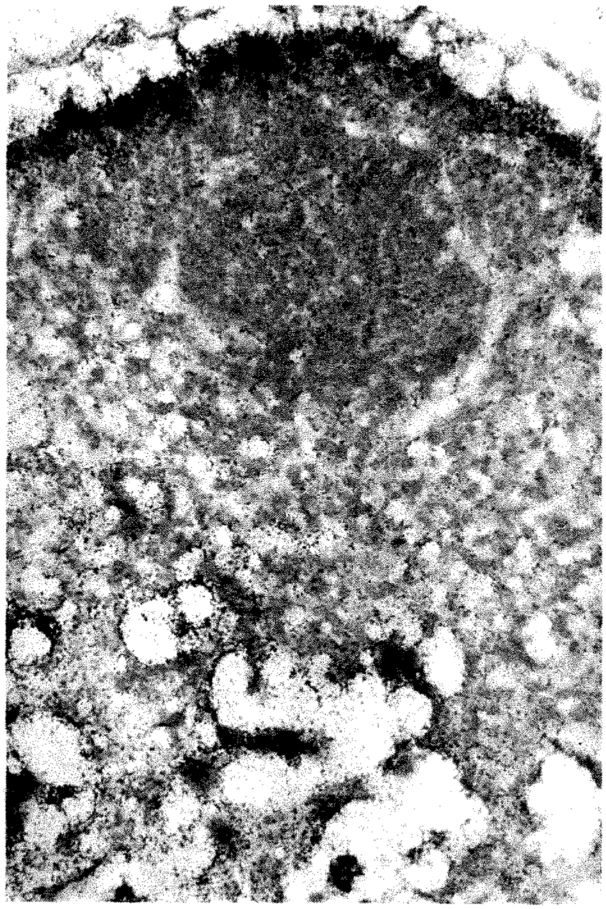

Fig. 2. Photomicrograph of an autoradiograph exposed for 33 days showing a section of a popliteal node from a germ-free rat 8 h after injection of flagella labelled with iodine-125. Although the phagocytic cells of the sinuses are as heavily labelled as those in the node from the conventional rat (Fig. 1), the primary cortical follicle, in contrast, has only a small increase in concentration of labelling compared with the diffuse cortex. Methyl green-pyronin stain, × 60.

Neither consistent germinal centre formation nor plasmacytopoiesis occurred in the popliteal lymph nodes from the conventional or the germ-free rats in response to these doses of iodinated flagella. To establish the immunogenicity of the particular flagella preparation used, and to determine the immunogenicity of flagella in germ-free rats, new groups of conventional and germ-free rats were injected with non-iodinated but otherwise similarly handled flagella of the same batch in doses of 0·05 μg or 0·5 μg per hind foot pad. The rats were bled 42 days later and given secondary injections of 5·0 μg flagella/hind foot pad. The rats were bled again 7 and 14 days after the secondary injections. The titres of *Salmonella adelaide* immobilizing antibody[11] in the germ-free rats were higher than those in the conventional rats after both primary doses and secondary stimulation (Table 2), although not as high as expected on the basis of past experience with another strain of rats[12].

Table 2. AVERAGE TITRES OF IMMOBILIZING ANTIBODY IN GERM-FREE AND CONVENTIONAL RATS

	Primary	Secondary	Average log^2 titre (1 = 1/5 dilution)* 42 day primary	7 day secondary	14 day secondary
Germ-free	0·05 μg	5·0 μg	7·3	11·4	13·8
	0·5 μg	5·0 μg	9·2	12·5	14·0
Conventional	0·05 μg	5·0 μg	0·6	9·5	9·8
	0·5 μg	5·0 μg	5·4	11·8	12·2

* Six rats in each group. At the beginning of the experiment, germ-free rats were estimated to weigh an average of 100 g and conventional rats weighed an average of 75 g.

In conclusion, localization of *Salmonella* flagella in the lymphoid follicles but not in the medullary macrophages of rat lymph nodes is less efficient in rats raised in a germ-free environment than in conventionally raised rats. This is consistent with the hypothesis that localization of immunogens in follicles, and thus germinal centre formation, is dependent on the pre-existence of antibody. The fact that the germ-free rats of this experiment had the ability to retain some flagella in the follicles may have been caused by antibodies which were not highly specific, or to slight previous stimulation with specific antigens present in the food, the probable source of the *Clostridium* spores causing contamination of the isolator. Alternatively, the localization of the antigen in the follicles, obviously delayed in the germ-free rats, may have been caused by *de novo* antibody synthesis to the injected antigen. The dissociation of the degree of concentration of flagella in follicles and the antibody titres produced in the germ-free rats compared with the conventional rats might indicate that the surface-retained immunogen of lymph node follicles has little to do with the initiation of antibody production *per se*. Careful study of possible early differences in anti-flagella antibody production in germ-free and conventional animals would, however, be necessary to make this more certain.

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[1] White, R G., *The Immunologically Competent Cell Its Nature and Origin*, Ciba Found Study Group No 16 (edit by Wolstenholme, G E W, and Knight, J), 6 (Churchill, London, 1963)

[2] Nossal, G J. V., Ada, G L , and Austin, C M , *Austral J Exp Biol* , **42**, 311 (1964)

[3] Miller, J J , and Nossal, G J V , *J Exp Med* , **120**, 1075 (1965)

[4] Mitchell, J , and Abbot, A , *Nature*, **208**, 500 (1965)

[5] McDevitt, H O , Askonas, B A , Humphrey, J H , Schechter, I , and Sela, M , *Immunology*, **11**, 337 (1966)

[6] Humphrey, J H , and Frank, M M , *Immunology*, **13**, 87 (1967)

[7] Ada, G L , and Lang, P G , *Immunology*, **10**, 431 (1966)

[8] Lang, P G , and Ada, G L , *Immunology*, **13**, 523 (1967)

[9] Ada, G L , Nossal, G J V , and Pye, J , *Austral J Exp Biol* , **42**, 295 (1964)

[10] Williams, G M , *Immunology*, **11**, 467 (1966)

[11] Nossal, G J V , *Immunology*, **2**, 137 (1959)

[12] Nossal, G J V , Ada, G L , and Austin, C M , *Austral J Exp Biol* , **42**, 283 (1964)

BIOLOGY

Nutritional Evaluation of Bacterial Diets in Growing Rats

There has recently been interest in the potential of bacteria (single cell protein) as a food for populations on a diet deficient in protein[1,2] and for astronauts on long space flights There seems to be no literature on the growth of animals fed for extended periods on bacteria as the only nitrogen source or as a large proportion of the diet This communication reports the results obtained when weanling rats were fed on diets containing acetone-killed *Escherichia coli B* or *Hydrogenomonas eutropha* as a minimal nitrogen source, and of feeding diets which utilize these bacteria as the chief source of calories The findings are useful in establishing the extent to which bacterial protein can replace conventional protein and the degree to which a diet containing large amounts of whole bacteria is compatible with good nutrition and health *H eutropha* is of particular interest because it is under consideration as a possible source of regenerated food for use on long space flights[3]

The concept that bacterial protein may be a useful nutritional supplement is not new It has been shown[4,5] that the addition of 5 per cent by weight of purified *E coli* protein to a diet containing only sufficient protein for minimal growth produced the same or better stimulation of growth in weanling rats as that produced by 5 per cent fishmeal protein Animals fed on whole cells killed by heat responded as well as those fed on purified protein

There have been studies of the nitrogen balance of bacteria over a short period The protein of mixed rumen bacteria killed with ethanol has a biological value of 81 per cent and a digestibility of 74 per cent[6,7] in rats

It has also been shown[8] that the protein of live *E coli* cells has a biological value of 69 per cent and a digestibility of 81 per cent These values were not changed noticeably after freeze drying the cells, sonically disrupting the dried cells or altering the growth medium The biological value for *Lactobacillus arabinosis* was found to be 52 per cent with a digestibility of 84 per cent

There are two potential problems related to growth over a long period on diets containing *H eutropha* First, the bacterial protein in whole cells may not be available for digestion or, if digested, the distribution of amino-acids may not be compatible with good nutrition A relatively good indicator of protein value is the "Essential Amino Acid Index"[9] When this index is calculated for casein, *Pseudomonas* (*Hydrogenomonas*) *saccharophilia*[10] and *H eutropha*[11], values of 88, 75 and 78, respectively, are obtained Thus *H eutropha* protein should have a biological value somewhat lower than casein but still be of relatively high quality

The second problem which might arise relates to the amount of urine which must be excreted to eliminate compounds containing nitrogen from the body The limited ability of the kidney to concentrate urea and uric acid indicates that when large amounts of protein are eaten, more water must be ingested It must therefore be shown whether the increased volumes associated with diets containing these bacteria as the chief calorie source will be too large to preclude their use in this manner It is significant that good nutritional practice avoids diets containing more than 45 to 50 per cent protein because of this and associated problems[12]

There were difficulties in obtaining an adequate amount of *H eutropha* A preliminary study was therefore done with a single lot of *E coli B* grown out to 3/4 log phase on phosphate-buffered minimal medium, washed successively with saline and acetone and then air dried The resultant dried powder contained no viable *E coli* and contained 78 per cent protein (N × 6 25) These dried cells were incorporated at levels of 10 7 and 71 8 per cent by weight into diets containing 10 per cent corn oil, 4 per cent of the Wesson modification of Osborn and Mendel's salt mixture, vitamins, and the remainder potato starch. Similar control diets were prepared containing 8 0 and 66 0 per cent casein An equal weight of water was blended into the 8 per cent casein and 10 7 per cent *E. coli* diets to give a paste Twice this amount of water had to be added to the 66 per cent casein and 71 8 per cent bacteria diets to give pastes of the same consistency The nitrogen content on a dry basis of these diets as determined by analysis was 1 74 per cent for the low protein diets and 9 04 per cent for the high protein diets This is equivalent (N × 6 25) to 7 75 and 57 0 per cent protein The true protein content of the bacteria containing diets, however, is probably reduced by 8 to 10 per cent of the stated value because of the presence of nucleic acids All diets were stored at 4° C and fed fresh to the rats daily for 14 days The male Sprague–Dawley rats (66 ± 3 g) were caged as pairs, food and water consumption was recorded daily

Table 1 RESPONSE OF MALE WEANLING SPRAGUE–DAWLEY RATS TO DIET CONTAINING ACETONE-KILLED BACTERIA

Material	Per cent	No of animals	Total per animal during experimental period ± S E M Weight gain (g)	Food (g)	Water (g)	Urine (ml)	Faeces (g)
			14 day study with *E coli*				
Casein	8 0	8	33 ± 5	214 ± 14	432 ± 35		
Casein	66 0	6	42 ± 1	138 ± 3	460 ± 27		
E coli	10 7	4	41 ± 7	222 ± 2	526 ± 105		
E coli	71 8	4	37 ± 4	117 ± 0	625 ± 30		
			26 day study with *H eutropha*				
Casein	12 0	4	85 ± 8	300 ± 18	460 ± 41	143 ± 15	33 ± 2
Casein	50 0	4	135 ± 6	315 ± 8	668 ± 23	329 ± 28	36 ± 1
H eutropha	17 0	4	101 ± 9	326 ± 11	545 ± 16	236 ± 24	43 ± 1
H eutropha	72 0	4	72 ± 11	266 ± 4	896 ± 124	483 ± 17	130 ± 10

When the results of this feeding study (Table 1) were examined, the only statistically significant changes observed between groups at the same level of dietary protein were the food and water consumption on the high protein diets The rats ate less of the *E coli* diet ($P < 0\ 05$) (*t* test) and drank more water ($P < 0\ 05$) This experiment verified the hypothesis that diets containing very large amounts of bacteria are not very harmful to the animals after they have been fed on the diets for 14 days.

Subsequently, we made a longer study with *H. eutropha*. The bacteria were grown heterotrophically as a single batch in a 250 gallon fermentor on a medium containing salts, casein hydrolysate, protopeptone, yeast extract and sucrose (Grain Processing Corporation, Iowa). After the cells had reached full growth (24 h), they were prepared as described for *E. coli*. We formulated the diet in a slightly different way from that with *E. coli* in the hope of increasing acceptability. Thus 17 and 72 per cent dry weight of cells were mixed with 10 per cent cottonseed oil, 10 per cent sucrose, 5 per cent *USP* XIV salt mixture (plus 16.5 mg $ZnSO_4$/100 g diet), 2 per cent α-cellulose, vitamins, and the remainder corn starch. Similar control diets were prepared containing 12 and 50 per cent casein. In this case, an equal weight of water was added to all the diets. The rats (67 ± 3 g) were kept as pairs in metabolic cages. The diets were kept at 4° C and were fed fresh to the rats daily for 26 days; food and water consumption and urine and faeces excretion were recorded daily.

When 17 per cent *H. eutropha* was compared with 12 per cent casein in the diet, growth, food and water consumption, and urine excretion were the same (Table 1); excretion of faeces was increased. These data agree with the findings with *E. coli* that whole bacterial cells are digested and provide protein with approximately the same biological value as casein. When *H. eutropha* was fed at a much higher level (72 per cent), however, there was a highly significant ($P < 0.01$) decrease in growth rate and food consumption when compared with the casein control. In addition, the excretion of urine and faeces was significantly increased ($P < 0.05$) with the diet containing *H. eutropha*. The increased production of faeces is probably related to non-digestible components of the bacteria such as cell walls. The ash content of the *H. eutropha* diets was appreciably greater than the corresponding controls and may explain in part the increased urine excretion.

On the basis of these experiments, bacterial cells may be an adequate sole source of protein for extended periods when present at low levels in the diet. When fed at a high level for a period of 26 days, *H. eutropha* does not seem to provide as good nutrition to weanling rats as does a conventional protein. Consideration will have to be given to the problems of increased waste production in any speculation about the use of bacteria as a food for space flights of long duration, as man excretes the less soluble uric acid as a catabolite of the purines rather than as allantoin as does the rat.

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[1] Bunker, H. J., in *Biochemistry of Industrial Microorganisms* (edit. by Rainbow, C., and Rose, A. H.), 34 (Academic Press, New York, 1963).

[2] Johnson, M. J., *Science*, 155, 1515 (1967).

[3] Jenkins, D. W., in *Proc. Sixteenth Intern. Astronaut. Cong.*, 4, 229 (Gordon and Breach, New York, 1966).

[4] Roberts, R. S., *Nature*, 172, 351 (1953).

[5] Roberts, R. S., *Brit. J. Nutrit.*, 8, 353 (1954).

[6] McNaught, M. L., Smith, J. A. B., Henry, K. M., and Kon, S. K., *Biochem. J.*, 46, 32 (1950).

[7] McNaught, M. L., Owen, E. C., Henry, K. M., and Kon, S. K., *Biochem. J.*, 56, 151 (1954).

[8] Kaufman, B., Nelson, W. O., Brown, R. E., and Forbes, R. M., *J. Dairy Sci.*, 40, 847 (1957).

[9] Oser, B. L., in *Protein and Amino Acid Nutrition* (edit. by Albanese, A. A.), 281 (Academic Press, New York, 1959).

[10] Young, H. L., Callahan, P. X., and Klein, H. P., NASA TM X-1353 (1967).

[11] Foster, J. F., and Litchfield, J. H., *Biotechnology and Bioengineering*, 6, 441 (1964).

[12] Hegsted, D. M., in *Nutrition*, 1 (edit. by Beaton, G. H., and McHenry, E. W.), 115 (Academic Press, New York, 1964).

Brood-protection in a Deep-sea Holothurian, *Oneirophanta mutabilis* Théel

BROOD-PROTECTION is known to occur in thirty species of holothurians belonging to the orders Dendrochirota and Apoda. All are littoral–sublittoral and most are inhabitants of cold seas, especially the Antarctic. The brood-protecting Dendrochirota usually carry their young on the body-surface or in pockets invaginated from the body-surface. Some Dendrochirota and most of the brood-protecting Apoda have a coelomic incubation, the juveniles passing through a free-swimming stage in the body-fluid of the mother. The Antarctic synaptid *Taeniogyrus contortus* (Ludwig) breeds the young within the ovaries, which they leave at a length of 3 mm (the adult specimens measure 20–45 mm) (ref. 1).

To this hitherto isolated case of intra-ovarial brood-protection in holothurians can now be added that of the deep-sea holothurian, *Oneirophanta mutabilis* (family Deimatidae, order Elasipoda). Brood-protection in this species was found during dissection of twenty-seven specimens, 40–95 mm long, from the Galathea st. 716 off the Pacific coast of Central America (9° 23′ N., 89° 32′ W., 3,570 m deep).

Five females had between one and six young, 7–30 mm long, present in the ovaries (Fig. 1). The smallest specimen with young was 70 mm long and contained three juveniles measuring 7–8 mm. The two largest young, both 30 mm long, were present in a 75 mm long specimen.

Eggs were absent in the ovaries of the females which were bearing young. The other females contained very scattered and small eggs in the ovarial wall, in one female some eggs reached a size of 0.2 mm. The absence of intermediate stages between ovaries in an early stage of egg-formation and ovaries with young in an advanced stage suggests the existence of a reproductive periodicity in this deep-sea species. The females with no young are up to 90 mm long and most of them are probably at the beginning of a new reproductive cycle after the young have been born.

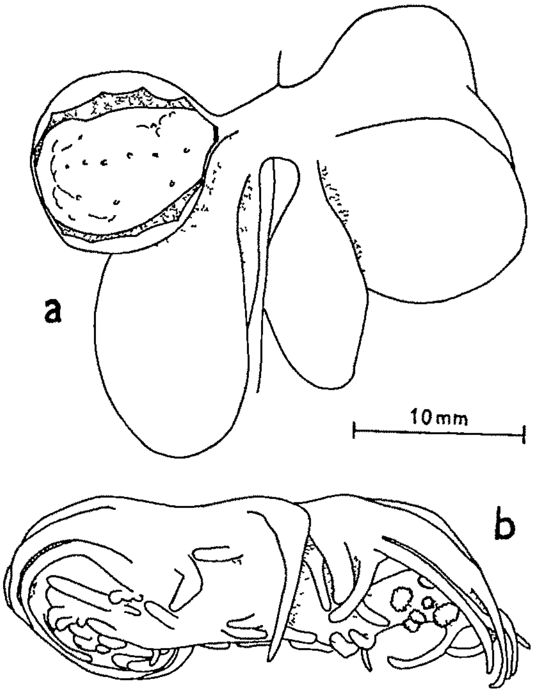

Fig. 1. *Oneirophanta mutabilis*. *a*, Ovary, ventral view, one sac opened to show a young. *b*, Large young from another ovary.

The smallest young had few and minute tubefeet and papillae and five oral tentacles, while the largest had fully developed tubefeet and papillae and ten oral tentacles These juveniles differ from the free-living specimens by the feeble development of the calcareous spicules of the skin and by the lower tentacle-number, the number in free-living specimens being eighteen to twenty

In the earlier stages, the young may feed on the unfertilized eggs The fact that the young increase in size from 7 to 30 mm in ovaries emptied of eggs suggests, however, that they are nourished by a nutritive fluid secreted into the ovaries

An intra-ovarial brood-protection exists also in a few Antarctic species of ophiurans[2,3], but is otherwise unknown in the echinoderms

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[1] Ludwig, H , *Ergebn Hamburger Magalhaensischen Sammelreise*, 1 (1898)

[2] Mortensen, T , *Studies of the Development and Larval Forms of Echinoderms* (Gad, Copenhagen, 1921)

[3] Mortensen, T , *Discovery Reports*, 12, 207 (1936)

Production of Hydrogen Sulphide by a Thermophilic Blue-green Alga

LIGHT has been found to stimulate the production of hydrogen sulphide in the unicellular blue-green alga *Synechococcus lividus* Copeland (axenic clone *Y* 52) The culture was initially isolated in 1964 from one of the Fairy Springs, Lower Geyser Basin, Yellowstone National Park The cells were collected at 52° C and the thiosulphate ion content of the water was probably about 9 0 p p m (ref 1)

Although no other organism has been found to exhibit a light stimulated production of hydrogen sulphide gas, several other dissimilatory reductions have been described which may be similar to the production of hydrogen sulphide Among these are the evolution of hydrogen gas in *Rhodospirillum rubrum*[2], *Scenedesmus*[3], *Chromatium*[4] and spinach chloroplast preparations[5] *Chromatium* reduced elemental sulphur to hydrogen sulphide in the dark, but returned to sulphur oxidation when illuminated[6] The non-photosynthetic sulphur bacterium *Desulfovibrio* is a dissimilatory sulphate reducer which can utilize sulphate as its terminal electron acceptor in anaerobic respiration[7] Certain organisms are capable of using nitrate as a terminal electron acceptor A respiratory nitrate reductase has been described for several bacteria and fungi[8] and for the green alga *Chlorella pyrenoidosa*[9] This communication describes preliminary experiments designed to clarify the relationship of hydrogen sulphide production in both light and darkness to the various reductions mentioned

Experimental cultures were grown at 800 lm/ft ² (tungsten) and 45° C in the following defined medium (medium *C*) 1 l of distilled water, 0 1 g of nitrilotriacetic acid; 0 5 ml of trace element solution (1 l of water, 3 0 ml of concentrated hydrochloric acid, 2 0 g of manganese chloride 4 H_2O, 0 5 g of zinc nitrate 6 H_2O, 0 5 g of boric acid, 0 025 g of cupric chloride 2 H_2O, 0 025 g of sodium molybdate 2 H_2O, 0 025 g of cobaltous chloride 6 H_2O, 0 025 g of vanadyl sulphate 16 H_2O), 1 0 ml of ferric chloride solution (0 2805 g of ferric chloride in 1 l of water), 0 0825 g of calcium nitrate 4 H_2O, 0 1023 g of magnesium nitrate 6 H_2O, 0 0077 g of sodium chloride, 0 1032 g of potassium nitrate, 0 6888 g of sodium nitrate, 0 1111 g of sodium mono H phosphate, and 0 9862 g of sodium thiosulphate The *p*H was adjusted to 8 2 with sodium hydroxide, and was about 7 5 after autoclaving The substitution of 0 6 g of calcium sulphate 2 H_2O and 0 0988 g of magnesium sulphate 7 H_2O for sodium thiosulphate produced a good general medium for the growth of thermophilic blue-green algae (medium *A*).

For the experiments, cells were concentrated by centrifugation (5,000*g* at 0° C) The cells were resuspended and washed three times in Sigma buffer (Table 1) The hydrogen sulphide evolved was trapped in 4 M sodium hydroxide placed in the centre well of 15 ml conical Warburg flasks and determined to be sodium monosulphide by the colloidal lead method[10]

Table 1 PRODUCTION OF HYDROGEN SULPHIDE AS μg/g DRY WEIGHT/h $\left(Q^{N_2}_{H_2S}\right)$ IN THE PRESENCE AND ABSENCE OF THIOSULPHATE ION

	Thiosulphate present	Thiosulphate absent
Light	932–1,044 ($\bar{x}$ = 1,024, five trials)	0 (four trials)
Dark	152–244 (x = 168, four trials)	0 (four trials)

The Warburg flasks contained whole cells of *S lividus* in 3 ml of the following Sigma buffer (expressed in μmoles/3 ml of water) 100 μM *tris* HCl buffer (*p*H 6 8), 3 5 μM magnesium chloride 6 H_2O, 10 μM potassium chloride, 10 μM potassium monohydrogen phosphate, 40 μM sodium thiosulphate (when used) The light intensity was 2,600 lm/ft ² and the temperature was 45° C with nitrogen as the gas phase

The cells evolved hydrogen sulphide gas in the light and dark when incubated under nitrogen with either sodium sulphate or thiosulphate Fig 1 shows that the production of hydrogen sulphide gas in the light was constant over the experimental period, that the rate of gas production when the culture was supplied with thiosulphate was twice that of the sulphate culture, and that with either salt the rate in the light exceeded the respective dark rate by a factor of ten

Several bacteria[11] and fungi[12] have been shown to evolve hydrogen sulphide gas by the action of cysteine desulphydrase on L-cysteine, so *S lividus* was assayed for this enzyme Assays in both the clarified and broken-cell preparations were negative[13]

The formation of hydrogen sulphide gas required an exogenous source of sulphur in both the light and dark (Table 1) Sulphur reduction occurs in micro-organisms of nearly all nutritional types, and may occur independently of photosynthesis Nevertheless, it is possible when sulphur reduction, either assimilatory or dissimilatory, and photosynthesis take place in the same organism

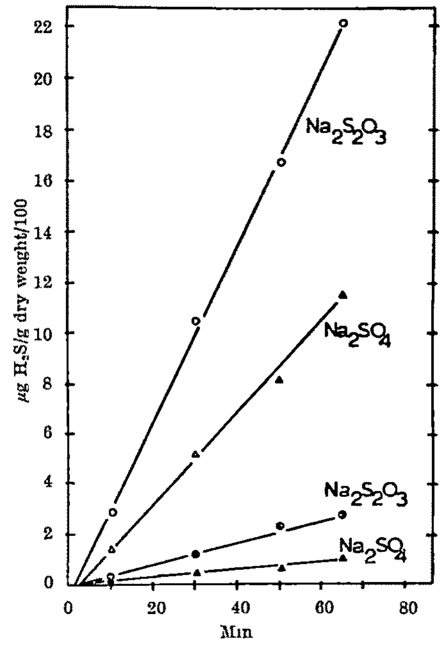

Fig 1 The production of hydrogen sulphide by *S lividus* in the light and dark The flask contained 3 ml of Sigma buffer (see Table 1) with either sodium sulphate or thiosulphate, 40 μM/3 ml The centre well contained 0 3 ml of 4 M sodium hydroxide as a sulphide trap The temperature was 45° C, the light intensity was 2,600 lm/ft ² and the gas phase was nitrogen ○ and △, Light, ● and ▲, dark

that the two processes are closely linked as in the reduction of molecular nitrogen[14], nitrate[15,16] and sulphate[17,18]

If water serves as the electron donor in anaerobic dissimilatory sulphate photoreduction, then the addition of a substitute electron acceptor in the form of sulphate or thiosulphate should result in an increased rate of oxygen evolution The maximum stimulation should be obtained when other reducible substances, such as carbon dioxide, are excluded and when the light is saturating for photosynthesis. Fig 2 compares the rates of oxygen evolution during the reduction of thiosulphate or sulphate with a control lacking an exogenous sulphur electron acceptor Clearly, the addition of either sulphate or thiosulphate stimulated the rate of oxygen evolution, both serving equally well as shown by the nearly identical rates, approximately double that of the control

Fig 3 illustrates the same effect using a Clark silver-platinum oxygen electrode The cells, initially in anaerobic conditions, responded to the injection of an anaerobic thiosulphate solution with an increased rate of oxygen evolution, approximately double that of the preceding rate

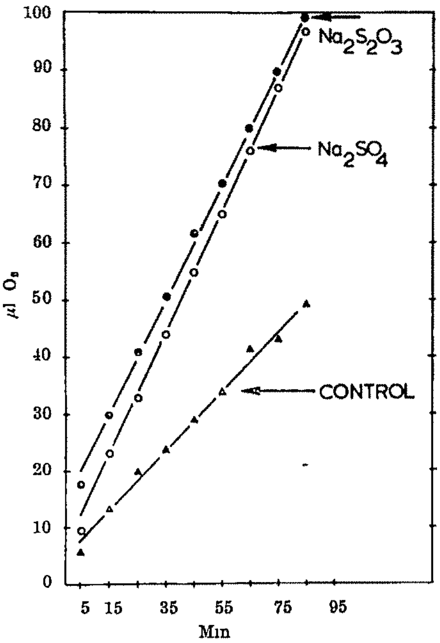

Fig 2 The effect of exogenous sulphate and thiosulphate on oxygen evolution in *S lividus* The flask contained 3 ml of Sigma buffer (see Table 1) with or without sodium sulphate or thiosulphate, 40 μM/3 ml The centre well contained 0 3 ml of 4 M sodium hydroxide as a sulphide trap The temperature was 45° C, the light intensity was 2,600 lm/ft^2 and the gas phase was nitrogen

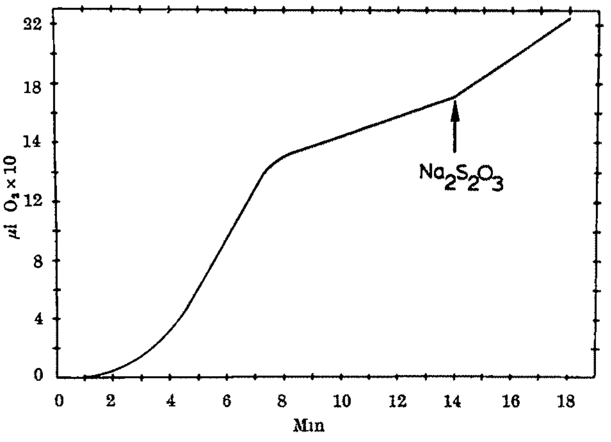

Fig 3 Oxygen production (corrected for respiration) by *S lividus* before and after the addition of sodium thiosulphate, 40 μM/3 ml The reaction mixture was Sigma buffer (see Table 1), the temperature was 45° C, the light intensity was 2,600 lm/ft^2 and the gas phase was nitrogen The curve shows an initial oxygen burst after the onset of illumination at time zero, a subsequent equilibrium rate and a higher equilibrium rate after the addition of thiosulphate

The stoichiometric relationship of sulphur reduction in *S lividus* to photosynthesis and respiration in anaerobic conditions will be reported later

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[1] Allen, E T, and Day, A L, *Hot Springs of the Yellowstone National Park*, Carnegie Inst Washington, Publ No 466, 277 (1935)
[2] Gest, H, Ormerod, J G, and Ormerod, K S, *Arch Biochem Biophys*, **97**, 21 (1962)
[3] Bishop, N I, and Gaffron, H, in *Photosynthetic Mechanisms in Green Plants*, Publ 1145, US Nat Acad Sci, National Res Council, 441 (1963)
[4] Losada, M, Nozaki, M, and Arnon, D I, in *A Symposium on Light and Life* (edit by McElroy, W D, and Glass, B), 570 (Johns Hopkins Press, Baltimore, 1961)
[5] Mitsui, A, and Arnon, D I, *Plant Physiol*, **37** (Suppl), iv (1962)
[6] Hendley, D D, *J Bacteriol*, **70**, 625 (1955)
[7] Peck, H D, *J Bacteriol*, **82**, 933 (1961)
[8] Nason, A, *Bacteriol Rev*, **26**, 16 (1962)
[9] Warburg, O, and Nagelein, E, *Biochem Z*, **110**, 66 (1920)
[10] Delwiche, E A, *J Bacteriol*, **62**, 717 (1951)
[11] Barker, H A, in *The Bacteria* (edit by Gunsalus, I C, and Stanier, R Y), **2**, 151 (Academic Press, New York and London, 1961)
[12] Kikuchi, T, *Plant Cell Physiol*, **6**, 195 (1965)
[13] Smythe, C V, in *Methods in Enzymology* (edit by Colowick, S P, and Kaplan, N D), **2**, 315 (Academic Press, New York and London, 1955)
[14] Fogg, G E, and Than-Tun, *Biochim Biophys Acta*, **30**, 209 (1958)
[15] van Niel, C B, Allen, M B, and Wright, B E, *Biochim Biophys Acta*, **12**, 67 (1953)
[16] Losada, M, and Paneque, M, *Biochim Biophys Acta*, **126**, 578 (1966)
[17] Wedding, R T, and Black, M K, *Plant Physiol*, **35**, 72 (1960)
[18] Ashi, T, *Biochim Biophys. Acta*, 58 (1964)

Effect of Seed Extract of *Trifolium subterraneum* on Germination and Seedling Growth Rate

Seeds may contain water-soluble substances which inhibit or retard germination[1] This communication reports the effects of a water-soluble extract from the seed of *Trifolium subterraneum* L (subterranean clover) on the germination and growth of subterranean clover and wheat

A water-soluble extract was prepared by soaking 200 g of commercial seed of subterranean clover (cultivar 'Mt Barker', 208 seeds/g) in 300 ml of distilled water for 24 h at 30° C The solution was then evaporated at 100° C and the resulting crystals were finely ground A quantity of extract was also freeze-dried The effect of the freeze-dried solution on subsequent germination and growth of subterranean clover at 30° C was similar to that of the material evaporated at 100° C The results only refer to an extract evaporated at 100° C.

Seeds of subterranean clover ('Mt Barker') were germinated in the dark at 18°, 23° and 30° ± 1 5° C All combinations of the three temperatures, and five relative concentrations (0, 25, 50, 75 and 100, where 100 = 1 g of extract/16 ml of water) were tested, using five replicas each of 100 graded seeds (135 seeds/g) The seeds were placed in Petri dishes, so that they were partially immersed in 10 ml. of the solution, and the Petri dishes were hermetically sealed After 3 days, germination, based on emergence of the radical, was recorded The length of the radical of germinated seeds was used as a measure of the initial rate of growth By this time and in these conditions the hypocotyl had not elongated To test the extract for non-specificity, its effects on the germination of growth rate of wheat (variety 'Falcon') were examined at temperatures of 18°, 25° and 30° ±1 5° C Fifty seeds were used in each replicate. A wider range of concentration of extract was tested as shown in Fig 2 Germination was recorded based on the emergence of both coleoptile and primary root, and lengths of these organs gave a measurement of growth

The results of the germination tests for subterranean clover are given in Fig 1*a*. There was no significant effect of the extract on germination at 18° C, but with increasing temperature and concentration germination was markedly reduced Fig. 1*b* shows the effect of temperature and concentration of the extract on the length of the radicle There was no effect of temperature in the absence of the extract At 18° C and 23° C the length of the radicle decreased with increasing concentration, with little differential effect caused by temperature At 30° C there was a particularly marked effect at relative concentration 25, but increasing the concentration had no further effect

Many of my experiments have shown that the germination and growth rate of subterranean clover in hermetically sealed dishes at minimum moisture tension are unaffected by temperatures in the range 18°–30° C The finding that the percentage of germination of control material at 30° C is not significantly reduced differs from the results reported by Ballard[2]. A typical experiment showed that the percentage of germination of imbibed seed in these conditions was reduced by at least 50 per cent when dishes were not sealed. Differences in the methods of germination may be responsible for the apparent discrepancy with Ballard's results

The effects of temperature and concentration of extract on the germination of wheat are shown in Fig 2*a* Again, the extract was most effective at 30° C and least effective at 18° C At 30° C relative concentrations 0 5, 1, 2 5, 5 and 10 had a stimulatory effect on germination. Fig 2*b* shows the effect of temperature and concentration of an extract on the length of the coleoptile In the absence

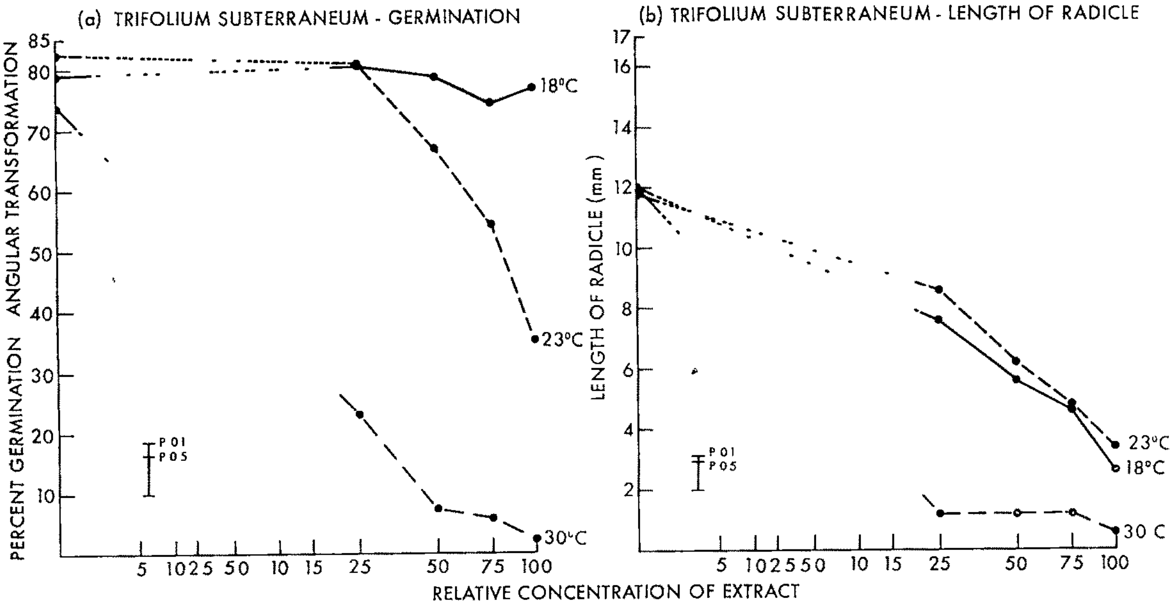

Fig 1 Effect of temperature and concentration of extract on ,germination of subterranean clover, *b*, radicle length of subterranean clover

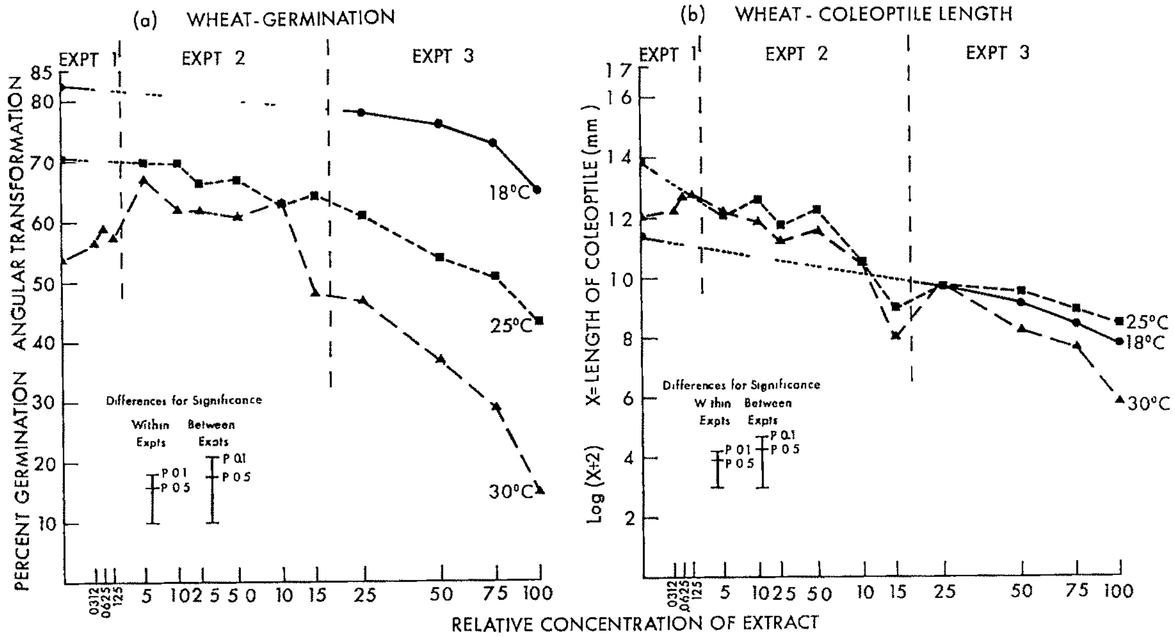

Fig 2 Effect of temperature and concentration of extract on *a*, germination of wheat, *b*, coleoptile length of wheat

of extract, growth rate is less at 18° C and 30° C than at 25° C At all temperatures there was a marked effect of the extract on the length of the coleoptile at relative concentrations of 25 and over The effect of temperature and concentration of extract on the length of the primary root was usually similar to that for coleoptile length

The osmotic pressure of the solution at a relative concentration of 100 was only 5 4 atm as determined by the vapour pressure method At 30° C, pressures of 5 atm have been shown to have only a slight effect (about 10 per cent reduction) on the germination of subterranean clover seed (personal communication from R M Moore) There is no effect of such an osmotic pressure at lower temperatures The reduction of about 75 per cent in germination of subterranean clover at 23° C and 30° C obtained in this work can be attributed therefore to an inhibitory effect of the extract

The effect of the extract on both subterranean clover and wheat is maintained only if the seed remains in contact with the solution The viability of seeds which had not germinated after three days was tested by washing thoroughly in distilled water, and germinating at a favourable temperature The seeds from all treatments germinated readily, even after they had been soaked for three days at relative concentration 100 at 30° C It was observed, however, that there was a residual effect of the extract on the growth rate of the radicle of subterranean clover, the effect increasing with increasing concentration

The results show that seed of subterranean clover contains a water-soluble substance or substances which at high concentrations adversely affect germination and growth This inhibitory effect is strongly dependent on temperature and seems to be non-specific

A recent paper[3] has been brought to my attention since this publication was prepared The hypothesis of Taylor and Rossiter that a water-soluble inhibitor of germination is involved in the regulation of germination of new (dormant) seed is extended by the results of the present study The magnitude of the inhibitory effect obtained in this study may vary with cultivar and seed-lot as suggested by Taylor and Rossiter

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[1] Evenari, M , *Bot Rev* ,15, 153 (1949)
[2] Ballard, L A T , *Aust J Biol Sci* ,14, 173 (1961)
[3] Taylor, G B , and Rossiter, R C , *Nature*,216 ,384 (1968)

Taxonomic Significance of Carotenoids

Goodwin[1], reviewing the distribution of carotenoids in non-photosynthetic tissue, came to the conclusion that it was very difficult to see their taxonomic significance Rubixanthin seemed to be restricted to *Rosa* spp [2] and it was suggested that, if a carotenoid mixture contained significant amounts of rubixanthin, then one could say with some confidence that the source of rubixanthin was the hips of a species of *Rosa*[1] Rubixanthin, however, has been identified in several other plants, namely, *Cuscuta salina*, *C subinclusa*[3] and *Gazania rigens*[4] Valadon[5] has also identified this pigment in the orange peel fungus, *Peziza* (*Aleuria*) *aurantia*, but Jensen[6] failed to confirm this result and had found a new pigment With a view to finding whether rubixanthin was restricted to some members of the higher plants, we have reinvestigated the carotenoid composition of the orange peel fungus

A number of specimens of *Peziza aurantia* were extracted in methanol and the carotenoids were separated and identified by our usual procedures[7] The following pigments were characterized β-carotene, γ-carotene and aleuriaxanthin ester, lycopene and 3,4-dehydrolycopene, in agreement with Liaaen Jensen's findings[6] Several other pigments, however, were identified α-carotene, neurosporene and rubixanthin, which were not in Jensen's specimens There were also four other carotenoids that could not be positively identified because they were found in too small an amount One such pigment was strongly adsorbed on the MgO–'Celite' column, was found as the top band, had the same absorption spectrum as γ-carotene and might be aleuriaxanthin[6] Rubixanthin was present and it was identified by its absorption spectrum and its position on an MgO–'Celite' column It also did not separate from pure rubixanthin using circular chromatography This was achieved with paper loaded on 22 per cent silica gel and eluted with 1 per cent acetone in *n*-hexane The amounts of carotenoids separated from *Peziza aurantia* are shown in Table 1

Table 1 CAROTENOID COMPOSITION OF *Peziza* (*Aleuria*) *aurantia*

Carotenoids	Amount (μg/g)	Per cent
α-Carotene	2 0	0 3
β-Carotene	300 5	43 9
Unknown (418, 443, 470)	4 3	0 6
γ-Carotene	106 4	15 5
Neurosporene	28 8	4 3
Rubixanthin	67 7	9 9
Aleuriaxanthin	146 9	21 5
Lycopene	11 8	1 7
3,4-Dehydrolycopene	5 5	0 8
Unknown (475, 486)	5 0	0 7
Unknown (416, 440, 467)	3 2	0 5
Unknown (435, 460, 487)	2 5	0 3
Total carotenoids	684 6	100

It is well known that plants from different geographical habitats and of different species differ with respect to their carotenoid composition[1,7] Fungi seem to be no exception It has been shown that ultraviolet irradiation can alter the pigment composition in fungi[8] and one may conclude that in different conditions of light and temperature it is possible to have changes in the quantities as well as in the qualities of the pigments involved It is not unexpected therefore to find different pigment composition in the same species of fungi in different parts of the world The evidence presented shows that carotenoids may not be useful as a taxonomic character

We thank Mrs Pat Andrews for the specimens of *Peziza aurantia*

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[1] Goodwin T W , in *Comparative Phytochemistry* (edit by Swain, T (Academic Press, London, 1966)
[2] Goodwin, T W , *Biochem J* 62, 346 (1956)
[3] Mackinney, G , *J Biol Chem* ,112, 421 (1935).
[4] Zechmeister, L , and Schroeder, W A , *J. Amer. Chem Soc* , 65, 1535 (1943)
[5] Valadon, L R G , *Biochem J* 92, 19*P* (1964).
[6] Liaaen Jensen, S , *Phytochemistry*, 4, 925 (1965)
[7] Valadon, L R G , and Mummery, R S , *Phytochemistry*, 6, 983 (1967).
[8] Valadon L R G , and Heale, J B , *Nature*, 202, 512 (1964), *J Gen. Microbiol* , 41, 341 (1965)

MICROBIOLOGY

Action of Bacteriophages obtained from *Corynebacterium diphtheriae* on *C. ulcerans* and *C. ovis*

The value of phage-typing in studying the strains of bacteria responsible for certain infections raised the question of whether phage-typing might also be valuable in epidemiological studies of diphtheria Early investigations on this subject have already been reported[1–6]. Most of these earlier studies were restricted by the very small numbers of bacteriophages that had been isolated from *C. diphtheriae* up to that time, and of suitable sensitive indicator strains for demonstrating and propagating new phages. The decline in the incidence of diphtheria in many countries after the adoption of widespread prophylactic vaccination has reduced the incentive to pursue these lines of investigation. In some countries, however, diphtheria epidemics continue to be an important public health problem. This fact, together with the predominance of one biotype of *C. diphtheriae* in certain regions over a number of years—for example, type *gravis* in Romania and type *intermedius* in the German Democratic Republic (East Germany)[7]—has stimulated further studies in phage-typing as a possible means of providing more detailed information concerning the characteristics of strains involved within a prevailing biotype[7-10]. Arising from studies on some thousands of strains isolated over the period 1954–64 in the Diphtheria Department of the Cantacuzino Institute, Bucharest, Romania, Saragea and Maximesco proposed a provisional scheme of phage-typing of *C. diphtheriae*[7]. This involves the use of twenty-four diphtheria phage preparations by means of which nineteen lysotypes of *C. diphtheriae* have been recognized, not by the lysis of strains of *C. diphtheriae* by single phages, but on the basis of the characteristic pattern of their reactions to one or more test phages.

Saragea and Maximesco[7] and Meitert *et al.*[11] failed to obtain evidence of lysis when their diphtheria phages were tested on a series of strains of pathogenic *Corynebacteria* other than *C. diphtheriae*, including seventeen strains of *C. pyogenes*, thirteen of *C. equi*, nine of *C. renale*, two of *C. murium*, two of *C. ovis*, and seven unclassifiable strains from bronchopneumonia in pigs. These observations suggested that the diphtheria phages used in Saragea and Maximesco's proposed scheme of phage-typing were specific for *C. diphtheriae*.

In the course of our own studies on the relationship between *C. diphtheriae*, *C. ulcerans* and *C. ovis* (*C. pseudotuberculosis*), the opportunity was offered, through a gift from Dr Saragea, of the twenty-four test phages derived from *C. diphtheriae* used in her phage-typing scheme, to test the lysosensitivity to these phages of fifty-two strains of *C. ovis* (sixteen ovine, twenty caprine, fourteen equine and two bovine), and fifty-six strains of *C. ulcerans* (forty-nine human, seven bovine).

The method of testing was that used by Saragea[7] of spotting phage preparations on lawns of young cultures of the bacterial strains to be tested. All tests were carried out with the same preparations of phages which throughout the period of these experiments showed no significant loss of activity on their sensitive indicator strains of *C. diphtheriae* which were included as controls with most tests.

Some strains of *Corynebacteria* of all groups of both *C. ovis* and *C. ulcerans* showed lysis by one or more of the twenty-four diphtheria phages, the proportion of sensitive strains ranging from 44 per cent in ovine strains of *C. ovis* to 86 per cent in both equine strains of *C. ovis* and bovine strains of *C. ulcerans*.

Every one of the twenty-four diphtheria phages produced lysis of some of the strains tested, but at no time did the pattern of lysosensitivity of any strain of *Corynebacterium* exactly match that of any of the lysotypes of *C. diphtheriae* included in Saragea and Maximesco's proposed scheme of phage-typing.

Some degree of uniformity of pattern of lysosensitivity was evident within lysosensitive strains belonging to certain groups of the *Corynebacteria* tested. There were also differences between such patterns in different groups. Assessment of the validity and significance of such apparent patterns must await further observations on additional strains and a wider range of phages. The results suggest, however, the possibility that significant differences exist between these closely related *Corynebacteria*. The reactions to nine of these diphtheria phages are of special interest because of the high proportion of sensitive strains among caprine and equine strains of *C. ovis* and in both human and bovine strains of *C. ulcerans*. Furthermore, of these nine phages, five produced a high proportion of complete or almost complete (clear) lysis, whereas most plaques with other strains were of the cloudy type.

These observations indicate that although Saragea and Maximesco[7] and Meitert *et al.*[11] found no evidence of lysosensitivity to their diphtheria phages among fifty strains from more than five species of pathogenic *Corynebacteria* of animal origin, when these same phages were tested on a more extended range of strains of *C. ovis* and *C. ulcerans* (two species not well represented in their series), numerous lysosensitive organisms were revealed. It is therefore clear that the twenty-four phages isolated from *C. diphtheriae* which have been employed by Saragea and her colleagues are not completely specific for *C. diphtheriae*.

The part played by bacteriophage infection in determining toxinogenicity in *C. diphtheriae*, first revealed by Freeman[12], raises a number of interesting questions concerning the occurrence and effects of cross-infections by bacteriophages between different species of pathogenic *Corynebacteria*. The foregoing observations were made as part of a general study of this question and full details will be reported elsewhere.

I thank Dr Alice Saragea and Dr H. Rische for diphtheria phages and sensitive indicator cultures. I also thank Professor R. I. N. Greaves for facilities in his department. This work was supported by a grant from the Medical Research Council.

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[1] Keogh, E. V., Simmons, R. T., and Anderson, G., *J. Path. Bact.*, **46**, 565 (1938).

[2] Toshach, S., *Can. Publ. Hlth. J.*, **41**, 332 (1950).

[3] Fahey, J. E., *Can. Publ. Hlth. J.*, **43**, 167 (1952).

[4] Hewitt, L. F., *Lancet*, ii, 272 (1952).

[5] Thibaut, J., and Fredericq, *CR Soc. Biol.*, Paris, **150**, 1039 (1956).

[6] Ortalli, A. V., Princivalle, M., and Zampieri, A., *Atte IX Congr. Naz. Microbiol. Palermo*, 214 (1956).

[7] Saragea, A., and Maximesco, P., *Arch. Roum. Path. Exp. Microbiol.*, **23**, 817 (1964).

[8] Endemann, D., and Rische, H., *Colloquium über Fragen der Lysotypie*, Wernigerode, 173 (1960).

[9] Rische, H., and Endemann, D., *Arch. Roum. Path. Exp. Microbiol.*, **21**, 337 (1962).

[10] Saragea, A., Maximesco, P., Meitert, E., Diaconu, J., Marion, M., Olinesco, E., and Calab, G., *Arch. Roum. Path. Exp. Microbiol.*, **21**, 391 (1962).

[11] Meitert, E., Bica-Popii, V., and Saragea, A., *Arch. Roum. Path. Exp. Microbiol.*, **25**, 95 (1966).

[12] Freeman, V. I., *J. Bact.*, **61**, 673 (1951).

CYTOLOGY

Polymelanosomes of the Melanocyte and their Substructures

THE importance of studying visceral melanocytes has already been emphasized[1-4] and the liver of *Amphiuma tridactylum*—a rich source of melanocytes—is a suitable material to use. The melanoprotein of the liver of *Amphiuma* is very similar to that of human liver melanoma[5], and the melanin polymer (free from protein) closely resembles that of human substantia nigra pigment and synthetic catecholamine pigment formed by incubating the various amines with a mitochondrial preparation containing monoamine oxidase[4]. Structural and functional studies of the hepatic melanocytes of *Amphiuma* may therefore have a bearing on other pigmentary systems. We have reported (refs. 1 and 6 and unpublished work of Prasad) that the melanocytes of *Amphiuma* constitute about 20 per cent of the liver cell population (hepatocytes and melanocytes). Most of these melanocytes (70–80

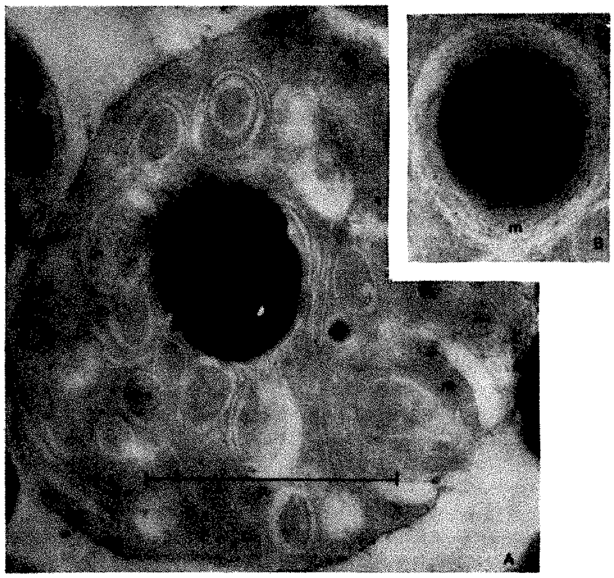

Fig. 1. *A*, An isolated polymelanosome from *Amphiuma* liver. Note the unmelanized structures which probably represent melanosomes (ms). Concentric membranes (m) surround both melanosomes and melanin granule. (× *c*. 33,260.) *B*, A melanin granule surrounded by several concentric membranes (m).

per cent of total pigment cell population) are lightly pigmented and have an abundance of melanin-containing organelles which we named polymelanosomes[6]. The darkly pigmented cells contain closely packed granules lying freely in the cytoplasm, but no polymelanosomes. There is some morphological evidence that melanin-synthesis occurs in the polymelanosomes, and the light melanocytes which have an abundance of these organelles probably represent the younger form[6]. Polymelanosomes possess three mitochondrial enzymes, monoamine oxidase, cytochrome oxidase and succinic oxidase[7].

We have studied isolated polymelanosomes of *Amphiuma* liver melanocytes in greater detail. Recently negative-contrast staining has been used to visualize membrane structures of mitochondria[8], and we have used a similar technique to study the membrane structures of polymelanosomes. The question also arises as to whether or not polymelanosomes are unique to *Amphiuma* liver melanocytes; thus we have investigated melanocytes in the skin and eye of *Amphiuma*, the liver of *Necturus maculosus* (mud-puppy), frog and alligator and the substantia nigra of monkey and human.

Polymelanosomes and their membrane structures were isolated by the method of VanWoert *et al.*[7] with a slight modification[6]. This procedure gave a good yield of polymelanosomes. (The polymelanosome fraction was not contaminated with mitochondria and polysomes, but occasionally a nucleus or partly broken cell was present. Thus our polymelanosome fraction was as pure as that of Van Woert *et al.*[7].)

For negative staining, the isolated polymelanosomes were suspended in 2–3 ml. of standard buffer which contained no sucrose, and were broken either by sonication at 1 mA for 20 s or by homogenization in a Potter-Elvehjem homogenizer, and centrifuged at 1,000*g* for 5 min to remove melanin granules. The supernatant, melanin granules and unbroken polymelanosomes were negatively stained by placing a small drop of each fraction on a grid and blotting the excess fluid. It was stained in 1 per cent uranyl acetate for 10–20 s and examined with an electron microscope at 100 kV. To investigate the presence of polymelanosomes in other organs, samples of skin and eye of *Amphiuma*, liver of *Necturus maculosus* (mud-puppy), frog and alligator, and substantia nigra of monkey and human were fixed and stained for electron microscopy as described earlier[7]. The human substantia nigra was fixed within 3 h after death. The monkey brain was fixed *in vivo* by perfusing glutaraldehyde through the carotid artery and the pigmented portion of the substantia nigra was excised and processed for staining as described earlier.

Fig. 1*A* is an electron micrograph of an isolated intact polymelanosome of a hepatic melanocyte of *Amphiuma* which shows a single melanin granule and several less electron-dense areas probably representing melanosomes. Two to seven concentric membranes surround both the melanosomes and melanin granule. Fig. 1*B* shows a melanin granule surrounded by concentric membranes. On the basis of morphology, it seems as though melanin polymer is being laid down on the melanosomes. This observation is consistent with the present concept of formation of the melanin granules. Such a clear demonstration of the morphological apparatus of melanogenesis has not been encountered, however, in other tissues. The part played by the concentric membranes in melanogenesis, if any, remains uncertain.

We have studied the membrane structures of polymelanosomes by negative staining. The isolated polymelanosomes were broken by homogenization or sonication and the melanin granules were removed before staining. Many structures are present in the supernatant fraction (Fig. 2), and small spherical bodies are associated with large ones. With a few exceptions, the average diameter of the small bodies is 430 Å (ranging from 250 Å to 600 Å). A single melanin granule is also visible but polysomes or ribosomes were not seen in this preparation. Polymelanosomes broken by sonication or by homogenization have the same substructure. The melanin granule pellet does not contain any spherical bodies. The fraction containing unbroken polymelanosomes shows several melanin granules and a few small bodies; the negative staining does not show the internal structures of the intact polymelanosomes. We do not know whether these large bodies are an integral part of the polymelanosomes *in vivo* or are formed during preparation of the fraction. The uniformity of the small bodies and the presence of structures similar to those of the large bodies in the section of an isolated polymelanosome indicate that the latter is a possibility. The significance of these substructures in polymelanosomes, if any, remains obscure.

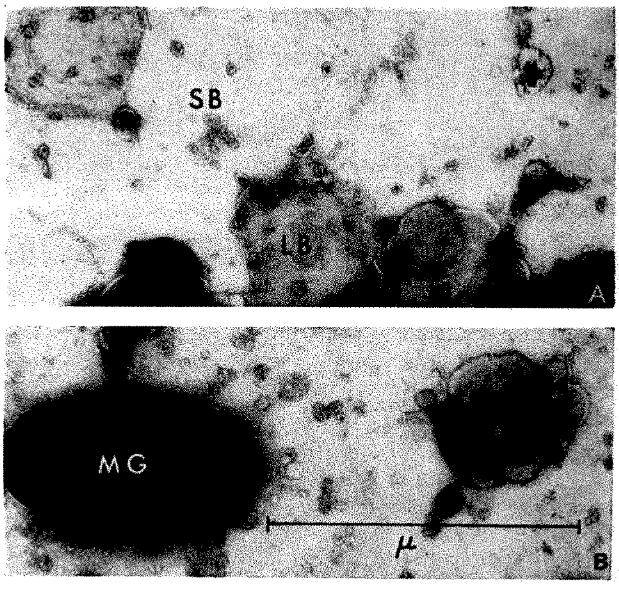

Fig. 2. Substructures of the polymelanosome. Polymelanosomes were disrupted by homogenization, and the supernatant fraction obtained after removal of melanin granules was stained with 1 per cent uranyl acetate. Many small spherical bodies (SB) are associated with large ones (LB). A single melanin granule (MG) is also seen. (× *c*. 41,670.)

Polymelanosomes similar to those in lightly pigmented melanocytes of *Amphiuma* liver have not yet been demonstrated in melanocytes of the skin and eye of *Amphiuma*, liver of *Necturus maculosus* (mud-puppy), frog and alligator, or the substantia nigra of monkey and human. This should not be surprising, because melanocytes in these organs resemble the dark melanocytes of *Amphiuma* liver which have melanin granules but no polymelanosomes. Thus the possibility still remains that polymelanosomes similar to those in the lightly pigmented melanocyte of *Amphiuma* liver may be found in the younger stage of melanocytes of these organs.

Arrangements of melanin granules bearing some similarities to those of *Amphiuma* liver have been seen before in a variety of tissues as phagocytic vacuoles[9], as conglomerate granules[10], as melanin-building centres[11], as melanin spheres[12], as irregular inclusions[13] or as compound granules[14]. These structures are not as abundant in the cytoplasm of their respective cells as are the polymelanosomes in *Amphiuma* liver melanocytes. They also do not have complex membrane structures similar to those of polymelanosomes. Nor is it known whether these various organelles can maintain their structural integrity after isolation as the polymelanosomes of *Amphiuma* liver melanocytes do.

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[1] Prasad, K. N., Johnson, H. A., and Cotzias, G. C., *Nature*, **205**, 526 (1965).
[2] Hays, G. B., Lyle, C. B., and Wheeler, C. E., *Arch. Dermatol.*, **90**, 471 (1964).
[3] Wassermann, H. P., *Nature*, **213**, 282 (1967).
[4] VanWoert, M. H., Prasad, K. N., and Borg, D. C., *J. Neurochem.*, **14**, 707 (1967).
[5] Hack, M. H., and Helmy, F. M., *Proc. Soc. Exp. Biol. and Med.*, **116**, 348 (1964).
[6] Prasad, K. N., and Johnson, H. A., *Radiat. Res.* (in the press).
[7] VanWoert, M. H., Nicholson, A., and Cotzias, G. C., *Nature*, **208**, 810 (1965).
[8] Lehninger, A. L., *The Mitochondrion*, 215 (1964).
[9] Charles, A., and Ingram, J. T., *J. Biophys. Biochim. Cytol.*, **6**, 41 (1959).
[10] Wellings, S. R., and Siegel, B. V.. *J. Nat. Cancer Res.*, **24**, 437 (1960).
[11] Weissenfels, N., *Z. Zellforsch.*, **45**, 60 (1956).
[12] Vogel, F. S., and McGregor, D. H., *Lab. Invest.*, **13**, 767 (1964).
[13] D'Agostino, A. J., and Luse, S. A., *Neurology*, **14**, 529 (1964).
[14] Stäubli, W., and Loustalot, P., *Cancer Res.*, **22**, 84 (1962).

Ability of Haematopoietic Spleen Colonies to form Macrophages *in vitro*

THERE is substantial evidence that blood monocytes are the immediate precursors of macrophages in inflammatory exudates[1,2] and in the peritoneal fluid[3], and that bone marrow is a potential source of these macrophages[3-5]. In previous work using the chromosome marker technique, and *in vitro* cultivation of macrophages, it was shown that in mouse radiation chimaeras, bone marrow is a source of alveolar macrophages and of macrophages which can be cultured from different organs[6]. This work also suggested that only haematopoietic tissues contain precursor cells for macrophages.

In the work reported here the relationship of macrophage origin to the granulocytic and erythrocytic series of differentiation was investigated by establishing macrophage cultures from haematopoietic spleen colonies[7] of different cellular composition. The yield of macrophages was found to be correlated to the type of colony, and dividing macrophages had the same genotype as the donor cells used for the production of the colonies.

Adult male *CBA* mice were given 820 r. using a dual source ^{137}Cs irradiator at a dose rate of 180 r./min. Within 6 h of irradiation the mice were injected with 20,000 nucleated bone marrow cells from $CBA.T_6T_6$ donors. The surviving mice were killed 13–16 days later and their spleens were removed. Individual colonies which were well separated from neighbouring colonies were dissected and cut in half. Imprint preparations were made on sterile slides from the cut surfaces and stained with May–Grünwald–Giemsa (MGG). Half of each colony was dispersed by aspirating in a plastic syringe, incubated in the presence of 0·5 µg/ml. of colcemid and processed for chromosome preparations[8]. The other half was cut into smaller pieces, where necessary, and used for organ cultures as previously described[6,9]. After 2 days the coverslips underlying the organ explants were removed from the grid culture system, and the coverslip cultures were grown separately in small dishes. To induce macrophages to divide, medium in the separate coverslip cultures was supplemented with 50 per cent of used medium from *L* cell cultures, known to contain growth factor(s) for macrophages[10]. Coverslip cultures were processed after 3–4 days for chromosome preparations as described earlier[6]. When duplicate cultures were available, these were stained with MGG for morphological observations.

During the first 2 days, all organ cultures of spleen colonies produced large numbers of round, free-floating cells which dropped on the coverslip underlying the explants. During 3–4 days of incubation in separate culture dishes, most cells degenerated. In most cultures, however, a variable number of cells became attached to the coverslips. These large, stretched cells had the morphological characteristics of macrophages[6], judged by observations of living cultures using an inverted microscope with phase optic and of MGG-stained preparations. The capability of colony explants to produce macrophages was scored visually from + + + (crowded monolayer of macrophages) to – (less than 100 macrophages/coverslip) by observing the living cultures. The type of colony was determined from an imprint preparation stained with MGG. A colony was scored as "erythrocytic" if less than 3 per cent of the cells could be identified as immature or mature granulocytic cells, and vice versa. The rest of the colonies were called "mixed". Erythrocytic colonies showed little ability to produce macrophages. Granulocytic and most of the mixed colonies produced large numbers of macrophages (Table 1). Satisfactory chromo-

Table 1

Colony No.*	Age of colony (days)	Type of colony	Incidence of T_6 cells in whole colony	Formation of macrophages *in vitro*	Incidence of T_6 marker in macrophages
165A	13	Erythrocytic †	N.D.§	–	0/0¶
172A	14	,,	N.D.	+	0/0
182A	14	,,	N.D.	–	0/0
187B	16	,,	N.D.	–	0/0
188A	16	,,	9/9	+	5/5
166A	15	Mixed	30/30	+ + +	30/30
166B	15	,,	30/30	+	11/11
167A	15	,,	30/30	+	11/11
167B	15	,,	30/30	+ +	21/21
172B	14	,,	N.D.	+	0/0
172C	14	,,	22/22	+ +	7/7
173A	15	,,	30/30	+ + +	30/30
174A	15	,,	30/30	+ + +	30/30
174B	15	,,	30/30	+ +	30/30
174D	15	,,	N.D.	+	0/0
186B	15	,,	2/2	+	19/19
187A	16	,,	5/5	+ +	30/30
188B	16	,,	30/30	+ +	16/16
165B	13	Granulocytic ‡	30/30	+ + +	15/15
174C	15	,,	N.D.	+ +	0/0
186A	15	,,	24/24	+ + +	30/30
188C	16	,,	30/30	+ +	22/22

* Colonies with the same number were isolated from the same spleen.
† Less than 3 per cent granulocytic cells.
‡ Less than 3 per cent erythrocytic cells.
§ Not done.
¶ No well spread metaphases found.

some preparations could be obtained only from coverslip cultures with large numbers of macrophages. In all examples, the dividing macrophages had the T_6 marker chromosomes of the bone marrow donor, as did all the dividing cells of the individual colonies (Table 1); thus there was no contribution from the host spleen to the macrophage population.

Spleen colonies seem to result from the proliferation of single stem cells[11], which are pluripotent and capable of giving rise both to differentiated erythrocytic, granulocytic and megakaryocytic cells, and to new colony-forming cells[12]. The present findings suggest that the colony-forming stem cell is also capable of producing progeny which differentiates into macrophages *in vitro*. Macrophage formation seems to be related to the granulocytic series of differentiation. It would be important to establish if the presence of some specific cell type(s) in granulocytic colonies could be correlated to the capability of the colonies to form macrophages.

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[1] Volkman, A., and Gowans, J. L., *Brit. J. Exp. Path.*, **46**, 60 (1965).

[2] Spector, W. G., Walters, M. N-I., and Willoughby, D. A., *J. Path. Bact.*, **90**, 181 (1965).

[3] Volkman, A., *J. Exp. Med.*, **124**, No. 2, 241 (1966).

[4] Balner, H., *Transplantation*, **1**, 217 (1963).

[5] Volkman, A., and Gowans, J. L., *Brit. J. Exp. Path.*, **46**, 62 (1965).

[6] Virolainen, M., *J. Exp. Med.* (in the press).

[7] Till, J. E., and McCulloch, E. A., *Radiat. Res.*, **14**, 213 (1961).

[8] Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).

[9] Jensen, F. C., Gwatkin, R. B. L., and Biggers, J. D., *Exp. Cell Res.*, **34**, 440 (1964).

[10] Virolainen, M., and Defendi, V., *The Wistar Institute Symposium Monograph No. 7* (edit. by Defendi, V.), 67 (The Wistar Institute Press, Philadelphia, 1967).

[11] Becker, A. J., McCulloch, E. A., and Till, J. E., *Nature*, **197**, 452 (1963).

[12] Fowler, J. H., Wu, A. M., Till, J. E., McCulloch, E. A., and Siminovitch, L., *J. Cell Physiol.*, **69**, 63 (1967).

Hypothesis to explain Synapsis of Meiotic Chromosomes

THERE is so far no satisfactory explanation for the synapsis of homologous chromosomes at meiosis[1]. In the past, the process has been attributed to various factors such as electrostatic forces[2], resonance bonding[3], hydrodynamic forces[4] and antigen-antibody formation[5]. The criticisms of these hypotheses have been reviewed by Riley and Law[1]. In this communication I have attempted to explain this phenomenon in molecular terms.

I suggest that the molecular process which, according to Whitehouse[6], results in crossing over is also responsible for synapsis. This process is the formation of hybrid DNA between broken single strands of homologous chromatids, which gives precision of pairing and at the same time establishes bonds between the homologues. Initial contact of the homologues would be made at the chromocentre or more rarely at other places on the chromosomes[7]. From these points of initial contact, the formation of hybrid DNA would then proceed along the chromosomes in both directions in zipper-like fashion. Synaptic (hybrid DNA) bonds would arise from single (primary) breaks in the homologous DNA molecules, while crossing over occurs later, through another set of (secondary) breaks in the other strand of the same DNA helices. Synaptic bonds not involved in crossing over would be removed through an excision-repair process as found in thymine dimer removal[8]. There is still the question of the nature of the initial force which attracts the homologous centromeres to each other. A controlling element in the heterochromatin near the centromeres might have this recognizing and attracting property[9].

This model differs from that of Whitehouse[6] only in the assumption that hybrid DNA itself is responsible for synapsis. For this to be correct the primary breaks must occur before synapsis. This would, in fact, be so if the assumption[10] were correct that the same breaks were involved both in transcription and crossing over; experimental evidence on this point is contradictory[11]. If the same breaks are involved in both processes, then single polynucleotide strands would have to persist from interphase (when transcription occurs) until prophase (when synapsis and crossing over take place). Single strands of DNA are known to be degraded by DNases[12], but such enzymes have so far been detected only in bacteria, and may not be present universally in interphase nuclei.

Various unexplained phenomena of synapsis and crossing over could be fitted into this model of synapsis. Synaptinemal complexes are usually found only in regions of association of chromosomes in the bivalents[13] and may represent regions of hybrid DNA as seen in the electron microscope. The central core could be the region of hybrid DNA itself, while the thin connecting chromatin strands could be single stranded portions of the DNA helices. The lateral components are more difficult to explain.

Several authors[14] have suggested that mutagens may induce mitotic or meiotic crossing over by facilitating pairing of homologues where it is weak or, as in *Drosophila* spermatozoa, does not occur. With the proposed hypothesis, the inducing agents might do this by providing more single strand breaks.

Genetic control of synapsis is a well known phenomenon[15]. Conceivably this, too, may act by means of control of the breaks available for synapsis. Pontecorvo[16] has suggested that in the course of evolution a reduction of crossing over has occurred in somatic cells from a primitive condition in which crossing over was very frequent. One of the evolutionary mechanisms for suppressing crossing over may be shortage of breaks at the appropriate time either because fewer breaks occur or more are repaired before prophase.

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[1] Westergaard, M., *CR Trav. Lab. Carlsberg*, **34**, 359 (1964). Whitehouse, H. L. K., *Towards an Understanding of the Mechanism of Heredity*, 63 (Edward Arnold, London, 1965). Riley, R., and Law, C. N., *Adv. Genet.*, **13**, 57 (1965).

[2] Darlington, C. D., *Recent Advances in Cytology*, second ed., 671 (Blakiston, Philadelphia, 1937).

[3] Delbrück, M., *Cold Spring Harbor Symp. Quant. Biol.*, **9**, 122 (1941).

[4] Fabergé, A. C., *J. Genet.*, **43**, 121 (1942).

[5] Hinton, T., *Biol. Bull.*, **88**, 144 (1945).

[6] Whitehouse, H. L. K., *Nature*, **199**, 1034 (1963).

[7] Swanson, C. P., *Cytology and Cytogenetics*, 207 (Macmillan and Co., London, 1963).

[8] Boyce, R. P., and Howard-Handers, P., *Proc. US Nat. Acad. Sci.*, **51**, 293 (1964).

[9] Novitski, E., *J. Cell. Comp. Physiol.*, **45**, Suppl. 2, 151 (1955). Crouse, H. V., *Genetics*, **45**, 1429 (1960).

[10] Jones, K. W., and Truman, D. E. S., *Nature*, **202**, 1264 (1964). Whitehouse, H. L. K., *Nature*, **211**, 708 (1966).

[11] Luzzati, M., thesis, Paris (1965), quoted by Whitehouse, H. L. K., *Nature*, **211**, 708 (1966). Shestakov, S., and Barbour, S. D., *Genetics*, **57**, 283 (1967). Helling, R. B., *Genetics*, **57**, 665 (1967).

[12] Lehman, I. R., and Richardson, C. C., *J. Biol. Chem.*, **239**, 233 (1964).

[13] Meyer, G. F., *Proc. Eur. Reg. Conf. Electron Microscopy*, **3**, 461 (1964). Westergaard, M., and von Wellstein, D., *CR Trav. Lab. Carlsberg*, **35**, 261 (1966).

[14] Auerbach, C., *Proc. Roy. Soc. Edin.*, Sec. B, **62**, 120 (1945–46). Sobels, F. H., and van Steenis, H., *Nature*, **179**, 29 (1957). Holliday, R., *Genetics*, **50**, 323 (1964). Zimmermann, F. K., and von Laer, U., *Mutation Res.*, **4**, 377 (1967).

[15] Wald, H., *Genetics*, **21**, 264 (1936). Miller, O. L., *Genetics*, **48**, 1445 (1963). Riley, R., *Sci. Prog., Oxf.*, **54**, 193 (1966).

[16] Pontecorvo, G., *J. Linnean Soc., Lond.*, **44** (Zool.) and **56** (Bot.), 116 (1958).

GENETICS

Cyclopism as a Hereditary Malformation

CYCLOPISM is a malformation which has been recognized for many years and which may have furnished the model for the Homeric monsters. It has been suggested that this developmental disorder is hereditary, but until recently there has been no proof of this.

One of us reported an extra, very small chromosome in 30 per cent of fibroblasts from a cyclops[1]. Some weeks after the report was published the same hospital announced another cyclops whose mother was an aunt to the mother of the first. Attempts to grow fibroblast cultures from the child at birth were unsuccessful. At the same time we started lymphocyte and fibroblast cultures from the mother and initiated an examination of the family. Its pedigree is shown in Fig. 1. Careful discussion of the family history with its different members revealed the birth of two other cyclopes (cases 15 and 17) to two sisters of case No. 7, one being the grandmother (case 5) of the 1966 cyclops (case 26). Both cyclopes had been born at home in 1939 and 1942, respectively, and were described by the mothers. Cases 23 and 26 had been delivered in a hospital. In addition there had been eleven abortions and other proved or probable malformations, and there were only twelve healthy births in the family.

Chromosome counts were carried out for all members of the family except two, and the number was found to be 46. Fig. 2 shows one of twenty karyograms of the mother (case 7) of the most recently born cyclops. A missing third chromosome and a supplementary chromosome in the *C* group are characteristic features. In the best karyograms there is one *C* chromosome as large as the *B* chromosomes, but its short arm is distinctly larger and its long arm a little shorter than the arms of a chromosome of the *B* group. None of the arms resemble a normal third chromosome. So far we have not been able to decide whether this *C* chromosome is really changed or only less spiral. Detailed autoradiographic studies and control of secondary constrictions will perhaps solve this problem.

Identical karyograms are found in the three healthy sisters (cases 3, 5 and 6), their father (case 1) and in the third generation in the mother (case 13) of the 1966 cyclops. Fifteen to twenty karyograms were controlled from each person.

The most probable explanation is that a translocation from a third chromosome to a *C* chromosome and eventually to the *X* chromosome is balanced in the healthy persons 1, 3, 5, 6, 7 and 13. Case 1 transmitted it to his four daughters. One male and one probably male abortion could be unbalanced. The quadrivalent ring or chain formation in the meiotic division of the daughters led to meiotic accidents, such as the extra very small chromosome of the 1966 cyclops[1]. The deletion of a small chromosome segment probably led to cyclopism four times. Other distributions of the structurally changed chromosomes could explain at least some of the abortions and different malformations.

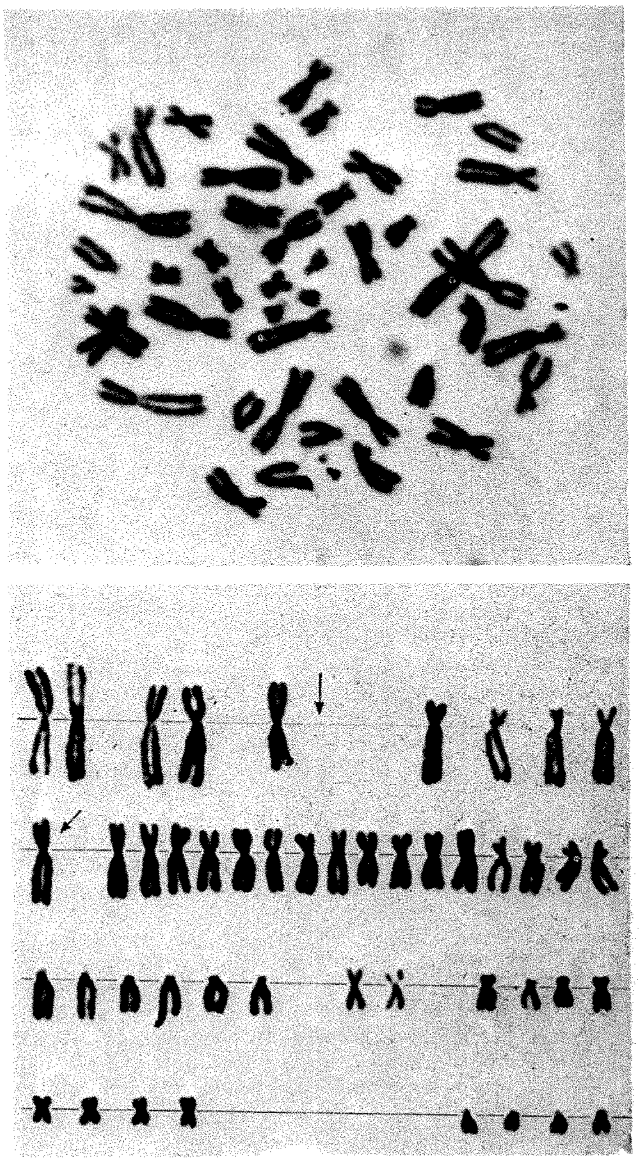

Fig. 2. Karyogram of one of the mothers of the cyclopes (case 7) (× 1,300).

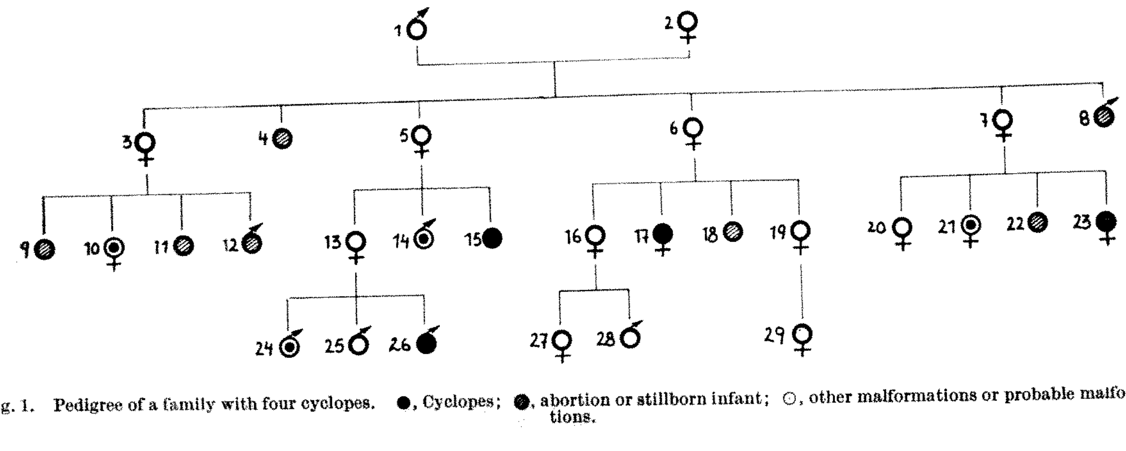

Fig. 1. Pedigree of a family with four cyclopes. ●, Cyclopes; ◍, abortion or stillborn infant; ○, other malformations or probable malformations.

Together with the twins of Ellis[2], the two brothers of Klopfstock[3], the three children in the family described by Dominok and co-workers[4], the arrhinencephalic two sisters of DeMyer and co-workers[5], this is another case of family cyclopism or related malformations, the four different mothers being relatives and showing the same chromosomal aberration. The hereditary character of the malformation seems to be beyond doubt in our case. We hope that other translocation patterns will allow localization of the cyclopism-locus on one of the two involved chromosomes one day.

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[1] Pfitzer, P., *Lancet*, ii, 102 (1967).
[2] Ellis, R., *Trans. Obstet. Soc.*, 7, 160 (1865).
[3] Klopfstock, A., *Mschr. Geburtsh. Gynäk.*, 56, 59 (1921).
[4] Dominok, G. W., and Kirchmaier, H. H., *Z. Kinderheilkunde*, 85, 19 (1961).
[5] DeMyer, W., Zeman, W., and Palmer, C. G., *Neurology*, 13, 913 (1963).

PATHOLOGY

Sarcomas in Afghanistan

SARCOMAS in general receive much less attention than carcinomas. There are two reasons for this: first, sarcomas are considerably outnumbered by carcinomas in most populations and, second, environmental, that is, avoidable, factors have been more often aetiologically related to carcinomas than to sarcomas. For these reasons I have made this investigation. In Afghanistan, sarcomas are not greatly outnumbered by carcinomas and I shall suggest that there may be an aetiological relationship between some sarcomas and an environmental factor, namely, arthropod vectors.

During the period from September 1965 until December 1967, forty sarcomas of the soft tissues (site No. 197)[1] were received in the Department of Pathology of the Faculty of Medicine of the University of Kabul. Of the malignant neoplasms received during this period, this site ranked third (after skin and lymphomas), and accounted for 8·0 per cent of all cancers—7·0 per cent in males and 10·1 per cent in females. Of the forty tumours, twenty-three were in males and seventeen in females. Fig. 1 shows the age distribution for thirty-nine of these tumours. The average age of the males was 36 years, and the range was 6–75. The average age of the females was 37, and the range was 4–60. The incidence was highest between the ages of 36 and 45.

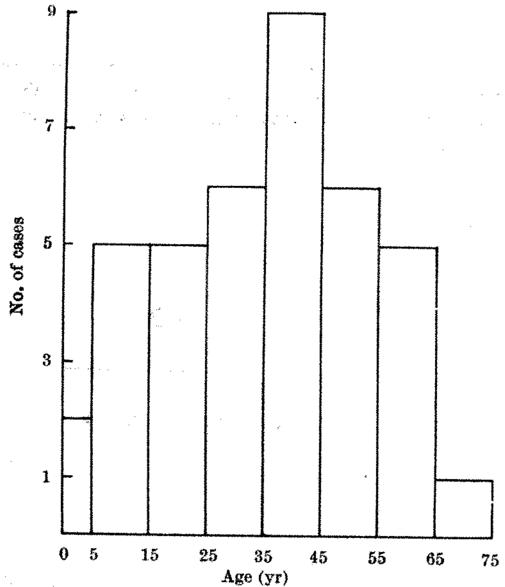

Fig. 1. Age distribution of sarcomas in Afghanistan.

Table 1. DISTRIBUTION OF SITES OF SOFT TISSUE SARCOMAS

Site	Head	Trunk	Upper limb	Lower limb
ICD No.	197.0	197.1	197.2	197.3
Males	3	4	2	14
Females	2	7	1	7
Total	5	11	3	21
Percentage	12·5	27·5	7·5	52·5

Table 2. DISTRIBUTION OF SOFT TISSUE SARCOMAS BY HISTOLOGICAL TYPE

Histological type	Fibrosarcoma	Rhabdomyosarcoma	Liposarcoma	Leiomyosarcoma	Small cell sarcoma	Haemangiosarcoma	Myxosarcoma	Unclassified
Males	9	1	1	2	3	–	2	5
Females	6	2	1	1	2	2	1	2
Total	15	3	2	3	5	2	3	7
Percentage	37·5	7·5	5·0	7·5	12·5	5·0	7·5	17·5

Table 1 shows the distribution of the sites of the sarcomas. More than 50 per cent were on the lower extremities, and almost half of these were on the thigh (ten cases). Table 2 shows the histological types of the tumours. Fibrosarcomas accounted for almost 40 per cent. Liposarcomas were rare compared with American and European series of tumours[2,3].

Undoubtedly, the relatively large proportion of soft tissue sarcomas in this series was influenced by the ease of resection and the obvious disfigurement caused by tumours at this site compared with deeper malignancies. The comparative youthfulness of the population in Afghanistan is another factor which might explain a high proportion of soft tissue tumours. Data provided by the National Malaria Eradication Project in Afghanistan showed that 52 per cent of the rural population were under 15 years of age. In the series which I studied, almost 50 per cent of the patients were less than 35 years old.

The aetiology of neoplasms of the soft tissues is unknown, although trauma is sometimes implicated. The connective tissues are usually protected from the environmental influences that affect the skin and other epithelial surfaces, for example, solar radiation, chemical agents, irritating foods and smoke. The subepidermal connective tissues, however, are particularly exposed to arthropods which pierce the epidermis.

The transmission of a variety of infectious diseases—viral, bacterial and protozoal—by arthropods is well known, and these animals have also been associated with the transmission of neoplastic diseases. Suspensions of adult and larval beetles administered orally or intraabdominally have produced leucosis in fowls[4]. The hamster reticulum cell sarcoma, *TM*, has been transmitted by the mosquito, *Aedes aegypti* (L), by a transfer of tumour cells. The cells remained viable within the vector for up to 8 h after ingestion[5]. The Shope fibroma virus can be transmitted by the bites of fleas and mosquitoes, causing neoplasms in the hosts, and may remain active in the vector for several weeks[6]. Squirrel fibroma virus can be transmitted by blood-sucking vectors, which remain infective for at least 16 days[6]. The Yaba monkey virus, which produces benign histiocytomas, is also thought to be transmitted in nature by blood-sucking arthropod vectors[6].

The experimental subcutaneous administration of tumour-inducing agents, both viral[7] and chemical[8], often leads to the development of sarcomas at the sites of inoculation.

The distribution of bites from crawling arthropods, for example, fleas, ticks and bedbugs, is similar to the distribution of our soft tissue sarcomas, that is, they are most abundant on the lower extremities and rarely found on the palms or soles.

The almost equal occurrence of the tumours in males and females may indicate an equal risk of exposure. Contact with vector-borne carcinogenic agents could begin very early in life and may thus account for the lower average age incidence for soft tissue sarcomas compared with other types of cancers. Viral carcinogenesis is more easily produced experimentally in young than in older animals[9].

The penetrating part of an insect is most likely to encounter the more superficial and abundant fibrous tissue of the dermis than the deeper fat and skeletal muscle or the comparatively sparse smooth muscle. This might explain why fibrosarcomas are most prevalent. Thus there is good reason for further examination of the hypothesis that soft tissue sarcomas may be caused by carcinogenic agents introduced through the skin by arthropod vectors.

This work was carried out while I was WHO professor of pathology at the Faculty of Medicine, University of Kabul.

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[1] *WHO Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death*, seventh revision (WHO, Geneva, 1957).

[2] Stout, A. P., *Tumors of the Soft Tissue, Atlas of Tumor Pathology*, Section II—Fascicle 5 (AFIP, Washington, 1953).

[3] Ringertz, N., Ericsson, J., Sjöstrom, A., and Swenson, D., *Cancer Incidence in Sweden 1961* (National Board of Health, the Cancer Registry, Stockholm, 1965).

[4] Eidson, C. S., Schmittle, S. C., Goode, R. B., and Lal, J. B., *Amer. J. Vet. Res.*, **29**, 1053 (1966).

[5] Banfield, W. G., Woke, P. A., and MacKay, C. M., *Cancer*, **19**, 1333 (1966).

[6] *WHO Tech. Rep. Ser.*, No. 295, 16 (WHO, Geneva, 1965).

[7] Trentin, J. J., Yabe, Y., and Taylor, G., *Science*, **137**, 835 (1962).

[8] Willis, R. A., *Pathology of Tumours*, third ed., 35 (Butterworths, London, 1960).

[9] Rich, M. A., Karl, S. C., and Clymer, R., *Nature*, **216**, 270 (1967).

PHYSIOLOGY

Time Relationships of Spermatozoon Penetration into the Egg of the Rhesus Monkey

IN some mammalian species, spermatozoa must be exposed to the female reproductive tract for some time before they acquire the ability to penetrate the egg[1,2]. During this time the spermatozoa are thought to undergo an essential physiological change—capacitation[3]—which enables them to pass through the mass of cumulus cells surrounding the recently ovulated egg, and then to penetrate the zona pellucida[4]. It is possible that capacitation is an essential prerequisite for fertilization in all mammals, but there is no direct evidence that capacitation occurs in the human or any sub-human primate.

In the species investigated so far, the need for capacitation has been established by a variety of experimental techniques, all of which depended on a detailed examination of the time relationship between insemination and the penetration of the spermatozoon[5]. We have been able to study the time relationships of spermatozoon penetration in the rhesus monkey.

Thirty observations were made on twenty-eight mature rhesus monkeys subjected to laparotomy between menstrual days 11 and 15. The monkeys all showed an established menstrual periodicity, and a deliberate attempt was made to synchronize the laparotomies with the time of expected ovulation in each monkey. At laparotomy, seventeen monkeys had ovulated and in the others the ovaries were in a pre-ovulatory condition. A suspension of epididymal spermatozoa was injected into the uterine lumen of the monkeys that had ovulated, and they were then maintained under surgical anaesthesia for about 6 h. During this time, their body temperature was maintained by using a heated blanket, and the initial pentobarbitone sodium ('Nembutal', Abbott Laboratories, Ltd.) anaesthesia, which had been adequate for the laparotomy, was supplemented with phencyclidine hydrochloride ('Sernylan', Parke Davis and Co.).

The eleven sperm suspensions for insemination were obtained from six normal, fully mature males that were being killed or castrated for other experiments. The contents of a single cauda epididymis, and as much of the vas deferens as was attached to it, were expressed into 2·5 ml. of medium *TC* 199. The resulting suspension was immediately inseminated into the uterus. Approximately 10^8 vigorously motile spermatozoa were inseminated in $<0·4$ ml. of medium.

Between 5·5 h and 6·5 h after insemination the original laparotomy wound was reopened so that the fallopian tubes could be flushed with physiological saline. The flushes were carefully examined for the presence of eggs and spermatozoa.

A total of nine one-celled eggs was recovered from the seventeen monkeys inseminated. All the eggs were naked and one was completely degenerate. Six eggs had not been penetrated by a spermatozoon although three had spermatozoa embedded in their zonae pellucidae. The numbers of embedded spermatozoa were four and twenty-six on two seemingly normal eggs, and six on the wholly degenerate egg. When the five, apparently normal, one-celled eggs were examined after fixation and staining[5] they were all found to contain a first polar body and their chromosomes were at the second meiotic metaphase.

Three eggs had been penetrated by spermatozoa. One egg was at the pro-nuclear stage and showed two well formed pro-nuclei (Fig. 1*a*), a first and second polar body and one spermatozoon embedded in the zona pellucida. The tail of the penetrating spermatozoon could be identified in the vitellus. This egg was recovered 6 h after insemination and was examined within 20 min of recovery. The other penetrated eggs both showed a swollen spermatozoon head (Fig. 1*b*) and an associated tail lying within the vitellus. A first polar body was present in each case and one and two spermatozoa, respectively, were embedded in the zonae. These eggs were recovered 6·5 h after insemination and examined within 20 min; in one egg the chromosomes were still at the second meiotic metaphase, whereas those of the other egg were at anaphase.

Spermatozoa were only detected in three flushes from the fallopian tube, and it was estimated that a total of <50 sperm were present in each of them. Thus, although a large number of spermatozoa had been inseminated directly into the uterus, the number recovered from the fallopian tube was similar to that observed after natural mating (unpublished work of Kelly and Marston).

It is not yet possible to determine the exact time of ovulation in the naturally cyclic rhesus monkey. In this study, it was not feasible to remove the ovaries that had ovulated so as to determine the morphological age of their corpora lutea. Thus the actual post-ovulatory age of the eggs we recovered cannot be estimated. Some of them may have reached the end of their fertilizable life

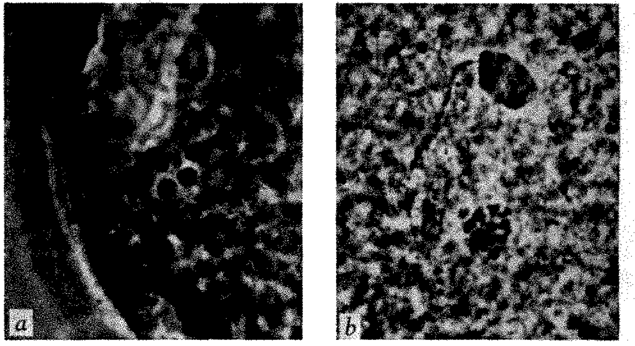

Fig. 1. Rhesus monkey egg after fixation and staining. *a*, Male pro-nucleus; *b*, swollen spermatozoon head and part of second meiotic anaphase figure.

before they were exposed to spermatozoa. The fact that three out of the nine eggs which we recovered had been penetrated suggests that, if capacitation is essential for spermatozoon penetration in the rhesus monkey, it must be completed within 6 h of insemination. By comparison with the rate of development in other mammals, it is possible that the pro-nuclear egg was actually recovered about 2–3 h after penetration. If this estimate is accepted, the process of capacitation may be completed within 3–4 h.

Experimental proof that capacitation is essential in the rhesus monkey can be obtained by showing that eggs are more rapidly penetrated by spermatozoa that have already undergone preliminary incubation in the female reproductive tract. Because our results suggest that the interval between insemination with epididymal spermatozoa and penetration of the egg is very short, it may not be practicable to show the superiority of supposedly capacitated spermatozoa over epididymal spermatozoa. Should this be the case, the need for capacitation in the rhesus monkey can only be inferred from indirect evidence: for example, by successful *in vitro* fertilization of monkey eggs with spermatozoa previously incubated in the female reproductive tract, and complete failure of *in vitro* fertilization with epididymal spermatozoa.

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[1] Austin, C. R., *Austral. J. Sci. Res.*, **4**, 581 (1951).
[2] Chang, M. C., *Nature*, **168**, 697 (1951).
[3] Austin, C. R., *Nature*, **170**, 326 (1952).
[4] Austin, C. R., *Intern. J. Fertil.*, **12**, 25 (1967).
[5] Marston, J. H., and Chang, M. C., *J. Exp. Zool.*, **155**, 237 (1964).

Relation of Epithelial Ion Transport to Corneal Thickness and Hydration

THE control of corneal thickness is a complex process involving the chemistry of the stromal tissue and the barrier properties of the two bounding membranes. Fluid dynamics are intimately involved, for corneal thickness is linearly related to hydration[1], which in turn is affected by ion transport. Also, it was shown recently that in the rabbit cornea the epithelium is the source of an active sodium transport system, and the endothelium shows only a passive behaviour in relation to the passage of ions[2].

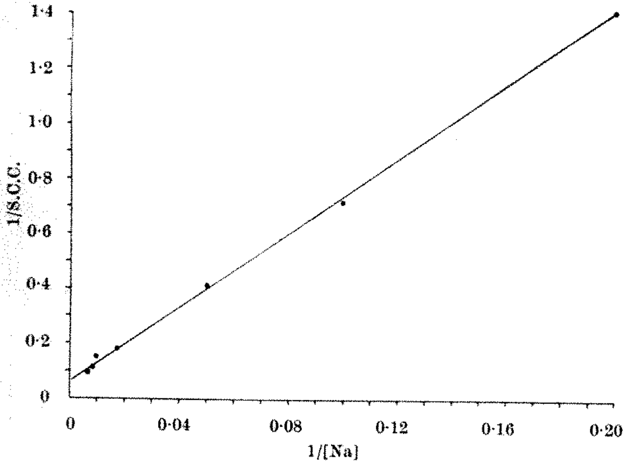

Fig. 1. Lineweaver–Burke type plot of short-circuit current (S.C.C.) against sodium concentrations in the fluid bathing the epithelial surface of the rabbit cornea.

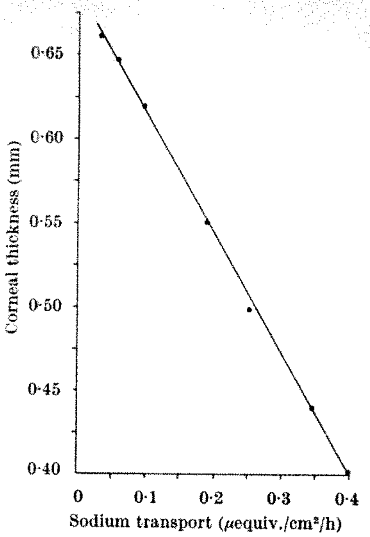

Fig. 2. The stable corneal thickness (mm) at each epithelial sodium concentration is plotted against the stable short-circuit current (in µequiv./cm²/h) at that sodium concentration.

To investigate the effect of the sodium transport system on corneal thickness and hydration, an external voltage was applied through the cornea in opposition to the standing potential (endothelium normally positive); the resultant increase in corneal thickness was proportional to the applied voltage[3]. It was reasoned that if the applied voltage controlled the active sodium transport, the variations in corneal thickness resulted from different rates of sodium transfer into the stroma. Correlated with the increases in thickness, decreases in the sodium concentration of the stroma were found; but no effect on other ions such as K^+ or Cl^- was seen.

Our experiments were made on isolated corneas mounted in chambers either for the measurement of electrical activity[4] or corneal thickness[3] (using a Haag–Streit slit lamp as modified by Maurice and Giardini[5]). Using Krebs–Ringer bicarbonate to bathe the cornea, the sodium concentration of the solution bathing the epithelial surface was decreased while the total chloride concentration and osmolarity of the solution were kept constant with the addition of choline chloride. The sodium-ion concentrations used to bathe the epithelial surface were 143, 118, 100, 60, 20, 10 and 5 mM, while the endothelial surface was bathed in 118 mM sodium throughout the experiment.

When the sodium concentration of the epithelial bathing solution was decreased, the short-circuit current, which indicates the rate of sodium transport, also decreased. When epithelial sodium concentration was plotted against the stable short-circuit current obtained with each of the various concentrations of bathing solution, a saturation-type curve was obtained. Fig. 1 shows the straight-line relationship seen in double-reciprocal plottings.

Corneal thickness was also seen to increase as the epithelial sodium concentration was decreased. When the solution bathing the epithelium was changed to contain less sodium, there was an increase in thickness dependent on the difference in sodium concentration. At all concentrations there was a stabilization of thickness after 80–100 min. If each stable thickness is plotted against the stable short-circuit current (converted to µequiv./cm²/h) at the particular epithelial sodium concentration, a straight-line relationship is obtained (Fig. 2).

In contrast, when the solution bathing the epithelium was maintained at 143 mM sodium and the sodium concentration of the endothelial solution was varied, the short-circuit current remained virtually unaltered. The rate of sodium transport was initially stimulated after these changes, but then stabilized at the original value.

Corneal thickness also showed little change when the endothelium was bathed in solutions of differing sodium concentrations.

These experiments reveal a direct relationship between the rate of transport of sodium ions and corneal thickness: as the rate of sodium transport decreases, corneal thickness increases. It was suggested from the previous work involving the use of applied voltages[3] that the rate of transport of sodium ions controls the sodium content of the stroma (which is primarily an anionic mucopolysaccharide) and in this way exerts a control on corneal hydration. The results reported here show that such a relationship between sodium ion transport and corneal hydration exists even without the application of extraneous (for example, electrical) forces. The transport–hydration relationship is physiologically fundamental to the control of corneal hydration and thickness. These findings also substantiate the role of the epithelium in the maintenance of corneal thickness.

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[1] Hedbys, B. O., and Mishima, S., *Exp. Eye Res.*, **5**, 211 (1966).
[2] Green, K., *Exp. Eye Res.*, **6**, 79 (1967).
[3] Green, K., *Life Sci.*, **5**, 2309 (1966).
[4] Green, K., *Amer. J. Physiol.*, **209**, 1311 (1965).
[5] Maurice, D. M., and Giardini, A., *Brit. J. Opnthalmol.*, **35**, 169 (1951).

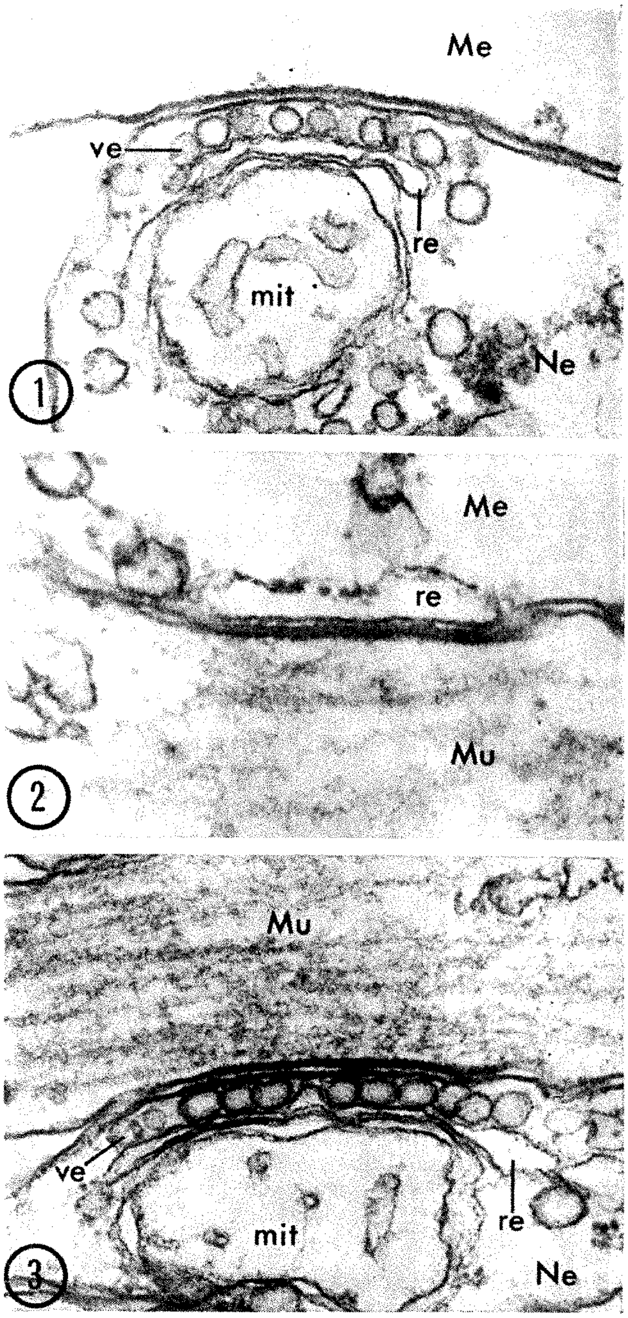
Specialized Connexions between Nerve Cells and Mesenchymal Cells in Ctenophores

THE mesogloea in ctenophores, as in certain coelenterates[1,2], is essentially a third cell layer containing muscle cells, nerve cells and so-called "amoeboid" cells[3,4]. Our unpublished studies on *Beroe* suggest that the "amoeboid" cells do indeed undergo a migratory phase during regeneration, but that there is no evidence of migration in the normal animal. Histological preparations show the cells with very long, thin processes connected together in the form of a net. We prefer to call them "mesenchymal" cells. We now have evidence from electron microscopy of specialized junctions between these mesenchymal cells and neighbouring muscle and nerve cells in *Beroe forskälii* and *B. ovata*.

At the neuro-mesenchymal cell junction the membranes are in close contact—separated by a gap of 130–150 Å—and show an obvious, densely staining thickening (Fig. 1). The nervous side of the junction is characterized by a row of vesicles tightly packed between the cell membrane and a sac of smooth endoplasmic reticulum, itself flattened against a single, huge mitochondrion (Fig. 1). The diameter of the vesicles ranges from 300 to 900 Å, with an average of 660 Å, which slightly exceeds the average size of "synaptic vesicles" of higher animals. After osmic fixation followed by block-staining with phosphotungstic acid (PTA) and section-staining with uranyl acetate and lead citrate, the vesicles look clear or are unevenly filled with a grey, amorphous mass. After fixation with glutaraldehyde, however, most of them show an electron-dense core of variable diameter.

Ultrastructural specializations such as those just described are also found at interneural, neuro-muscular (Fig. 3), neuro-glandular and neuro-sensory junctions in Beroids, Cydippids and Lobates (my unpublished results) and they correspond in essential features to the structures earlier described by Horridge[5,6] as synapses. If it is correct to regard them as such (and we assume that it is), it seems that the junctions between nerve cells and mesenchymal cells are also synapses, which implies that mesenchymal cells are effectors. It is noteworthy that mesenchymal cells receive large numbers of such synapses, in some cases receiving more than adjacent muscle cells. A single neurite may make synaptic connexions with both muscle and mesenchymal cells, which suggests that the two effectors could be excited at the same time.

In the absence of physiological evidence it would be inappropriate to speculate about the spread of excitation within or beyond the mesenchymal cell, or about possible effector action resulting from such excitation. It is of



Figs. 1–3. Electron micrographs from tangential sections of *Beroe forskälii* below the lip. Tissue fixed with OsO_4, buffered according to the method of Palade; embedded in 'Epon'; block stained by PTA; sections stained with uranyl acetate followed by lead citrate. 1, Neuro-mesenchymal junction (×59,000). 2, Junction between a mesenchymal cell and a muscular fibre (×82,000). 3, Neuromuscular junction (×59,000). *Me*, Mesenchymal cell; *mit*, mitochondrion; *Mu*, muscle cell; *Ne*, neurite; *re*, endoplasmic reticulum; *ve*, synaptic vesicles.

interest that specialized junctions of a distinct type occur between mesenchymal cells and muscle cells (Fig. 2) and between the processes of contiguous mesenchymal cells. These junctions show thickening and close approximation of the membranes (Fig. 2), but there is no accumulation of vesicles in the vicinity and the configuration is thus a symmetrical one. In junctions between mesenchymal cells and muscle cells a sac of endoplasmic reticulum does occur within the mesenchymal cell in direct apposition to the plasma membrane (Fig. 2). There is no evidence of membrane fusion and the junctions cannot be regarded as a type of "nexus"[7]. This does not, however, preclude the possibility that the junctions are somehow involved in information transfer between the cells in question.

From the point of view of comparative histology, it is instructive to align these observations of connexions between mesenchymal cells and nerve cells and between mesenchymal cells and muscle cells with evidence of similar connexions involving glio-interstitial cells in molluscs[8] and interstitial cells in rodents[9] and man[10].

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[1] Hyman, L. B., *The Invertebrates*, I, *Protozoa through Ctenophora* (McGraw-Hill Book Co., Inc., New York and London, 1940).
[2] Pavans de Ceccatty, M., and Buisson, B., *CR Acad. Sci.*, **259**, 3611 (1964).
[3] Hertwig, R., *Jenaische Ztschr. Naturw.*, **14** (1880).
[4] Pavans de Ceccatty, M., Hernandez, M. L., and Thiney, Y., *CR Acad. Sci.*, **254**, 3241 (1962).
[5] Horridge, G. A., *Quart. J. Microsc. Sci.*, **105**, 163 (1964).
[6] Horridge, G. A., *Proc. Roy. Soc.*, **162**, 333 (1965).
[7] Dewey, M. M., and Barr, L., *Science*, **137**, 670 (1962).
[8] Nicaise, G., *Nature*, **216**, 1222 (1967).
[9] Taxi, J., *Ann. Sci. Nat., Zool.*, **7**, 413 (1965).
[10] Honjin, R., Takahashi, A., and Tasaki, Y., *Okaj. Fol. Anat. Jap.*, **40**, 409 (1965).

Prostaglandins and Toad Spinal Cord Responses

PROSTAGLANDINS have a wide distribution in the vertebrate body and have been shown to be pharmacologically active on many types of vertebrate tissues. Evidence that these compounds have a neurohumoral role is suggested by several findings. Prostaglandins are widely distributed in the central nervous system[1], and in the rat cerebral cortex they are localized in synaptic vesicles[2]. They are released from the feline cortex[3] and possibly cerebellum and ventricles[4,5]. In addition, prostaglandins have effects on the central nervous system of cats and chickens[6,7]. We decided to investigate the effects of prostaglandins on the isolated toad spinal cord because the preparation is convenient for studying direct actions of drugs on a neuronal pool. Furthermore, prostaglandins are released from the frog spinal cord on stimulation of the hind limbs[8], suggesting that they are involved in synaptic transmission in amphibians.

Spinal cords of the toad *Bufo marinus* were isolated, hemisected and perfused with oxygenated amphibian Ringer solution. Experiments were performed during the Australian summer. The methods have been described in detail elsewhere[9,10]. The eighth dorsal root was stimulated every 10 s and a.c. or d.c. recordings were made from the ninth dorsal and ventral roots using Ag/AgCl electrodes. A d.c. preamplifier and 'Grass Polygraph' were used to record changes in potential level. Prostaglandins PGE_1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (supplied by Dr J. E. Pike) were prepared as 10 mg/ml. solutions in 95 per cent ethanol. Stoichiometric conversion to the sodium salt was effected by adding 0·9 ml. of a 0·2 mg/ml. solution of sodium carbonate for each 1 mg of prostaglandin. Immediately before application the stock solutions were diluted to an appropriate concentration with amphibian Ringer solution. L-Glutamic acid was applied regularly to the preparation in order to assess its level of excitability.

The three prostaglandins, PGE_1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$, all produced distinct changes in reflexes and polarization level when perfused over the toad spinal cord for periods of 0·5–2 min. Simultaneous d.c. records from dorsal and ventral roots were comparable except that drug-induced effects recorded from the dorsal root were more pronounced than those from the ventral root.

Typically, the prostaglandins caused a slowly developing, long-lasting depolarization of both dorsal and ventral roots. During the application of PGE_1, this was frequently preceded by a hyperpolarization of both roots. This is illustrated in Fig. 1*A*. Two minutes before the application of PGE_1, the spinal cord was perfused for 30 s with L-glutamic acid (5×10^{-5} g/ml.) which induced a rapid depolarization with a simultaneous reduction in size of the dorsal and ventral root potentials. Recovery was complete within 1 min of termination of the glutamate application. PGE_1 (5×10^{-5} g/ml.) caused a hyperpolarization during its 50 s application, followed by a depolarization which lasted for several minutes. The dorsal root polarization level had not reached control value 20 min after the application. During the hyperpolarizing phase of the response, polysynaptic reflex discharges in the ventral root were markedly reduced. Tracings *B* and *C* in Fig. 1 were recorded from the same preparation. $PGF_{1\alpha}$ and $PGF_{2\alpha}$ induced a slowly developing and long-lasting depolarization which began during the application.

PGE_1 had the most pronounced effects, usually causing a greater depolarization than L-glutamate applied in the same concentration. $PGF_{1\alpha}$ was usually more potent than $PGF_{2\alpha}$. Desensitization occurred with repeated applications. Thus, although the first application of a prostaglandin always had a significant effect, a second or third application a few minutes afterwards had little or no effect. On washing for 0·5–1 h, the prostaglandin again had a significant effect.

The results of this investigation, together with those of Ramwell *et al.*[8], suggest that prostaglandins have a

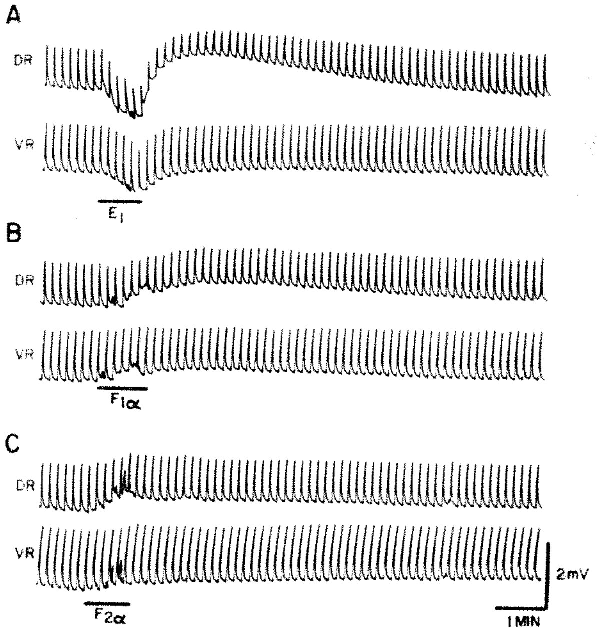

Fig. 1. d.c. Recording (negativity upwards) from dorsal and ventral roots of isolated hemisected toad spinal cord. *A*, *B* and *C* illustrate effects of three different prostaglandins on the same preparation, each applied in a concentration of 5×10^{-5} g/ml. Each pair of tracings represents simultaneous recordings from the ninth dorsal and ventral roots. The eighth dorsal root was stimulated at intervals of 10 s. Temperature was maintained constant at 20° C.

synaptic role in the amphibian spinal cord, but it is difficult to speculate on their mechanism of action. The extremely slow rate of recovery (their effects sometimes last for 0·5 h or more) may mean that they are not transmitters but that they affect the release of transmitter, as suggested by Ramwell *et al.*[8].

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[1] Horton, E. W., and Main, I. H. M., *Brit. J. Pharmacol. Chemother.*, **30**, 582 (1967).
[2] Kataoka, K., Ramwell, P. W., and Jessup, S., *Science*, **157**, 1187 (1967).
[3] Ramwell, P. W., and Shaw, J. E., *Amer. J. Physiol.*, **211**, 125 (1966).
[4] Coceani, F., and Wolfe, L. S., *Canad. J. Physiol. Pharmacol.*, **43**, 445 (1965).
[5] Feldberg, W., and Myers, R. D., *J. Physiol.*, **184**, 837 (1966).
[6] Avanzino, G. L., Bradley, P. B., and Wolstencroft, J. H., *Brit. J. Pharmacol. Chemother.*, **27**, 157 (1966).
[7] Horton, E. W., and Main, I. H. M., *Brit. J. Pharmacol. Chemother.*, **30**, 568 (1967).
[8] Ramwell, P. W., Shaw, J. E., and Jessup, R., *Amer. J. Physiol.*, **211**, 998 (1966).
[9] Phillis, J. W., and Tebecis, A. K., *Comp. Biochem. Physiol.*, **23**, 541 (1967).
[10] Tebēcis, A. K., and Phillis, J. W., *Comp. Biochem. Physiol.*, **23**, 553 (1967).

Evidence favouring the Existence of a Single Adrenergic Receptor

THE fact that adrenaline can act differently in various organs has often been explained by assuming that there are different kinds of receptors[1]. A simpler explanation of these differences in adrenaline action is that a single receptor, influenced in different ways by the different metabolic milieu in various organs, is sufficient to interpret all kinds of adrenaline effects. If this assumption is correct, the well defined receptor quality of the adrenaline effect, as tested by the effectiveness of different types of receptor blocking agents, can be transformed into another receptor quality in one organ, by changing its metabolic milieu.

This hypothesis is supported by the fact that the positive inotropic and chronotropic effects of adrenaline can be blocked by the alpha blocking agent ergotamine in the isolated heart of a frog[2], although myocardial effects of adrenaline in mammals are usually considered to be specifically connected with beta receptors[1]. If this difference is caused by the *a priori* lower metabolic rate of the frog heart, then increasing the metabolic level of the frog myocardium may result in the transformation of the quality of the adrenaline effect from alpha to beta. On this assumption, by lowering the metabolic rate of the mammalian heart, the adrenaline effect must lose its beta quality.

To test the validity of this assumption we altered the metabolic rate of both isolated amphibian and mammalian hearts by increasing or decreasing the temperature of the perfusion fluid. Frog hearts were isolated according to Straub. Rat hearts were isolated according to Langendorff and perfused at 38° C with Locke solution, containing 10 per cent of washed canine red blood cells. Adrenaline sensitivity (effective threshold dose for positive inotropic and chronotropic effects) was repeatedly determined in each preparation. To determine the adrenergic receptor quality of the myocardium, 3×10^{-8}–2×10^{-7} M phentolamine ('Regitin', Ciba) was used as an alpha blocking agent, and 2–4×10^{-8} M pronethalol ('Alderlin', ICI) or 1–3×10^{-8} M propranolol ('Inderal', ICI) was applied as beta receptor blocking agents. The blocking capacity was estimated on the basis of the decrease in adrenaline sensitivity after the administration of the blocking agent. Contractions of the heart were recorded by an isotonic lever.

The capacity of an alpha blocking agent to block the adrenaline effect in the frog heart[2] was confirmed in these experiments, and 3×10^{-8}–2×10^{-7} M of phentolamine was effective in blocking the adrenaline effect in the winter frog heart; that is, it decreased adrenaline sensitivity in twelve out of thirteen experiments at room temperature. When the metabolic rate of the winter frog heart was increased by raising the temperature to 30°–34° C, the blocking capacity of the same dose of phentolamine diminished, or disappeared completely, as illustrated by a typical experiment in Fig. 1. Adrenaline sensitivity of a winter frog heart was 0·0001 µg at 22° C (Fig. 1*A*). After 10 µg of phentolamine had been added, adrenaline sensitivity was decreased to 1 µg, that is, phentolamine produced a 10,000-fold blockade. At 32° C (Fig. 1*B*) adrenaline sensitivity of the same preparation was 0·001 µg. After the addition of 10 µg of phentolamine it remained unchanged, that is, the blocking capacity of phentolamine disappeared.

The blocking capacity of phentolamine, however, could not be observed at all either in the summer frog heart at room temperature or in the rat heart at 38° C, both preparations representing an *a priori* higher metabolic rate than in the winter frog heart. Decreasing the metabolic rate of the summer frog myocardium by cooling to 5°–10° C and by administering 10^{-7} M dinitrophenol resulted, however, in five out of six cases, in the re-establishment of the phentolamine blockade which was initially lacking. Thus the capacity of an alpha blocking agent to produce adrenergic blockade in the myocardium is apparent only at lower metabolic rates.

The opposite correlation was found when beta blocking agents were applied. Thus 2–4×10^{-8} M pronethalol, or 1–3×10^{-8} M propranolol, which could block the effect of adrenaline in both summer and winter frog hearts at room temperature (sixteen experiments), as well as in rat hearts at 38° C (six experiments), lost their blocking capacity when the temperature was lowered to 5°–10° C in the summer frog heart or to 27° C in the rat heart, that is, when the initially higher metabolic rates of these two preparations were lowered. This phenomenon is illustrated in Fig. 2. In a rat heart 10 µg of propranolol completely blocked the positive inotropic and chronotropic effects of 2 µg of noradrenaline at 38° C (Fig. 2*A*). At 27° C (Fig. 2*B*) the same dose of propranolol produced no blocking effect at all: 2 µg of noradrenaline had the same effect as before propranolol was added. When the perfusion fluid was reheated to 38° C (Fig. 2*C*) the blocking capacity of propranolol was re-established; once

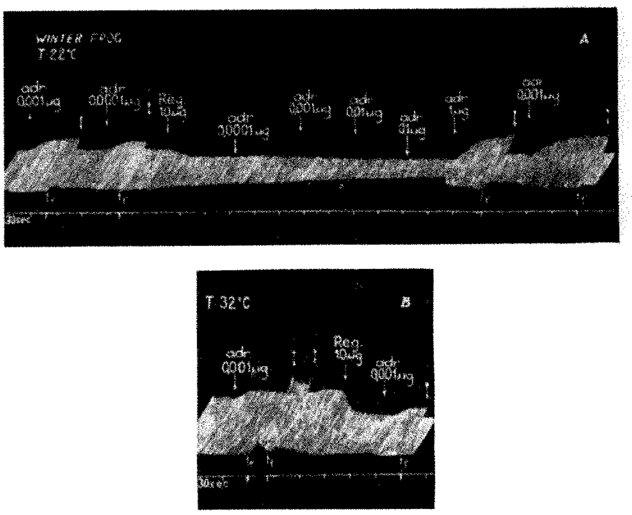

Fig. 1. Winter frog heart. Influence of change of temperature on the blockade of the adrenaline effect by 10 µg of phentolamine. Time signal: 30 s. ↓ designates washing out of substances. For explanation, see text.

again it completely blocked the effects of 2 µg of noradrenaline. Two minutes later, when propranolol had been washed out, 2 µg of noradrenaline was again effective.

When, on the other hand, the temperature was increased to 30°–34° C in the winter frog heart, the blocking capacity of the beta blocking agent pronethalol remained unchanged, or even increased in some cases, as compared with the diminishing or complete disappearance of the blockade produced by the alpha blocking agent phentolamine in the same experiments.

The principal trends induced by altering the metabolic milieu are summarized in Fig. 3. The degree of blocking capacity of the antagonists is expressed in all cases in terms of the log of the dose ratio, which is the ratio of effective threshold dose of adrenaline before and after blockade. When the temperature is lowered from 22°–24° C to 5°–10° C in the summer frog heart (Fig. 3*A*), the beta blocking capacity disappears, as shown by a significant decrease in the blockade, from $1{\cdot}95 \pm 0{\cdot}43$ to $0{\cdot}67 \pm 0{\cdot}39$ produced by pronethalol. In all cases, the dose of blocking agent remained unaltered in one and the same experiment. On the other hand, raising the temperature from the initial level to 30°–34° C (Fig. 3*B*) resulted in a decrease of the alpha blocking capacity, shown by a decrease in the blockade produced by phentolamine from $1{\cdot}18 \pm 0{\cdot}17$ to $0{\cdot}48 \pm 0{\cdot}13$.

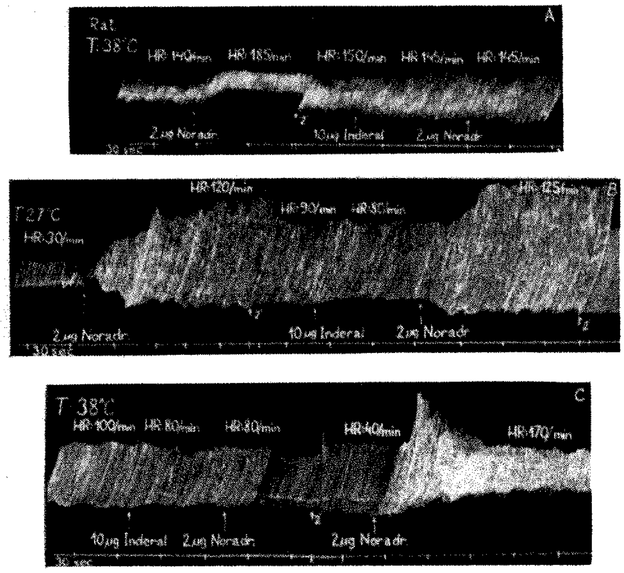

Fig. 2. Isolated rat heart. Influence of change of temperature on the blockade of the myocardial effects of noradrenaline by 5 µg of propranolol. Time signal: 30 s. HR, Heart rate. For explanation, see text.

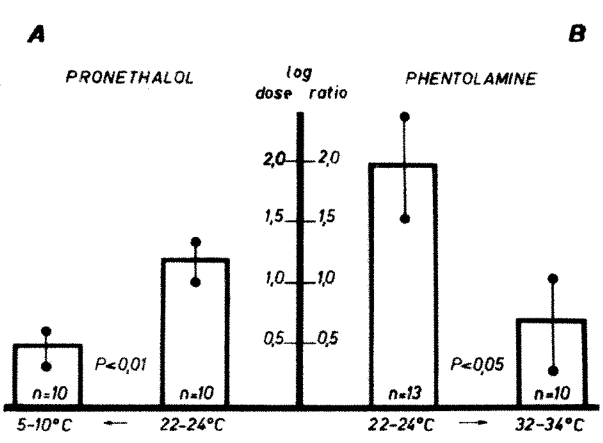

Fig. 3. *A*, Degree of blockade of adrenaline effect by $2\text{–}4 \times 10^{-8}$ M pronethalol at 22°–24° C and 5°–10° C in the summer frog heart. *B*, Degree of blockade of adrenaline effect by $3 \times 10^{-8}\text{–}2 \times 10^{-7}$ M phentolamine at 22°–24° C and 32°–34° C in the winter frog heart. Ordinate: log (dose ratio) of adrenaline sensitivity before and after blockade. *n*, Number of experiments; *P*, probability ratio. Vertical lines: standard error.

If these two trends are the results of different sensitivity of a single receptor, then there must be a state in which both types of blocking agents are equally and separately active; this was found in the case of the winter frog heart at 22°–24° C. This latter finding is incongruous with another possibility, that tissues can contain both alpha and beta receptors, with one predominating. If this were so, the greatest difference in the two kinds of blockades should have been found at room temperature, the temperatures at which frogs were previously kept and to which they had presumably become adapted. In this hypothetical case, only the combined administration of the two types of blocking agents could have produced an inhibitory effect instead of acting separately.

It can therefore be stated that the prerequisites for the existence of a metabolically influenced single adrenergic receptor—as far as myocardium is concerned—have been fulfilled in the present experiments.

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[1] Ahlquist, R., *J. Pharmaceut. Sci.*, **55**, 359 (1966).

[2] Amsler, C., *Pflüger's Arch. Ges. Physiol.*, **185**, 86 (1920).

GENERAL

Recognition of Syntactic Structure by Computer

The recent article concerning computer simulation[1] is refuted by its first sentence. As the first condition for an acceptable simulation of the way in which human beings recognize syntactic structure, the authors say "an analyser must not be dependent on looking up every word of an input sentence in a dictionary". To meet this condition a dictionary is used which contains information "only about those words with fixed syntactic functions and about suffixes". "With this dictionary alone, infinitely many English sentences can be analysed." Unfortunately, there are also "infinitely many" sentences which cannot be analysed in this way. The first sentence of the article is one such sentence.

This sentence is: "An essential part of the capacity to understand utterances lies in the ability to recognize syntactic structure". Any English-speaking reader can refute the article by playing the following simple game. First, rewrite the sentence keeping the pronouns, conjunctions and prepositions and substituting for other (non-fixed syntax) words an *X* plus the endings -s, -ed and -ing (if they appear). Second, the reader must try to replace *X*s by words which have a different syntactic function from the word in the original sentence. For example, the sentence is rewritten as "An *X* *X* of the *X* to *X* *X*s *X*s in the *X* to *X* *X* *X*". For the first two *X*s the noun–verb combination "actor knew" can be substituted for the adjective–noun combination "essential part". Whenever such a substitution can be made, the reader wins and the computer programme loses.

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[1] Bratley, P., Dewar, H., and Thorne, J. P., *Nature*, **216**, 969 (1967).

BOOK REVIEWS

HONOUR TO A PHYSICIST

Gustav Hertz in der Entwicklung der Modernen Physik

Festschrift zum 80 Geburtstag von Gustav Hertz am 22 Juli, 1967 (Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin—Klasse fur Mathematik, Physik und Technik, Jahrgang 1967, Nr 1) Pp 205 (Berlin Akademie-Verlag, 1967) n p

THIS is a "Festschrift" in the usual German style in honour of Gustav Hertz's eightieth birthday. It consists of a collection of specialized papers of high standard, but very heterogeneous, they are related in very varying degrees to Hertz's sphere of interest Only two short papers recall the main contributions of Hertz to atomic and nuclear physics, and draw a picture of his attractive personality Hertz won fame at an early stage of his career through his fundamental discovery, in collaboration with J Franck, of the excitation of quantal transitions in atoms by electron collisions a phenomenon that gave powerful support to the ideas just proposed by N Bohr Later he turned his attention to the processes of gas diffusion and developed a successful method of isotope separation based on these processes In the teaching and organization of physics in Germany he has been very influential, both before and after the Second World War; and his handling of the practical and human problems he had to face was characterized by the same common sense and directness of approach as his attitude towards the fundamental issues of science L ROSENFELD

ATMOSPHERE IN RUSSIA

Optical Instability of the Earth's Atmosphere

By N I Kucherov Translated from the Russian Pp viii+174 (Jerusalem Israel Program for Scientific Translations, London Macdonald—Oldbourne Press, 1966) 54s

THIS is a collection of papers, mainly the proceedings of a conference held at Kiev in 1962 (the Third All-Union Conference) It is unfortunate that such conference reports, and collections of papers in general, are presented with little or no connecting material In this case the topic is quite specific, and many of the papers are closely related there are twenty articles on the effect of the atmosphere on optical images obtained in astronomy, and it is easy to see how they could have been connected or even combined The advantages would be that a useful introduction to the whole subject would be created and that different approaches to the problems and comparable results would be brought together

First, a fair introductory chapter could be assembled from the various papers that establish the terminology, give physical explanations of the various phenomena, give fundamental theory, and give discussions of previous work The seven papers on image pulsation (position fluctuations observed with, for example, a 30 cm telescope) could easily be connected by a joint introductory and summarizing chapter Similarly, there are three papers in which optical instabilities are analysed in terms of meteorological phenomena this is an aspect of the subject in which progress has been difficult, and this could be emphasized by a brief general review of previous work It would be particularly interesting to have a comparative summary of the six papers which report on the magnitudes of the effects observed in various areas in tests of possible sites for new observatories The remaining four papers could not be combined quite so advantageously, although three of them are related to automatic observing. In addition, an index would have been useful

The experimental techniques described in some of the papers deserve special attention In a paper on "Pulsation of an Artificial Light Source in the Ground Layer" by V M Bovsheverov and others, there is an account of image pulsation observations made in known conditions, and resulting in a remarkably good verification of the theoretical treatment in terms of temperature and wind speed gradients O B Vasil'ev and V. S Sumin describe in detail their instruments for measuring image pulsation from photographic star trails, and it is clear that they have constructed a very successful machine Z N Kuteva and Yu A Sabinin describe a programmed automatic telescope which uses rotating transformers both for storing star co-ordinates and reading the position of the telescope, resulting in a simple and reliable installation

The topics covered in these papers receive considerable attention in the Soviet Union and this book is a useful progress report on this research R G BINGHAM

STATISTICAL PHYSICS

Statistical Physics

By A F Brown (Edinburgh University Publications—Science and Mathematical Texts, 7) Pp x+307 (Edinburgh Edinburgh University Press, 1968) 45s net

THIS book lies between an orthodox course on the properties of matter and a systematic treatment of statistical mechanics Professor Brown develops from first principles the concepts of probability distributions, and applies them to a selection of physical problems, mainly from the kinetic theory of gases and from solid state physics The general level is that appropriate to first-year university students of physics or chemistry Comprehensive coverage is not attempted and, in making the mathematics self-contained, many topics have had to be omitted There is, for example, no discussion of the various types of atomic interactions, of radiation, nor of Fermi and Bose statistics, even at a qualitative level Within the limits set by the author, the presentation is clear and straightforward, although more thermodynamics is assumed than is perhaps usual at a first year level

The first two chapters develop the elementary kinetic theory of gases Apart from misprints in equations (1 3 1) and (1 10 1) these should offer the student no difficulty, although I would have liked to see some mention of the distinction between averaging over particles and averaging over time The use of Clarendon type for the number density **n** seems rather arbitrary, because this is not done for other dimensional quantities

The theories of probability distributions and averaging in general are deferred to the third and fourth chapters, the latter also introducing the mean free path concept.

The fifth chapter contains as thorough a discussion of the Maxwell–Boltzmann distribution for gases as is possible at this level, later extended to more general systems. The sixth chapter contains a good short discussion of random walk and applications to diffusion problems The seventh chapter consists of statistical treatment of experimental results Again the presentation is clear, but this chapter is really a digression, and I feel it would be better moved to the end of the book In the eighth chapter the statistical concept of entropy is introduced For such an important topic it is surprising that this chapter should occupy only seven pages and be one of the only two in the book without problems or references

The ninth and tenth chapters contain various solid state topics, including melting, the Einstein specific heat model, order–disorder theory and diffusion processes in semiconductors The discussion here is lucid and well sustained. The final two chapters form essentially a statistical and mathematical appendix which might have been merged with the third chapter, although sections 12 1, 12 4 and 12 8 are so standard that their inclusion seems hard to justify

Misprints are remarkably few, and the price seems reasonable With the reservations mentioned, it should prove a useful first year book, although in subsequent editions some rearrangement of the chapters might be considered R COLLINS

MAGNETIC RESONANCE

Magnetic Resonance and Relaxation

Edited by R Blinc With the co-operation of D Hadzi and M Osredkar (Proceedings of the 15th Colloque Ampère, Ljubljana, 6–11 September 1966) Pp cv+1241 (Amsterdam North-Holland Publishing Company, 1967) 420*s*

As usual, the Colloque Ampère of September 1966 covered the whole broad spectrum of magnetic resonance and relaxation The proceedings consist of short contributed papers on rather specialized topics and in addition some invited longer papers often in the form of a concise review of a broader topic Much of the material of the short papers has since appeared in expanded form in the journals so that the value of the volume today lies largely in the review articles that it contains The subject matter for these longer papers (there are about thirty of them) ranges from molecular reorientation and nuclear relaxation in liquids to Jahn–Teller effects in solids and the Mossbauer emission from atoms in relaxing localized modes Their level is such that they can be readily understood by anyone with a background in magnetic resonance and serve in many cases as a good introduction to a given topic It would be difficult to find anyone in the field who did not find several of the articles of interest Personally, I found many of considerable interest, among them those dealing with nuclear acoustic resonance, nuclear resonance in superconductors, spin–spin relaxation, and on the mechanisms of dielectric relaxation

The price of the volume is such that few individuals will feel inclined to buy it, but any institute with an interest in magnetic resonance may well find it a worthwhile investment D. T EDMONDS

THEORETICAL CHEMISTRY

The Fundamentals of Theoretical Chemistry

Wave Mechanics Applied to the Study of Atoms and Molecules By Raymond Daudel Translated by R. E Watts Translation edited by J M Wilson Pp xx+211 (Oxford, London and New York Pergamon Press, 1968) 80*s*

THIS is an English translation of a French volume written in 1952 It is one of a series of three volumes by the same author, who is one of the leaders in French theoretical chemistry The first half of the book is devoted to a study of atoms, at a relatively simple level (but not for a complete beginner) The second half deals with molecules, concentrating chiefly on the method of molecular orbitals

It may well be asked whether the publication in 1968 of a book of this kind, written sixteen years ago, is justified Inevitably many new developments have taken place in these years, with the result that the volume has a rather "old-world" character Topics such as the Pekeris discussion of the helium atom, the Kolos–Roothaan work on molecular hydrogen, the Pariser–Parr approximation, and all mention of ligand-field theories, are missing The chief value of the book lies in the account—believed to be the only full account in any textbook—of Professor Daudel's concept of a loge This is a volume within which we have a fairly large probability of finding exactly one electron (or sometimes two, with opposed spins), and represents one of the best ways of localizing electrons in a physical picture

The translation is good, but the price of the book is ridiculous C A COULSON

AROUND LANGHOLM

The Geology of the Neighbourhood of Langholm

(Explanation of One-Inch Geological Sheet 11) By G I. Lumsden, W Tulloch, M F Howells and A Davies Palaeontology by R W Wilson, M A Calver and J D. D Smith Petrography by R W Elliot and M F Howells (Natural Environment Research Council–Institute of Geological Sciences Memoirs of the Geological Survey, Scotland) Pp viii+255+8 plates (Edinburgh and London HMSO, 1967) 52*s* 6*d* net

THE dust cover of this book has an attractive photograph of Langholm and its surrounding hills the blurb notes that the memoir traces the story of the deposition of the rocks from the oldest strata to the present day, refers to periods of major earth movement during which the rocks were thrown into great folds, and records at least four episodes of violent volcanic activity and two of the advance of great masses of ice And then

> "The Memoir contains much scientific detail about thousands [!] of outcrops, many boreholes and the more important natural sections, sedimentary structures and the petrology of selected strata and includes exhaustive lists of fossils"

from which the avid reader of Geological Survey memoirs will realize that the wrapping merely disguises the old familiar offering of painstaking, detailed work in which every outcrop appears to have been described, every fossil identified, and the petrography of each rock slice recorded

The six-inch geological maps of the area are already available, the one-inch is in the press Why then almost eighty pages of such detail as (page 33)

> "Green Cleuch is the stream which joins the Meikledale Burn on the east bank 450 yd upstream from Swingill A small asymmetrical anticline plunging west at 27 degrees is exposed about 210 yd upstream from the stream junction [37069536] The beds on the north limb are vertical and young north, while on the south limb the dip is south-south-west at 43 degrees Movement along the bedding has obviously taken place at this locality"

and (page 64)

> "An igneous intrusion is associated with the Enthorn Fault where it crosses the River Esk (p 204) near Glencartholm, 350 yd downstream from the mouth of the Hag Burn [37867897] Its outcrop in the Archer Beck, 400 yd W 43° S of Millstead [42008096]" etc

There are more than three hundred fossil localities pinpointed with such duplicated precision as (page 20)

> "Crue Gill, tributary of Wolfhope Burn draining the north-east slopes of Longgrain Head at head of stream 1600 yd S 63° W of Rigfoot [35769248] ?*M riccartonensis* Zone"

This kind of detail riddles the memoir and is precisely what geological maps, symbols and National Grid references were intended to obviate

Four geologists with supporting scientific staff have been engaged in this project on and off during the past sixteen years Their results are or will be embodied in six-inch sheets, one-inch map, memoir, scientific papers and sundry data including borehole records on open file So far as this consumer is concerned much of the detailed information in the memoir should have been restricted to the maps or put on open file Indeed, why not go one stage further and adopt the USGS custom of publishing a

12,000 word explanation of the geology of the area on a folding sheet containing the one-inch map? The need for some memoirs might then disappear, with resulting saving of effort and money

This preoccupation with what goes where and how should not obscure the fact that a useful survey has been made The work tends to confirm that the Southern Uplands consist of complex faulted monoclines Perhaps more importantly the memoir provides the first full description and classification of the Carboniferous rocks of the district G Y CRAIG

NOTODELPHYID DEVELOPMENT

Development and Systematics of Some Pacific Marine Symbiotic Copepods

A Study of the Biology of the Notodelphyidae, Associates of Ascidians By Patricia L Dudley Pp v+282 (Seattle and London University of Washington Press, 1966) 56s

ASCIDIANS provide a number of ecological niches for a great variety of invertebrate parasites and messmates Of the ascidicolous copepods the Notodelphyidae exhibit the greatest morphological diversity, many of the species are known only from adult females Although in 1892 Canu illustrated some developmental stages of five European species and succeeded in rearing some of their free-living copepodid stages, no complete life cycle of a notodelphyid was known In recent years the author has succeeded in culturing the free-living stages of all eight species, representing five genera, that occur in the Puget Sound region and has obtained from the hosts later commensal or parasitic copepodids to complete the life cycle of four species

Short sections on material and methods and on terminology follow the introduction, while collecting and culture data are given in the appendices Pre-eclosional development, eclosion of the nauplii and their escape from the host are described Post-eclosional development is considered under four main headings the nauplii, the copepodid stages, a comparison of the copepodids, and sexual dimorphism There are five naupliar stages in each of the seven commensal species and only four in the parasitic one The internal anatomy of the nauplii is described Development of the post-mandibular appendages proceeds gradually throughout the naupliar phase In these, and other yolky cyclopoid nauplii reared for comparison, the delicate transparent cuticle is never wholly in contact with these forming appendages and the exuviae therefore show little or no trace of these changes The author suggests that here a different mode of cuticle formation may be involved For each naupliar stage the superficial subexuvial structures are described as well as the exuvia In metal-contaminated sea water normal swimming movements and normal ecdysis may be arrested although differentiation of appendages and internal organs continues, in the parasitic species production of exuviae continued but the larvae were enclosed in two or even three skins

All eight species could be reared to the second copepodid, which is probably the invasive, stage, only in species of the genus *Doropygus* did any of these moult to the third copepodid stage A kind of metamorphosis occurs during the last naupliar stage, when copepodid features become more highly developed beneath the cuticle At the moult to the third copepodid a second metamorphosis occurs This third stage and subsequent stages are as useful in the taxonomy of the species as are the adults Sexual dimorphism is first apparent in the fifth copepodid stage in all the commensal species, in the highly modified parasitic species, it is first apparent in the fourth, and becomes marked in the fifth, stage The sixth stage is the adult for all the species and ecdysis ceases Since the females had been described in a recent work by Illg, only additional notes are given The much rarer males are fully described One species of *Doropygus* proves to have dimorphic males Non-swimming anamorphic males, differing only slightly from male fifth copepodids, are commonly found in ascidians The actively swimming metamorphic males were obtained direct from male fifth copepodids which were removed from the host and placed in culture dishes So far, only two metamorphic males have been found in ascidians

The relationship of the Notodelphyidae to other groups of copepods is discussed, utilizing first the adult characters then those of the developmental stages The naupliar characters of free-living copepods are enumerated, to show that there is a basic deviation in development between calanoids and cyclopoids, and to serve as a basis for comparison of notodelphyid nauplii with those of other commensal and parasitic forms Trends in reduction of mouthparts differ in the true poecilostomous cyclopoids like *Mytilicola* from those shown by Enterocolidae, Botryllophilidae and the aberrant parasitic notodelphyids These all show relationships with the gnathostomes

The book is well written and well arranged and will doubtless become the standard work on notodelphyid development The excellent line drawings are arranged as plates, labelled "Figure 1" to "Figure 51", inserted together with their legends between pages 164 and 269 There is no index, so the reader must rely on a short table of contents I GORDON

INTRODUCING BEHAVIOUR

An Introduction to Animal Behaviour

By Aubrey Manning (Contemporary Biology) Pp viii+208 (London Edward Arnold (Publishers), Ltd, 1967) 42s net, boards, 21s net, paper

THE study of behaviour has progressed far enough for the stage of pure description of behaviour patterns to be superseded by discussions of the facts by which the patterns can be explained, or for, at least, some sort of reasonable approach to a theoretical background to begin to be assembled Rightly, therefore, Dr Manning has not approached the subject from the standpoint of the biological function of particular patterns, like courtship, prey catching or the like, but reviews the principles on which behaviour is founded Not that natural selection is not one of these producing, as it does, biologically useful patterns, this is the other side of the coin, but an unusual coin, for its two sides are not starkly separated by a hard edge Nor is this a text in which the same behaviour in various groups is compared, although this evolutionary approach is one which has always commended itself to the zoologist as opposed to the psychologist The truth is that, apart from that derived from a few groups of closely allied species, the amount of comparative behavioural work is not very great It is striking that the examples of this approach are the same as would have been quoted in a book written ten years ago, there is no doubt that the immediately post-war work by the European behaviourists was classical in the sense that it will be quoted with advantage for a long time to come

In his preface, the author excuses the selection of topics explaining that the book was to be short and that he preferred to deal with those topics which he felt to be important This surely makes for a lively, interesting book instead of one recounting, dryly and dutifully, subjects which are dull to the author This book is stimulating and very thoroughly does what the author set out to do, that is, to introduce the subject by reviewing the current interests of ethologists and others

Manning's chosen subjects are "Reflexes and Complex Behaviour", "Development of Behaviour", "External Stimuli", "Motivation", "Conflict Behaviour", "Hormones and Behaviour" and "Evolution and Learning" It is plain from this list of chapter headings that Manning reflects the investigations of many workers today in the physiology of behaviour and draws, in his own researches, on the genetical approach That the genes act on behaviour by changes mainly in physiology rather than in detectable macrostructure is nowadays evident, although the difficulties of genetical analysis in this field are very great

Manning's selection of work ranges widely, as is made evident by almost 300 references listed at the end of the text, but sensibly, wherever possible, he has cited reviews rather than original papers For the student reader, at whom the book is directed, this is the only practical approach The book is well produced and illustrated, there is no reason why it should not be highly appreciated by its readers J D. CARTHY

BOOK OF A MONKEY

Biology of the Howler Monkey (*Alouatta caraya*)

(Bibliotheca Primatologica, No. 7) Edited by Manuel Rene Malinow Pp viii+232 (Basle and New York S Karger, 1968) 48 Sw francs, 88*s* ; $11 50

PEDANTRY is a tiresome stick with which to belabour anybody but, in the name of Linnaeus, a sensitive reviewer must protest against the nomenclatural error in the title of the book *Alouatta*, the howler monkey, is a genus containing five good species only one of which, *A. caraya*, is dealt with in this book that purports to be a "Biology of the Howler Monkey" This is most misleading

It is also somewhat misleading to call this book a "Biology" which suggests a comprehensive, systematic approach that this eclectic compilation does not attempt to achieve. Ecology, evolution, systematics and free-ranging behaviour are either wholly omitted or, at best, perfunctorily acknowledged One curious omission is any reference to the voice of the black howler monkey or to its extraordinary vocal anatomy, this is rather like making an apple pie without apples.

There are, however, a number of extremely useful independent chapters on the anatomy and pathology of this so far little known species Particularly valuable are chapters on growth, age determination and pre and post-natal development, some of which have been published elsewhere Malinow's contribution to the natural history of arterial disease in free-ranging primates is of great interest, especially his observations relating to the reduced incidence of atherosclerosis in pregnant animals Grand's analysis of the functional anatomy of the upper limb is a careful and thoughtful contribution

The good old-fashioned one-man monograph with its synthetic treatment of data and its total biological approach is becoming a rarity these days Its place in scientific literature has been taken by the multi-authored volume which neither covers the field nor attempts to present an integrated, balanced picture of the central organism Only too frequently, as in the present instance, many of the articles are reprints One questions the value of such compilations particularly at £4 8*s* and in paperback form JOHN NAPIER

CONTROLLING SPEECH

Research in Verbal Behaviour and Some Neurophysiological Implications

Edited by Kurt Salzinger and Susanne Salzinger. Pp xvii+510 (New York Academic Press, Inc ; London, Academic Press, Inc (London), Ltd , 1967) 88*s*

WHAT goes on in the brain when we speak, or are otherwise linguistically active, is a tantalizing problem, for it bears directly on the biological status and prospects of Man But many approaches and methods commonplace in the study of other "higher" functions of the brain are unavailable in the case of language. Animals do not speak and we cannot systematically cut away parts of the human brain to find out the effects on language Instead we have to wait on "Nature's experiments" and her techniques are casual and unsuited to scientific convenience. Opportunities of stimulating and recording from the intact brain while it is occupied in linguistic activity are scarce, and the results so far unrevealing Starting from the other end, analyses of language itself on its own terms, and of the human behaviour involved in its use, have made some progress But as they become more sophisticated their conclusions all too often seem remote from the concepts of contemporary neurophysiology Modern grammatical theory, for instance, is replete with patterned structures of one kind and another Which of these should we pick on to lay alongside any observable pattern of functioning in the nervous system ?

It is very tempting to try to bridge a gap such as this, and not always unprofitable even when there is more make-believe than masonry in the construction. As it happened, however, this symposium stopped short of any actual bridge building and devoted itself largely to laying down approach roads The first section, "Verbal Behaviour Facts and Theories", which occupies half the book, comprises a good deal of material familiar to readers of previous psycholinguistic symposia, though it breaks fresh ground in places Even here, however, as for example in Staats's paper on emotions and images in language, in the two essays by Lambert and by Fillenbaum on verbal satiation or in Lambert and Preston's note on the bilingual's two languages, there is evidence of undue anxiety to force the material into preconceived theoretical or methodological moulds But there are also examples, such as Starkweather's paper on the vocal characteristics of depressed patients, of a more straightforwardly empirical approach

The second section, "Verbal Behaviour as a Function of the State of the Organism", concentrates very largely on the effects of drugs on verbal and vocal behaviour, and Waskow contributes a general summary of work in this field to date Perhaps naturally, there has been much concentration of drugs in current psychiatric vogue, notably LSD, chlorpromazine and amphetamine. The outcome of this work is on the whole disappointing, but new drugs and new knowledge about their biochemical modes, and anatomical sites, of action are coming along fast, and thoughtful use of these may go far to establish some of the neural correlates of speech behaviour

The last section, "Neurophysiological Bases of Verbal Behaviour", in which the innocent reader might expect to find some evidence of bridge building on the other side of the chasm, is short and frankly disappointing. It contains four essays, by Luria (who was unfortunately unable to be present at the symposium), Geschwind, Davis Howes and Chase Luria's contribution, alas, contains only what he has already published in English, notably in two articles in *Word* (1959). Geschwind's paper, though mildly stimulating, is too brief and discursive to have any real hitting-power Howes, who in an earlier paper in the symposium had presented his well known data on word-frequency distributions in aphasia, again examines the implications of these findings for a theoretical view of the language mechanisms Chase does his best to bring the whole symposium to a focus in such a way as to catch hold of any neurophysiological implications there might be, but these remain potential rather than apparent He is clearly hampered by the mass of doubtfully relevant material assembled by the symposiasts, and his effort only seems to reveal the marginal utility of this kind of scientific communication in a field unsuited to it R C OLDFIELD

APOTHECARIES AND THEIR PAST

The Worshipful Society of Apothecaries of London

A History, 1617–1967 By W S C Copeman Pp 112+19 plates (Oxford, London and New York Pergamon Press, 1967) 63*s*

Last year members of the Worshipful Society of Apothecaries of London celebrated the three hundred and fiftieth anniversary of their foundation as a City Livery Company In 1617, James I granted them a Royal Charter and Dr Copeman's book commemorates this foundation and gives a very brief, but attractively illustrated, account of the society's history The development of the society from activities such as the growing and compounding of drugs to a body which after 1815 was made responsible for the training of the general practitioner is engagingly retold There are also potted biographies of a few of the more famous apothecaries, such as the founder of the society, Gideon de Laune, the poet John Keats, John Hughlings Jackson, Sir Charles Dodds, Sir Arthur Porritt and others

The present Apothecaries Great Hall which was built during the period 1668 to 1670 is itself of note, for it is claimed to be the only Livery Hall out of thirty-six existing in 1939 to escape unscathed the ravages of the Second World War, it is said to be "the oldest surviving hall of any City Livery Company and the oldest medical building still in use in the country" (page x) Dr Copeman has included a list of the paintings it possesses as well as a roll of past masters of the society The superb photographs demonstrate the hall's beauty and elegance, as well as showing some of its possessions

This book will serve as a useful résumé of the authoritative and scholarly history of the society compiled by C Wall, H C Cameron and E A Underwood, the first volume of which appeared in 1963 It will also help to emphasize the important part that the apothecaries have played in the creation of our medicine of today and the part they will continue to take in the prevention and cure of disease in the future

Edwin Clarke

HOW TO FIND A SCIENTIST

Who's Who in Science in Europe

A New Reference Guide to West European Scientists Vol 1 Pp 1–560 Vol 2 Pp 561–1144 Vol 3 Pp 1145–1747 (Guernsey, C I Francis Hodgson, Ltd, 1967) 200*s* , $29 each volume

At first glance, intending users of this new reference book may be misled into thinking that it covers scientists in all European countries A second look will confirm that it does in fact cover only West European scientists to the exclusion of all those in the "eastern bloc" Nevertheless, it is the first guide to West European scientists to be published, and as such should serve a useful purpose in libraries, particularly now that there are several joint scientific organizations in Europe and more and not less co-operation is likely in the future

It is stated in the introduction that there are more than 30,000 entries covering all the "more important European scientists and technologists and those who have interests in the field of research or who are interested in encouraging or promoting science and technology" It is not clear what criteria were used in the selection of entries Help was apparently sought from various international and national organizations, government departments, universities and science-based industrial firms Questionnaires were also used This claims to be a "Who's Who", rather than a "Directory", and so one can hardly expect the comprehensiveness of the national directories of scientists, for example the *Directory of British Scientists* (*DOBS*) and its equivalent in other countries *DOBS*, for example, includes school teachers and research students—categories obviously excluded from the book under review The scope of *Who's Who in Science in Europe* appears, however, to be wider than *DOBS* in some respects, for it includes important doctors, technologists and industrialists—which *DOBS* appears not to do, perhaps because these persons are covered sufficiently well in other British directories

The British representatives in the book appear to be of the level of those in *Who's Who* [in Britain]—professors, Fellows of the Royal Society, and so on Readers in university departments do not appear to be included, but heads of industrial and government research departments, chairmen and council members of the important scientific societies are The comprehensiveness of a book like this, however, can only really be discovered through use. It is also not easy to judge the representation of scientists in countries other than Britain, although superficial examination shows no very marked British bias There must have been difficulties in the selection of entries just on the grounds that titles and status of, for example, professors vary from one country to another

Biographical details given vary in length from name and address only, to details of degrees, subject interest, higher education, recent appointments, society membership, and scientific interests Publications have not been included, but it is useful to have the person's year of birth in some (but not all) of the entries The entries are easy to read—a minor fault is the lack of bold type to distinguish each name An unfortunate omission is that there is no explanation of abbreviations used Not many are used apart from degrees, but a scientist overseas might want to know what FRCS, ACORD and MRS mean

Sarah Bunney

OBITUARIES

Dr Shuhei Fujitaka

Dr Shuhei Fujitaka, who died in Tokyo on November 26, 1967, at the age of sixty, was a brilliant high voltage engineer and educationalist

Fujitaka was born in 1907 at Nara, Japan In 1931 a year after graduating in electrical engineering from Tokyo Imperial University, he became assistant professor at the Tokyo Institute of Technology at Ōokayama In 1943 he became a full professor of Tokyo Imperial University and was attached to the second engineering department

Early in his career Fujitaka was involved in high voltage engineering, and worked on transmission problems After the end of the Second World War the power shortage in Japan was at its worst—the domestic electricity supply dropped to seventy-five volts—and the national policy was to reconstruct the power industry Fujitaka was involved in the development of the 215 kV Shinhokuriku line, from Shinaimoto to Hirakata, which was one of the most important power sources in the town of Kurobe

Fujitaka also participated in national electrification schemes, notably the Jyoetsu line, from Ueno to Niigata He was the first to employ a one step transformer connexion from 66 kV to 1 5 kV, a system which is economical in railway electrification and is now employed by the Japanese National Railways at their substations Fujitaka had recently been working on the new 500 kV line which crosses Japan from east to west

Fujitaka had taught high voltage engineering since joining the Tokyo Institute of Technology in 1931 He also held positions as vice-chairman of the Institution of Electrical Engineers of Japan, as a member of both the regular committee of the International Conference on Large Electric High Tension Systems and the Japanese National Committee of the International Electrotechnical Commission

Sueo Yamakura

University News:

Birmingham

Dr John Hardwicke and Dr Kenneth Walton, both at present readers in the Department of Experimental Pathology, have been appointed to the newly established additional chairs of experimental pathology

Keele

Professor R J Frankenberg, reader in sociology in the University of Manchester and at present on secondment as professor of sociology and dean of the School of Humanities and Social Sciences in the University of Zambia, has been appointed professor of sociology and head of the Department of Sociology

Sheffield

Dr P N Robson, at present reader in electrical engineering in University College, London, has been appointed to the second chair of electronic and electrical engineering

Appointments

The Minister of Technology has appointed the following new members to the Advisory Council on Technology *Dr J M Hill*, chairman of the UK Atomic Energy Authority, *Sir Frank Schon*, part-time member of the Industrial Reorganization Corporation and of the National Research Development Corporation and a non-executive director of the Associated Portland Cement Manufacturers, Ltd, *Sir William Swallow*, chairman of the Shipbuilding Industry Board, *Lord Wright of Ashton-under-Lyne*, general secretary of the Amalgamated Weavers' Association and present chairman of the Trades Union Congress.

Sir John Henniker-Major, at present assistant under-secretary at the Foreign Office, supervising the African departments, has been appointed director-general of the British Council in succession to Sir Paul Sinker

Announcements

The Royal Geographical Society has made the following awards *Founder's Medal* to W B Harland, Sedgwick Museum, Cambridge, for Arctic exploration and research, *Patron's Medal* to Professor A Gansser, Federal Institute of Technology, Zurich, for geological exploration and mapping of the Himalaya

The Seventh James B Macelwane Award is to be presented to Michael B McElroy of Kitt Peak National Observatory, near Tucson, Arizona, at the annual meeting of the American Geophysical Union This award is given each year to a young scientist in recognition of his work in geophysical research

Meetings

Stereochemistry of Macromolecules, May 21–24, Venezia (Dr Giuseppe Zerbi, Istituto di Chimica Industriale del Politecnico, Piazza L da Vinci 32, Milano)

Surging Glaciers, June 6–8, Banff (C M Lockwood, Assistant Director, Department of Extension, University of Alberta, Edmonton, Alberta, Canada)

New Olefin Reactions, June 25–26, University of Manchester Institute of Science and Technology (Professor R N Haszeldine, University of Manchester Institute of Science and Technology, PO Box No 88, Sackville Street, Manchester 1)

Carbon and Graphite, July 2–5, University of Newcastle upon Tyne (Dr T E O'Hair, Department of Physical Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne 1)

Molecular Crystal Symposium, July 8–12, Technical University "Twente", Enschede (Professor J Kommandeur, Laboratory for Physical Chemistry, The University, Bloemsingel 10, Groningen, The Netherlands)

Chemistry of Natural Products, July 8–13, London (The Secretary, 5th International Symposium on the Chemistry of Natural Products, c/o The Chemical Society, Burlington House, London W1)

Crystal Growth, July 15–19, University of Birmingham (Professor A. D McQuillan, Department of Physical Metallurgy and Materials Science, University of Birmingham, Birmingham 15)

Pattern Recognition, July 29–31, National Physical Laboratory, Teddington (The Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2)

Erratum In the article "Histochemical and Biochemical Study of Synaptic Lysosomes" by Mildred K Gordon *et al* (*Nature*, 217, 523, 1968) the name of one of the authors, Klaus G Bensch, was misspelt

Erratum The address of Dr R Spitzer, the author of the communication "Possible Explanation of Anomalies in Neutral *K* Phenomena" (*Nature*, 217, 943, 1968), should be Lawrence Radiation Laboratory, Livermore, California

Erratum In the introduction to the article "Urinary Metabolites in Parkinson's Disease" by Frederick A Kuehl, jun , *et al* (*Nature*, 217, 136, 1968), the word tryptamine should read tryptophan

Erratum In the communication "Effect of Cocaine, Desipramine and Angiotensin on Uptake of Noradrenaline in Tissues of Pithed Rats" by D T Pals and F D Masucci (*Nature*, 217, 772, 1968), the second sentence of the fourth paragraph should read "The variation of radioactivity recoverable was probably a result of the presence of different numbers of sympathetic nerve endings in these tissues" In the final paragraph the words facilitated and facilitation should read potentiated and potentiation, respectively

CORRESPONDENCE

The Magic of Numbers

Sir,—In your leading article of March 2 (*Nature*, 217, 793, 1968) you surmise that the 87 5 per cent of all scientists who, according to the "First Law of Research on Research", have ever been alive are alive now would be a ratio which would also probably have been valid in 450 BC In fact, if this law is true at all this ratio can be shown to have been valid almost always

Assume that the number of scientists increases by a ratio q (>1) per decade Then n decades after the first scientist, say Archimedes, there will therefore be q^n scientists alive The total number of scientists who have ever lived will be $(q^n-1)/(q-1)$ The ratio of those living in the nth decade to those who have ever lived will be $q^n(q-1)/(q^n-1)$ When n is large enough, say several tens of decades (q not being too near to 1), this ratio becomes equal to $(q-1)$ and is no longer dependent on n

If $q-1=0\ 875$, this would mean that scientists increase by a factor of 1 875 per decade But why per decade? Or better, which is the right unit of time to choose?

Yours faithfully,

K. Schiller

97 Repton Road,
West Bridgford,
Nottingham

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 18

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 10 a m —Colloquium on "Threshold Extension Techniques"

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, SW1), at 5 p m —Mr J A R Bates "A Review of Governmental Control in Various Countries"

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, NW1), at 5 p m —Professor J R Buchanan (Pennsylvania) "Radiology of the Canine Heart" *

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "The Class Structure of the British Single-phase House Service Meter" opened by Mr A J Baggott *

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College London, Gower Street, London, WC1), at 5 30 p m —Professor Horst Kunze (Director General of the German State Library, East Berlin) "The Training of Scientific Librarians and Documentalists in Eastern Germany" *

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, INSTRUMENTATION AND CONTROL GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 6 p m —Professor H H Rosenbrock "Control Past, Present and Future" (Inaugural Meeting)

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2), at 6 p m —Professor Owen Lattimore "China To-day—Some Social Aspects" (first of three Cantor Lectures)

Tuesday, March 19

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the I Mech E , at Savoy Place, London, WC2), at 10 a m —Colloquium on "Engineering Aspects of Satellite Design"

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (at 14 Belgrave Square, London, SW1), at 10 a m —Meeting on "Soil Analysis and Fertilizer Recommendations"

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, NW1), at 5 p m.—Professor James W Buchanan (University of Pennsylvania) "Cardiovascular Surgery in Dogs" *

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Lecture Theatre of the Science Museum, Exhibition Road, London, SW7), at 5 30 p m —Mr W F Grimes "The Development of Archaeological Techniques"

ROYAL INSTITUTION (at 21 Albemarle Street, London, W1), at 5 30 p m —Professor R L F Boyd "Recent Studies of the Planet Venus" (Lecture for Sixth Form Pupils from Schools in London and the Home Counties To be repeated on March 20, 26 and 27)

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, SW7), at 5 30 p m —Professor T R E Southwood "The Abundance of Animals" (Inaugural Lecture) *

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, COMMUNICATIONS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 6 p m —Mr W D Benson and Mr B Buckingham "Integrated Circuits for Radio Receivers"

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Teaching of Materials Science"

Wednesday, March 20

INSTITUTION OF MECHANICAL ENGINEERS, MANUFACTURE AND MANAGEMENT GROUP (at 1 Birdcage Walk, Westminster, London, SW1)—All-day Discussion Meeting on "A New Approach to Machine Tools in Relation to the Components They Make"

BRITISH COMPUTER SOCIETY (at Queen Elizabeth Hall, London, SE1), at 9 a m —Symposium on "Production Control by Computers—Where are We Going ?"

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, SW1), at 3 p m —Dr D E Karwath "Navigation of Supersonic Transport"

UNIVERSITY OF LONDON (at the Institute of Diseases of the Chest, Brompton Hospital, London, SW3), at 5 p m —Dr D P Winstanley "Pulmonary Embolism" *

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, WC1), at 5 15 p m —Discussion Meeting on "Regression Methods"

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 5 30 p m —Mr W J Quill "Satellite Communications"

Thursday, March 21

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W1)—Professor L Nirenberg "Pseudo-differential Operators"

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at Victoria Halls, Bloomsbury Square, London, WC1), All-day Meeting on "Some Modern Laboratory Techniques Applied to the Study of Road and Building Materials"

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, WC2), at 2 30 p m —Colloquium on "The Role of the Computer in Device, Circuit and Equipment Design"

ROYAL SOCIETY (at 6 Carlton House Terrace, London, SW1), at 4 15 p m —Meeting for the election of Fellows 4 30 p m —Dr H Kronberger, FRS "Hydrostatic Extrusion" (Review Lecture)

INSTITUTION OF MINING AND METALLURGY (at the Geological Society of London, Burlington House, Piccadilly, London, W1), at 5 p m —Mr R Jeffrey "Physical Metallurgy and Safety in Mines", Mr R H Lamb and Mr L R Verney "Investigations into RST Group Concentrator Practices", Dr M J Gallagher "Determination of Molybdenum, Iron and Titanium in Ores and Rocks by Portable Radioisotope X-ray Fluorescence Analyser"

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W1), at 5 30 p m —Mr R B Hyde "Abnormal Formation Pressures—Their Origin, Distribution, and Control Whilst Drilling"

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Role of Research and Development in the Support of Design"

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the Royal Army Medical College, Millbank, London, SW1), at 7 30 p m —Laboratory Meeting Chairman Professor P C C Garnham, CMG, FRS

Thursday, March 21—Friday, March 22

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP and OILS AND FATS GROUP (at the School of Pharmacy, Brunswick Square, London, WC1)—Symposium on "The Functions of Natural and Synthetic Surface-Active Lipids in Foods"

Friday, March 22

ARBORICULTURAL ASSOCIATION (in the Lecture Room, Royal Horticultural Society's New Hall, Greycoat Street, London, SW1), at 6 30 p m —Dr Francis Rose "Woodland Ecology and Conservation"

Monday, March 25

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the Automatic Control Group of the I Mech E , at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Fluidics and Integrated Circuits" opened by Dr R C Foss and Dr K Foster

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "The Impact of Proposed Vehicle Noise Regulations"

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, WC2), at 6 p m —Dr C H G Oldham "China To day—Science" (Second of three Cantor Lectures)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned

LECTURERS and ASSISTANT LECTURERS IN CHEMISTRY—The Registrar, University of Manchester, Manchester, 13, quoting Ref 20/68/Na (March 22)

ASSISTANT LECTURER (with an honours degree in pharmacology, physiology, biochemistry or pharmacy, and preferably a higher degree or research experience) IN PHARMACOLOGY—Clerk to the Council, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1 (March 25)

LECTURER or ASSISTANT LECTURER IN THE PHILOSOPHY OF SCIENCE—The Secretary, The University, Aberdeen, Scotland (March 25)

SENIOR EXPERIMENTAL OFFICER in the DEPARTMENT OF APPLIED PHYSICS to work on the crystallographic perfection of photoconductive and electroluminescent materials using a JEM-120 metallurgical electron microscope—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (March 25)

SENIOR LECTURER, LECTURER or ASSISTANT LECTURER (three vacancies) in PSYCHOLOGY—The Secretary, The University, Aberdeen, Scotland (March 25)

LECTURER (with a degree in sociology, social anthropology or psychology, and experience of industrial research) in INDUSTRIAL SOCIOLOGY—The Registrar, University of Warwick, Coventry, CV4 7AL, Warwickshire (March 29)

SENIOR LECTURER (graduate in physiology, biochemistry or related subjects, or with a medical qualification registrable in the United Kingdom) in PHYSIOLOGY—The Registrar, University of Manchester, Manchester 13, quoting Ref 25/68/Na (March 29)

LECTURER and an ASSISTANT LECTURER in the PHYSIOLOGY DEPARTMENT —The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W8 (March 31)

PRINCIPAL LECTURER, SENIOR LECTURER and a LECTURER (Grade II) in SOCIOLOGY—The Academic Registrar, Hatfield College of Technology, Hatfield, Herts (April 1)

CHAIR in the DEPARTMENT OF MATHEMATICS in the Faculty of Science, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Kuala Lumpur, April 8)

VISITING LECTURER in CHEMISTRY at the University of Canterbury, Christchurch New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (April 8)

SENIOR LECTURER or LECTURER in MORBID ANATOMY in the DEPARTMENT OF PATHOLOGY, University of the West Indies, Jamaica—The Inter-University Council, 33 Bedford Place, London, WC1 (April 10)

PROFESSOR (with interests in some field of physical geography, such as quaternary geomorphology, physical geography in its application to planning, particularly conservation of natural resources, quantitative analysis in physical geography) in the DEPARTMENT OF GEOGRAPHY—The Registrar, Room 39, ORB, The University, Reading (April 11)

LECTURER IN PHILOSOPHY at Massey University, Palmerston North, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, April 15)

SECRETARY (senior experienced administrator preferably with a scientific or technological background) of the BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE—The President, Dame Kathleen Lonsdale, DBE, FRS, University College London, Gower Street, London, WC1 (April 30)

ASSISTANT LECTURER or LECTURER (biochemist or microbiologist) in the DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY—The Registrar, University College of Wales, Aberystwyth

BASIC GRADE BIOCHEMIST for routine chemical pathology laboratory—The Assistant Secretary, University College Hospital, Gower Street, London, WC1

CHAIR OF CHILD HEALTH at King's College Hospital Medical School—The Academic Registrar (A), University of London, Senate House, London, WC1

MASTER to teach MATHEMATICS at all levels, including the Sixth Form—The Master, Dulwich College, London, SE21

SRC RESEARCH ASSISTANT (graduate in chemistry) to Dr G D Parfitt in the DEPARTMENT OF CHEMISTRY, for work involving an experimental study of the coagulation of colloidal systems involving mixtures of solid particles (hetero-coagulation)—Dr G D Parfitt, Department of Chemistry, The University, Nottingham

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Medical Research Council Monitoring Report No 15 Assay of Strontium-90 in Human Bone in the United Kingdom, Results for 1966, Part 2, with some further Results for 1965 Pp iii+24 (London H M Stationery Office, 1967) 3*s* net [41

Philosophical Transactions of the Royal Society of London Series B Biological Sciences No 785, Vol 253 (4 January, 1968) The Morphology and Histochemistry of the Echinoid Axial Organ By N Millott and H G Vevers Pp 201–230+plates 9–12 (London The Royal Society, 1968) 16*s*, $2 05 [41

The Royal Society British National Committee for Geodesy and Geophysics United Kingdom Report on Research Activities in Seismology, 1963–67 Pp 56 United Kingdom Report on Research Activities in Geomagnetism and Aeronomy, 1963–67 Pp 81 United Kingdom Report on Research Activities in Volcanology 1963–67 Pp 70 United Kingdom Report on Research Activities in Physical Oceanography 1963–67 Pp 49 United Kingdom Contribution to the Upper Mantle Project—1967 Progress Report Pp 103 (London The Royal Society, 1967) [51

The Ordnance Survey Annual Report 1966–67 Pp iii+7+13 appendices and 8 plates (London H M Stationery Office, 1967) 7*s* 6*d* net [51

The Systematics Association Publication No 1 Bibliography of Key Words for the Identification of the British Fauna and Flora Edited by G J Kerrich R D Meikle and Norman Tebble Third edition Pp vii+186 (London Systematics Association, 1967) 15*s* , library copy, cloth bound, 20*s* [81

Imperial College of Science and Technology (University of London) Sixtieth Annual Report of the Governing Body, 1966–67 Pp vi+151 (London Imperial College, 1968) [81

Hopkin and Williams, Ltd Monograph No 71 Salicylideneamino-2-Thiophenol Reagent for Tin Pp 4 (Chadwell Heath, Essex Hopkin and Williams, Ltd , 1968) [91

Proceedings of the Royal Society Series A Mathematical and Physical Sciences Vol 302, No 1470 (9 January 1968) Including a Discussion on the Applications of Electron Spin Resonance in the Study of Reactive Intermediates in Chemical Reactions, organized by W A Waters, FRS Pp 287–436 (London The Royal Society, 1968) 22*s* 6*d* , $2 90 [91

Scientific Proceedings of the Royal Dublin Society Series A Vol 3, No 5 Some Aspects of Single Electron Response and Afterpulsing in Photo- and Electron Multipliers By C F G Delaney and J A Harwood Pp 57–68 5*s* Vol 3, No 6 A Small Mass Spectrometer for the Analysis of Residual Atmospheres in Vacuum Systems By D J Reid Pp 69–74 5*s* Vol 3, No 7 Biogenic Carbonate in Beach Sediments of the West Coast of Ireland By Raymond Keary Pp 75–86 6*s* Series B Vol 2, No 11 Studies of the Clay Minerals of Irish Soils Influence of Pedogenic Factors on the Clay Mineral Status of County Wexford Soils By M J Gardiner and J P O'Callaghan Pp 87–98 5*s* (Dublin The Royal Dublin Society, 1967) [101

The Association of Commonwealth Universities Fourth Annual Report of the Council, 1966–67 Pp 51 (London The Association of Commonwealth Universities, 1967) [101

Bulletin of the British Museum (Natural History) Zoology Vol 15, No 8 *Calgius longicaudatus* Brady, 1899 (Calgidae Copepoda) By R R Parker Pp 353–368 8*s* Vol 16, No 1 Earthworms (Acanthodrilidae and Eudrilidae Oligochaeta) from Gambia By R W Sims Pp 1–43+9 plates 22*s* 6*d* (London British Museum (Natural History), 1967 and 1968) [101

BBC Lunch-time Lectures Sixth Series—3 Working in Colour By Ian Atkins Pp 15 (London British Broadcasting Corporation, 1967) [111

Ministry of Overseas Development Annual Report for the year ended 31st March 1967 of the Directorate of Overseas Surveys Pp 60 + maps (London H M Stationery Office, 1967) 10*s* net [111

Ministry of Agriculture, Fisheries and Food Fishery Investigations, Series II, Vol XXV, No 6 The Hydrography of the Estuary of the River Blackwater By J W Talbot Pp 92 (London H M Stationery Office, 1967) 32*s* 6*d* net [111

Other Countries

United States Department of the Interior Geological Survey Water-Supply Paper 1841 Geology and Ground Water of the Savannah River Plant and Vicinity, South Carolina By George E Siple Pp vi+113+plates 1–6 Water-Supply Paper 1881 Quality of Surface Waters of the United States, 1961 Parts 1 and 2 North Atlantic Slope Basins and South Atlantic Slope and Eastern Gulf of Mexico Basins Prepared under the direction of S K Love Pp xi+412 $1 50 Professional Paper 520 Geology and Uranium Deposits of the Ralston Buttes District, Jefferson County, Colorado By Douglas M Sheridan, Charles H Maxwell and Arden L Albee Pp iii+121+plates 1–8 Professional Paper 522 Area Geology of the Western Mojave Desert, California By Thomas W Dibblee, Jr Pp vii+153+plates 1–4 Professional paper 524-G The Gulf Series in the Subsurface in Northern Florida and Southern Georgia By Paul L Applin and Esther R Applin Pp iii+35+plates 1–8 Professional Paper 530 The Geological Occurrence of Monazite By William C Overstreet Pp viii+327+plates 1 and 2 Professional Paper 562-B Flume Experiments on the Transport of a Coarse Sand By Garnett P Williams Pp iv+31 $0 30 (Washington, D C Government Printing Office, 1967) [11

United States Department of the Interior Geological Survey Professional Paper 566 Younger Precambrian Geology in Southern Arizona By Andrew F Shride Pp iv+89 $0 65 Professional Paper 583-A Some Silicified Middle Ordovician Brachiopods from Kentucky Pp iii+14+plates 1 and 2 $0 30 Professional Paper 583-B Calymenid and Other Ordovician Trilobites from Kentucky and Ohio By Reuben James Ross, Jr Pp iii+15+plates 1–5 $0 45 Professional Paper 593-A Permian Tethyan Fusulinids from California By Raymond C Douglass Pp iii+13+plates 1–6 $0 40 (Washington, D C Government Printing Office, 1967) [11

National Museum of Canada Bulletin No 214 (Anthropological Series No 78) Contributions to Anthropology—Linguistics 1 (Algonquian) Pp vii+162 $2 50 Bulletin No 222 (Biological Series No 78) The Birds of Southeastern Victoria Island and Adjacent Small Islands By David F Parmelee, H A Stephens and Richard H Schmidt Pp ix+229 (10 plates) $2 (Ottawa Queen's Printer, 1967) [21

United States Department of the Interior Geological Survey Water-Supply Paper 1846 Ground Water in the Vicinity of American Falls Reservoir, Idaho By M J Mundorff Pp iv+58+plates 1–5 (Washington, D C Government Printing Office, 1967) [21

Australia Commonwealth Scientific and Industrial Research Organization. Report of the Division of Horticultural Research 1965–67 Pp 87 (Adelaide Commonwealth Scientific and Industrial Research Organization, 1967) [21

American Museum Novitates No 2289 (April 24, 1967) A Review of the Genera of Grouse (Aves, Tetraoninae) By Lester L Short, Jr Pp 39 No 2294 (June 28, 1967) Speciation in Colombian Forest Birds West of the Andes By Jurgen Haffer Pp 57 Bulletin of the American Museum of Natural History, Vol 136, Article 6 Revision and Probable Evolution of the *Myiarchus* Flycatchers of the West Indies By Wesley E Lanyon Pp 329–370+plates 22–36 $3 (New York American Museum of Natural History, 1967) [31

The United Republic of Tanzania Ministry of Industries, Mineral Resources and Power Annual Report of the Mineral Resources Division, 1965 Pp iii+66 (Dodoma Asst Commissioner for Mineral Resources, 1967) Shs 5/80

Illinois Natural History Survey Bulletin, Vol 29, Article 3 Hybridization of Four Species of Sunfishes (Centrarchidae) By William F Childers Pp iii+159–214 (Urbana, Ill Department of Registration and Education, Natural History Survey Division, 1967) [51

Australia Commonwealth Scientific and Industrial Research Organization Annual Report of the Division of Mechanical Engineering, 1966–67 Pp 62 (Melbourne Commonwealth Scientific and Industrial Research Organization, 1967) [81

NATO and Science Facts about the activities of the Science Committee of the North Atlantic Treaty Organization, 1959–1966 Pp 142 (Paris NATO—Scientific Affairs Division, 1968) [81

World Health Organization Technical Report Series No 375 Chemotherapy of Malaria—Report of a WHO Scientific Group Pp 91 3 Sw francs, 8*s* , $1 25 No 380 Current Problems in Leptospirosis Research—Report of a WHO Expert Group Pp 32 4*s* , $0 60 (Geneva World Health Organization, London H M Stationery Office, 1967) [81

Australia Commonwealth Scientific and Industrial Research Organization Research Report of the Division of Nutritional Biochemistry, 1966–67 Pp 48 (Adelaide Commonwealth Scientific and Industrial Research Organization, 1967) [81

Western Australia Report of the Government Chemical Laboratories for the year 1966 (Extract from the Report of the Department of Mines) Pp 43 (Perth Government Laboratories, 1967) [81

American Phytopathological Society Monograph No 4 Stewart's Bacterial Wilt of Corn By Evan H Pepper Pp 36 (Berkeley Committee on Phytopathological Monographs and Reviews, University of California, 1967 Available from the Business Manager, Dr M F Kernamp, 1955 University Avenue, St Paul, Minnesota, USA) $1 50 [101

Fisheries Research Board of Canada Bulletin 157 An Economic Appraisal of the Canadian Lobster Fishery By J B Rutherford, D G Wilder and H C Frick Pp x+126 (Ottawa Queen's Printer, 1967) $2 50 [111

Geological Survey of Western Australia Bulletin No 117 The Geology and Iron Deposits of the Hamersley Range Area, Western Australia By Dr W N MacLeod Pp 170+11 plates 1 250,000 Geological Series Explanatory Notes Sheet SF/50–12 Roy Hill, Western Australia Compiled by W N MacLeod and L E de la Hunty Pp 25+map Sheet SF/50–16 Newman, Western Australia Compiled by J L Daniels and W N MacLeod Pp 24+map Mineral Resources of Western Australia Pp 84. (Perth Geological Survey of Western Australia, 1965 and 1966) [111

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Professor Watson's Memoirs

THE publication in the United States of Professor J D Watson's book *The Double Helix* has established a claim on polite conversation which is not entirely stifled even by public concern about the relationship between gold and the dollar or even by the drama of the first manoeuvres in the election of a new president That, to say the least of it, is a proof of the importance of a book intended to provide a full account of the way in which, in the early fifties, Watson and Crick felt or fumbled their way towards a correct structure for the DNA molecule Professor Watson's book is not, however, a formal history but rather a personal recollection of events—a memoir Many will dissent from his assertion, in a preface, that "no-one will ever be able to write a definitive history of how the structure was established", but in any case this is not Professor Watson's chief concern He is quite honestly, and quite properly, concerned to convey the flavour of how scientific research is ' done", to use his own words In doing so he has necessarily flown in the face of several conventions, literary and social This in turn has no doubt lost him his place on a good many Christmas card lists, but that is his concern

In the circumstances, an objective appraisal of the value of the book as literature or as history is probably too much to hope for Most critics so far have been deprecatory and somewhat patronizing, but they have usually also been in positions to feel threatened, directly or otherwise, by what Professor Watson has to say It will be easier to tell when *The Double Helix* is eventually published in Britain two months from now, after what must seem an absurd delay In the meantime, however, there is no doubt that Professor Watson has written a rattling good yarn, quite spellbinding. It bounces along like a detective story and will probably be a great success This is a tribute to Professor Watson's engaging candour He might have been better than Boswell and certainly funnier

There remains the question of how the issues of principle raised by the book are to be dealt with The first thing to acknowledge, however, is that there should be no difficulty at all in what seems most commonly to have been the offence—the way in which this personal account of events is necessarily subjective and even distorting If, for example, one of the participants is repeatedly presented as over-ebullient or over-cautious, this is to be read more as a comment on the relationship between Professor Watson and the people concerned than as a truth that the qualities described are real If some people seem larger than life, or smaller than life, it may well be that the fault lies in Professor Watson and not his cast of characters To be sure, publication of the book will be a chastening experience for many molecular biologists, and some of them, no doubt, will be offended, but that is one of the hazards of this kind of literature

The way in which the book is larded out with tales of Professor Watson's pursuit of young women at Cambridge may also seem to other participants to be undignified Others, however, may recognize that unmarried graduate students often share the same weaknesses In reality, these and other incidents in the book are one of the sources of its credibility and a valuable clue to Professor Watson's estimation of his own part in the problem of DNA If, of course, his picture is seriously awry, then other people are free to protest and even have a duty to do so It is not enough simply to resolve never again to invite Professor Watson to tea and biscuits

It is in passing worth remarking that the techniques which have been used in constructing this book are commonly and rightly eschewed by professional reporters as a means of getting at the truth What Professor Watson has done is to write down the often unguarded statements of those concerned with the working out of DNA The trouble, of course, is that statements like these may be misleading If X complains angrily of Y, for example, it does not follow that the complaint is justified, or even that X is habitually hostile to Y To know the truth, both X and Y must be cross-examined by some third party and even then the truth may be elusive

These, however, are almost technical objections and certainly do not suggest that *The Double Helix* should never have been published The way in which this enthralling book provides non-professional readers with an occasional unseemly view of science and of research is another issue likely to lose Professor Watson friends But is he wrong ? A good deal of what he has to say, for example, about the sense of competition between the group at Cambridge and its rivals—real and imaginary—elsewhere may come as something of a shock to those outside But is competitiveness all that uncommon ? And is it sensible of professional scientists sometimes to pretend that feelings like this never sully the state of science ? In fact what Professor Watson has done is to provide a much more real appraisal of the character of science in the laboratory than other published statements of a more formal kind He has done something to ensure that science will become less pompous than it is For all the faults which may be found in *The Double Helix*, this is a considerable public service

Time for a Change of Policy

THE worst epidemic of foot and mouth disease in the history of British farming seems to have run its course, although a fresh outbreak of the disease, confirmed last Monday, has delayed yet again the lifting of all restrictions on the movement of livestock. 423,029 cattle, sheep, pigs and goats have been slaughtered since last October, and the British Government has paid £26·5 million in direct compensation. Added to this are countless consequential costs, so the total bill could be as high as £100 million. Have any lessons been learnt? It is early days yet, but judging from the Government's actions to date the answer must be very few.

The Reid report (see *Nature*, **217**, 894; 1968) came to the conclusion that lamb imported from the Argentine was the most likely source of the infection. Tourists, the birds, the wind, hay and straw were all exonerated. It is true that the evidence for this conclusion is only circumstantial, and quite naturally the Argentinians, with an annual £40 million worth of meat exports at stake, dispute it, but it is in keeping with the findings of the Gowers Committee of 1952–54. Why then has the British Government, in the face of vehement opposition from the farmers and apathy from everyone else, decided to resume importing beef, but not mutton, from Argentina from April 15? It must believe that it is in the national interest once again to risk the chance of an epidemic, which will always be present so long as meat is imported from South America under the existing conditions of inspection, so as to obtain inexpensive beef when home supplies are at their lowest. The Government may win its gamble, and everybody hopes that it will. But if its intention is to prevent re-infection, the decision to ban mutton while importing beef is completely absurd. Both animals are affected by the disease—in the past more outbreaks have been caused by contaminated beef than mutton—and it can only be fortuitous that mutton rather than beef caused the recent epidemic.

As part of the grand post-mortem Mr Fred Peart, the Minister of Agriculture, has asked the Duke of Northumberland to be chairman of a seven man committee "to review the policy and arrangements for dealing with foot and mouth disease in Great Britain and to make recommendations", and the committee intends to collect all its evidence by May 31. The Gowers Committee of 1952–54, which was the last to consider this, came out firmly in favour of maintaining the traditional slaughter policy and prohibiting vaccination. As a result, the no vaccination policy has become an inflexible national dogma—a very expensive dogma as it has turned out—not a policy. What is even worse in the long term is that research into vaccines, methods of vaccination and the epidemiology of foot and mouth disease has been woefully under-financed. Perhaps, now it is too late, some thought will be given to stepping up research to the critical point.

The claim is that once vaccination is permitted, foot and mouth disease will become endemic and that vaccination of all the livestock in Britain is hopelessly expensive. (In 1954 the Gowers Committee estimated it would cost £25 million to vaccinate sheep, pigs and cattle three times a year.) Recent experience in France, however, seems to suggest otherwise and the Northumberland Committee would do well to recognize this.

FRANCE

Year	Individual outbreaks	Totals of livestock slaughtered	Vaccine strains
1959	6,188	116,016	—
1960	7,381	130,880	—
1961	2,627	65,468	—
1962	191	11,790	O_2A_5C
1963	28	1,129	O_2A_5C
1964	56	5,016	—
1965	10	1,608	OO_2A_5C
1966	59	4,190	OO_2A_5C
1967	17	2,045	OO_1,O_2A_5C
1968	3 so far		

The French National Herd comprises 21·184m cattle, 9·186m sheep, 9·840m pigs and 1·018m goats.

GREAT BRITAIN

Year	Individual outbreaks	Total livestock slaughtered
1959	45	7,000
1960	298	70,000
1961	103	25,000
1962	5	1,000
1963	nil	
1964	nil	
1965	1	200
1966	34	45,700
1967	2,210	390,000
1968	Over 100 so far	130,000 so far

The British National Herd comprises 12·342m cattle, 28·885m sheep and 7·107m pigs.

Nobody can seriously claim that foot and mouth disease is endemic in France where, since 1962, all cattle over six months old, but not sheep and pigs, have been compulsorily vaccinated just once a year. Indeed, as the table shows, since compulsory vaccination began there has been a hundredfold decline in the incidence of the disease. What about cost? Each vaccination costs about 3·5s., of which the French Government pays a little less than half. When an outbreak occurs, however, all livestock on the infected farm is destroyed and livestock movement is controlled as in Britain. The total annual cost of this combined vaccination and slaughter policy has, since its introduction, averaged out at about £1·5 million, and the French National Herd of cattle is about 21·184 million head compared with the British 12·342 million head. On this basis, for the price of the recent epidemic, all the cattle in Britain could have been vaccinated for 20 to 25 years. Alternatively, the cattle and those of the 28·885 million sheep in Britain that are readily accessible could have been vaccinated for something like 10 years.

The French also have an answer to the naive argument that it is impossible to vaccinate against all fifty-six strains of foot and mouth virus and so there is no point in any vaccination at all They follow continually the epidemiology of the various strains present in Europe and the countries with which they trade, and progressively change to the appropriate strains No doubt they are now producing vaccine against A_{22}, a strain loose in the USSR

The fact that Holland, Belgium and Germany also have combined vaccination and slaughter policy argues strongly for it Is it not time for Britain to follow suit ? Even if the Northumberland Committee decides that vaccination of the entire National Herd is not practical, voluntary vaccination and compulsory vaccination to cordon off centres of infection should be used in the future The folly of prohibiting vaccination while risking infection by importing South American meat ought by now to be apparent to everyone

Mr Peart should now waste no time in tightening the inadequate inspection control arrangements for imported meat As a starting point, there is the recent and overdue Argentinian proposal for an exchange of British and Argentinian experts to see what can be done to improve checks for the disease A three man British veterinary mission will leave for Buenos Aires on March 17 The present arrangements with the Argentine date from the Bledisloe agreement of 1928 and provide only for a visual inspection, by local veterinary officers, of cattle on the farms and at the slaughter and packing plants Any animal which is either incubating the disease or is a carrier will simply not be detected by visual inspection without quarantine, so there will always be a risk that meat is infected Furthermore, because of local conditions, a declaration from the farmer is accepted instead of veterinary inspection at the farm These forty year old arrangements are just not good enough The disease must be detected before slaughtering by stringent inspection, for it is certainly hopeless to try systematically to check for foot and mouth virus in the bones of refrigerated carcasses

A Space Policy for Europe?

by Angela Croome

THE report of the Causse Advisory Committee on Programmes to the European Space Conference (ESC) may well set the pattern for European space developments for the next 10 years After being leaked in *Le Monde* (March 13) the report was released at the end of the meeting of the Alternates Committee of the European Space Conference (not of ESRO as reported last week) in Paris on March 13–14 The report should be followed by action at the next ministerial meeting of the ESC some time this summer —which will mean that a mere two years have elapsed between the setting up of the ESC and the adoption of a programme One spokesman for the British aerospace industry remarked that "it's a great pity that such objectives were not adopted when the two main European space organizations were set up in the early sixties"

The aim of the advisory committee's proposals is a "balanced programme", by which it means a suitable combination of scientific and "useful" satellites and the appropriate means of launching them "European activity in space should be orientated towards direct objectives, namely, increased knowledge and worthwhile applications Rather than seek the 'power' objective that has characterized other space policies, Europe should above all demonstrate her determination to be independent Rather than indulge in illusory competition, she should seek to practise the closest collaboration with the other space powers "

The committee was constrained on the one hand by the level of investment that can reasonably be supported by Western Europe, and on the other by the nature of the proposals actually put forward by European groups These have included candidate projects sponsored by ESRO, ELDO, CETS, Eurospace and Eurovision (the European Broadcasting Union) All these have been assessed by the Technical Subcommittee of five independent experts, after which the economic implications were scrutinized by a larger economic sub-committee representing 13 of the member states of the European Space Conference

The programme that has evolved allows for three options, one of which should be chosen by the ESC for its immediate target (that is, to come to fruition in the early seventies) The options range from one in which priority is given to an ambitious science programme with the Large Astronomical Satellite as its centre-piece and a minimal applications programme, to one with a fairly comprehensive applications content and a minimal scientific satellite programme (averaging 2 scientific satellites a year, each carrying 8–10 sophisticated experiments) This covers the launching period 1973–76 The Large Astronomical Satellite (LAS) gets rather little attention, and seems to be considered a major technological liability, despite its acknowledged scientific potential The technical sub-committee believes that it cannot be attempted by adhering to the ESRO budget for its first 8-year period (1964–72) and that it may be outmoded by US activity by the time it is practicable

The whole programme should be possible if a growth rate of 10 per cent is allowed The analysis concludes that this is well below 1 per cent of the European GNP in the years ahead, assuming an annual growth of 4 per cent (It is estimated that the US is spending roughly 1 per cent of its GNP on space activity) The actual figures are equivalent to $150 million in 1967 against a plateau of $240 million from about 1972 For this, a scientific launching programme of two satellites a year, plus the development of a geostationary TV relay satellite for Eurovision use in the 1970s, is envisaged There would be enough slack to start on one other type of applications vehicle—navigational meteorological or earth resource are all of interest It is pointed out that by dropping LAS, a meteorological satellite can be introduced "giving European meteorologists a chance of taking part in the World Weather Watch in 1973 with a satellite of European construction", and that meteorology incorporates an element of research so that the sacrifice may not seem so bitter

A feature of all the options, and a distinct break with the past, is that all the launchings employ the

European launcher based on the British Blue Streak The adoption of ELDO launchers for the scientific payloads as well as for the applications satellites will put launcher production on a rational basis (an average requirement of four a year) The report points out " . the present situation is indeed unsatisfactory the programme as a whole is neither coherent nor balanced It does not include any applications satellites and the launcher projects are mainly justified by applications satellite projects " It also calls for a sustained development programme for the ELDO vehicle, involving strap-on boosters, liquid hydrogen upper stages and possibly electric propulsion, for the tasks of the later seventies The possible ELDO-4 launcher, for instance, would have about double the capacity of the present ELDO-1 specification and could place 2 tons in geostationary orbit, enabling direct-broadcast colour television to be supplied to all of Europe as well as Africa and the Middle East by 1978. Blue Streak is seen as perfectly suitable for the booster stage throughout the period

A number of decisions are called for by the report, but, the authors point out, four of them are urgent and must be taken by the ministers this year—the future of the LAS, a start on the Eurovision satellite, increase in production of ELDO launchers and statutory bodies to handle the problems of organization But what is needed most is a firm decision to pay ESRO has been living from hand to mouth for two years now

CERN Meeting

THE 37th meeting of the Council of CERN, held last week in Geneva, discussed the progress being made towards the building of the 300 GeV accelerator Most of the progress is at the organizational level, but it now seems clear that a final decision on the project should emerge by the end of this year, probably in December There were also hopeful signs for supporters of the machine that fewer governments are dragging their heels than was the case at the end of last year The United Kingdom delegation, in particular, expressed itself completely satisfied with the answers given on design, cost, management and collaboration, and said that there was now no reason why these issues should be reopened The German delegation was rather more subdued, for reasons of its own, but put forward no major objection

It is now also clear that a decision about the siting of the laboratory should be made in the summer of this year, and a short list of four sites should be ready by June At the moment, nine sites are still under consideration, and technical reports have now been published on each one They are Aspropyrgos in Greece, Doberdo in Italy, Drensteinfurt in Germany, El Escorial in Spain, Focant in Belgium, Gopfritz in Austria, Le Luc in France, Mundford in the United Kingdom and Uppsala in Sweden The final decision will be taken in two stages, a preliminary vote will be used to reduce the number of sites to four, and the delegates last week agreed that this vote should be taken in open ballot, though it is possible that it could be done in closed session But, whatever the choice is to be, it will have to await promises of money which represent a substantial proportion of the cost of the accelerator In other words, countries will have to commit themselves to the project before they know where it is to be built

At the organizational level, the delegates agreed to the formation of a steering committee to work on the 300 GeV project until an advisory committee for the project is formed The function of the advisory committee would be to assist the director-general of the new laboratory, and it might also exercise some form of control As a stopgap arrangement, the steering committee has been set up to fulfil this function, the membership is Professors Amaldi and Amman of Italy, Dr J B Adams and Professor Merrison of the UK, Dr Levy-Mandel of France, Professor Paul of Germany, and Drs Hine and Zilverschoon of CERN, the director-general of CERN is an ex-officio member Several delegates emphasized the need for speed, particularly once the decision to go ahead has been taken Professor Puppi of the Science Policy Committee, for instance, said that the name of a proposed director-general could be put forward very quickly The feeling seems to be that the second the decision is taken, the delegates should dash from the meeting to lay the foundation stone, before anybody can change their minds

Engineers of the World Unite

NATIONAL engineering organizations from all over the world got together recently under the auspices of Unesco to form a World Federation of Engineering Institutions At a meeting in Paris from March 4 to 7, representatives from 60 countries and four regional organizations gave sanction to a plan to set up the federation The hope is that it will do for engineering what the International Council of Scientific Unions does for science—a process of organization, co-operation and, to a limited extent, unification

The World Federation will replace or supplement a number of other international bodies which for reasons of representation or organization were unable to do the job There have been limited groupings of national institutions for a number of years—there was one in Europe, one for the Commonwealth, and another covering the Americas These organizations will no doubt co-exist with the new one quite happily The only organization whose continued existence is threatened is the Union des Associations Techniques Internationales, based in Paris, which has for some time been regarded as something of a lame duck Unesco has been pressing for a new organization for some years, and it seems that its gentle prompting met with a ready response from the national engineering institutions At the Paris conference the federation was set up by a unanimous vote

The first president of the federation will be the Swiss engineer Dr Eric Choisy, and Mr R Gibrat of France will be vice-president It seems likely that much of the executive work of the federation will be done at the Institution of Electrical Engineers in London—the secretary of the institution, Dr G F Gainsborough, has been appointed first secretary-general of the federation The federation itself will consist of national members, which will be the representative engineering body for each country (in Britain the Council of Engineering Institutions), and some international members, representing existing federations throughout the world

Dr Gainsborough is enthusiastic about his new responsibilities Already, he says, two projects have been started The first is to set up a committee to examine the qualifications and continuing development of engineers throughout the world. The ultimate aim, still a long way off, is international recognition of qualifications, so that an engineer from Poland, say, can have his qualifications recognized in Ethiopia. This committee will also help to speed the exchange of ideas in the education of engineers, and should help developing countries which are setting up departments of engineering. The other project started by the federation is to examine the possibilities of a world-wide information system, for dissemination and retrieval. This committee will be collaborating with the abstract board of ICSU. The federation has also started on a more abstract project, that of drawing up an international code of conduct for professional engineers.

The general work of the federation will be financed from contributions from member countries. The special committees, on the other hand, are each charged with responsibility for finding sources of funds for their investigations. Dr Gainsborough is hoping that Unesco, OECD and other international organizations will see the federation as a body worthy of support. There seems every likelihood that this hope will be justified.

No Student Power at Oxford

from our Oxford Correspondent

It was too much to expect the Hebdomadal Council to comment other than adversely on the petition submitted to the Privy Council for various reforms in government of the University of Oxford. One of the three chief proposals of the petition, intended to give undergraduates direct representation in university affairs, would indeed have been without precedent in many less staid institutions. In its letter to the Privy Council, the Hebdomadal Council took an optimistic view of relations within the colleges between undergraduates and senior members, and justified its rejection of the proposal because the Student Representation Council is not highly regarded by the undergraduates themselves. The argument is circular; the SRC is not even recognized by, let alone allowed to participate in, the university government. Its lack of prestige among students is a reflexion of this. But in any case, the council's dismissal of the proposition will doubtless be justified shortly by the anarchic demise of the London School of Economics whose Board of Governors has decided to admit students to certain committees (see *Nature*, **217**, 699; 1968).

A second proposal in the petition is that the convocation of the university's MAs should be made into an active forum, rather than consigned to impotence as it will be under the Franks Commission's recommendations. This proposal indeed seems "unnecessary and undesirable". As the Hebdomadal Council points out, many members of convocation live abroad, and most are too busy to attend sessions.

The rejection of the third proposal of the petition, outlined at some length in *Nature* (**217**, 500; 1968), is not so easily defended. The Hebdomadal Council does not deny that, in the disciplinary procedure of the university, the proctors act both as judges and policemen, so that there is a possibility of bias in the judgment of students' misdeeds. Yet the council (which, it is worth noting, includes proctors) announces its satisfaction with the present arrangements. The council's claim that students are made aware of their right to appeal against proctorial decisions to an independent committee is indeed true. But this has no bearing on what is requested by the petition. If the proctors confined themselves to the carrying out of petty university regulations, such as the licensing of student's cars, then the reform of proctorial procedure would scarcely be worth the attention of the Privy Council, but the Oxford proctors, unlike their Cambridge counterparts, show little reluctance to act in cases where junior members have broken the law of the land, rather than simply that of the university. The proctors' terms of reference are wide and their punishments sometimes severe, and it is only reasonable that the slightest possibility of bias should be eliminated.

Among the signatories of the petition, senior members of the university outnumber juniors by 101 to 29. In its letter to the Privy Council the Hebdomadal Council doubts whether the request of 100 out of the 1,600 members of the congregation (the "parliament of senior members") is enough to justify discussion of major constitutional changes. Yet it is well known that few senior members attend the congregation. Important issues have been decided with less than 200 people present. Outside observers might be expected to be surprised that so great a demand still exists for new reforms 18 months after the Commission of Inquiry issued its report. It will be interesting to see how this consideration weighs with the Privy Council as it studies the petition and the reply.

More Teachers

The latest volume of *Statistics of Education*, 1966 (published by HMSO, price £1) starts on a cheerful note, for between October 1965 and October 1966, the number of students training to be teachers in England and Wales rose by more than 12,000, bringing the total to 88,823. This represents an increase of nearly 75 per cent over the figure of 50,942 for 1962. In addition there were 1,308 men and 654 women in one-year supplementary or advanced courses, and courses of one year for the teaching of handicapped children also showed a marked increase for the same period. Less reassuring is the decline in the proportion of students successfully completing training in both junior and secondary courses, even though the numbers and proportions in infant/junior and junior/secondary courses increased. Another unsatisfactory trend was the fall between 1963 and 1966 in the numbers of courses for secondary school teachers and students for them.

The increase in the total teaching force from 321,000 in 1962 to 349,000 for 1966 was most striking in the colleges of education, where the number of teachers was more than doubled. A 70 per cent increase for further education establishments is also a striking feature. Up to March 31, 1966, 20 per cent of teachers in maintained primary and secondary schools were graduates—the proportions ranging from 75 per cent of teachers in grammar schools to some 4 per cent in primary schools. About 33 per cent of teachers in establishments of further education were graduates, and graduates also accounted for more than 60 per cent of teachers in direct grant grammar schools.

But the figures showing the wastage of qualified teachers are dismally high. Thus between 1965 and 1966, 9,000 qualified men teachers entered maintained primary and secondary schools, and in the same period 7,000 left the schools. Wastage was greatest among those under thirty, and was also high among untrained graduates under 25. The comparable figure for women was even higher—representing 13 per cent of those qualified and in service, and wastage was again high among untrained graduates. Significant proportions of this wastage could, however, be attributed to transfer from one teaching establishment to another, although among qualified women teachers only 1,000 out of a total of 20,000 transferred in this way.

NUMBERS OF STUDENTS IN COLLEGES OF EDUCATION AND UNIVERSITY DEPARTMENTS OF EDUCATION (other than supplementary courses)

Numbers of students	1956–57	Oct 1962	Oct 1964	Oct 1965	Oct 1966
In university departments of education	2,512	3,260	3,739	3,695	3,192
In colleges of education	26,039	47,682	62,112	72,856	84,911

The last page in the report lists the salaries of teachers in colleges of education at March 31, 1966. For male graduates the average salary was £1,539 for those under thirty, rising to an average of £2,674 for those aged 60 and over; figures for women graduates were very similar. Male non-graduates received some £100 less under the age of 30, and about £140 less over the age of 60.

Change on Blood Tests

THE British Home Office seems to be modifying slightly its attitude to the tests by which motorists in Britain can now be convicted of driving under the influence of alcohol. A recent paper in *Nature* by Professor J. B. Payne, Dr D. W. Hill and Mr D. G. L. Wood of the Royal College of Surgeons (**217**, 963; 1968) suggests that the methods used by police authorities for carrying out the tests are far from accurate. The method by which the amount of alcohol in the bloodstream is estimated from the amount in the urine seems particularly suspect. So far the Home Office has not been forced to act, because no motorist accused of driving under the influence of drink has quoted Professor Payne's work in his defence.

Although the Home Office has not changed its views on the urine test, there does seem to have been a shift of emphasis in the blood tests. Originally, after advice from the medical associations, police surgeons were advised to take small samples of capillary blood for use in the test, although it was also open to them to take venous blood if they preferred. The work at the Royal College of Surgeons suggests that the latter is likely to give more accurate results, because of the way in which the alcohol tends to become more concentrated in the plasma than in the red blood cells. Blood taken from capillaries can often contain more plasma than blood from veins, so that the capillary method can give anomalously high readings, with the possibility of conviction for a driver who is in fact under the limit.

The Home Office has now sent a circular to police authorities pointing out that it is within their discretion to take venous rather than capillary blood. The police authorities are advised by the Home Office always to have available a supply of syringes with which venous blood samples can be taken. The motorist can still decide which method he prefers, though the doctor will be at liberty to advise the syringe method if he thinks fit. The circular also points out that the motorist has the right to keep a sample of his own blood for independent analysis, and that these samples should be sealed, stored in a cool place, and analysed with the minimum of delay.

So far, this is no more than a recommendation from the Home Office to the police authorities. Both methods of taking blood are still legal, and the circular says nothing of the inaccuracies of the urine test. But in the event of a driver being acquitted because of the inaccuracies which Professor Payne and his colleagues have discovered, the Home Office would doubtless have to act very much more decisively. Whether the work provides new grounds for appeal for those already convicted under the existing law has yet to be tested, but there is an urgent need to clarify the situation. Sooner or later the Home Office will be forced to set about the task.

Preserving the Bastard Toadflax

THE Nature Conservancy has announced two new nature reserves and an extension to another, bringing the total number of national nature reserves to 124, covering 257,239 acres.

One of the new reserves is in the central part of the range of chalk hills forming the northern scarp of the Vale of Pewsey seven miles south-west of Marlborough. It is a fine example of unreclaimed chalk downland, and it has long been known to botanists and entomologists for its rich plant and insect life. Extending from Milk Hill in the west to Knap Hill in the east, the reserve covers 188 acres and it has been established by agreement with New College, Oxford, and the agricultural tenant. The summit of Milk Hill (964 ft) is the highest point in Wiltshire. Examples of plants found in the reserve are round-headed rampion, field fleawort, bastard toadflax and several orchids. The Chalk Hill Blue and the Brown Argus are two of the butterflies found. There are also several features of archaeological and historical interest. One of the best examples of a neolithic causeway camp lies on Knap Hill, and there is also a chambered long barrow known as Adams Grave on Walkers Hill.

The other new reserve is at Ebbot Gorge, Somerset,

North part of Hartland Moor, Isle of Purbeck (Crown copyright).

on the southern face of the Mendip Hills near Wells It was presented to the National Trust by Mrs Gerard Hodgkinson in 1967 in memory of Sir Winston Churchill and it is being managed by the conservancy under a lease The reserve covers 101 acres and includes a dry gorge of carboniferous limestone There are several small caverns in the gorge where the remains of reindeer, bears, wolves and lemmings have been found together with those of Neolithic Man

The Nature Conservancy has also leased an area of 423 acres on the Isle of Purbeck which increases the Hartland Moor Reserve to 637 acres The extension is characterized by its fen type vegetation Black Bog rush occurs in the north-eastern part while sundews and bladderworts grow on the middle and lower bogs There are several local plants including the Dorset Heath along the bog edges The rest of the reserve consists of dry heathland

Wellcome Initiative

THE Wellcome trustees announced this week that they are proposing to allocate up to £2 5 million to medical scientists in universities in Great Britain and Northern Ireland, with the aim of encouraging more high-quality men and women to stay in Britain The new grants will enable medical research workers to continue their research programmes which might otherwise be threatened Letters have been sent to vice-chancellors in Great Britain and Northern Ireland asking them to inform the appropriate departments of the proposal Applications will have to be sponsored by the university concerned, and the final selection will be made by the Wellcome trustees Each grant will be allocated to meet the special needs of an individual and may be as large as £20,000 over a period of about five years

How Children Choose

OCCUPATIONS, and the factors which affect children's choices of subjects and careers, were both involved in the Dainton enquiry, because of their effect on the flow of candidates in science and technology into higher education To provide information on this subject a critical review was commissioned and has now been published (*Occupational Choice*, J R Butler, HMSO, 3s 6d) Mr Butler, a research fellow in sociology at the University of Manchester Medical Care Research Unit, has reviewed the literature which covers the formation of decisions on occupations in Britain, and summarizes the factors that appear to be most important Emphasis is laid on science and technology, because of the requirements of the Dainton enquiry, but non-scientific occupations had to be included in the investigations so that the influences working specifically towards occupations in science and technology could be picked out

The subject is approached from a sociological point of view, in so far as conclusions on occupational choice are assessed in terms of concepts which are linked with sociological or psychological theory, but the pattern of choice which emerges has practical significance Psychological, motivational, economic and sociological factors work together over a period of several years to predispose an individual towards a particular type of occupation, while his opportunities in other directions become limited by his education

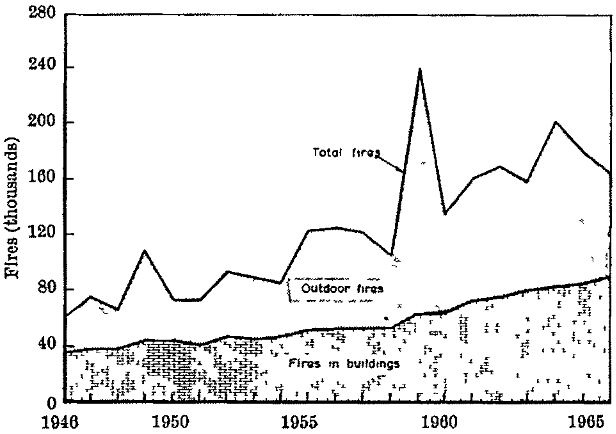
Scientific interests are often obvious as early as eight or ten, and by the mid-teens the choice between science or arts has usually been made. Because of the way the educational system is organized, revision of choice of occupation becomes less possible as an individual progresses up the school Although the specialized teaching helps those who have a definite career in mind, it has the disadvantage of setting limits on the choices of those who are uncommitted Thus the broad area of occupation is often decided early in the school career, while the final decision is a result of interactions between personal and external factors Personal interests and aptitudes are matched against ideas of the value set on various jobs, orientated towards people, extrinsic rewards or self-expression, external influences such as school, family and social contacts also have their effect The guidance of parents and friends is believed to be more important than more formal career advice received at school There are conflicting opinions about the effects on subject choices of personalities of teachers and the ways in which subjects are presented

Mr Butler's recommendations are put forward as guide lines for future investigators rather than as detailed research proposals He suggests that a multidisciplinary approach would provide interesting results, and recommends following the progress of a group of children from the age of eight until they enter the labour market To get the greatest value from such research a comprehensive theory must be developed to test findings An experimental programme of early and frequent vocational guidance to a group of children might yield valuable results

Incendiary Statistics

FIRES in Great Britain killed 780 people, three of them firemen, during 1966 This figure is in line with the increase that has, regrettably, been going on over the past six years, but does not reach the abnormally high figure of 818 which was recorded in 1963, the year of the hard winter Standing at 165,053, the total number of fires in 1966 was less than that for any of the three previous years, although the number of fires in buildings, 88,162, increased as it has done for the past 20 years The recently published *United Kingdom Fire Statistics for 1966* (HMSO, 15s) gives these figures, and statistical analyses by the Ministry of Technology's Fire Research Station, Borehamwood, of reports of fires attended by the 147 local authority fire brigades The statistics cover when, where and how fires were started, how they spread, and how they were fought Details of casualties are also given together with ways of escape of survivors, and correlations of casualties with sources of ignition of the fire

The number of fires in dwellings seems to be levelling off at about 34,000 a year, putting the rate of incidence at 20 fires per thousand dwellings at risk The rate of incidence in the distributive trades also appears to be levelling off, but in the manufacturing industries there is still an upward trend Outdoor fires have been fewer in the past couple of years, but the general trend since 1946 has been upward Refuse disposal is a particular problem, and fires from this source have more than doubled in the past four years Although these fires do not cause very much damage, they are expensive in terms of fire brigade time Smoking materials such as matches and naked lights caused 131 fatal fires, 25 per cent of the fatal fires from known causes. Electrical appliances and fires in grates each cause 19 per cent, and although children with fire caused only 5 per cent, this proportion is increasing. The number of incidents in which more than one person has died has been increasing over the past six years, and stands at 78 for 1966. Fires in buildings were more frequent in winter than in summer, while the reverse was true for fires not in buildings. The largest fire loss for a single month was estimated at more than £10 million, and the smallest at about £4 million. A computer was used for the first time to produce the 1966 tables and it is hoped that in future results will be produced more quickly.



Annual incidence of fires in the United Kingdom (from *United Kingdom Fire Statistics for 1966*)

Design for a Fusion Reactor

THE major problem of using nuclear fusion to produce power remains that of stabilizing a high temperature plasma for long enough for the reactions to produce a net gain of power. Although this has yet to be achieved, some rather speculative studies have been made of the engineering characteristics of fusion reactors. Mr R. Carruthers, Dr P. A. Davenport and Dr J. T. D. Mitchell of the Culham Laboratory of the United Kingdom Atomic Energy Authority have written the most recent of these, called *The Economic Generation of Power from Thermonuclear Fusion* (HMSO, 4*s* 6*d*.)

All, or almost all, designs for fusion reactors use magnetic fields as a means of keeping the very hot gases together. The authors of this report do the same, and suggest that the best geometry for the reactor is toroidal—doughnut shaped. The plasma, at a temperature of 7×10^8 °K, is contained in what amounts to a cylinder with the open ends joined to form a closed loop. The fuel used is a 50/50 mixture of deuterium and tritium. To get the reactor started, both fuels would be injected into the reactor, but once it was operating it would generate it own tritium in a blanket of molten salt surrounding the plasma. The blanket, containing lithium salts, would generate tritium by the Li(n,t) reaction, and would also act as a means of extracting the useful heat from the reactor. Thus the blanket would be circulated through salt pumps and heat extractors, and the lithium replenished. In this sense, the real fuels for the reactor would be deuterium and lithium.

The reactor chosen for the study is one of 5,000 MW (thermal), or 1,250 MW (electrical). The authors estimate that the capital cost of such a system would be between £68·5 and £74 per kW, the greater part of this (£29 per kW) being spent on conventional equipment, which includes the salt recirculating pumps, and the necessary electrical equipment. The second most expensive item is the superconducting magnet which would be used to keep the plasma in the right place, the reactor vessel and "blanket" would be reasonably cheap, only £4 per kW. On the basis of a 25 year life at 80 per cent load factor, and interest rates of 8 per cent, the generating costs of such a system would be 0·23 to 0·25 pence per kWh, of which the vast majority is attributable to the capital costs of the station. Fuel charges would be only 0·004 pence per kWh, and operation and maintenance 0·017 pence per kWh.

But the authors concede that there are a number of problems outstanding before this type of reactor can be built. The most obvious one, of course, is that of plasma containment, but there are others, the properties of the vacuum wall, for example, would need to be quite unusual. Fuel injection and exhaust extraction would also be a problem. It would even be quite hard to get the reactor started. For all that, the report is striking evidence of the continued optimism of those involved in fusion research.

Crystal Growing

THE Science Research Council has awarded a grant of £14,505 to Dr J. N. Sherwood, of the University of Strathclyde, Glasgow, to set up facilities for the growth of organic molecular crystals. Molecular solids are attracting a great deal of attention at present because of their uses in solid state devices. In particular, the electro-optical properties of many organic crystals suggest applications in radiation detectors—a familiar example is the use of certain aromatic phosphors in scintillation counters.

The electrical, optical and mechanical properties of these solids frequently depend on the structure of the material as well as its chemical composition. This is why there is a demand for high quality single crystals of organic substances.

Dr Sherwood will use the SRC grant to provide a crystal growing service for the universities and industry. This seems to be a desirable step. Crystal growing can require specialized equipment, and is frequently time-consuming. Because many research workers need only a few samples of the crystals they are studying, it is logical to have a laboratory with the facilities and the skill to provide the service.

Effects of Malnutrition

ONCE again the need to produce more food to keep up with trends in increasing population and changing social habits has been stressed. This time it is the joint FAO/WHO Expert Committee on Nutrition in its seventh report (published with two alternative covers as *WHO Tech. Rep. Series* No. 377, and *FAO Nutrition Meetings Rep. Series* No. 42).

The report reviews the state of knowledge in relation to nutritional requirements, and includes a discussion of various health problems which arise from malnutrition The consequences of infection are made worse by malnutrition Weanling diarrhoea, which is almost universal in developing countries, is caused by a combination of malnutrition and reduced resistance to infection in an environment with poor sanitation The committee recommends that the relationship between malnutrition and resistance should be investigated and stressed in the education of medical and public health workers

Iron deficiency is one of the causes of anaemia, and a programme of study initiated by WHO in 1961 is in progress to investigate this problem The contribution of factors such as loss of blood caused by hookworm infection is also being investigated Experiments with iron-59 have shown that iron from wheat is less readily absorbed than haemoglobin, ferritin or ferrous ascorbate, and people deficient in iron absorb less iron from wheat than do healthy people The committee recommends that, while such studies should continue, more emphasis should be placed on anaemia in infancy and childhood, times when it is possible that iron deficiency could cause irreparable damage to the blood and other tissues such as the gastro-intestinal tract Vitamin A deficiency is another serious problem, causing various diseases of the eye in infants and young children in Asia, Africa and Latin America In South and East Asia such diseases constitute an important cause of preventable blindness An effective preventive treatment for vitamin A deficiency is likely to be the enrichment of foodstuffs with the vitamin The committee recommends investigation of the stability of vitamin A in enriched foods, and the effects of cooking on its biological activity

Rickets, caused by vitamin D deficiency and lack of exposure to sunshine, is prevalent in many tropical and subtropical countries—in North Africa a WHO consultant found signs of the disease in between 45 and 60 per cent of children between birth and 5 years old It has been suggested that rickets influences infant mortality by lowering resistance to infections and aggravating the course of pneumonia and bronchopneumonia As measures to prevent rickets, the committee recommends supplementing breast milk with vitamin after the third or fourth month, treatment with vitamin D during the first 2 years of life as a public health measure, and education of mothers to expose their children to sufficient sunlight Daily administration of vitamin D will not be easy to achieve, and other approaches such as the use of periodic massive doses may be tried

Progress is also being made in the control of endemic goitre by the introduction of iodized salt to counteract iodine deficiency In the Himalayan endemic goitre belt there has been a striking reduction—from about 40 per cent to 15 per cent—in the prevalence of the condition in areas receiving salt fortified with potassium iodide or iodate

There is also some evidence that malnutrition in early childhood can adversely affect mental development, although there are so many complicating social factors that the results of intelligence tests are very difficult to assess in these terms The committee recommends that studies should be encouraged to assess the effects of malnutrition on mental development, learning and behaviour of young children, and that, when sufficient data have been accumulated, possibly within 5 years, an expert group should be convened by WHO to discuss the evidence

Alerting Chemists Quickly

It is becoming increasingly difficult for the information section of an organization to keep research staff informed of the literature Scanning journals for articles of likely interest to research staff, and attempting to keep up to date with their changing interests and projects, can put a great strain on the information staff While the ratio of information to research staff in the USA is 1 10, in Britain 1 30 is more usual, and 1 500 is not unknown Use of printed current awareness journals can help to some extent, particularly in small organizations or in those with restricted subject interests A new innovation is the use of computers for current awareness, providing what is known as a Selective Dissemination of Information service (SDI), that is, a service aimed at alerting research staff to material relevant to their subject interests The application of computers to SDI is only just beginning in Britain, but there are a few research programmes in progress now, and there is growing experience of the use of magnetic-tape outputs from large literature-handling services such as MEDLARS and Chemical Abstract Services

The main computer-based service operating at present is the Chemical Society Research Unit in Information Dissemination and Retrieval at Nottingham University supported by the Office for Scientific and Technical Information This unit is currently running searches (on a KDF 9 system) of the magnetic tape versions of *Chemical Titles* (*CT*), *Chemical Biological Activities* (*CBAC*) from the Chemical Abstracts Services, for a selected user population of more than 300 chemists Two other mechanized SDI services for chemists are those being run at the UKAEA laboratory at Aldermaston and the Shell laboratory, Sittingbourne These are also based on taped *CT* Outlines of these services, which are still in the experimental stage, were given by Mr L Corbett of Aldermaston and Mr P Gallagher of Sittingbourne at a meeting of the Institute of Information Scientists on March 13 Their talk was entitled, "Using Commercially Available Literature Tapes and 'Package' Computer Programmes for Current Awareness Services" Mr Corbett spoke of the advantages, limitations, potential and user reactions, and effects of the mechanized literature services operated at Aldermaston, and Mr Gallagher concentrated on features and problems of the programmes and machine operations

At Nottingham, the unit has developed its own programmes for the university's computer The Aldermaston and Shell services, however, use IBM 360/40 computers with the "1401 package" programmes of *CT* Experimental runs on issues of *CT* were begun at Aldermaston in 1966 Twenty issues have now been run The maximum throughput is 150 questions consisting of approximately 700 search words These have to be processed in two "passes" through the computer for each *CT* issue There are now more than 40 users of the service The machine time per issue is 1½–1¾ hours, and the number of references distributed is about 800 On the usefulness of the references or "hits" sent to users, Mr Corbett illustrated a sample analysis of ten questions Results of these showed an average relevance (precision) of 52 7 per cent The size of this figure is not as important in current literature alerting as in retrospective information retrieval It is in fact not desirable to have too narrow a field for current awareness—there should be room for "idea stimulating" information, developments in neighbouring fields, and so on The general relevance level of the references responded to (about 75 per cent) is 20 to 30 per cent, the average level for a question being about 35 per cent

Other "literature on tape" services were also discussed at the meeting Both Mr Corbett and Mr Gallagher spoke of the effects of SDI on the research staff, who certainly want it and like it Given a regular service, they show a tendency to alter and improve their "literature-use" habits individually and as "teams", this also applies to research/information staff communications

Defence Research

THERE was a mood of intense self-questioning in Britain about the national investment in research and development, Professor P M S Blackett told the Select Committee on Science and Technology on March 14 The dominant feeling was that the investment had not been wisely made, despite expenditures much greater than those in other countries, Britain's growth rate was slow But Professor Blackett was optimistic that now there was intense activity, centred on the Ministry of Technology, which would help to change all this The most important thing, he thought, was to persuade more scientifically qualified people to go into management—engineers were particularly important. "Historically we have failed to match management with technology," he said

Although the committee is launched on an investigation of the defence research establishments, it made little progress towards its objective in this session Professor Blackett's recent experience as an adviser to the Ministry of Technology and the cogent memorandum which he presented to the committee were both in the civil rather than the military field Often he frankly admitted that the questions were outside his competence In consequence, the session was spent in discussion of rather generalized notions of how Britain's economic performance could be improved Briefly, this meant a discussion of the twin doctrines of size and selectivity

In his memorandum, Professor Blackett provided a compelling argument in favour of large firms Since few firms can afford to spend more than 5 per cent of their turnover on research and development, it followed that a firm's turnover must be at least twenty times its research expenditure It was worthless to employ less than about five to ten qualified scientists and engineers, each of whom cost £10,000 a year to keep going For a team of ten scientists, then, the firm must have a turnover of £2,000,000 a year Without this, it would be unlikely to survive for long in a rapidly advancing technology, although if it were exceptionally lucky it might get along for a little while As for selectivity, Professor Blackett said that it was inescapable, because Britain had reached the limit of what could be done by broad fiscal means If the work of government laboratories was to be used, it had to be offered to one firm on the basis of exclusivity, or perhaps to two firms If the results were thrown open to all, nobody took them up, he said

Conservative members expressed some anxiety about this approach Sir Ian Orr-Ewing suggested that civil servants were not sufficiently well-equipped to make decisions of this sort Professor Blackett denied that the National Research Development Corporation or the Industrial Reorganization Corporation were part of the civil service As for the Ministry of Technology, there was a new section, concerned with industry, which he thought was well able to make these decisions And government purchasing power should also be more widely used as a selective mechanism It might be necessary to look at the whole company structure—exports, research and development structure, and the like—before awarding contracts This would mean that the Government would not always accept the lowest price offered

Parliament in Britain

by our Parliamentary Correspondent

Fuel Policy

MR ARTHUR PALMER and Sir Harry Legge-Bourke, both members of the Select Committee on Science and Technology, pressed the Minister of Power to make clear his policy on fuel Mr Palmer suggested that there were as many fuel policies as there were chairmen of nationalized industries, but Mr Marsh, Minister of Power, denied this He said that the Government's policy was not in conflict with the report of the select committee on the nuclear power industry in Britain, and that he and the Minister of Technology were both discussing this "large and important" document They hoped to come to Parliament soon with their conclusions

High Temperature Reactors

MR R FREESON, for the Ministry of Power, refused a request from Mr Eric Lubbock that he should direct the Central Electricity Generating Board to seek tenders for high temperature reactors for future power stations Mr Freeson said it was not a matter in which a general directive was appropriate Mr Lubbock asked sharply whether Mr Freeson was aware that the tender for the Hartlepools power station by the Nuclear Power Group, using a high temperature reactor, was several million pounds cheaper than the next quotation Was he also aware that electrical utilities in the United States published all details of tenders ? Mr Freeson said that these were matters for the CEGB

Dainton Report

MR PATRICK GORDON WALKER, Secretary of State for Education and Science, refused to be drawn on the subject of the Dainton report Mr Christopher Price, who asked the minister for a statement, wanted to know if he agreed that all children should study mathematics until they left school If so, he asked, where were the mathematics teachers to come from ? Mr Gordon Walker said that most of the recommendations of the report were directed at local authorities, universities and schools, and he did not think that he was yet called on to give a view But he said that he was having consultations with various bodies and associations concerned, and was gratified that "there has been a general acceptance that the swing away from science is wrong, and should be reversed" He would, he said, make a statement in due course

NEWS AND VIEWS

Magnetic-Faunal Boundaries

from a Geophysics Correspondent

EVER since Uffen (*Nature*, **198**, 143, 1963) suggested that removal of the geomagnetic field would produce an increase in ionizing radiation at the Earth's surface, thereby affecting the evolution of organisms, the problem of faunal extinction at field polarity transitions has rested in confusion Uffen's argument was essentially that, if during a geomagnetic polarity reversal the field strength passes through zero, the Earth will no longer be shielded from sterilizing cosmic rays, the Solar Wind and particles trapped in radiation belts These will then be free to bathe the Earth and thus produce drastic changes in mutation rates

Two years later, Sagan (*Nature*, **206**, 448, 1965) observed that even in the absence of the geomagnetic field, atmospheric absorption would remove most of the harmful radiation, but by this time Harrison and Funnell (*Nature*, **204**, 566, 1964) had discovered that the most recent reversal coincided with a faunal boundary in deep sea cores In the light of subsequent research we may now say that there is a close correlation between the Brunhes–Matuyama magnetic boundary and the χ–ψ Radiolaria boundary

Confusion arose through the conflict between Uffen's and Sagan's ideas together with a lack of precise knowledge of the magnitude of the radiation increase during a reversal, or the effect this increase would have on organisms In the past few months, three important papers have appeared independently, each of which indicates that faunal extinction cannot be related to known sources of radiation because the increase in such radiation during a field reversal is extremely small

In the first of these papers, Waddington (*Science*, **158**, 913, 1967) notes, after Sagan, that few primary particles impinging on the upper atmosphere will avoid nuclear interaction, and so any increase in radiation dose at sea level will be due entirely to secondary particles On this basis, he calculates that removal of the geomagnetic field will increase the secondary cosmic ray dosage by a maximum of only six mrad/year and the dosage of isotopes produced by cosmic rays and the Solar Wind by less than two mrad/year Each of these is negligible compared with normal background radiation Furthermore, there will be little or no increase in Antarctic latitudes, from which regions most of the deep sea cores exhibiting faunal extinction coincident with the latest reversal boundary have come The effect of the dumping of radiation belt particles will also be negligible because this process will occur only slowly as the magnetic field gradually weakens

In the third paper to appear, Harrison (*Nature*, **217**, 46, 1968) reaches essentially the same conclusions as Waddington In addition, however, he points to recent work of King and Kohl and of King-Hele and Allan, which indicates that certain meteorological phenomena may be partially controlled by the geomagnetic field The suggestion is that climatic change may be the causal intermediary between geomagnetic field reversals and faunal extinction, although Harrison admits that at the present time this hypothesis rests on insufficient evidence

The most impressive of the three contributions, however, is that of Black (*Earth and Planet Sci Letters*, **3**, 225, 1967) In an excellent paper, this author considers, quantitatively and in detail, the effects of cosmic rays, radiation from solar flares, the dumping of Van Allen belt particles, exposure of the Earth to the Solar Wind and the production of radioisotopes from the various radiation sources In each case, increased dosages during a polarity transition are shown to be negligible, and thus incapable of drastically affecting evolution—least of all, causing faunal extinction Even the increase in the concentration of ozone produced by the impact of the Solar Wind on the upper atmosphere is extremely small At least one source of climatic modification during a reversal is thus eliminated

In his conclusion, moreover, Black raises a significant general point He asks, in effect, whether there really is a causal magnetic–faunal boundary correlation In only one case, the Brunhes–Matuyama boundary, has a correlation been proved In a second case, the beginning of the Olduvai event, a correlation has been suggested by some workers but not confirmed by others Further, at least one of the faunal boundaries within the period covered by the last nine reversals lies away from any magnetic boundary If both the faunal and magnetic boundaries are distributed randomly in time, the probability of one coincidence within 10,000 years is ten per cent, but falls to one per cent for two coincidences Until a second correlation is confirmed, therefore, the first may only be a "coincidence" in the more colloquial sense of the word.

A DNA Restriction Enzyme

from our Cell Biology Correspondent

IN this issue of *Nature* (see page 1110) Meselson and Yuan report the isolation and partial purification, from *E coli* strain K, of an intriguing enzyme, endonuclease III K This enzyme can recognize and degrade foreign DNA introduced into the cell either as a bacteriophage genome or during abortive mating with another strain of *E coli* In other words the enzyme causes host and mating restriction by preventing infection of the cell with certain phages and mating with other *E coli* strains

When phages, such as λ, are grown in a particular strain of *E coli*, their DNA can be modified by modification enzymes in the host cell It seems that the modification enzymes methylate the phage DNA at specific sites When the phage reinfect a second cell of the same strain they replicate, but if they infect a different strain of *E coli* the phage DNA may be degraded by restriction enzymes like the one Meselson and Yuan have isolated These restriction enzymes probably recognize the pattern of methylation of DNA molecules If the introduced DNA has the same pattern of methylation as that of the host cell it is not degraded but if the pattern is different it is In one sense the DNA modification and restriction enzymes provide a recognition system analogous to an immune response which allows recognition of "self" from "non self"

Endonuclease III K requires ATP, Mg^{++} ions and, strikingly, S adenosylmethionine (SAM) SAM is a donor of methyl groups and because modification probably involves methylation of DNA it is possible that the one enzyme, endonuclease III K, can act either as a modification enzyme or as a restriction degrading foreign DNA molecules In this context, it is significant that Meselson and Yuan found that hybrid (heteroduplex) DNA molecules made with one strand from DNA of phage λ grown on *E coli* strain K (this DNA is not degraded by endonuclease III K) and the other strand from DNA of λ grown on *E coli* strain C (this DNA is degraded) are resistant to the restriction enzyme In order to function as a nuclease, endonuclease III K must recognize the pattern of methylation on both strands of the DNA molecule, if one is "self" and the other "non self", it does not function This, of course, provides an explanation of how the bacterial chromosome can replicate in a cell which contains endonuclease III K Because of semi-conservative replication, a newly replicated DNA molecule will be a heteroduplex in the sense that the parental strand will be methylated whereas the newly synthesized strand will not So the cell's restriction enzyme will be inhibited and this could allow time for the modification enzymes to methylate bases of the newly synthesized DNA strand at sites complementary to the methylated bases on the parental strand

Indeed, if endonuclease III K has both modification and restriction functions it is possible that the enzyme attaches to heteroduplex DNA, including the newly replicated DNA of the cells, completes methylation and then either degrades or leaves the DNA depending on the pattern of methylation

This, of course, is speculation, but Meselson and Yuan have already defined the chief characteristics of the restriction activity of endonuclease III K They proved that the enzyme is an endonuclease by showing that it digests circular λ phage DNA molecules As these have no ends, the enzyme must be an endonuclease attacking DNA molecules internally rather than at their ends The digestion product is double-stranded DNA with no single chain breaks In order to break double-stranded DNA, however, the enzyme first causes the scission of one of the two strands and then, a few seconds later, breaks the complementary strand either directly opposite or nearly opposite the first break They cannot say as yet whether one enzyme molecule catalyses the breakage of both strands or whether the two chains are attacked independently by two enzyme molecules However, the enzyme requires ATP, SAM and Mg^{++} ions for both double and single chain scission

New Insecticides

The Association of Applied Biologists held a symposium in the Natural History Museum on Friday, March 15, entitled "New Insecticides and their Application in Public Health and Industry"

The only new insecticides discussed were the synthetic pyrethroids, related to the natural pyrethrins, synthesized recently by Dr M Elliott and his colleagues at Rothamsted with financial assistance from the National Research Development Corporation The biological performance of these compounds was the subject of papers read by Mr A W Farnham (Rothamsted) and Mr J C Wickham (Cooper Technical Bureau) These compounds have been described elsewhere (*Nature*, **213**, 493, 1967, *Proceedings of the Fourth British Insecticide and Fungicide Conference* 1967, 437), but this was the first time that their biological properties have been critically presented

The synthetic pyrethroids, designated NRDC 104, 105, 106 and 107, were compared against several insect species with natural pyrethrins, other common insecticides and the other synthetic pyrethroids allethrin and 'Neopynamin' Their toxicities were found to be superior to all of these, and NRDC 107 (5-benzyl-3-furyl-methyl (+)-*trans* chrysanthemate) came out best of all Much time was spent comparing them with the natural pyrethrins, and particular reference was made to the fact that they are not synergized to the same extent, and do not knock down insects so rapidly It was, however, pointed out that synergism and knock down are not important in all fields of insect control These compounds should be investigated for applications where the properties of extremely high insecticidal activity and short persistence, coupled with complete safety in respect of mammalian toxicity, are desirable

Dr A S Hadaway (Ministry of Overseas Development) referred briefly to the interest which had been shown in these synthetic pyrethroids for vector control, and dealt with the large international operation being staged to find insecticides which would be suitable for the control of the vectors of tropical diseases and would replace DDT, to which may insects are now resistant The process of selection of insecticides from initial laboratory screening tests, through single hut and whole village trials, to large-scale epidemiological studies was outlined Emphasis was placed on the careful mammalian toxicity checks necessary, particularly during the field use of the insecticides

The remaining papers were concerned with the use of well-known insecticides for the control of pests of stored products Mr W Burns Brown (Pest Infestation Laboratory) discussed the growing interest in the use of phosphine for the control of insects in stored grain

This very effective fumigant is now available in a safe formulation that does not present a fire hazard when it is used New and interesting applications for dichlorvos were outlined by Mr A A Green (Pest Infestation Laboratory) and Mr E D Heath (Shellstar) It seems that although dichlorvos is no longer a new insecticide, new and safer ways of using it are being found A 1 per cent formulation in oil eliminates the hazard involved in diluting concentrates and the uncertainty of performance caused by hydrolysis in the water of aqueous formulations. This can be applied with safety by mist blowers and motorized knapsack sprayers

After these papers on new insecticides and on fundamental studies of the safer and more effective use of dichlorvos and phosphine, the audience was brought down to earth by the more mundane and essentially practical problems of infestation control Mr J Gradidge, deputizing for Mr R H Thompson (MAFF Infestation Control Laboratory), told of the trials and tribulations of ridding ships' holds, and government property, of vermin Buildings such as London Airport, which are in constant use and continuously illuminated, present special problems of insect control If the time spent in the final discussion is a guide, the problems of London Airport aroused the most interest of all the topics discussed during the day

Milk Production

from our Medical Biochemistry Correspondent

Babies in developed countries are no longer dependent on their mothers' milk, but for most of the world's population lactation is a matter of life or death for the baby. To the biochemist it is a fascinating synthetic process which produces a specific disaccharide (lactose), a specific protein (casein) and large quantities of fats All these activities are very rapidly "switched on" at birth and cease abruptly when lactation ends

The final step in the production of lactose is the reaction of UDP-galactose with glucose to form lactose and UDP This reaction is catalysed by the enzyme lactose synthetase, and Kuhn has now shown that, in rats, lactose synthetase activity increases very rapidly at birth and is probably the factor limiting lactose production (*Biochem J*, **106**, 743, 1968) There was no measurable lactose synthetase activity until 24 hours before birth, but if bovine α-lactalbumin was added the measured activity increased and there was some slight lactose synthetase activity before birth Studies of the lactose synthetase in milk have shown that it consists of two separable proteins, the *A* and *B* proteins. Tanahashi, Brodbeck and Ebner (*Biochim Biophys Acta,* **154**, 247, 1968) have recently shown that the *B* proteins from several species will react with bovine *A* protein to give an active enzyme, but only enzyme reconstituted with the *B* proteins from ruminants behaved like the normal bovine enzyme immunologically The bovine *B* protein is α-lactalbumin, and the stimulation of lactose synthetase in Kuhn's experiments may indicate that rat mammary gland has a relative deficiency of the *B* protein of lactose synthetase

Greenbaum and Pinder (*Biochem J*, **107**, 63, 1968) have studied the changes in activity of the enzymes synthesizing the nicotinamide nucleotides NAD and NADP during pregnancy and lactation in the rat Activity of the enzyme catalysing the synthesis of nicotinamide mononucleotide from nicotinamide (NMN pyrophosphorylase) and NADP from NAD (NAD kinase) followed the pattern found for many enzymes during pregnancy They were low on the tenth day of pregnancy, but increased fairly rapidly in the latter half of pregnancy and again at birth NMN pyrophosphorylase activity was high throughout lactation, falling sharply in the first two days of involution, but NAD kinase activity continued to increase in the second half of lactation when the NADP concentration increases Activity of the enzyme NMN adenylyltransferase which forms NAD from NMN appeared to be correlated with the occurrence of cell division rather than with coenzyme concentrations It was high at mid-pregnancy, rose slightly in the latter half of pregnancy and again doubled at birth The maximum activity was five days after birth, and the activity then fell steadily The authors think that this may be related to Morton's suggestion that the NAD concentration triggers cell division, while reduced NADP is needed for the synthesis of the fats in milk

Recent work on the synthesis of casein has shown that the lactating mammary gland must have arginase activity. Verbeke and co-workers (*Biochem. J.*, **106**, 719, 1968) showed that ^{14}C-ornithine was incorporated into the proline of casein through deamination of the ornithine to glutamic semi-aldehyde When ^{14}C-arginine was added to a solution perfusing an isolated lactating sheep udder, it was found that as much as 9 per cent of the proline in casein came from arginine The specific activities of plasma arginine, ornithine and proline indicated that the arginine was first converted to ornithine by arginase They did not, however, investigate whether arginase is always present or is rapidly synthesized at birth like lactose synthetase

Magnetic Storms

from our Magnetosphere Correspondent

A model of the magnetosphere known as the "reconnexion model" leads to the simple prediction that the southward component of the interplanetary magnetic field should be correlated with magnetic activity on the Earth Other models assume that the interplanetary magnetic field has no important effect on this activity The reconnexion model is based on the notion that interplanetary field lines can merge with terrestrial field lines On the solar side of the magnetosphere, field lines from the Sun go through the process of reconnexion near the neutral point, in such a way that interplanetary lines of force become connected to the polar regions of the Earth Because the Solar Wind sweeps lines of force from the polar regions into the tail of the magnetosphere, these interplanetary field lines later reconnect again on the night side of the Earth This has the effect of detaching the interplanetary field lines from the polar regions

Over the past few years, several tests of the prediction of the reconnexion model have been made using direct measurements of the interplanetary field from spacecraft The positive results found in all of them provide one of the most important reasons for the current wide acceptance of the reconnexion model Fairfield and Cahill (*J Geophys Res*, **71**, 155, 1966) made a detailed comparison with a small amount of data Wilcox and co-workers and Fairfield have also made statistical comparisons with the activity index

K_p. Recently Rostoker and Fälthammar (*J. Geophys. Res.*, **72**, 5853, 1967) made an extensive detailed comparison and, apart from clinching the close correlation, discovered a relation with the main phases of magnetic storms as well as the more common substorms seen by Fairfield and Cahill.

Rostoker and Fälthammar used hourly means of the interplanetary field and, because the duration of a typical substorm is about half an hour, the true correlation may be even stronger than the one they found. Main phases last several hours, and hourly means should be adequate for them, but it should be kept in mind that the interplanetary field can change completely several times in an hour. Rostoker and Fälthammar find that, for about 90 per cent of their cases, substorms are associated with a single hourly mean southward component of the interplanetary field of more than about $3\gamma (1\gamma = 10^{-5}$ G), while main phases are associated with two or more consecutive positive values, which need not be quite so strong. They also find that, when the recovery from the main phase is simple, it always occurs when the southward component decreases.

The study of magnetograms both from the ground and from space immediately shows a degree of complication comparable to meteorology, but, just as in meteorology there are some simple associations between kinds of weather and wind directions, there is clearly an association between magnetic storms and the southward interplanetary field.

Cell Wall Amino Alcohols

from our Microbiology Correspondent

LAST year, Tomasz (*Science*, **157**, 694, 1967) reported the occurrence of choline in the cell walls of pneumococci. The precise relationship of choline to the other wall constituents is unresolved, but it appears to be linked co-valently to teichoic acid or polysaccharide entities. The nutritional requirement of pneumococci for choline has been known for many years, but its discovery in cell walls, instead of in phospholipid, has allowed a number of physiological phenomena to be analysed. Tomasz and Mosser have discussed previously the requirement of choline metabolism for genetic "competence", the process whereby exogenous DNA is absorbed and the bacteria are transformed (*Proc. US Nat. Acad. Sci.*, **55**, 58, 1966). In the latest phase of this investigation, Tomasz (*Proc. US Nat. Acad. Sci.*, **59**, 86, 1968) has examined the effects of choline analogues on various cellular properties. The fate of both the mono- and di-methylamino derivatives and the parent member of the series, ethanolamine, has been followed and it seems that pneumococci are unable to methylate ethanolamine or the derivatives to choline. Although ethanolamine (EA), mono-methylamino ethanolamine (MEA) and di-methylamino ethanolamine (DEA) are incorporated into wall material, the physiological properties of the altered walls show a trend towards the normality of the choline-containing cells in the order DEA > MEA > EA.

An obvious effect of ethanolamine on pneumococci was the prevention of cell separation. Long chains of several hundred cells were produced, whereas in choline-supplemented media single and two-celled arrangements were predominant. Furthermore, stationary phase populations of EA-containing cells did not autolyse but remained stable for several weeks, "normal" cells autolysed within 20 h of the onset of stationary conditions. The autolytic enzyme system from choline-grown pneumococci was without action on the EA cells. Pneumococci are sensitive to low concentrations of deoxycholate and lysis follows this treatment; again, the EA cells proved to be resistant, although higher levels of the detergent adversely affected their viability. Perhaps the most interesting data relate to the incapacity of EA cultures to undergo genetic transformation—in other words, loss of competence. All of these abnormal properties could be removed by addition of choline, provided that the culture was still growing: deoxycholate sensitivity reappeared after 10 min, competence after 2 h and cell separation after about 6 h.

All aspects of choline and analogue incorporation into pneumococcal cells walls appear to be identical, which suggests that the amino alcohols are incorporated into a similar wall complex at a finite number of sites. The mechanism of the ethanolamine effects has not been elucidated. It may involve an all-inclusive primary effect inhibiting the action of the autolytic system, or a general disorientation of the wall components which disturbs several independent surface properties. The blocking of competency in cells containing EA is significant as the first unequivocal correlation between a primary wall structure and genetic transformation. Addition of the competence activator protein did not induce competence in the EA cells, neither did such cells produce the activator. Thus these EA-incompetent pneumococci are functionally analogous to the heritable incompetent mutants described previously by Tomasz.

Muscle Building

from our Molecular Biology Correspondent

UNTIL quite recently, the only tractable cell-free protein synthesizing system of mammalian origin was the reticulocyte. This had the great advantage of producing essentially one protein as the end product, which could be isolated and identified. Since then a few other systems have been made to function with reasonable efficiency, the limiting factor in general being the concentration of nucleases present in the tissue. One of the most satisfactory, by virtue of low nuclease activity, and because the ribosomes are not attached to a membrane, is chick embryo muscle. This was shown by Rich and his associates to produce myosin with high efficiency.

The same workers (Heywood and Rich, *Proc. Nat. Acad. Sci. US*, **59**, 590, 1968) now describe a spectacular experiment in which synthesis of the three most prevalent muscle proteins could be separately observed *in vitro*. This result stems from the observation that the extracted polysomes give rise to several populations in a sucrose density-gradient sedimentation. Four fractions were isolated, the heaviest consisting of polysomes made up of 50–60 ribosomes, the second of 15–25 ribosomes, the third of 5–9 ribosomes and the lightest of monomers and dimers. The first three fractions incorporated amino-acids with high efficiency. If it is now assumed that the polysomes are joined by intact messenger RNA, the length of the latter turns out to correspond roughly to the length of *m*RNA molecules specifying polypeptides of molecular weights equivalent to single myosin, actin and tropomyosin chains respectively. The inference was put to the test by examining the products of the cell-free systems stimulated by the four fractions. Non-radioactive muscle proteins were added as carriers, and the resulting material was examined by electrophoresis in polyacrylamide gel in a urea medium. It is to be expected that the results of such an experiment would be partly obscured by the presence of incomplete chains on the one hand, and of other muscle proteins on the other. Nevertheless, the product from the largest polysomes has its major component in the position corresponding to single myosin chains, the products from the other two populations of polysomes show some overlap, but it is clear that the first contains a prominent peak of radioactivity in the actin position, as well as two unidentified peaks, and the second contains two major peaks, one corresponding to tropomyosin and the other to one of the components that accompanies the actin. The final category of polysomes—monomers and dimers—gives no single recognizable product.

To strengthen their conclusions, Heywood and Rich applied the standard procedures for purification of myosin, actin and tropomyosin to the three products, diluted as before with carrier protein. In all cases the specific radioactivity followed the degree of purification. This strongly suggested that the products had been correctly identified. The final proof, as the authors recognize, will depend on chemical characterization—for example, fingerprinting—but the case is already strong.

A further interesting aspect is the change in relative concentrations of the different polysome populations which accompanies the development of the embryo: at ten days there is a preponderance of the actin polysomes with little myosin, at fourteen days a detectable increase in the myosin polysomes occurs and by eighteen days there is a large rise in the concentration of the tropomyosin group. This (as one by now expects) is consistent with morphological observations on the developing myofilaments: the thin actin filaments predominate throughout the early development, and only later do the Z-bands, in which the tropomyosin is thought to reside, become apparent.

Evidently then the protein sub-units are each synthesized under the control of a monocistronic messenger. This work must surely be seen as a most encouraging portent in the area of differentiation.

No Data on Sonic Booms

THE probability of serious material damage being caused by sonic booms of aircraft is very small so long as the aircraft are operated in a safe and normal manner. But with the current wholly inadequate understanding of the response of building materials to sonic boom pressure loadings it is just not possible to assess accurately what kinds and amounts of physical damage will occur. That is the conclusion of the report by the subcommittee on physical effects of the US National Academy of Sciences Committee on supersonic transport-sonic boom. The report was published on March 5.

The subcommittee set out to answer two questions: first, what kinds and amounts of physical damage might be anticipated from sonic booms which are "acceptable" to people and, second, what information is necessary to assess equitable compensation for any such damage. It found that the first question cannot be answered beyond saying that the damage is likely to be very small, because alarmingly little is known about the response of building materials to sonic booms or, for that matter, environmental parameters such as thunder claps, wind pressures and temperature changes. As a result the subcommittee has been principally concerned with how to collect more data. By far its most urgent recommendation is that instead of resuming overflight test programmes with supersonic aircraft, which cannot yield reliable and reproducible data because of uncontrollable atmospheric variations, at least two sonic boom simulators should be built immediately for controlled and reproducible testing of the effects of sonic boom forces. The simulators, one small and one large, should be capable of simulating the characteristic signature, the positive and negative pressures, the pulse duration and the rate of pressure rise and decay of sonic booms, ideally over a temperature range of − 65° F to + 150° F. The subcommittee recommends that about $300,000 should be spent over 2 years on this work exclusive of the cost of building the simulators. It is clear that until this is done there is no hope of establishing the cumulative effect of small pressure overloadings and the general failure criteria of potentially critical building materials.

Glass has already proved to be the most easily damaged building material. Furthermore, the manufacturers' data are apparently both inadequate and misleading for the analysis of sonic boom damage. The subcommittee therefore recommends an extensive investigation of the strength and responses of glass in the simulators. How the thickness of glass affects the probability of damage, whether or not the net boom overloading is the critical parameter, what effect various forms of mounting have and how the age of glass affects its response to sonic booms are a few of the questions that need to be answered. Apart from the straightforward tests, the report urges more theoretical research into glass failure and into the way the size, spacing and height of buildings influence constructive interference and reflexion of sonic booms.

It is not even clear how much glass is used in buildings. The subcommittee has called for a census of window panes, particularly in Albuquerque, Chicago, Dallas–Fort Worth and Denver—metropolitan areas which were subjected to sonic booms during the testing of the USAF aeroplane, the SR-71—and also Oklahoma City, where other sonic boom studies have been made. Censuses of other selected cities and towns would prove how climatic variation and city size affect glass usage. From the results of censuses it should be possible to plot the glass pane population of the United States and hence the best route for overflights.

Needless to say, not enough is known about the propagation of sonic booms, and the subcommittee urges, as a matter of high priority, the acceleration and expansion of the efforts of the Environmental Science Services Administration. ESSA has been working on the effects of atmospheric conditions on the sonic boom signatures of various aircraft recorded at ground level. This work should be expanded to include analysis of atmospheric and sonic boom data recorded at sites, especially urban centres, with different weather and topographical characteristics.

The subcommittee also calls for increased study of the effects of wind gusts and thunder claps, which in many ways resemble sonic booms, on buildings and building materials Such data ought to be useful in making realistic appraisals of the natural lifetime of structures likely to be damaged by sonic booms Under certain conditions booms might also cause failure of earth structures, such as coal tips and cuttings and foundations on unstable sand or clay strata So, as a matter of lower priority, the report suggests that small exploratory studies, both theoretical and in simulators, of earth structures should be made Apart from providing new information, these would provide some indication of the degree of potential danger

Anticipating the legal wrangles that are sure to follow regular overflights by supersonic aircraft, the subcommittee is concerned that not enough attention is being given to the problems of assessing progressive and incipient damage It points out that before any equitable law for adjudicating damage claims can be written, criteria are needed to assess claims for progressive damage that arises not from a single sonic boom but from the cumulative effect of many, and also for damage from incipient failures which would have occurred anyway with the next gust of wind The lawyers and civil engineers will have to put their heads together over this Furthermore, it may be necessary to set up a nation-wide sonic boom monitoring system to verify claims, to determine which aircraft was responsible and why it should have caused an excessive boom The subcommittee therefore recommends that a series of study contracts, worth $50,000 each, should be offered to competent companies so they can explore feasible monitoring systems and estimate their cost

The subcommittee is to be congratulated on its clear thinking and for making the ignorance of the effect of sonic booms so abundantly clear The US Government recently delayed the start of the Boeing supersonic airliner project by a year, and with luck many of the questions the report raises will be answered before that aeroplane comes into service In contrast, as far as can be gathered, Britain, which is pressing ahead with the Anglo-French Concorde, has so far made no systematic tests of the effects of sonic booms or any concise proposals of how such tests should be made. The Ministry of Technology coyly says some tests on the physical effects are in progress at the National Physical Laboratory and elsewhere, and the results of last year's test of public reaction to eleven sonic booms have yet to be announced

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Drifting and Rifting of Africa

by

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The development of the East African Rift System is related to the palaeomagnetic evidence for the continental drift of Africa.

RECENT studies of the East African Rift System have emphasized the need for looking at its evolution in terms of the continental drift of Africa There are two aspects for study first, the geometry of the rift system and, second, its evolution in time The East African Rift System extends from Zambia in the south to the Dead Sea in the north It is most well developed in the northern half, that is, the Red Sea and Gulf of Aden which is considered here.

Geometrical Considerations

One of the impressive features of the Red Sea which has been noticed for some time[1-3] is its symmetry This applies to the bathymetry, the gravity anomalies and the magnetic anomalies There is also further evidence that the youngest parts of the Red Sea and Gulf of Aden are along their centres For example, the accurately determined epicentres of earthquakes recorded since the introduction of the World Standard Seismograph Net (1963) for the Gulf of Aden and southern part of the Red Sea concentrate along their centres It seems that the centres of the Red Sea and Gulf of Aden are still active with crustal expansion still taking place Further, Vine[4] has re-interpreted the magnetic anomalies over the Red Sea in terms of the spreading sea floor hypothesis and this also requires symmetry with the newest crust in the centre

In addition to symmetry, it has been noticed[2] that the Red Sea and its various features widen towards the south and the Gulf of Aden widens towards the east This, with other evidence[2], has led to the suggestion that Arabia has moved northwards and rotated anticlockwise It is noted that the symmetry observations require that Africa has rotated clockwise

The structural evidence based largely on geophysical data is summarized in Figs 1 and 2 In Fig 1, the newest crust coincides with the largest magnetic anomalies In the Gulf of Aden, Laughton[5] suggests that widening took place in a north-easterly direction as indicated by the transform faults The transform fault movement has been confirmed by first motion studies of recent earthquakes[6] In the Red Sea, no transform faults have been found This does not mean that some might not exist A study of the topographic and geological maps suggests the possibility of a transform fault at about 19° N (Fig 1) Furthermore, it is clear from the dotted lines connecting both sides of the primary crack that there is a dominant N E -S W direction of movement This direction persists through the Red Sea to the Aqaba–Dead Sea rift where there is a possible transform motion of 90 km (ref 7)

Fig 2 shows a section across the Red Sea (15 5° to 16 5° N) incorporating the Vine interpretation of the axial magnetic anomalies with normally and reversely magnetized rocks becoming progressively older from the centre At this latitude, the anomalies extend over a reversal timescale just greater than three million years giving an average spreading rate of about 1 cm/yr Towards the north, the anomalies become progressively simpler until an anomaly associated with a single normally magnetized intrusion is found. The crack therefore developed in the south and spread northwards associated with the greater tension in the south and rotational movements

The Red Sea and Gulf of Aden are not exactly similar For the Red Sea, seismic refraction work[3,8] shows the presence of continental material on either side of the large, axial magnetic anomalies, whereas in the Gulf of Aden all seismic refraction profiles give oceanic structure[5,8] This includes a profile close to the Somali shore It is therefore possible that the amount of crustal separation in the Gulf of Aden may exceed the amount of separa-

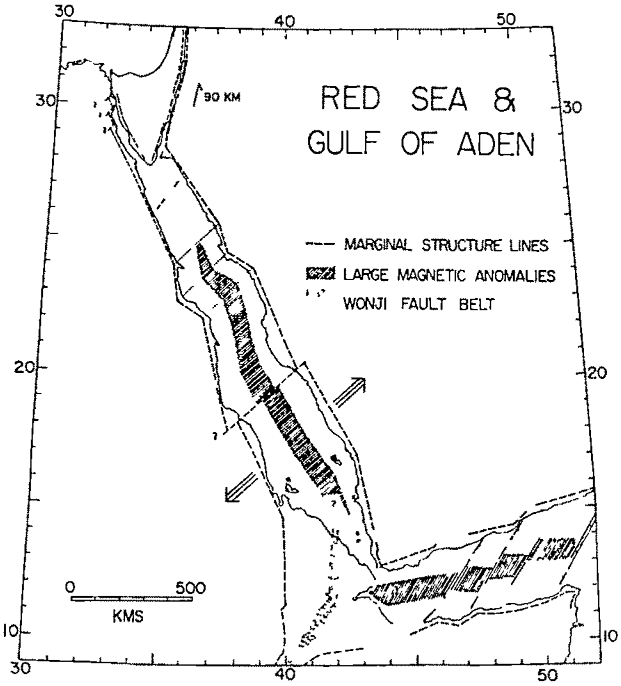

Fig 1 Map of the Red Sea and Gulf of Aden showing the possible movement of Arabia and Africa as inferred from transform faults and the interpretation of magnetic anomalies Mohr's Wonji fault belt in Ethiopia is also shown

tion in the Red Sea by a factor of three Because Arabia seems to have moved as one unit, this implies the horn of Africa must have moved clockwise further than the rest of Africa Further work in the junction area of the Red Sea and Gulf of Aden should help to solve this problem

Geological History of Rifting

This discussion suggests that the present activity has been proceeding for at least 5 million years Presumably the tensional stresses must have been in progress for much longer than this to cause sufficient crustal thinning to result in fracture and injection of igneous material

Geological evidence suggests that rifting has taken place in a series of phases For example, Baker[9] from his work in Kenya suggests a late Pleistocene phase which is still in progress (0–1 million years), a late Pliocene–early Pleistocene phase (? 4–2 million years) and a late Miocene phase (? 12–18 million years) Other workers[10] suggest several phases in the Miocene (the time of the Alpine orogeny) and an early phase of rifting at the end of the Cretaceous (about 70 million years). Evidence for rifting before this time seems to be controversial It seems that in most places the rift system has developed in a series of phases during the past 70 million years

The question now arises as to how these movements and phases of rifting are related to the continental drift of Africa For Africa most palaeomagnetic results are of particularly good quality First, many of the data are from igneous rocks and, second, in many cases the rocks are well dated radiometrically The data available covering the past 600 million years have been assembled using the original references where possible The pole positions have been computer checked and some minor discrepancies have been corrected

African Palaeomagnetic Poles

The recomputed palaeomagnetic poles for Africa are given in Table 1 Most of the ages are radiometric ages from the original references Where absolute ages are not available, such as for sedimentary rocks, they have been estimated from the Geological Society of London Phanerozoic time-scale[30] In each case the latitude and longitude of the palaeo-north pole is given together with the polarity of the rocks The method of computing mean poles from site mean poles has been preferred where the data are available Nearly all the data are from rocks which were subjected to a c or thermal demagnetization The data from rocks not so treated (included to fill large gaps in the time-scale) are marked by an asterisk

The best polar wander curve, using poles only from treated samples, is shown in Fig 3 The poles may be considered in three groups (*a*) The poles for rocks less than 50 million years old spread from a position close to the present axial dipole to a position 73° N. 145° E , most of them being to the east of the Greenwich meridian (*b*) The poles for rocks with ages ranging from 110 to 260 million years form a tight cluster with a mean at 64° N , 101° W , and circle of confidence[31] $\alpha_{95} = 4\ 6°$, suggesting that there was no continental drift of Africa and no polar wander during this period (*c*) The few results available for rocks older than 250 million years suggest there were phases of large scale continental drift and/or polar wander from 250 to 550 million years.

Palaeolatitudes of Nairobi

For some purposes, such as relating the drift of Africa to rifting and other aspects of geological history, it is more convenient to express the palaeomagnetic results in

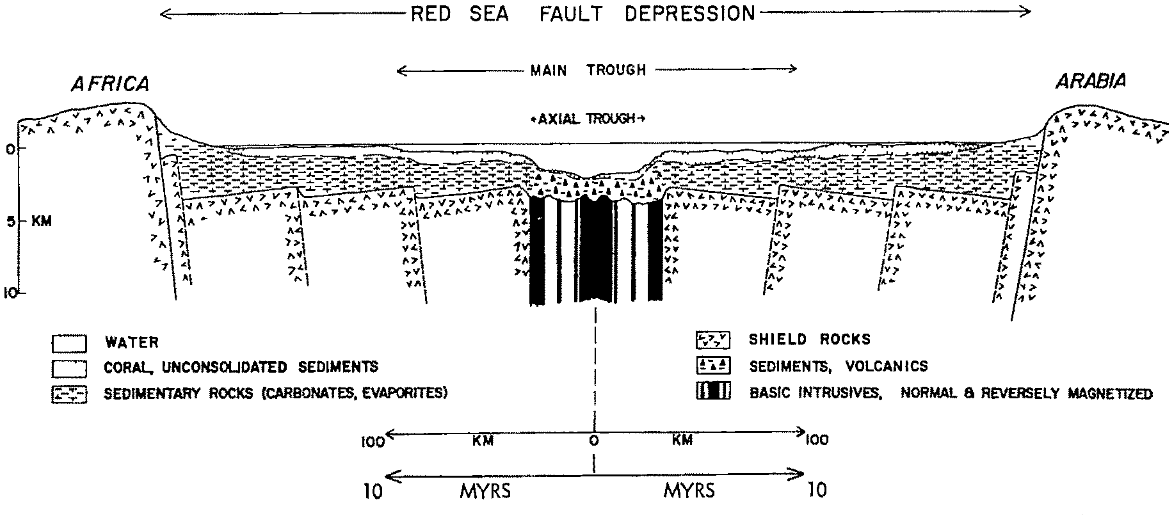

Fig 2 Structural section of the southern part of the Red Sea modified after Drake and Girdler (1964) and incorporating Vine's interpretation of the axial magnetic anomalies The igneous rocks in the axial zone have been intruded progressively over the past 3 to 4 million years

Table 1

Age (million years)	Rock type	Polarity	N Pole	Az	Lat.	Ref
< 2	Kenya trachytes	R	77 1° N , 127 1° E	+13°	2° S	11
1 8	Olduvai basalts	N	84 7° N , 101 0° E	+5°	0°	12
1 9	Olduvai trachyandesite	N	89 0° N , 107 4° W.	0°	2° S	13
4 ± 3	Nairobi basalts	N, R	87 6° N , 115 7° W.	−1°	4° S	14, 15
4 ± 3	Rungwe lavas	N, R	73 1° N , 146 9° E	+16°	7° S	11
4 5 ± 2 5	Kerichwa tuffs	N	78 0° N , 140 8° E	+12°	5° S	11
16 ± 10	Basalts and phonolites	N, R	82 7° N , 105 3° E	+7°	0°	11
22 5 ± 10 5	Turkana lavas	N, R	85 4° N , 170 5° W.	+2°	6° S	15
45 ± 18	Ethiopian lavas	N	85 8° N , 173 3° E	+3°	5° S	16
> 109	Lupata volcanics	N	61 8° N , 101 0° W	−20°	22° S	17
122 ± 12	Mlanje syenite	N	59 5° N , 96 9° W	−22°	22° S	18
172 ± 18	Karoo dolerites	N	65 4° N , 104 9° W	−16°	21° S	19, 20, 11
190 ± 40	Stormberg lavas	N, R	70 5° N , 91 4° W	−17°	13° S	21
190	Nuanetsi igneous	N, R	62 1° N , 92 0° W	−22°	18° S	22
205 ± 10	Red s s Zambia	N	68 1° N , 130 4° W	−7°	22° S	23
209 ± 16	Shawa Ijolite	N	64 2° N , 94 4° W	−20°	18° S	24
260 ± 20	Ecca Red s s	R	38 2° N , 115 1° W	−31°	45° S	25
260 ± 20	Ecca Red s s	R	58° N , 106° W	−21°	26° S	11
302 ± 23	* Dwyka Varve Clay	N, R	14 5° N , 158 5° W	+48°	70° S	11
415 ± 20	* Lower Table Mt Series	R	51 1° S , 168 4° E	+148°	25° S	26
500 ± 17	Hook intrusives	R	14 1° S , 156 8° E	+107°	28° S	27
527 ± 13	* Morocco basic flows	N, R	53° S , 146° W	+178°	36° S	28
555 ± 15	* Ntoya Ring Complex		28° S , 165° E	+124°	32° S	29

Az , palaeodeclination of Nairobi +, measured clockwise from present north, −, measured anticlockwise from present north Lat , palaeolatitude of Nairobi

* May be unreliable because of lack of cleaning or incomplete information

terms of palaeolatitudes and palaeoazimuths Fig 4 shows the palaeolatitudes of Nairobi computed from the poles of Table 1 It is seen that the site of Nairobi has moved fairly rapidly north from 20° S during the past 110 million years During the period 110 to 250 million years ago the palaeolatitude was constant at 20° S Before this time, there was a rapid movement of Africa across the polar regions to about 25° to 30° S some 400 million years ago Such a movement across the polar regions is consistent with the evidence for the existence of glaciations in the Permo-Carboniferous (about 300 million years ago)[32,33]

The orogenic revolutions of Holmes[34] are also shown in Fig 4 along the same timescale. It is seen that the time interval from 110 to 250 million years was a particularly quiet time in the Earth's history The correlation of this part of the two curves is so good that it is tempting to extrapolate the palaeolatitude curve and suggest that Africa remained in the same position from 250 to 70 million years ago The two phases of rapid continental drift of Africa occur at the same times as the Hercynian and Alpine orogenies It is during the latter northward movement of Africa that the development of the East African Rift System was most impressive

Palaeoazimuths Rotation of Africa

The palaeo-declinations or azimuths for Nairobi have also been computed and included in Table 1 The azimuths and rotation of Nairobi are presented in diagrammatic form in Fig 5 In order to gain a representation of the way in which Nairobi has rotated, the azimuths have to be plotted with opposite sign

For the past 50 million years, eight of the nine declinations range from 0° to +16° (that is, east of north) and for clarity smoothed values are plotted in Fig. 5 It is seen that there is some suggestion of a change in direction of rotation of Africa some time between 5 and 15 million years ago, from anticlockwise to clockwise This is consistent with the interpretation of the magnetic anomalies over the Red Sea which suggests that the last phase of the development of the Red Sea was associated with the anticlockwise rotation of Arabia and clockwise rotation of Africa.

The azimuths for the period 110 to 250 million years are shown by the shaded zone, there being no significant differences for this period By analogy with Fig 4 it seems likely that the azimuth of Nairobi (N 19° W) remained constant from 250 to 70 million years ago when Africa began to rotate anticlockwise and move northwards

Because Africa crossed the polar region some time between 300 and 400 million years ago (Fig. 4), the azimuths for this interval are liable to large errors After crossing the pole the azimuths become "reversed" This does not, of course, imply a 180° rotation of the continent Because of this, the azimuths for 500 and 550 million years are shown separately

Does Polar Wandering Exist ?

These observations cast doubt on the existence of polar wandering First, from the African palaeomagnetic results, it seems there was no appreciable polar wander for at least 140 million years and maybe for 180 million years (from 250 to 70 million years ago) Second, the tentative agreement between the movement of Africa as required from palaeomagnetic results and structural arguments (for example, the symmetry of the Red Sea and Gulf of Aden) suggests that continental drift is sufficient to explain the palaeomagnetic results The possibility, however, of polar wandering synchronous with, or following, continental drift due to redistribution of mass on the sphere, is not excluded (the wandering beetle of Gold[35,36]) The palaeomagnetic results are not sensitive enough to resolve this possibility

Implications for Mantle Convection Theories

The large-scale movements of Africa (before 250 million years ago and from 70 million years ago to the present)

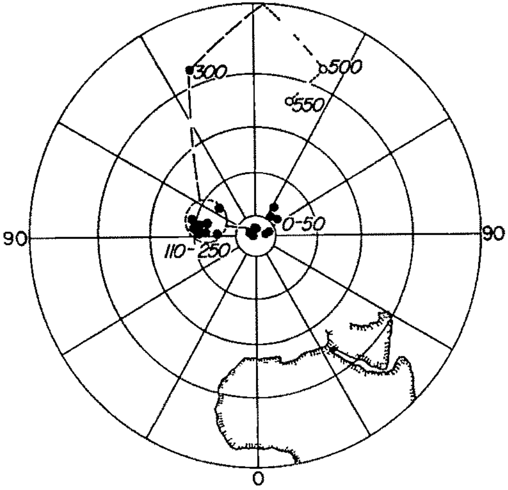

Fig 3 Provisional polar wander curve for Africa The circle groups the poles from rocks 110 to 250 million years old and is not a circle of confidence During this period the pole was relatively stationary

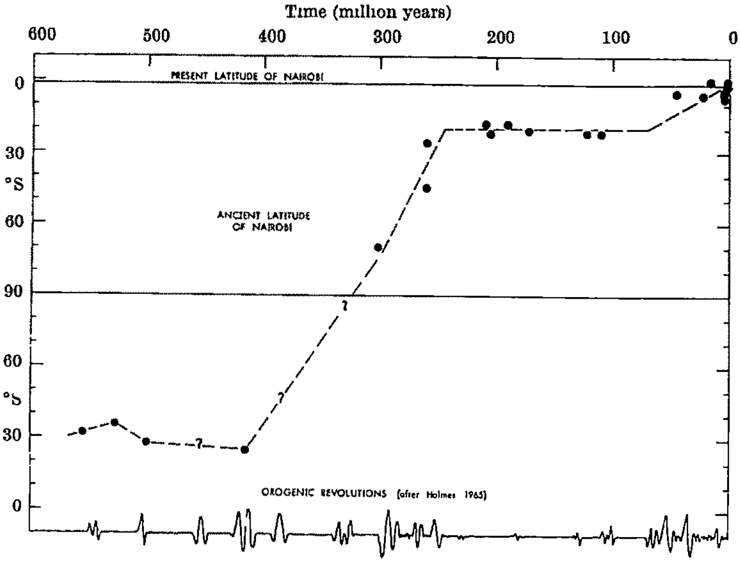

Fig 4 The palaeolatitude of Nairobi Africa crossed the polar regions about 300 million years ago Before 300 million years, there is a shortage of reliable data From about 250 million years to possibly 70 million years ago, Africa was stationary There were at least two phases of drift coinciding with the Hercynian and Alpine orogenies respectively

support the existence of some kind of convective motion in the mantle The fact that Africa remained stationary for at least 140 million years suggests two possibilities either the mantle convective motion stopped some 240 million years ago and restarted some 70 million years ago or Africa was conformably seated in an equilibrium position and the convection pattern changed about 70 million years ago The possible change in direction of rotation of Africa about 10 million years ago suggests the possibility of a further sudden change in the convection pattern at about that time

The available palaeomagnetic results make possible the following conclusions for the history of the African continent over the past 600 million years (1) From 600 to 300 million years there is a shortage of data. The palaeolatitude of Nairobi from 550 to 410 million years was about 30° S and at 500 to 550 million years the palaeoazimuth was 110° to 125° . (2) For a period just before 300 million years to about 250 million years ago, there was rapid continental drift when Africa passed across the polar regions (3) From 210 to 110 million years and possibly from 240 to 70 million years Africa was stable (no drift and no polar wander) The latitude of Nairobi was 20° S and the palaeoazimuth was N 19° W (4) During the past 70 million years, Africa moved north again and rotated anticlockwise, Nairobi changing latitude from 20° S to its present latitude of 1 3° S It was during this time that the East African Rift System developed probably in phases (5) There is some evidence for a change in direction of rotation of Africa from anticlockwise to clockwise during the past 10 million years or so This is consistent with the symmetrical development of the Red Sea and Gulf of Aden (6) The data raise doubts about polar wandering but cannot exclude the possibility of polar wandering during periods of continental drift

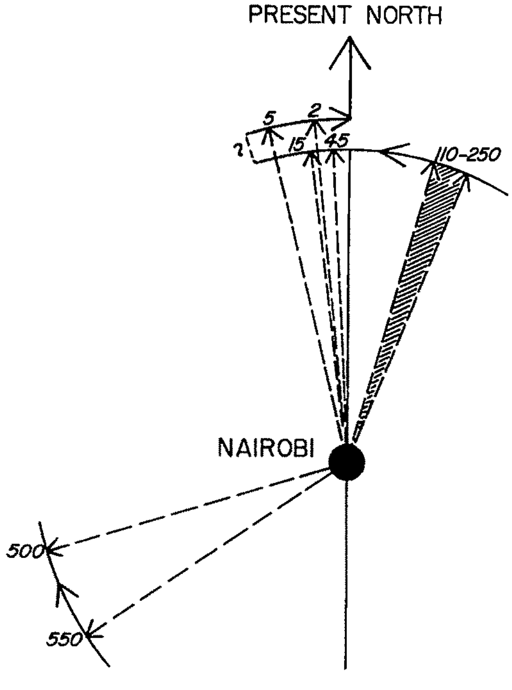

Fig 5 Palaeoazimuths and rotation of Nairobi The palaeoazimuths of Table 1 are plotted with opposite sign so as to give some representation of the way in which Africa has rotated During the past 70 million years Africa rotated anticlockwise and over the past 10 million years or so may have rotated clockwise

Since this work was carried out, two relevant papers have appeared[29,37] Ewing and Ewing[37], in their studies of the distribution of sediment on the ocean floor, have found a paucity of sediments over the central parts of the ocean rift system At a distance of 100 to 400 km from the ridge axes, they find an abrupt or discontinuous increase in sediment thickness On the spreading sea floor hypothesis, this increase is found in all cases to occur at approximately 10 million years This favours the idea that the sea floor restarted spreading about 10 million years ago after a quiet interval The Red Sea and Gulf of Aden are in continuity with the mid-ocean ridge system, so it is reasonable to conclude that the last phase of opening of the Red Sea and Gulf of Aden commenced at about the same time Briden[29] finds that drift in phases may apply to the other southern hemisphere continents and suggests there were at least four episodes of drift of Gondwanaland

Evidence seems to be accumulating in support of long quiet periods of Earth history with relatively sudden phases of activity, continental drift and mountain building. Within these active periods, there were shorter periods of quiescence and activity. The shorter timescale periods of activity produce phases of sea floor spreading and rifting such as we are experiencing today

I thank Mrs Marion Russell for computer checking the palaeomagnetic data and various members of the School of Physics, University of Newcastle, for helpful discussions

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[1] Girdler, R W., *Quart J Geol Soc* , **114**, 79 (1958)
[2] Girdler, R W , *Phys Chem Earth*, **5**, 122 (1963)
[3] Drake, C L , and Girdler, R W , *Geophys J Roy Astro Soc* , **8**, 473 (1964)
[4] Vine, F J , *Science*, **154**, 1405 (1967)
[5] Laughton, A S , *Phil Trans Roy Soc* , A, **259**, 150 (1966)
[6] Sykes, L R , *History of the Earth's Crust* (NASA Symposium)
[7] Freund, R , *Geol Mag* , **102** 189 (1965)
[8] R R S Discovery, *N I O Cruise Report Series, CR* 16 (1967)
[9] Baker, B H , *UMC/UNESCO Seminar*, Nairobi, April 1965, 82
[10] *UMC/UNESCO Seminar*, Nairobi, April 1965
[11] Nairn, A E M., *Overseas Geol and Min Res* , **9**, 302 (1964)
[12] Grommé, C S , and Hay, R L , *Nature*, **200**, 560 (1963)
[13] Grommé, C S , and Hay, R L , *Earth Planet Sci Lett* , **2**, 111 (1967)
[14] Mussett, A E , Reilly, T A , and Raja, P K S , *Proc East African Acad* , **2**, 27 (1964)
[15] Raja, P K S , Reilly, T A , and Mussett, A E , *J Geophys Res* **71** 1217 (1966)
[16] Grasty, R L , thesis, Univ. London (1965), quoted in ref 15.
[17] Gough, D I , and Opdyke, N D , *Geophys J Roy Astro Soc* , **7**, 457 (1963)
[18] Briden, J C , *Geophys J Roy Astro Soc* , **12**, 375 (1967)
[19] McElhinny, M W , and Jones, D L , *Nature*, **206**, 921 (1965)

[20] Graham, K. W. T., and Hales, A. L., *Phil. Mag.*, **6**, 149 (1957).

[21] Van Zijl, J. S. V., Graham, K. W. T., and Hales, A. L., *Geophys. J. Roy. Astro. Soc.*, **7**, 169 (1962).

[22] Gough, D. I., Brock, A., Jones, D. L., and Opdyke, N. D., *J. Geophys. Res.*, **69**, 2499 (1964).

[23] Opdyke, N. D., *J. Geophys. Res.*, **69**, 2495 (1964).

[24] Gough, D. I., and Brock, A., *J. Geophys. Res.*, **69**, 2489 (1964).

[25] Opdyke, N. D., *J. Geophys. Res.*, **69**, 2477 (1964).

[26] Graham, K. W. T., and Hales, A. L., *Geophys. J. Roy. Astro. Soc.*, **5**, 318 (1961).

[27] Brock, A., *Nature*, **216**, 359 (1967).

[28] Helsley, C. E., *Trans. Amer. Geophys. Union*, **46**, 67 (1966).

[29] Quoted in Briden, J. C., *Nature*, **215**, 1334 (1967).

[30] *The Phanerozoic Time-Scale* (Geol. Soc., 1964).

[31] Fisher, Sir Ronald, *Proc. Roy. Soc.*, A, **217**, 295 (1953).

[32] Irving, E., *Palaeomagnetism and Its Application to Geological and Geophysical Problems* (J. Wiley, New York, 1964).

[33] Girdler, R. W., in *Problems in Palaeoclimatology* (edit. by Nairn, A. E. M.) (J. Wiley, New York, 1964).

[34] Holmes, A., *Principles of Physical Geology* (Nelson, London, 1965).

[35] Gold, T., *Nature*, **175**, 526 (1955).

[36] Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth—A Geophysical Discussion* (Cambridge University Press, 1960).

[37] Ewing, J., and Ewing, M., *Science*, **156**, 1590 (1967).

Control of Growth in Two Populations of Elephant Seals

by

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Growth models can explain differences in growth patterns and time of onset of puberty in elephant seal populations at Macquarie Island and Falklands Islands. Elephant seals at Macquarie Island are permanently stunted—possibly because of retarded growth during the suckling period.

It has been said that gigantism in whales is an indication of over-specialization, and it can be developed to the degree in which it now occurs only in an aquatic mammal completely emancipated from the land, because in water the weight of the whale is counter-poised by the weight of water it displaces[1]. Whales washed up on beaches often die after collapse of the rib cage[2], which is not sufficiently strong to support, in air, the weight of tissue surrounding it. "The elephant [seal] ... now seems to be at the critical point in this regard. Some increase in size ... would likely render it unable to leave the water"[1].

The control of mature size in the elephant seal (*Mirounga leonina*, Linn.) seems to be most important for its survival. Although adapted for an aquatic life, it still hauls itself out onto land to breed and moult. Its structure must therefore be adequate to withstand the effects of gravity in spite of the fact that most of the annual cycle is spent in the sea.

Differences in the growth patterns of elephant seals at Macquarie Island and the Falklands Islands have been observed[3,4]. Macquarie Island seals have a slower rate of growth, later onset of puberty and smaller mature size than elephant seals of the Falklands Islands Dependencies. Elephant seals on Heard Island are also known to be smaller than those of the Falklands Islands[5]. Even though Lydekker[6] proposed the existence of several subspecifically distinct groups of elephant seals in the Southern Ocean, there is still no real justification for the recognition of such sub-species[7]. There is no justification therefore in assuming that the different growth and reproductive patterns of elephant seals at the different localities are caused by genetic factors, and the possibility of limitation of food supplies being associated with slower rate of development and delay of puberty of elephant seals at Macquarie Island and Heard Island has been dismissed[3].

I propose to explain, in terms of models proposed by Dickinson[8] and Tanner[9], possible reasons for the differences in growth and age at puberty of elephant seals at the different islands.

A hypothesis of mammalian growth, proposed by Dickinson[8], is represented diagrammatically in Fig. 1. The hypothesis has two limits to the growth curve, a maximum growth path permitted by a "juvenile growth potential", and a minimum growth path conforming to the genetic growth competence. In Fig. 1, the minimum growth curve represents the boundary between growth which reaches a normal end point, and abnormal growth which fails to reach the normal end point. The juvenile growth potential controls the maximum growth path which can occur when nutritional and other environmental conditions are good. When the maximum growth path is followed, variation in size as a result of growth competence is obscured until maturity, by other factors influencing the utilization of nutrients present in excess of the genetically controlled requirements. Dickinson[8] postulated that genetically the juvenile growth potential is unrelated to the genetic growth competence.

The application of this hypothesis to this study is shown in Fig. 2. It is seen that because of the very rapid growth rate of elephant seal pups from birth to weaning, any retardation of growth during this short suckling

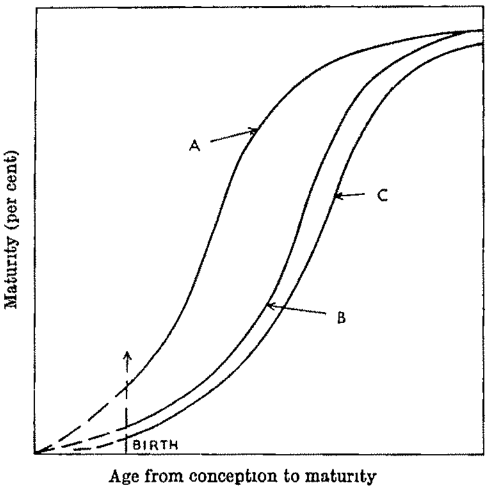

Fig. 1. Diagram illustrating Dickinson's hypothesis of mammalian growth[8]. *A*, Growth path making maximum use of juvenile growth potential; *B*, minimal growth path conforming to genetic growth competence; *C*, permanent stunting on a subminimal environment.

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period, shown to occur in Macquarie Island elephant seals[4], will have a profound effect on the remainder of the growth of the animal, if this retardation causes the growth curve to fall below the minimal growth path conforming to the genetic growth competence. The Falkland Islands elephant seals (F I) are most likely following a growth path which approaches that making maximum use of the juvenile growth potential, whereas the elephant seals of Macquarie Island (M I) probably follow a growth path on which there is permanent stunting as a result of subminimal environment during the suckling period.

Laws[10] demonstrated a close relationship between the attainment of a certain body size, and the onset of puberty in pinnipeds. Assuming that a certain minimum body weight must be attained before puberty is reached in elephant seals, the model explains the later onset of puberty in elephant seals at Macquarie Island (Fig 2).

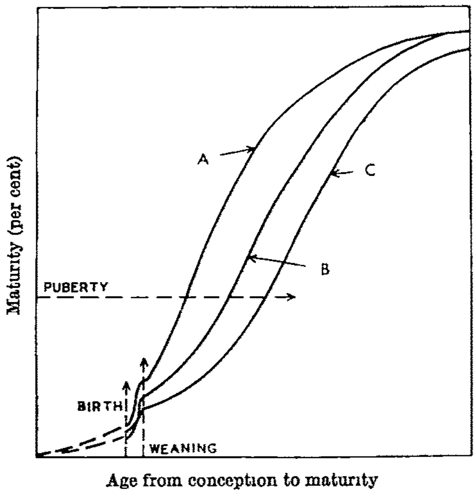

Fig 2 Application of Dickinson's model to the present study. *A*, *B* and *C* as in Fig 1

The growth path followed by Macquarie Island elephant seals resulting in permanent stunting is derived from Dickinson's hypothesis. This hypothesis, however, does not explain the physiological mechanism involved. The mechanism can be explained in terms of the model of growth postulated by Tanner[9]. In this model a time-tally mechanism, located somewhere in the brain—most likely in the hypothalamus—is envisaged. It is suggested that this mechanism is responsible for the recognition of, and interaction with, a growth inhibitor substance produced by the cells of the body. The example was given of experiments[11,12] in which rats poorly fed during the first 21 days after birth never caught up afterwards with their littermates' growth. In terms of Tanner's model, the malnutrition occurring while the brain was still growing relatively rapidly apparently resulted in permanent damage to the time-tally mechanism. Circumstantial evidence is available in support of this model. Schinckel[13] produced an approximation from results in the literature which indicated that the sensitivity to permanent stunting of lambs was most marked during late foetal life and the first few weeks only of postnatal life, and Fox[14] reported that the most dramatic structural changes in the brain of the dog during postnatal growth occurred between 2 and 4 weeks of age.

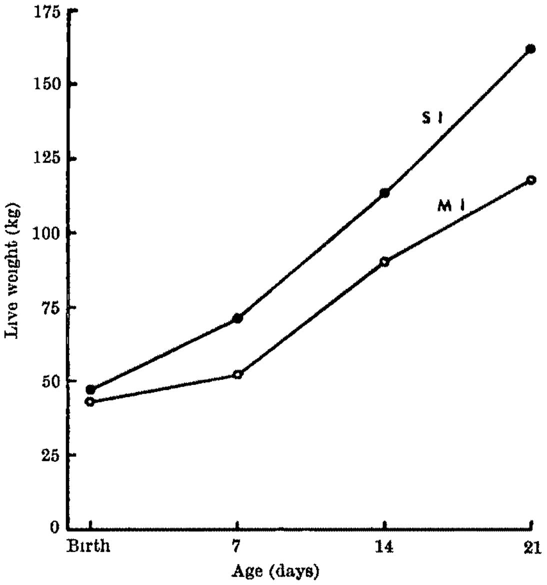

Fig. 3 Comparison of growth of elephant seal pups from birth to 21 days old at Macquarie Island (M I) and Signy Island (S I) (the latter after Laws[15])

The growth curves of elephant seal pups at Macquarie Island and Signy Island (the latter from Laws[15]) are shown in Fig 3. The birth weight of Macquarie Island pups is only slightly lower than that of Signy Island pups (and may not be significantly different[4]), but there is a very much greater difference in weights at weaning, which can be attributed, at least in part, to interference to suckling of Macquarie Island pups by disturbances in the harems[4]. Here then is a natural situation similar to that produced experimentally in rats by Widdowson and McCance[11] and Widdowson and Kennedy[12].

The growth curves of nutritionally independent elephant seals at Macquarie Island and the Falkland Islands Dependencies are shown in Fig 4. The Macquarie Island seal growth curves were made up from my own data; those of the Falkland Islands seals were obtained by fitting body length measurements to a weight–length curve[7].

Figs 5 and 6 show the growth of the brain of elephant seals during the suckling period, and brain growth after weaning. In females 24 per cent, and in males 15 per cent, of postnatal brain growth occurs during this approximately 3 week suckling period. Retardation of pups' growth therefore during this stage of their life must affect the growth of the brain to a considerable extent, even allowing for the fact that the brain has a high

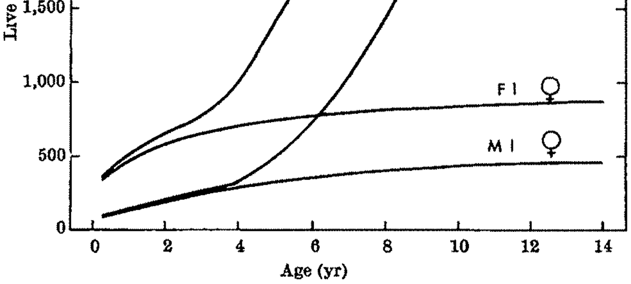

Fig 4 Postweaning increase of live weight of elephant seals at Macquarie Island (M I) and the Falkland Islands (F I). The Falkland Islands curves were drawn by fitting body lengths to a weight–length curve (Laws[7])

priority for growth The reduction in growth of suckling pups at Macquarie Island while their brains are so actively growing could result in permanent damage to the postulated time-tally mechanism

Comparison of body weight figures of mature animals at Macquarie Island and at the Falkland Islands (Table 1) indicates that mature elephant seal males at Macquarie Island are 94 per cent of the size of mature males at the Falkland Islands Mature females at Macquarie Island are only 60 per cent of the size of those on Falkland Islands The size of mature males, however, is difficult to compare, and the comparison is only approximate because sealing operations at the Falklands Islands Dependencies selectively remove the largest bulls, and it is unlikely that the full potential for growth of males is realized at this locality[7] In the light of Howell's statement[1] the possibility arises that, with the relief of social pressures caused by the sealing operations, some of the male animals may reach a size which would prevent them from living and moving comfortably on land should they reach full size

Table 1 COMPARISON OF MATURE WEIGHTS OF ELEPHANT SEALS AT MACQUARIE ISLAND AND THE FALKLAND ISLANDS DEPENDENCIES

	Mean weight (kg)	
	Macquarie Island	Falkland Islands*
Mature males	3,000	3,200
Mature females	400	680

* Weights derived by fitting body lengths to a weight–length curve[8]

It is seen from Fig 4 that Macquarie Island seals, which have been retarded early in life, grow very slowly after weaning, but they never catch up with the Falkland Islands seals This agrees with the model shown in Fig 2 Age at onset of puberty, greater in Macquarie Island than in Falkland Islands seals[3], seems to be related to attainment of a certain body size This also seems to agree with the Fig 2 model The age of onset of puberty of Macquarie Island seals is also more variable This is probably closely related to the greater variation in growth rate of these animals This supports the assumption that onset of puberty in the elephant seal is associated with the attainment of a certain minimum body size, which occurs more rapidly and with less variation in the faster and more uniformly growing Falkland Islands seals In a comparative study of growth in body length of various families of seals[16], it was shown that the slower growing species tended to take longer to reach a size necessary for reproduction (about 87 per cent of final body length) and so mature later This is most likely to occur within, as well as between, species

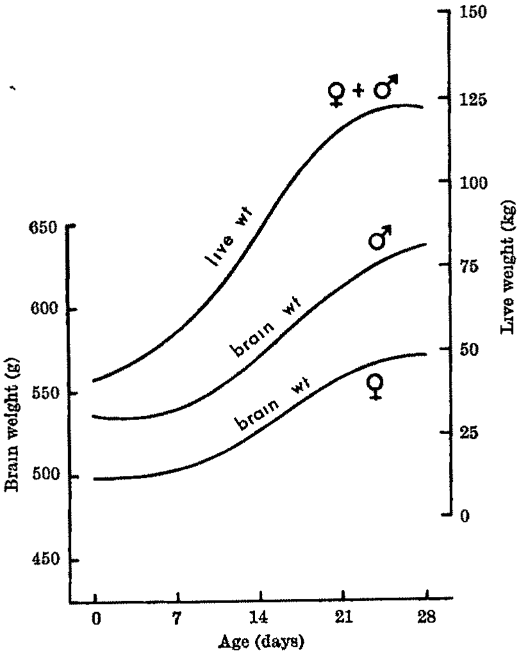

Fig 5. Increase in brain weight and live weight of elephant seal pups during suckling at Macquarie Island.

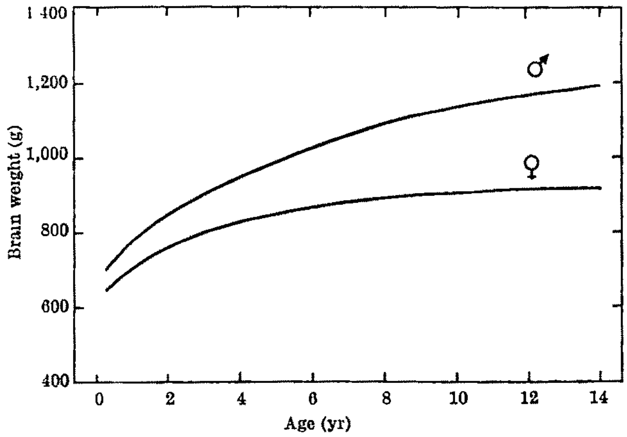

Fig 6 Postweaning increase in brain weight and live weight of elephant seals at Macquarie Island

Brody[17] has shown that puberty is associated with the attainment of a certain size, which corresponds to the point of inflection of the growth curve The latter occurs at over 60 per cent of the mature weight in man, slightly below 60 per cent of the mature weight in chimpanzees and about 30 per cent of the mature weight in laboratory and farm animals From the figures available from the Falkland Islands and Macquarie Island, it appears that puberty in elephant seals occurs at about 30 per cent of the mature weight in males, and at about 55 per cent in females, which corresponds to approximately 4 yr and 2 yr of age respectively in male and female Falkland Islands seals[18], and 5 to 8 yr and 3 to 5 yr respectively in male and female Macquarie Island seals[3]

The growth models offer an explanation of the difference in growth pattern from birth to maturity, different mature size and reproductive pattern of these two populations of elephant seals, and of the mechanism by which these differences are brought about, as a result of a difference in growth during the first three weeks of postnatal life Reduction in size of mature females most likely leads to depressed birth weight of pups as with other mammals[19]. This could further reduce postnatal growth if birth weight should fall below the minimum growth path conforming to the genetic growth competence

In this highly adapted aquatic species, it is apparent that the terrestrial part of the annual cycle which has been retained has an important effect on the total life history, particularly with regard to growth and reproduction

These observations were made while I was a member of the Australian National Antarctic Research Expeditions at Macquarie Island, 1964–66.

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[1] Howell, A B , *Aquatic Mammals* (C. C. Thomas, Baltimore, 1930)
[2] Harrison, R. J , *School Sci Rev* , 38, 191 (1957)
[3] Carrick, R , Csordas, S E , and Ingham, S E , *CSIRO Wildlife Res* , 7, 161 (1962)
[4] Bryden, M M , *Austral J Biol Sci* (in the press)
[5] Gibbney, L F , *ANARE Rep* (B), 1, 1 (1957)
[6] Lydekker, R , *Proc Zool Soc* , 600 (1909)
[7] Laws, R M , *Norsk Hvalfangsttid* , 49, 466, 520 (1960)
[8] Dickinson, A G , *J Agric Sci* , 54, 378 (1960)
[9] Tanner J M , *Nature*, 199, 845 (1963)
[10] Laws, R M , *Nature*, 178, 193 (1956).
[11] Widdowson, E M , and McCance, R A , *Proc Roy Soc* , B, 152, 188 (1960).
[12] Widdowson, E M , and Kennedy, G C , *Proc Roy Soc* , B, 156, 96 (1962).
[13] Schinckel, P G , *Proc First World Conf Anim Prod* , 1, 199 (1963)
[14] Fox, M W , *Growth*, 28, 135 (1964)
[15] Laws, R M , *FIDS Sci Rep* , 8, 1 (1953)
[16] Laws, R M , *Norsk Hvalfangsttid*, 48, 425 (1959)
[17] Brody, S , *Bioenergetics and Growth* (Reinhold, New York, 1945).
[18] Laws, R M , *FIDS Sci Rep* , 13, 1 (1956).
[19] Barcroft, J , *Researches on Prenatal Life* (Blackwell, Oxford, 1946)

Computers and Employment

A recent conference in West Germany discussed the effects of advancing technology on employment. Professor L R. B. Elton, of the Institute for Educational Technology at the University of Surrey, a participant at the conference, has prepared the following report.

FOR some years now, the German Metalworkers' Union (I G Metall) has concerned itself with the problems and opportunities that automation and technological progress are bringing to the worker and to the community This month, Dr G Friedrichs, director of the division on automation of I G Metall, organized the third large international conference in this field, this time on the subject of computers and employment, with special reference to the employment of technicians and other non-manual workers It took place at Oberhausen in the heart of the Ruhr and was attended by more than 1,000 delegates of whom about 130 came from 22 foreign countries, including the United States and the USSR Of the 33 main speakers, three were ministers of the German Federal Government and two were high international officials The rest were divided between academics, employers, workers and trade union officials

It is probable that nothing much was said at the conference that in one way or another was not known before We heard about the computer explosion, particularly in the United States, and the effect it is having on the employment of workers and operatives Surprisingly, perhaps, the effect at the technician level has been much less pronounced This may well be because the advent of a computer in an organization is at first likely to increase the need for technicians, and that at present computers are used mainly for data processing Once they are used widely for the solution of engineering problems and problems of design, technicians too will face the need for re-education and re-deployment On the whole, however, Arnold Weber, professor of industrial relations at Chicago, was reassuring and felt that automation would lead to a redistribution of employment, which could be manageable This presupposed, however, a generally healthy economy, planned programmes to deal with the problems of shifting workers from one job to another, and a general support and expansion of education

The need for more and wider education was a recurring theme of the conference and the one that created most passion Again and again it was stressed that this education had to be general, particularly in mathematics and in the basic sciences, in order to make subsequent re-education possible It was interesting that, compared with educational concerns, economic ones seemed to fade into the background as the conference proceeded It is clear that none of the European countries have found a satisfactory way to combine education and training for those who have left school, and the American solution—to leave students in full-time education for as long as possible through the creation of junior colleges —is not likely to prove economically viable in any other country Whether it is educationally sound was not discussed

The structure of the German system of secondary education came in for some hard knocks At present it is not unlike the tripartite system that was instituted in this country through the 1944 Act, and it was clear that the trade union members of the conference were much in favour of a change towards the comprehensive school The employers' side seemed decidedly less enthusiastic for change On the other hand, when it came to content, the recent proposals of the Dainton Committee would merely bring Britain into line with German reforms of the past few years, which have approached the problem of the swing away from science from the point of under-specialization An unfortunate consequence of the German reform appears to be that science in the upper school has lost ground in the curriculum compared with the study of languages. The point that a general education in mathematics and science should be part of the curriculum for all for as long as possible was again stressed in a televised discussion on further education and re-education, in which representatives of the Netherlands, Czechoslovakia, Hungary, West Germany and Britain took part In this discussion, the demand of the I G. Metall for sabbatical re-education leave for all as of right received qualified, although sympathetic support, but it was accepted that more could not be expected from employers Employers would, of course, provide courses which were in the direct interests of their concerns, but any wider forms of re-education—and it would have to be wide to be effective—would have to come through government action. In this connexion the flexible approach of the British technical college received praise

Another theme that received much attention was the division between white collar and blue collar workers In the Netherlands this division seems to have largely disappeared, and contributions from both the United States and the United Kingdom stressed that, in the logic of the situation, the divisions are decreasing and will eventually disappear everywhere. But this does not mean that there are not difficulties on the way, and much discussion centred on the problems of those employeers who are not members of a trade union, and are faced with rapidly changing employment conditions, including shift and piecework. Difficulties of a different kind, that face a small socialist country trying to liberalize both its economy and its society, were discussed in a very frank report by Professor Levčik of Czechoslovakia

An outstanding and forward looking contribution came from one of Germany's grand old men, Professor Salin, who stressed the need for a management and sociological revolution, without which the technological revolution would be stillborn, and contrasted the American "job" with its built-in flexibility, with the German "Beruf" that concentrated on pension rights This came at the end of the last day, and was a fitting close to a conference which on the very first day had been aroused by Professor André Philip from France with an impassioned plea to examine "le sens même de notre civilization" His peroration included the memorable statement. "mais on ne peut démocratiser les technocrates si l'on n'a pas d'abord technicisé les démocrates"

What was said at this very impressive conference was, however, less important than who had organized it and who took part in it That a trade union had taken the initiative in this field and was co-operating with employers and the government was immensely encouraging, and it was unfortunate that the British trade union movement was not represented The German I G Metall is showing in a very practical way how co-operation on both the national and international planes can be to the benefit of all The excellence of the contributions of its members illustrated the strength of the union's research effort in this field, and showed that it is taking a responsible attitude towards the problems that are arising for employers and employees, and for society, through the progress of technology

DNA Restriction Enzyme from *E. coli*

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An endonuclease which degrades foreign DNA has been isolated. The enzyme requires S-adenosylmethionine, ATP and Mg^{++}

MANY strains of *E. coli* can recognize and degrade DNA from foreign *E. coli* strains. Whether a foreign DNA molecule will be rejected can depend on non-heritable characteristics imparted to it by the cell from which it is obtained. Such characteristics are called host-controlled modifications[1-3]. For example, the ability of λ and several other bacteriophages to multiply on *E. coli* strain K depends on the bacterial host in which the phages were last grown. Phages grown in bacteria possessing the modification allele m_K multiply well, but phages grown in bacteria lacking m_K do not. Instead, their DNA is quickly degraded on entering cells of strain K. The ability of strain K to reject or "restrict" DNA from cells lacking m_K is itself under genetic control, the responsible allele being designated r_K (refs. 4 and 5).

More generally, cells with the restriction allele r_i can degrade DNA from cells lacking the corresponding modification allele m_i. Several different modification and restriction alleles are known[6,7]. As well as certain phage DNAs, bacterial DNA transferred between cells by conjugation or transduction is subject to host-controlled modification and restriction, suggesting that these phenomena play a part in regulating the flow of genetic information between bacteria.

There is evidence that the modification character of a DNA molecule is determined by its pattern of methylation[8,9]. The simplest hypothesis for the biochemical basis of restriction is that each restriction allele directs the formation of a nuclease specific for DNA lacking the corresponding modification character. We have detected, isolated and characterized such an enzyme; it is an endonuclease present in strain K that is specifically active against λ DNA from strains lacking m_K.

Restriction by bacterial extracts. Restriction by extracts of r_K bacteria was discovered as an ATP-dependent nucleolytic activity specific for DNA from λ grown on a m_K^- host. Fig. 1 shows the sedimentation profiles of ^3H λ K DNA and ^{32}P λ C DNA incubated without extract (*a*), with extract of the r_K strain *E. coli* 1100 (*b*), and with extract of its r_K^- derivative, strain 1100.293 (*c*). The sedimentation of λ K DNA is seen not to be affected by incubation with either extract. In contrast, the sedimentation rate of λ C DNA is specifically decreased by incubation with the r_K extract. We conclude that the activity is specific for unmodified DNA and occurs only in the restricting strain.

Purification of the enzyme. Purification of the restriction activity was at first frustrated by its apparent loss on dialysis of bacterial extracts. The difficulty was overcome when we found that both ATP and S-adenosylmethionine (SAM) are needed for restriction to occur. A role for SAM was suggested by the report that methionine, a biosynthetic precursor of SAM, is necessary for optimal restriction *in vivo* by methionineless bacteria (unpublished observations of W. B. Wood, cited in ref. 8).

Following the establishment of suitable assay conditions, the activity was purified as follows. *E. coli* 1100 was grown to 6×10^8 cells/ml. at 37° C in tryptone broth with 1 μg/ml. of thiamine hydrochloride. The cells were sedimented and stored at −20° C. 120 g of cells was suspended in 0.01 M *tris* *p*H 7.4, 2×10^{-4} M $MgCl_2$, 10^{-4} M EDTA, 2×10^{-3} M mercaptoethanol. All subsequent operations were conducted at approximately 4° C. The cells were extracted by blending with glass beads and the extract was centrifuged at low speed and then at 35,000 r.p.m. for two hours in the International *A*-170 rotor. The sediment possessed very little restriction activity and was discarded.

The high speed supernatant (300 ml.) was fractionally precipitated by adding dry $(NH_4)_2SO_4$. The material precipitating between 35 and 55 per cent saturation was dissolved in 100 ml. of 0.02 M potassium phosphate, *p*H 7.0, 10^{-4} M EDTA, 2×10^{-3} M mercaptoethanol (PEM), dialysed against the same buffer, and applied to a column of Whatman *DE* 52 DEAE cellulose 6 cm in diameter and 11 cm long that had been equilibrated with buffer. The column was washed with 600 ml. of buffer and eluted with a 1,000 ml. linear gradient of PEM running from 0.06 to 0.30 M potassium phosphate and collected in 50 ml. fractions. The restriction activity was found in two adjacent fractions with a mean phosphate concentration of 0.15 M.

The active DEAE fractions were combined, dialysed against PEM containing 0.02 M potassium phosphate, and applied to a 1 × 15 cm column of Whatman *P*11 phosphocellulose equilibrated with the same buffer. The column was washed with 40 ml. of buffer and eluted in 9 ml. fractions with a 300 ml. linear gradient running from 0.05 to 0.3 M phosphate. The restriction activity emerged in three adjacent fractions with a mean phosphate concentration of 0.16 M.

The pooled active phosphocellulose fractions were dialysed against 0.01 M potassium phosphate, *p*H 7.5, 10^{-4} M EDTA, and 5×10^{-4} M dithiothreitol, concentrated to 7.5 ml. by dialysis against dry 'Sephadex *G*-200', and dialysed again. The concentrate was applied in 2.5 ml. portions to 30 ml., 10–25 per cent glycerol gradients made up in the dithiothreitol buffer. After centrifugation at 25,000 r.p.m. for 20 h at 2° C in an International *SB*101 rotor, the activity was found in three adjacent 1 ml. fractions at a position corresponding to a sedimentation coefficient of approximately 12*S*. These were stored at −10° C and have been kept for nearly a year without noticeable inactivation. Table 1 summarizes the purification of the restriction enzyme.

Assays with a limiting amount of pure enzyme were conducted with and without the addition of extract of

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* Fellow of the Damon Runyon Memorial Foundation.

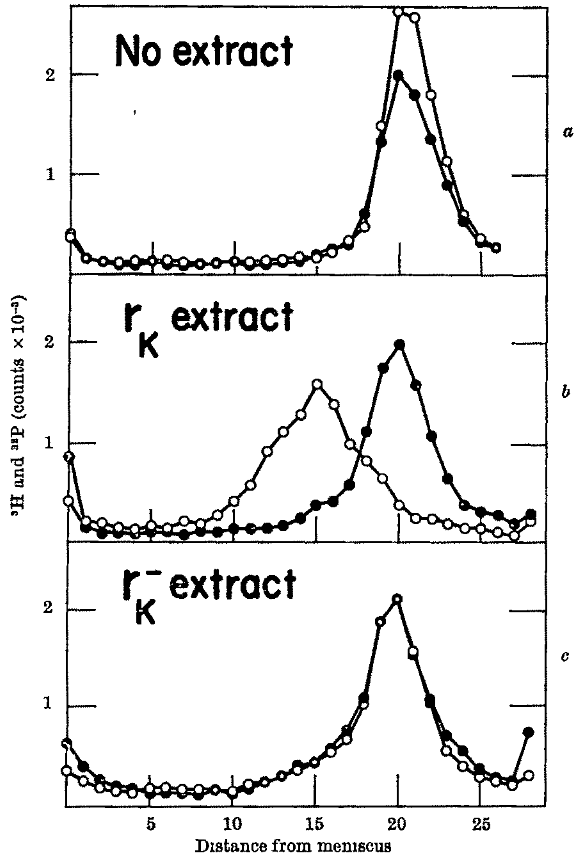

Fig 1 Sedimentation profiles of a mixture of ^{3}H λ K DNA and ^{32}P λ C DNA incubated for 10 min (*a*) without extract, (*b*) with extract of *E coli* strain 1100, or (*c*) with extract of strain 1100 293 Incubation mixtures (*b*) and (*c*) contained 1 mg/ml bacterial protein A 0 25 ml sample of each mixture was layered on a 3 5 ml 6–20 per cent sucrose gradient containing 0 01 M *tris* *p*H 8, 0 001 M EDTA, 0 04 per cent sodium dodecyl sulphate and centrifuged for 1 8 h at 55,000 r p m in an International *SB*405 rotor at 20° C Fractions were collected and counted by standard techniques ——●——, ^{3}H, ——○——, ^{32}P

the r_K^- strain 1100 293 The extract had no effect on the extent of the reaction, showing that the activity of r_K extract can be directly compared with that of the purified enzyme in computing the degree of purification achieved The failure of the r_K^- extract to inhibit the reaction also shows that the r_K^- phenotype results from a reduction in the amount or activity of the restriction enzyme rather than from the presence of an inhibitor

Requirements of the reaction Table 2 shows the effects of omitting various components of the reaction mixture. Restriction is measured as the inactivation of infectious λ C DNA Enzyme, ATP, Mg^{++} and SAM are all seen to be essential while gelatine, EDTA and mercaptoethanol are not Fig 2 shows the effect of lowering the concentration of ATP or SAM The requirement for SAM cannot be satisfied by l-methionine, 5′-thiomethyladenosine, S-adenosyl-*dl*-homocysteine or S-adenosylethionine ATP can be replaced by *d*ATP, although at limiting concentrations approximately twice as much must be used for the same degree of inactivation ATP cannot be replaced by GTP, ADP, AMP, adenosine, pyrophosphate or phosphate The optimal *p*H for the restriction reaction lies between 7 5 and 8 0

Table 1 PURIFICATION OF THE ENZYME

	Total protein (mg)	Activity
Low speed supernatant	8,000	~1
High speed supernatant	5,700	
$(NH_4)_2$ SO_4 precipitate	3,400	
DEAE eluate	260	
Phosphocellulose eluate	2	
Glycerol gradient fraction	⩽0·5	⪆5,000

Activity is expressed as the reciprocal of the mg/ml of protein required to break 90 per cent of λ C duplexes during a 10 min incubation under the assay conditions described in the text

Table 2 REQUIREMENTS FOR REACTION

	$\log_{10} \frac{\lambda K}{\lambda C}$
Complete system	2 0
–EDTA	2 0
–Gelatine	2 0
–Mercaptoethanol	2 0
–$MgCl_2$	0 0
–SAM	0 0
–ATP	0 0

The restriction enzyme is an endonuclease The action of the enzyme on ^{32}P λ C DNA liberates little or no non-sedimenting ^{32}P, showing that degradation is endo rather than exonucleolytic The action of the enzyme on λ twisted circles, DNA molecules without ends, confirms this Sedimentation analyses of a mixture of ^{3}H λ K and ^{32}P λ C twisted circles incubated with and without enzyme (Fig 3) prove that the enzyme readily degrades λ C twisted circles The enzyme, however, has no effect on λ K twisted circles, neither double nor single chain scissions are produced

The limit product is duplex DNA A mixture of ^{3}H λ K DNA and ^{32}P λ C DNA was incubated with enzyme, re-isolated by phenol extraction, dialysed against TNE, and again incubated with enzyme. Fig 4 shows that the second incubation caused little or no additional degradation A control incubation proved that intact ^{32}P λ C DNA added to the second incubation mixture was extensively degraded We conclude that the sedimentation profiles seen in the figure represent the limit product of the restriction enzyme under our reaction conditions The maxima of these profiles occur close to the position to which λ quarter molecules would sediment The sedimentation distribution of the limit product in 0 1 M salt was compared with that in 0 01 M salt No difference was observed Single DNA chains sediment about three times faster in the stronger salt solution, whereas duplexes sediment at the same rate in both solvents. We conclude that the limit product consists of duplexes containing little or no single stranded DNA

The limit product is without single-chain breaks. Clearly the enzyme is able to break the λ C duplex into a number of segments Conceivably, there are sites within these segments where the enzyme has broken only one of the two polynucleotide chains Such single-chain breaks would have little effect on the sedimentation behaviour of DNA duplexes They would, however, become apparent from a comparison of the molecular weight distribution of the reaction product before and after chain separation A mixture of ^{32}P λ C DNA and ^{3}H λ K DNA was extensively incubated with enzyme and then sedimented at 20° C on neutral and on alkali D_2O-H_2O gradients containing 0 9 M NaCl The alkali solvent dissociates duplexes into their component single chains Fig 5 shows the molecular weight distributions calculated from the sedimentation profiles in the two solvents Molecular weights

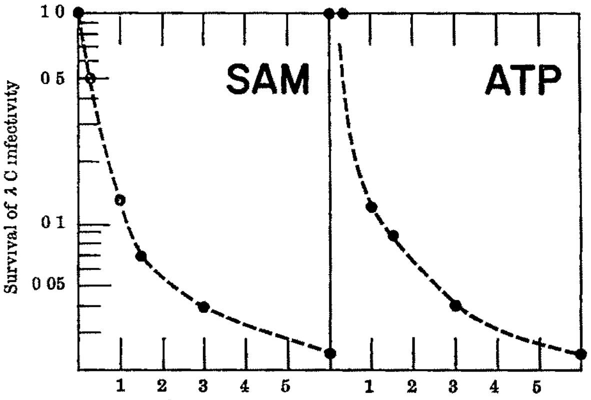

Fig 2 The dependence of restriction activity on SAM and ATP concentration.

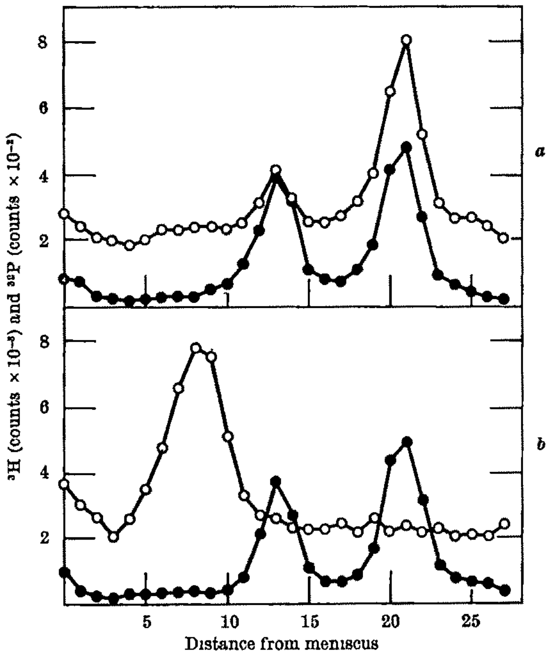

Fig 3 Sedimentation profiles showing the action of the restriction enzyme on twisted circular λ DNA molecules (*a*) A mixture of ^{3}H λ K and ^{32}P λ C DNA consisting mostly of twisted circular molecules but also containing the less rapidly sedimenting non-twisted circular form (*b*) The same mixture after 10 min of incubation with enzyme Samples were centrifuged for 1 h under the conditions given in the legend for Fig 1 ——○——, ^{3}H, ——●——, ^{32}P

are plotted as fractions of the weight of the intact duplex or single chain The near superposability of the two distributions shows that at least most of the limit product consists of duplexes without single chain breaks Only if located near the ends of the duplexes would any substantial number of single chain breaks have gone undetected by this test

Single chain scission precedes duplex cleavage. Prolonged action by the restriction enzyme breaks the DNA duplex into several pieces What is the timing with which individual chains of the duplex are broken ? Single and double chain scissions may be sensitively detected and distinguished by their effects on the twisted circular form of λ DNA (ref 18) Single chain scission converts twisted circles to the more slowly sedimenting non-twisted circular configuration. Double strand scission converts them to even more slowly sedimenting non-circular molecules Fig 6 shows the result of incubating a mixture of ^{32}P λ C twisted circles and ^{3}H λ K non-circular molecules with enzyme for 30, 60 and 120 s, after an initial lag, untwisted circles are produced before non-circular molecules appear Thus single strand scission precedes cleavage of the duplex The same conclusion has been reached by a comparison of neutral and alkali sedimentation analyses performed in parallel on samples taken at various times in the course of the reaction The occurrence of single chain scission early in the reaction, taken together with the paucity of single chain breaks in the limit product, indicate that the enzyme first breaks only one chain and then, a few seconds later, breaks the complementary chain at a point directly or nearly opposite Our results do not reveal whether a given enzyme molecule remains bound to catalyse the breaks on both chains or whether the two chains are attacked independently

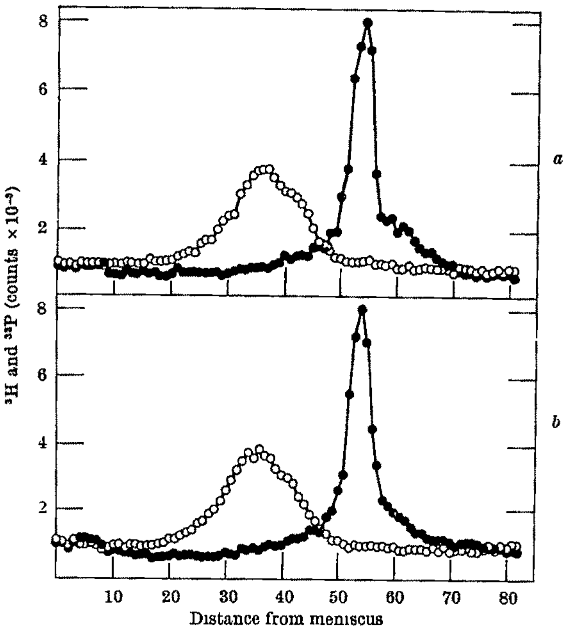

Fig 4 Sedimentation profiles of a mixture of ^{3}H λ K DNA and ^{32}P λ C DNA incubated with enzyme (*a*) once or (*b*) twice See text for details Samples of 0 4 ml were layered on 12 ml 15–28 per cent sucrose gradients containing 0 01 M *tris* *p*H 8 0, 0 001 M EDTA, 0 04 per cent sodium dodecyl sulphate and centrifuged for 17 h at 35,000 r p m in an International *SB*283 rotor at 2° C ——●——, ^{3}H, ——○——, ^{32}P

The infectivity assay employed to determine the requirements of the reaction is probably insensitive to single strand scissions, for λ DNA can be rather extensively nicked by pancreatic DNase without loss of infectivity[19]. We therefore reinvestigated the requirements for ATP, Mg^{++} and SAM by sedimentation analysis with ^{32}P λ C twisted circles as substrate We found that neither double nor single chain scission occurs if any one of the three components is omitted from the reaction mixture

Duplexes with only one modified chain are not attacked Separated polynucleotide chains of ^{3}H λ K DNA and ^{32}P λ C DNA were mixed in appropriate complementary combinations and annealed to obtain the two possible heteroduplexes (one chain modified, the other unmodified) and both homoduplexes Incubation with enzyme followed by sedimentation analyses on neutral and alkali gradients showed that unmodified homoduplexes are cleaved but that the enzyme does not act on modified homoduplexes

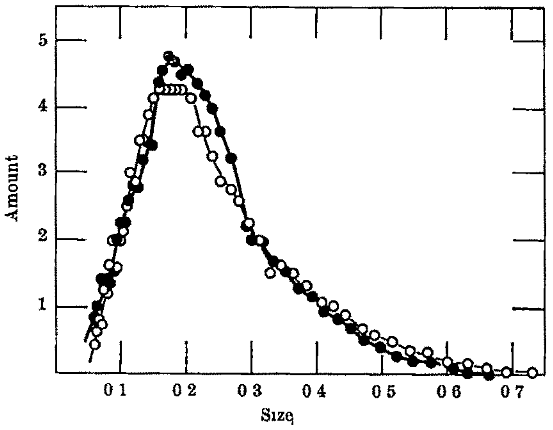

Fig 5 Molecular size distributions of the reaction product before (——○——) and after (——●——) strand separation Each point represents a fraction collected from the corresponding gradient A mixture of ^{3}H λ K DNA and ^{32}P λ C DNA was extensively incubated with enzyme and then analysed on 11 2 ml D_2O–H_2O gradients containing 0 9 M NaCl, 0 001 M EDTA and either 0 04 per cent sarcosyl *NL*97, 0 01 M *tris* *p*H 8 0 or 0 1 M NaOH The gradients were centrifuged in the *SB*283 rotor at 41,000 r p m for 3 3 h at 20° C, which moves the intact ^{3}H reference DNA about 85 per cent of the way down the gradient Sedimentation coefficients were computed for each fraction, taking into account the variation of acceleration, solution density, and viscosity along the gradient Molecular size relative to intact ^{3}H λ K duplexes or single chains taken as unity was obtained from the sedimentation coefficients[17] The ordinate for each point is the ^{32}P counting rate corrected for background and normalized to give the amount of DNA per unit molecular size No correction has been made for the effect of finite band width Parallel centrifugations of the DNA mixture incubated without enzyme showed the ^{32}P DNA to sediment in both neutral and alkali solution as a band with a half-width of three fractions at half its maximum concentration In the neutral gradient, the trailing edge was sharp while in alkali it displayed a tail indicating that approximately 1/4 of the polynucleotide chains were broken In neutral solution 10 per cent of the material sedimented slightly ahead of the leading edge while in alkali the leading edge was sharp

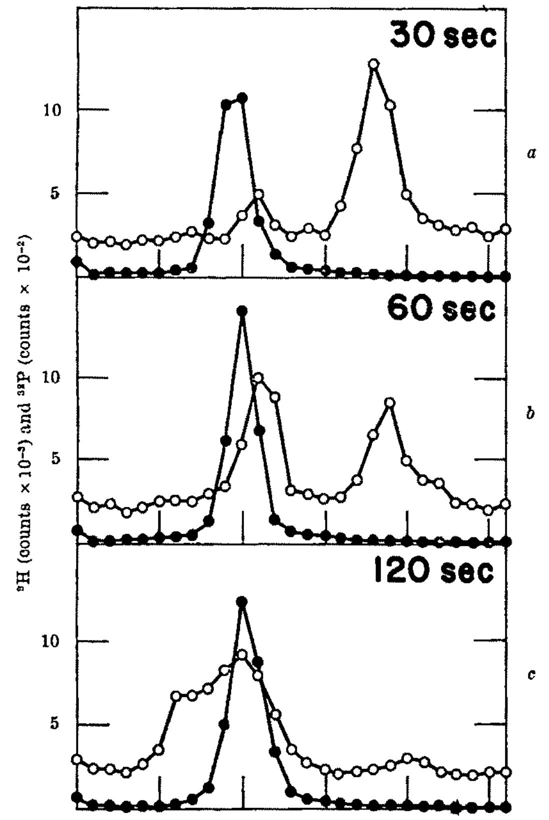

Fig 6 Sedimentation distributions of a mixture of ^{3}H λ K non-circular DNA and ^{32}P λ C twisted circular DNA after incubation with enzyme for (*a*) 30, (*b*) 60 and (*c*) 120 s The distributions of the untreated DNA mixture and of the mixture incubated with enzyme in a reaction mixture lacking ATP and SAM were essentially the same as that seen in (*a*) Samples were centrifuged for 1 1 h under the conditions given in the legend for Fig 1 ——●——, ^{3}H, ——○——, ^{32}P

or on either of the two heteroduplexes (Fig 7) Thus heteroduplexes are resistant even to single chain scissions

A restriction enzyme from P1 lysogenized cells We have partly purified a restriction activity from cells lysogenic for phage P1 which specifically breaks DNA duplexes of phage λ grown on strains not lysogenic for P1 Like the restriction enzyme from strain K, it requires Mg^{++}, ATP and SAM The product of its action also consists mainly or entirely of relatively large fragments of the λ duplex We propose that these enzymes be designated *E coli* endonuclease III K and III P, respectively

Discussion The degree of purification achieved and the diversity of the fractionation procedures employed make it most unlikely that the restriction activity we have isolated corresponds to more than a single molecular species, although it may well be a stable aggregate of two or more sub-units Indeed, the enzyme's high sedimentation coefficient, the complexity of its action and co-factor requirements suggest an aggregate

The ATP and SAM requirements of endonucleases III K and III P are unprecedented and we do not yet know whether either compound is consumed in the reaction or acts only catalytically, possibly as an allosteric effector The requirement for SAM is especially interesting because of the likelihood that modification corresponds to the specific methylation of DNA[8,9], presumably using SAM as a methyl donor It appears that the enzyme interacts directly with ATP, for it is rapidly inactivated when they are incubated together if SAM is absent

Takano, Watanabe and Fukasawa[20] have described a specific inactivation of unmodified infectious λ DNA by extracts of bacteria carrying certain restrictive episomes No ATP was added to their reaction mixtures In our experiments and in an earlier investigation (unpublished results of S Lederberg and M Meselson) using the λ infectivity assay system, neither K nor P restriction activity was detected in bacterial extracts without added ATP, the addition of SAM was unnecessary in our crude extracts It remains to be seen whether the enzymes associated with the restrictive episomes studied by Takano *et al* differ in their co-factor requirements from the K and P enzymes or whether there was adequate ATP (and SAM) naturally present in their extracts

The size distribution of the DNA fragments in the limit digest of the restriction enzyme poses several problems The very limited action of the enzyme *in vitro* contrasts sharply with the nearly complete and very rapid breakdown of unmodified DNA to acid soluble products seen *in vivo*[21,22] Perhaps the production of an end, or a certain type of end, quite generally exposes a DNA duplex to such breakdown within the cell, even though it is not clear that the known nucleases of *E coli* would be sufficient for this purpose[23] Although it seems very likely that the restriction enzyme attacks DNA at fixed sites, no clear indication of discrete species was seen in the sedimentation profiles of the limit product Depending on

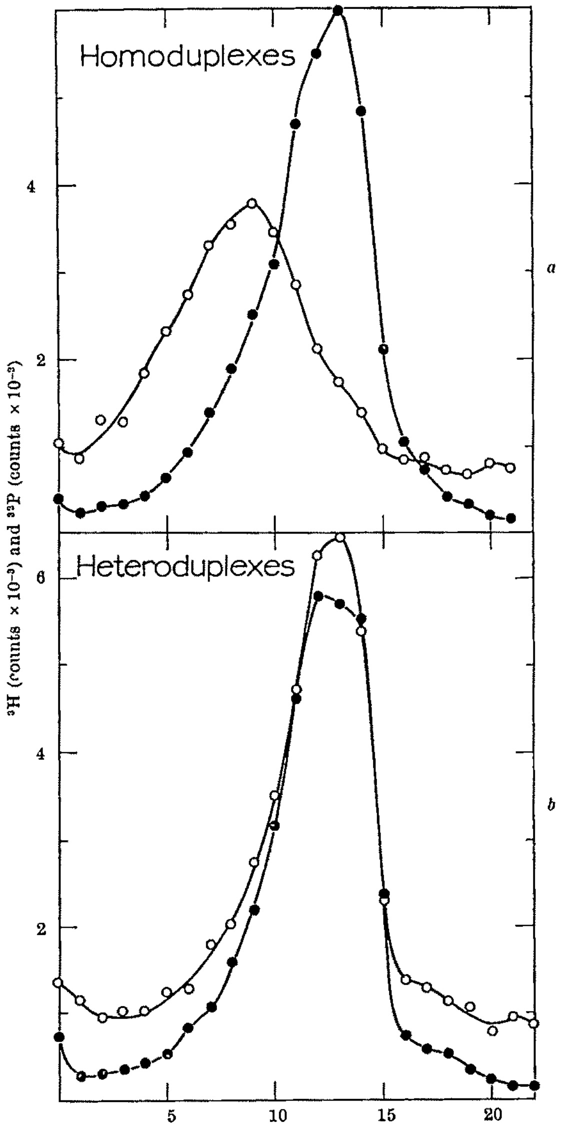

Fig 7 Alkali sedimentation profiles of (*a*) reconstituted ^{3}H λ K and ^{32}P λ C homoduplexes and (*b*) the two possible heteroduplexes after incubation with enzyme Samples of 0 2 ml were layered onto 3 5 ml 6–20 per cent sucrose gradients containing 0 005 M EDTA and 0 3 M NaOH and centrifuged 1 8 h at 55,000 r p m in the *SB*405 rotor at 20° C ——●——, ^{3}H, ——○——, ^{32}P

the distribution of susceptible sites, however, it is possible that different classes of fragments would not have been resolved under our conditions of analysis

The molecular size distribution of the limit product indicates that not all λ chromosomes are broken to the same degree For example, although there are segments of approximately 40 per cent length, they comprise much less than 40 per cent of the digest, as though an appreciable number of chromosomes did not react to give such segments If Fig 5 accurately portrays the molecular size distribution of the limit product, it may be that the initial population of DNA molecules was heterogeneous, some may already have been modified at one or a few sites. Alternatively, the restriction enzyme itself may occasionally render some sites resistant to cleavage Conceivably the restriction enzyme is also the modifying enzyme but with its modifying activity only weakly manifested in our assay system The presence of SAM in the incubation mixture, the close genetic linkage between r^- and m^- mutations, and the fact that a large proportion of r^- mutants are also m^- (refs 4, 5 and 24), are consistent with this interpretation, although other explanations can be given

It is definitely known that one, and somewhat less well established that both, of the two possible λ chromosomes with only one modified polynucleotide chain are able to infect and multiply normally in a restricting host[3]. Our finding that both types of λ heteroduplex are resistant to the restriction enzyme confirms these conclusions This shows that both polynucleotide chains of λ K DNA are modified at every site of potential attack The fact that heteroduplexes are resistant even to single chain scission indicates that the enzyme somehow takes account of the structure of both chains before cutting either The resistance of heteroduplexes may serve to protect newly replicated bacterial DNA from attack by the cells' own restriction enzyme, allowing time for modification of the newly synthesized chain Indeed, if restriction and modification are accomplished by the same enzyme, the choice between the two reactions may normally be governed by whether the substrate is an unmodified homoduplex or a heteroduplex, respectively The extreme destructive potential of the restriction system may have enforced selection for additional mechanisms to ensure that cellular DNA newly synthesized by replication or repair is modified before it can be restricted Various protective control mechanisms can obviously be devised involving the cofactor requirements for restriction and the sensitivity of the restriction enzyme to ATP when SAM is absent

If DNA synthesis in restricting bacteria could be continued beyond one generation after methylation has been blocked, the resulting unmodified homoduplexes should be vulnerable to cleavage and degradation by the cells' own restriction system Having made this prediction, we were struck by the report of Lark[25] that DNA synthesis in methionineless *E coli* 15T- halts one generation after methionine is replaced with ethionine or norleucine and that much of the cellular DNA may then become acid soluble Although the level of SAM may be very low during methionine deprivation, even one cleavage of the bacterial chromosome by restriction enzyme might stop replication and trigger extensive degradation According to this picture, DNA synthesis should continue beyond one generation and without degradation if Lark's experiments were repeated with a strain deficient in restriction

Finally, endonucleases III K and III P may provide a model for other systems that cleave duplexes or cut single chains at specific locations, not only in connexion with restriction phenomena, but possibly also in replication, recombination or transcription

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Materials and Methods *Bacteria and bacteriophages* Strain C600 (ref 10), its derivative CR34 *thy* (ref 11) and the DNA endonuclease I deficient strain 1100 (ref 12) have the restriction and modification properties $r_K m_K$ Strain 1100 293 is an $r_{K_1}^- m_K$ mutant of strain 1100 Strains C600 4 and C600 5 are $r_K^- m_K^-$ Bacteriophage λ is the wild type of Kaiser[14] and λ*c* is a clear plaque forming mutant Phages grown on m_K^- strains are designated λ C, following the custom based on the fact that *E coli* strain C is m_K^- Taking the plating efficiency on strain C600 4 as unity, that on all other sensitive r_K^- strains employed was 1 0 and on r_K^- strains was 1 0 for λ K and 10^{-4} for λ C

DNA preparations Stocks of λ K and λ*c* C were made on strains C600 and C600 4, respectively, by the agar layer method Stocks of ^{3}H-thymidine λ K and ^{32}P λ C were produced by ultraviolet light induction of CR34 (λ) and C600 4 (λ) in radioactive media Phages were purified by two cycles of differential centrifugation followed by equilibrium sedimentation in CsCl solution DNA was extracted with water-saturated phenol, extensively dialysed against 0 01 M *tris*, 0 01 M NaCl 2×10^{-4} M EDTA, *p*H 7 4 (TNE) and stored at 0° C Before use in assays, DNA solutions were kept at 55° C for 5 min in order to disassociate end-to-end aggregates Twisted circular λ DNA was prepared by infection of *E coli* C600 4 (λ) or C600 (λ) with 10 ^{32}P λ C or ^{3}H λ K per cell, respectively After incubation for 30 min at 37° C in tryptone broth, the infected cells were sedimented, washed with 0 03 M *tris* *p*H 8 0, 0 01 M NaCl, and resuspended in the same medium at 5×10^8 cells/ml The suspension was made 0 02 per cent in lysozyme, incubated for 1 min at 37° C, made 0 01 M in EDTA, incubated for an additional minute, and lysed by the addition of 10 mg/ml sodium dodecyl sulphate The lysate was sheared by vigorous passage through a No 26 hypodermic needle, extracted with water-saturated phenol, dialysed against TNE, and sedimented through a neutral sucrose gradient containing 10^{-3} M EDTA After shear treatment, host cell DNA sediments much more slowly than λ twisted circles, which are relatively insensitive to shearing The fraction of the gradient containing twisted circles was dialysed against TNE and stored at 0° C

Polynucleotide chain separation and annealing Separated polynucleotide chains were prepared according to Hradecna and Szybalski[15], using poly UG rather than poly IG Broken chains and poly UG were removed by sedimentation through an alkali D_2O–H_2O gradient Complementary mixtures containing approximately 10 μg/ml of purified single chains were annealed by overnight dialysis at 37° C against 0 001 M EDTA, 0 1 M NaCl, NaOH *p*H 10 5 followed by dialysis against TNE (private communication from W Doerfler and D Hogness) More than 80 per cent of the annealed material sedimented at the same rate as native DNA duplexes in neutral 0 01 M salt Sedimentation analysis in alkali showed that most of the annealed duplexes were free of single chain breaks

Enzyme assays Restriction activity was assayed in a mixture containing, per ml 50 μmoles *tris*, 5 μmoles $MgCl_2$, 0 2 μmoles EDTA, 5 μmoles mercaptoethanol, 100 μg gelatine, 0 2 μmoles ATP, 0 02 μmoles S-adenosylmethionine (SAM), approximately 10^{10} phage units of ^{32}P λ C DNA and ^{3}H λ K DNA, and the enzyme sample to be assayed SAM was purified from the commercial product by elution from 'Biorex 70' with acetic acid The *p*H of the reaction mixture was 7 7 at 20° C After incubation for the desired time at 37° C, 1/10 volume of 0 4 M EDTA *p*H 9 5 was added and the mixture was subjected to sedimentation analysis

In some assays, radioactive DNA was replaced with approximately 10^9 phage units of a mixture of λ*c* C DNA and λ K DNA After incubation, the reaction mixture was diluted 1 100 in TNE and assayed for infectivity Restriction was measured as a decrease in the ratio of clear plaques to turbid plaques, relative to the ratio for the untreated DNA mixture λ K DNA is not inactivated by the purified enzyme The infectivity assay was that of Kaiser and Hogness[16] except for the following strain C600 5 was used as recipient and C600 4 (λi^{434}) as indicator Recipient cells were prepared by 4 5 h growth at 37° C from a 1 3 dilution of an overnight culture Glycerol rather than glucose was used as carbon source The bacteria were sedimented, resuspended at 2×10^9/ml in 0 01 M $MgSO_4$ infected with 5 λi^{434} phages per cell, incubated for 7 min at 37° C, diluted 1 1 with iced 0 01 M *tris* *p*H 8 0, 0 01 M $CaCl_2$, 0 01 M $MgSO_4$, sedimented, and resuspended at 2×10^9/ml in the same solution The cells were kept at 0° C for 2–12 h before use The efficiency of the assay was approximately 0 01 plaques per phage unit of λ DNA Infectivity assays were useful for detecting restriction activity only after the phosphocellulose chromatography step in the purification of the enzyme Less purified enzyme preparations caused severe non-specific inactivation of infectivity

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[1] Luria, S E , *Cold Spring Harbor Symp Quant Biol* , 18, 237 (1953)
[2] Bertani, G , and Weigle, J J , *J Bacteriol* , 65, 113 (1953)
[3] Arber, W , *Ann Rev Microbiol* , 365 (1965)
[4] Colson, C , Glover, S W , Symonds, N , and Stacey, K A , *Genetics*, 52, 1043 (1965)
[5] Wood, W B , *J Mol Biol* , 16, 118 (1966)
[6] Eskridge, R W , Weingeld, H , and Paigen, K , *J Bacteriol* , 93, 835 (1967)
[7] Kerszman, G , Glover, S W , and Avonovitch, J *J Gen Virol* , 1, 333 (1967)
[8] Arber, W , *J Mol Biol* , 11, 247 (1965)
[9] Klein, A , and Sauerbier, W , *Biochem Biophys Res Commun* , 18 440 (1965)
[10] Appleyard, R K , *Genetics*, 39, 440 (1954)
[11] Okada, T , Homma, J , and Sonohara, H , *J Bact* , 84, 602 (1962)
[12] Durwald, H , and Hoffman-Berling, H , *J Mol Biol* (in the press)
[13] Meselson, M , *J Mol Biol* , 9, 734 (1964)
[14] Kaiser, A D , *Virology*, 3, 42 (1957)
[15] Hradecna, Z , and Szybalski, W , *Virology*, 32, 633 (1967)
[16] Kaiser, A D , and Hogness, D S , *J Mol Biol* , 2, 392 (1960)
[17] Studier, F W , *J Mol Biol* , 11, 373 (1965)
[18] Bode, V C , and Kaiser, A D , *J Mol Biol* , 14, 399 (1965)
[19] Heinemann, S , dissert , Harvard Univ (1967)
[20] Takano, T , Watanabe, T , and Fukasawa, T , *Biochem Biophys Res Commun* , 25, 192 (1966)
[21] Arber, W , Hattman, S , and Dussoix, D , *Virology*, 21, 30 (1963)
[22] Lederberg, S , and Meselson, M , *J Mol Biol* , 8, 623 (1964)
[23] Lehman, I R , *Ann Rev Biochem* , 36, 645 (1967)
[24] Boyer, H , *J Bact* , 88, 1652 (1964)
[25] Lark, C , *J Mol Biol* , 31, 401 (1968)

Strengthening of Silicate Glasses by Ion Exchange

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Surface defects are almost always present in glass, reducing its strength. One way of overcoming this is to replace cations in the surface layer with larger cations from a molten salt

A GENERAL characteristic of brittle solids is that surface cracks or other defects can lead to local stress concentrations and a fracture strength which is very much less than the intrinsic strength of the material Glass is a typical example—"ordinary glass" usually has a strength of the order of a few thousand lb /in ^{2} but, if special care is taken to avoid surface defects in manufacture, strengths of more than 300,000 lb /in ^{2} can be obtained even in rods[1], and very much higher strengths are observed with fibres

The presence of surface defects in glass must usually be accepted as inevitable Most of the successful methods for strengthening glass have involved the introduction of compressive stress in the surface layers to overcome the strength-impairing effects of surface cracks or other defects In thermal tempering, for example, compressive stress is introduced by rapid cooling Strength increases of up to 30,000 lb /in ^{2} can be achieved in this way

A most effective method of strengthening glass, known as chemical strengthening, has recently been developed In this method, small cations initially present in the glass are replaced by larger cations from a molten salt[2–6] The exchange process is confined to a thin layer next to the glass surface The strengthening treatment is carried out at a temperature below the annealing range so that the stresses introduced are not removed by relaxation On cooling, a permanently stressed layer with a thickness of from 50μ to a few tenths of a millimetre is obtained Strengths of more than 100,000 lb /in ^{2} can be attained which are not impaired by mild abrasion This method (or a suitable modification) should be applicable to other brittle solids and has indeed been applied recently to glass ceramics , the strength of nepheline glass ceramics was increased from less than 8,000 lb /in ^{2} to more than 200,000 lb /in ^{2} (ref 7)

Although the kinetics of ion-replacement processes in glasses have been investigated in some detail[6,8,9], there seems to be no published work on the nature of the ion-exchange equilibrium between the bulk glass and the molten salt The factors which determine the distribution of the exchanging ions between the glass and the molten salt would be expected to decide the ease with which one ion can be replaced by another and the compressive stress which can be achieved

In considering the ion-exchange equilibrium between a silicate glass and a molten salt, it will be assumed that the basic structure of the glass consists of an irregular three-dimensional network built up from silicon–oxygen tetrahedra in which each silicon ion is surrounded by four oxygen ions The tetrahedra are linked together at the corners by sharing a common oxygen ion In places, the silicon ions are linked to "non-bridging" oxygen ions instead of through —O— linkages to other silicon ions, thus providing sites for cations such as Na^+, K^+, Ca^{++} The alkali metal ions can diffuse through the structure and small ions in the glass (Na^+, Li^+, for instance) can be replaced by larger ions (such as K^+) from the molten salt In the strengthening process this ion replacement leads to an increase in volume This takes place against the elastic forces in the silicate network and the mechanical restraint imposed by the glass substrate which, if relatively thick, can be assumed to be rigid

If we consider the replacement of one univalent cation by another the exchange process can be represented by the equation

$$\bar{A}+B \rightleftarrows \bar{B}+A \tag{1}$$

where A and B represent the two cations in the molten salt and $\bar{A}$ and $\bar{B}$ represent the same cations in the glass

The electrochemical potential $\bar{\eta}_i$ of a species i in the glass will be given by

$$\bar{\eta}_i=\bar{\mu}_i+z_iF\bar{\varphi} \tag{2}$$

where $\bar{\mu}_i$, $\bar{\varphi}$, z_i and F are the chemical potential, the electric potential, the electrochemical valence and the Faraday constant, respectively

Assuming an isothermal system and splitting $\bar{\mu}_i$ into two parts—$\bar{\mu}_i'$ representing the composition-dependent part of the chemical potential and $\bar{\mu}_i''$ the contribution due to the change in mechanical strain energy when one ion is replaced by another—we have

$$\bar{\eta}_i=\bar{\mu}_i'+\bar{\mu}_i''+z_iF\bar{\varphi} \tag{3}$$

For the molten salt

$$\eta_i=\mu_i+z_iF\varphi \tag{4}$$

At equilibrium the electrochemical potentials $\bar{\eta}_i$ and η_i will be the same, so that because A and B are univalent cations ($z_i=1$) we have

$$F(\bar{\varphi}-\varphi)=\mu_A-\bar{\mu}_A'-\bar{\mu}_A'' \tag{5}$$

and

$$F(\varphi-\varphi)=\mu_B-\bar{\mu}_B'-\bar{\mu}_B'' \tag{6}$$

Introducing activities we obtain the relationship

$$RT\ln\left(\frac{\bar{a}_Aa_B}{\bar{a}_Ba_A}\right)=(\bar{\mu}_B''-\bar{\mu}_A'') \tag{7}$$

where a_A and a_B are the activities of A and B in the molten salt, and $\bar{a}_A$ and $\bar{a}_B$ are the corresponding activities in the glass

If we now define a selectivity coefficient K_B^A by the relation

$$K_B^A=\frac{\bar{c}_Ac_B}{\bar{c}_Bc_A} \tag{8}$$

where c_A and c_B are the concentrations of the ions in the molten salt and $\bar{c}_A$ and $\bar{c}_B$ are the corresponding concentrations in the glass, we have

$$\ln K_B^A=\ln\left(\frac{\bar{\gamma}_B}{\bar{\gamma}_A}\right)+\ln\left(\frac{\gamma_A}{\gamma_B}\right)+\left(\frac{W_m}{RT}\right) \tag{9}$$

where $W_m=(\bar{\mu}_B''-\bar{\mu}_A'')$ and γ_A, γ_B, $\bar{\gamma}_A$ and $\bar{\gamma}_B$ are the activity coefficients of the ions A and B in the molten salt and the glass, respectively

In this relationship the first two terms on the right-hand side represent the effect of chemical factors on the selectivity coefficient The last term represents the effect of the mechanical forces W_m is the mechanical work involved in the replacement of 1 mole of cation A by an equivalent amount of cation B, and includes any work which may be done against external forces

Some indication of the effect of the first two terms on the selectivity coefficient can be obtained from existing physico-chemical data The effect of the third term is considered below

The value of W_m in the absence of external restraints may be estimated by assuming that the glass is an elastic continuum and applying the "misfitting sphere" theory This approach has very obvious weaknesses but, as Eshelby[10] has pointed out, the limitations are perhaps more immediately obvious than the shortcomings of other approximate treatments. This type of approach, using the methods of classical elasticity, has been used with various modifications and with varying degrees of success in theories of the formation of lattice defects[10,11], precipitation hardening[12] and the thermodynamic properties of solid solutions of metals[13]. The following discussion of strain energy effects on ion exchange in glasses is based on the treatment of misfit energy in metallic solid solutions given by Eshelby[10] and Christian[13].

We assume that the glass is initially stress free and first consider the replacement of an ion A (radius r_A° and volume v_A°) by the larger ion B (radius $r_B^\circ = (1+\varepsilon)r_A^\circ$ and volume v_B°) in an infinite elastic continuum. We remove the ion A from the glass and expand the cavity to accommodate the ion B which can be assumed to be either a rigid or an elastic sphere. In the latter case, at equilibrium, the volume of the B ion will become v_B and its radius $r_B = (1+C\varepsilon)r_A^\circ$.

$$C = \frac{3K_B}{(3K_B + 4G_M)} \qquad (10)$$

where K_B is the effective bulk modulus of ion B and G_M is the shear modulus of the matrix. ($C=1$ and $v_B = v_B^\circ$ if the B ion is incompressible.)

For an infinite continuum there is no dilatation of the matrix (in other words, the density is constant). If we consider a closed surface in the continuum which encloses the A ion, it can then be shown that the volume enclosed by the surface will increase by Δv^∞ when the A ion is replaced by the B ion, and that

$$\Delta v^\infty = C\,\Delta v_{AB} \qquad (11)$$

where

$$\Delta v_{AB} = v_B^\circ - v_A^\circ = 4\pi\varepsilon(r_A^\circ)^3 \qquad (12)$$

When the exchange process takes place in a finite body of glass, the boundary conditions used in the previous solution are no longer applicable, but a solution can be obtained by superimposing a set of "image" displacements caused by surface tractions. For the case of a sphere (with the A ion at the centre) there is a uniform dilatation of the matrix and the increase in volume caused by image forces is

$$\Delta v^i = (4G_M/3K_M)\,\Delta v^\infty \qquad (13)$$

where K_M is the bulk modulus of the matrix.

The total increase in volume of the system is

$$\Delta v = \Delta v^\infty + \Delta v^i = \left(\frac{C}{C'}\right)\Delta v_{AB} \qquad (14)$$

where

$$C' = \frac{3K_M}{(3K_M + 4G_M)} \qquad (15)$$

This result for the change in volume has been shown by Eshelby[10] to be valid for any external surface. If the effective bulk modulus of the ion B is assumed to be equal to that of the matrix, then it follows from equations (10) and (15) that $C = C'$ and

$$\Delta v = \Delta v_{AB} = v_B^\circ - v_A^\circ \qquad (16)$$

The strain energy of the whole system is

$$W_s = 6G_M C\varepsilon^2 v_B \qquad (17)$$

and because $\varepsilon = \dfrac{\Delta v_{AB}}{3v_B}$

$$W_s = 2G_M\,C\,\frac{(\Delta v_{AB})^2}{3v_B} \qquad (18)$$

Equations similar to equation (17) have been derived by Frenkel[11] (for a rigid sphere) and by Friedel[14].

In a finite body of glass there is a dilatation of the matrix when ion A is replaced by the larger ion B, so the successive introduction of B ions becomes progressively easier. This effect can be expressed in terms of an interaction energy which will reduce the value of W_s as the fraction of B ions increases. In glasses, where the exchangeable ions occupy a relatively small proportion of the total volume, the effect is, however, expected to be small.

From the volume increase given by equation (16) we can estimate the compressive stress p which will be developed in a surface layer when A ions are replaced by B ions. If the underlying glass is assumed to be rigid so that expansion can take place in one direction only, it can readily be shown that the compressive stress is given by the approximate relationship

$$p = \left(\frac{\Delta v_0}{3v_0}\right)\left(\frac{E}{1-\rho}\right) \qquad (19)$$

where Δv_0 is the increase in volume of a volume v_0 of glass containing 1 mole of ion A, when the A ions are replaced by B ions. E is Young's modulus and ρ is Poisson's ratio.

The effect of external mechanical restraint on the ion-exchange equilibrium has not so far been considered. The work W_e done against the external forces when the glass is free to expand in one direction only can be estimated using equation (19), and is given by

$$W_e = \left(\frac{2}{3}\right)p\,\Delta v_0 \qquad (20)$$

If we know the appropriate values of v_A° and v_B°, we can now obtain W_s from equations (17) or (18). W_e can be obtained from equation (20) and the value of the selectivity coefficient K_B^A can be estimated from equation (9) if we assume that the effect of the first two terms on the right-hand side is relatively unimportant. We can also obtain the increase in volume from equation (16) and the compressive stress developed in a surface layer from equation (19).

The difficulty in the application of these relationships lies in the selection of appropriate values for v_A° and v_B°. It is clearly unlikely that the size of the cavity is determined by ionic radii equal to those in crystals. Indeed, if W_s is calculated for the replacement of Na ions by K ions assuming that $r_{\mathrm{Na}} = 0.95$ Å, $r_{\mathrm{K}} = 1.33$ Å, $E = 7\times10^5$ kg cm^{-2} and $\sigma = 0.25$, then $W_s = 13.9$ Cals mole^{-1} which would mean that the replacement of Na ions by K ions would be practically impossible because $K_{\mathrm{K}}^{\mathrm{Na}}$ would be of the order of 7×10^4 at 400° C. It is most likely that the size of the cavity will be determined by steric factors involved in the arrangement of the silicon–oxygen tetrahedra around the alkali metal ions and that the cavity may be similar to those in cristobalite. We can, however, assume an arbitrary effective radius for the cavity which gives the required volume expansion and then use this value to estimate the selectivity coefficient and the compressive stress which would be attained in the strengthening process.

To illustrate the application of these relationships to an actual system, we will consider the replacement of Na ions by K ions in a glass initially containing 1 mole of Na ions in 60 cm^3 of glass. If we assume that the Na ion is situated in a cavity with an effective radius of 1.15 Å, that the glass is initially stress-free and that the Na ion is replaced by a K ion with a radius of 1.33 Å, then the change in volume of the system will be 2.1 cm^3 mole^{-1}. This is in agreement with values of 1.25 to 3.5 cm^3 mole^{-1} calculated from the approximate data given by Burggraaf[15] for the increase in thickness of a surface layer after ion exchange. (It may be noted that these values are considerably less than the value of approximately 7 cm^3 mole^{-1} which would be expected from the difference in the densities of sodium and potassium glasses of similar composition prepared by melting.) From the volume increase and using equation (19) we can estimate the compressive stress which would be developed in a surface layer. Assuming $E = 7\times10^5$ kg cm^{-2}, $\sigma = 0.25$ and $v = 60$ cm^3, we obtain a value of 11,000 kg cm^{-2}, in reasonably good agreement with the value of 8,400 kg cm^{-2}

obtained experimentally by Burggraaf[16] with a glass containing 20·15 per cent by weight of Na_2O. From equation (17) a value of 2·65 Cal mole^{-1} is obtained for W_s. This corresponds to a value of 7·3 for K_K^{Na} at 400° C if we assume that $\bar{\gamma}_{Na}/\bar{\gamma}_K = \gamma_{Na}/\gamma_K$. Avalue of 0·36 Cal mole^{-1} is obtained for W_e from equation (20) assuming that $p = 11{,}000$ kg cm^{-2} and $\Delta v = 2{\cdot}1$ cm^3 mole^{-1}. The value of K_K^{Na} would therefore be expected to increase from 7·3 towards 9·5 as compressive stress is developed in a surface layer. A value of approximately 9 for K_K^{Na} would mean that there would be some difficulty in replacing Na ions by K ions but that 90 per cent replacement could be achieved if the ion-fraction of Na in the molten K salt is not allowed to exceed 1·2 per cent. Although the effect of the bi-axial compressive stress on the selectivity coefficient is relatively small, it would be expected that during the initial stages of the exchange process there would be a tendency for the K ions to form linear arrays perpendicular to the surface. (In a system with uniaxial compression a layered type of structure might be expected.)

In discussing the effect of the strain energy term on the ion-exchange equilibrium it has been implicitly assumed that the ions are situated in randomly distributed sites. There is, however, evidence that, in the caesium and rubidium silicate glasses, the non-bridging oxygens are closely associated in pairs and that with the potassium, sodium and lithium glasses, larger clusters may be formed[17,18]. This non-uniform distribution of exchangeable ions may be expected to have some effect on the validity of the model used in estimating the value of the strain energy term, but it would be expected that the model would still provide an approximate indication of the magnitude of the strain energy effects. It has also been assumed that the effect of the first two terms in equation (9) is relatively small. On the basis of Eisenman's theory of glass electrode behaviour[19] it would appear that the importance of these two terms relative to the strain energy term will depend primarily on the difference between the effective anionic radii in the glass and in the molten salt.

The model used in this discussion of ion replacement processes in silicate glasses is clearly an approximation, but the relationships derived show how the compressive stress achieved in the strengthening process will depend on the nature of the exchanging ions, on the concentration of ions introduced into the glass, on the elastic properties of the glass and on the conditions used in the replacement process. To achieve a high compressive stress in the strengthening process a high proportion of the replaceable alkali ions initially present in the surface layer have to be replaced by larger ions. If the ions differ considerably in size, the effect of the strain energy term on the selectivity coefficient will clearly become of considerable importance and may lead to a high value for K_B^A. The extent of exchange which can then be attained will be very sensitive to the presence of low concentrations of ion A in the molten salt and it will be difficult to achieve the required degree of replacement under practical conditions. The effect of the strain energy term may, however, be offset by the effect of the first two terms in the RHS of equation (9). By a suitable selection of the molten salt system or by a suitable modification of the glass (the use of an aluminosilicate glass, for example) it may be possible to increase the value of $\bar{\gamma}_B \gamma_A / \bar{\gamma}_A \gamma_B$ and thus reduce the effect of the strain energy term on the selectivity coefficient

Addendum

Further support for the theory of ion-exchange strengthening developed here is provided by the work of Rothermel[20]. In a study of the effect of stress on the durability of ion-exchanged surfaces, two aluminosilicate glasses were heated below their strain points in KNO_3 baths containing up to 10 per cent $NaNO_3$ as a contaminant. Glass F initially contained (on a mole per cent basis) 12·3 per cent Na_2O and 2·8 per cent K_2O; glass G contained 12·4 per cent Na_2O and 2·6 per cent K_2O. Treatment for 6 h at 525° C in 100 per cent KNO_3 led to approximately 90 per cent exchange at the surface and a modulus of rupture value of 70,000 lb./in.^{2} (90,000 lb./in.^{2} for unabraded samples). With abraded samples treated in KNO_3 containing 10 per cent $NaNO_3$, the exchange at the surface was approximately 55 per cent and the modulus of rupture was 40,000 lb./in.^{2}.

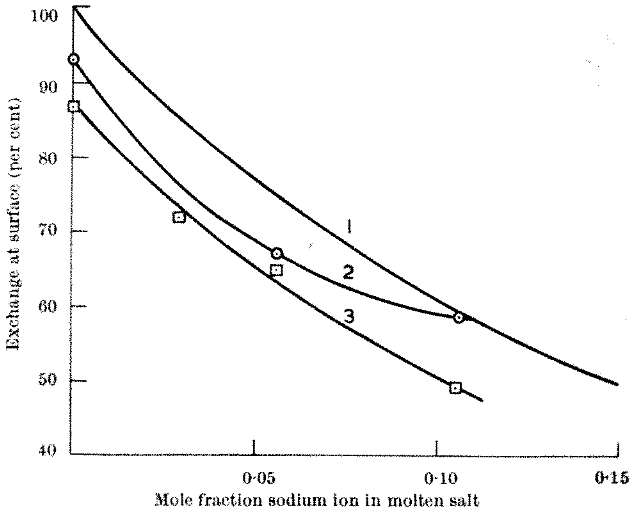

Fig. 1. Effect of sodium ion concentration in molten salt on ion exchange at a glass surface. 1, Theoretical relationship; 2, experimental (Rothermel, glass G); 3, experimental (Rothermel, glass F).

These experimental values may be compared with the theoretical values for a glass containing 11·6 per cent Al_2O_3, 12·4 per cent Na_2O and 76·0 per cent SiO_2. If we assume a density of 2·40 g cm^{-3}, the volume of glass containing 1 mole of Na^+ will be 109 cm^3. If we now take, as before, a value of 2·1 cm^3 mole^{-1} for the change in volume of the system on replacing Na by K ions and assume that $E = 7 \times 10^5$ kg cm^{-2} and $\sigma = 0{\cdot}25$, we obtain values of 76,500 lb./in.^{2} and 46,750 lb./in.^{2} for the compressive stresses introduced by 90 per cent and 55 per cent exchange, respectively. These theoretical values are rather higher than the modulus of rupture values for abraded samples, but the agreement is not unreasonable if it is assumed that the strength of untreated abraded samples is low. Because the value of W_s depends solely on the elastic constants and the volume change, it will have, as before, a value of 2·65 Cal mole^{-1}. This will correspond to a value of 5·3 for K_K^{Na} at 525° C if we assume that $\bar{\gamma}_{Na}/\bar{\gamma}_K = \gamma_{Na}/\gamma_K$. A value of 0·197 Cal mole^{-1} is obtained for W_e when the compressive stress is 5,990 kg cm^{-2}. This means that the theoretical value of K_K^{Na} will increase from 5·3 towards 6·0 as compressive stress develops in the surface. The theoretical relationship between the percentage exchange at the surface and the mole fraction of Na in the molten salt is shown in Fig. 1, which also includes Rothermel's results with the two aluminosilicate glasses. (It has been assumed that the mole fraction of Na ions in the molten salt in Rothermel's experiments can be calculated from the amount of added $NaNO_3$ and that the amount of sodium transferred from the glass to the molten salt is negligible.) It will be seen that the effect of added $NaNO_3$ on the extent of exchange at the surface is very similar to that which would be expected from the theory.

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[1] Proctor, B. A., *Appl. Materials Res.*, 28 (Jan. 1964).

[2] Kistler, S. S., *J. Amer. Ceram. Soc.*, **45**, 59, (1962).

[3] Nordberg, M. E., Mochel, E. L., Garfinkel, H. M., and Olcott, J. S., *J. Amer. Ceram. Soc.*, **47**, 215 (1964).

[4] Ward, J. B., Sugarman, B., and Symmers, C., *Glass Tech.*, **6**, 90 (1965).
[5] Sugarman, B., *J. Mat. Sci.*, **2**, 275 (1967).
[6] Burggraaf, A. J., and Cornelissen, J., *Phys. Chem. Glasses*, **5**, 123 (1964).
[7] Duke, D. A., MacDowell, J. F., and Karstetter, B. R., *J. Amer. Ceram. Soc.*, **50**, 67 (1967).
[8] Doremus, R. H., *J. Phys. Chem.*, **68**, 2212 (1964).
[9] Doremus, R. H., *Modern Aspects of the Vitreous State*, **2**, 1 (1962).
[10] Eshelby, J. D., *Solid State Phys.*, **3**, 79 (1956).
[11] Frenkel, J., *Kinetic Theory of Liquids*, 10 (Clarendon Press, Oxford, 1946).
[12] Mott, N. F., and Nabarro, F. R. N., *Proc. Phys. Soc.*, **52**, 86 (1946).
[13] Christian, J. W., *The Theory of Transformations in Metals and Alloys*, 161 (Pergamon Press, 1965).
[14] Friedel, J., *Advances in Physics*, **3**, 446 (1954); *Phil. Mag.*, **46**, 514 (1955).
[15] Burggraaf, A. J., *Philips Res. Rep. Suppl.* No. 3 (1966).
[16] Burggraaf, A. J., *Phys. Chem. Glasses*, **7**, 169 (1966).
[17] Forland, T., in *Fused Salts* (edit. by Sundheim, B. R.), 63 (McGraw-Hill, 1962).
[18] Everest, D. A., and Napier, E., *J. Inorg. Nucl. Chem.*, **28**, 1813 (1966).
[19] Eisenman, G., in *Advances in Analytical Chemistry and Instrumentation* (edit. by Reilley, C. N.), **4**, 213.
[20] Rothermel, D. L., *J. Amer. Ceram. Soc.*, **50**, 574 (1967).

Mechanism of Oxidation of Magnetite to γ-Fe_2O_3

by

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X-ray diffraction and kinetic measurements show that the rate of oxidation of magnetite crystals smaller than about 0.2μ to γ-Fe_2O_3 is controlled by the diffusion of iron cations. A model of the mechanism is successful in describing the kinetics of the process.

THE oxidation of Fe_3O_4 to γ-Fe_2O_3 has been investigated many times since the establishment of the close relationship between the structures of the two oxides, and since Hägg[1] first discussed the mechanism of the reaction. Feitknecht and Lehmann[2] showed that this oxidation was a one phase topotactic reaction. (We have used here the nomenclature of Mackay[3].) The oxide ion lattice remains cubic close packed and oxygen layers are added as the iron cations migrate to the surface. The results of Egger and Feitknecht[4] indicate that particle size not only determines the rate of the reaction but also whether the α or the γ form of Fe_2O_3 is obtained. When pure magnetite is oxidized below 220° C, particles less than about 3000 Å in diameter always yield γ-Fe_2O_3 while larger particles are first oxidized to a composition intermediate between Fe_3O_4 and Fe_2O_3, and then undergo disproportionation into Fe_3O_4 and α-Fe_2O_3. The Fe_3O_4, which has reappeared, is oxidized directly to α-Fe_2O_3 in the last stages of the reaction. Below 220° C, γ-Fe_2O_3, although metastable at these temperatures, remains unchanged for indefinite periods, but above about 250° C the transformation to the thermodynamically stable α-form may be observed. This transformation, which is quite a different phenomenon from the formation of α-Fe_2O_3 during the oxidation of the larger particles, has been the object of a separate study[5]. Because the oxidation of Fe_3O_4 is important in many fields, such as mineralogy and corrosion, and because the mechanism of the oxidation to the γ form has the appearance of being a special type of topotactic reaction[6], a comprehensive investigation employing X-ray and kinetic techniques was undertaken. This report describes the salient features of the study and proposes a model for the reaction mechanism.

The magnetite sample was prepared by a method[1,7] which produces uniform, monocrystalline cubes (see Fig. 1). The Fe^{2+} content was 24·6 per cent of the total iron content, which is the highest obtainable for particles of this size when prepared by this method. Electron micrographs showed that during oxidation the cubes remained intact and monocrystalline, and that the surface area as measured by the BET method, 6·2 m^2g^{-1}, remained virtually unchanged throughout. This and all previous evidence indicates that this system belongs to the topotactic class of solid state reactions.

Apart from some intensity changes and small line shifts the X-ray diagrams taken at successive stages of the oxidation are identical with those of stoichiometric magnetite, Fe_3O_4, down to a composition of about 22 per cent Fe^{2+} (see Fig. 2). Below this composition reflexions of the primitive lattice of the $LiFe_5O_8$ structure appear, indicating that cation holes are being created on the "lithium" sites of the structure. Between 8·5 and 6·5 per cent Fe^{2+} the tetragonal reflexions of γ-Fe_2O_3 emerge. The structure of γ-Fe_2O_3 is related to the $LiFe_5O_8$ structure but with holes and cations ordered in the sequence Fe □ □ Fe □ □ Fe □ □ Fe □ □ Fe along a spiral of the "lithium" sites, which gives rise to the tetrahedral symmetry[8]. Calculations show that at 9·09 per cent Fe^{2+} the number of occupied and empty "lithium" sites are equal and if ordered the cubic symmetry of the $LiFe_5O_8$ structure would be retained, but below this composition any kind of ordered sequence along the "lithium" spiral will result in a loss of cubic symmetry. Fig. 3 shows the variation in the intensity of the (111) reflexion, measured at successive stages of oxidation from 24·6 per cent to zero per cent Fe^{2+}, and during reduction with hydrogen from 24·6 to 30 per cent Fe^{2+}. At about 8 per cent there is a break in the curve which we believe is associated with the emergence of the tetrahedral reflexions at or near the same composition.

The profile of the α(440) reflexion was measured at different stages of oxidation after quenching to room temperature. The Bragg angle was found to move con-

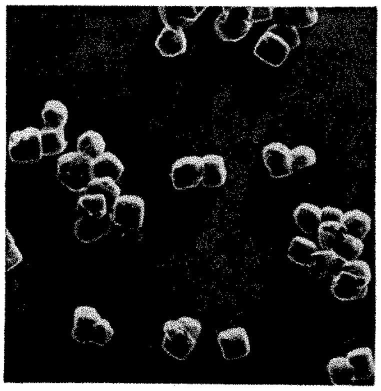

Fig. 1. Electron micrograph of a magnetite sample. The average length of the cube edge is 1940 ± 100 Å, which agrees closely with the value 1870 ± 30 Å calculated from BET surface area measurements.

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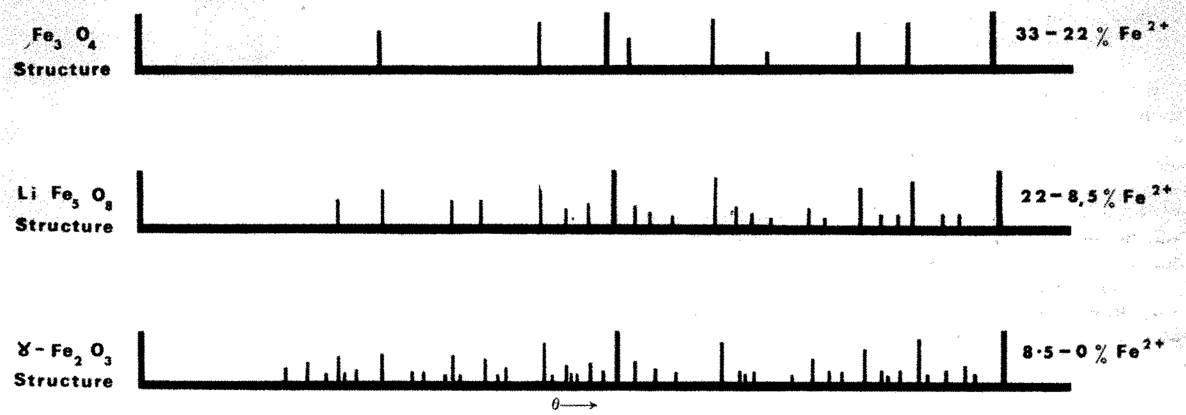

Fig. 2. Comparison of Guinier photographs taken at different compositions. New lines are always weak when they first emerge with decreasing Fe^{2+} content but increase in intensity to a maximum at the end of the composition range for that structure.

tinuously from 72·73° to 73·13°, the value for γ-Fe_2O_3, and the line profile at first gradually broadened asymmetrically to a maximum, and then slowly returned to its original width and shape towards the end of the reaction. If the reaction is interrupted the original width and shape (but not the Bragg angle) could be restored by annealing. The difference in asymmetric broadening between annealed and unannealed samples enabled the lattice strain to be calculated by the method of Kochendörfer[14] and was found to be a maximum at 16 per cent Fe^{2+}, but the change in line width and profile is much less than that expected from the concentration gradient normally associated with a diffusion mechanism. This point assumes considerable importance in the discussion of the mechanism.

For the kinetic investigations, 70–80 mg aliquots of magnetite were oxidized at 190° C to different compositions and annealed thoroughly. The kinetics of oxidation were measured on a Cahn electrobalance for all compositions at 190° C and the fractional uptake of oxygen plotted against $\sqrt{t}$ (Fig. 4) and against $t/t_{1/2}$, where $t_{1/2}$ is the time for half reaction (Fig. 5). Initially all the kinetic runs obey a parabolic law characteristic of diffusion when the semi-infinite solid conditions hold and when the boundary conditions of uniform initial concentration throughout the solid and constant surface concentration apply. The fraction F of the reaction over which the parabolic law is obeyed depends on the variation of D, the diffusion coefficient, but unless this changes discontinuously or extremely rapidly a well defined parabolic curve, usually up to at least $F = 0{\cdot}5$, is always found. For this reason, the experimental results of diffusion controlled reactions, measured under the boundary conditions given here (and in the absence of discontinuous changes of D), follow or almost follow the same curve up to $F = 0{\cdot}5$ when plotted on a reduced time scale, $t/t_{1/2}$. Reduced time plots are therefore of little value in distinguishing diffusion reactions in the region $F = 0$ to 0·5, but at higher F values they are much more sensitive to the type of diffusion mechanism prevailing.

The theoretical curve for diffusion into a cube under these boundary conditions and for a constant diffusion coefficient throughout was calculated from the analytical equation and is plotted as curve c alongside the experimental runs in Fig. 5. None of the runs followed this curve at all, but runs for an initial concentration of less than about 10 per cent Fe^{2+}, however, appeared somewhat similar. The symmetry of the crystal in this composition range is tetragonal and according to Neumann's principle diffusion is not necessarily isotropic but only limited by the restriction $D_x = D_y$ where D_x and D_y are the diffusion coefficients parallel to the a and b crystallographic axes, respectively. The possibilities for diffusion in a tetragonal crystal will fall into two categories.

(1) $D_x = D_y > D_z$ where D_z is the coefficient of diffusion along the c crystallographic axis. The two limits of this range are $D_z = 0$ and $D_z = D_x$. The former was computed and plotted as curve b in Fig. 5. The latter is the isotropic case and is therefore represented by curve c.

(2) $D_x = D_y < D_z$. The two limits here are $D_x = D_y = 0$ and $D_z = D_x$, the latter again being the isotropic case. The former corresponds to one dimensional diffusion in a plane sheet, the solution of which has been computed by McKay[15] and is plotted as curve a in Fig. 5.

The experimental points fall within the range of category (2) and correspond very closely to the upper limit of category (1), that is, to $D_z = 0$ (curve b).

The experimental curve thus has two interpretations. The interpretation according to category (2) requires a particular value of D_z/D_x, but we favour an interpretation which is a limiting case and which amounts to diffusion in two dimensions only. Unfortunately, it seems impossible at present to settle this problem by carrying out diffusion coefficient measurements along selected directions because of the instability of large single crystals in this composition range.

The agreement between the analytical solution for constant and equal values of D_x and D_y and the runs carried out over a narrow composition range (9·5–0 per cent Fe^{2+}), together with the gradual displacement of kinetic curves away from this solution for increasing initial compositions above about 10 per cent Fe^{2+}, suggested a model for reaction in which the diffusion coefficient is

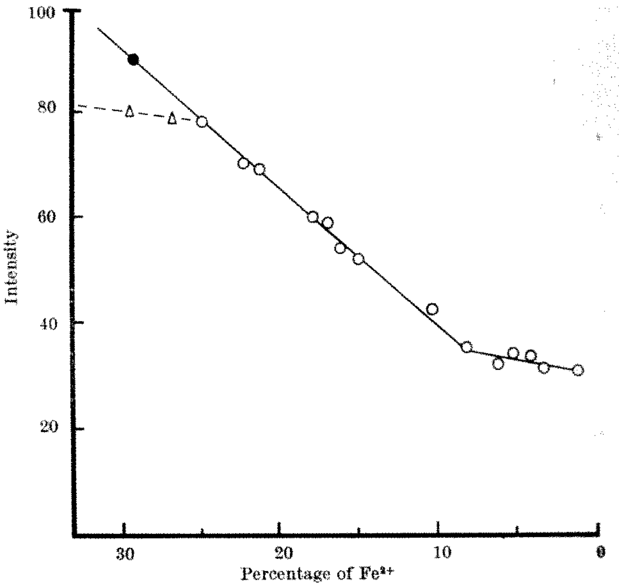

Fig. 3. Variation of the intensity of the (111) reflexion with composition.

concentration dependent and falls with decreasing Fe^{2+} content. To test this possibility diffusion coefficients for each starting composition were evaluated from the slopes of the plots in the parabolic region using the semi-infinite solid equation[6], and plotted against composition in Fig. 6. This plot indicates not only that D falls with decreasing concentration of Fe^{2+}, but also that below 9·3 per cent the diffusion coefficient is constant. This would explain why runs at or below this starting composition simulate curve *b* so well. (Below 9·3 per cent Fe^{2+} the diffusion coefficient measured in this way is the average of D_x, D_y and D_z.)

The uptake-time curve predicted by such a model was computed using Fick's law of diffusion

$$\frac{\delta c}{\delta t} = \frac{\delta}{\delta x}\left(D\frac{dc}{dx}\right)$$

which was linearized by introducing the variable

$$s = \frac{\int_0^c D dc}{\int_0^1 D dc}$$

and the resulting equation

$$\frac{\delta s}{\delta t} = D\frac{\delta^2 s}{\delta x^2}$$

was solved by numerical methods on an ICT 1905 computer for the case of a cube with the same boundary conditions as defined here, for initial compositions 24·6, 18·9 and 14·3 per cent Fe^{2+}. The computed curve for the 24·6 per cent Fe^{2+} case is shown as plot *d* in Fig. 5, yielding good agreement with the experimental points, but the fit of the theoretical curves for the other two cases was not so good. This feature will be discussed later. (The theoretical curves and experimental results of these two cases have been omitted from Fig. 5 to avoid confusion.)

The computer programme was designed to store the concentration profiles at successive points during the computation, which will correspond to successive stages during the reaction. These are presented in Fig. 7, together with profiles for the constant diffusion coefficient case for comparison. It is obvious that the profiles of the concentration dependent case are nearly horizontal in the "core" of the particle. The model thus predicts that as the chemical composition decreases it remains almost uniform over a considerable volume of the crystal, in contrast to the steep composition gradients predicted by the constant diffusion coefficient case. This phenomenon arises in the following way. During the first half of the reaction the greatest change in concentration in the magnetite crystal occurs in the surface layer. Because the diffusion coefficient will fall very rapidly in this layer during the early stages of the reaction it will behave like an insulator enabling the concentration in the "core" to remain near equilibrium during the whole of the reaction. The steep part of the gradient is confined to a smaller fraction of the volume of the crystal. This means that the broadening arising from strain induced by the change in lattice parameter with composition will be considerably less than that associated with the concentration gradient

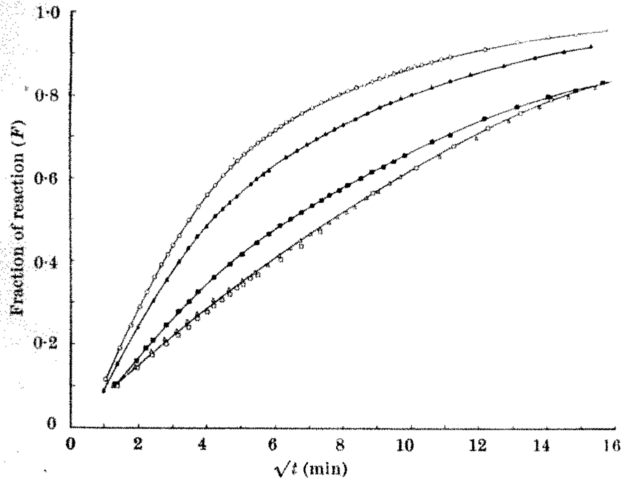

Fig. 4. Kinetics of oxidation of samples which had been previously oxidized to different compositions and then annealed at 190° C for more than 10 h. Kinetics were measured at 190° C and 150 torr O_2. Initial concentration (percentage of Fe^{2+}): ○, 24·6; ▲, 18·9; ■, 14·3; △, 9·5; □, 4·0.

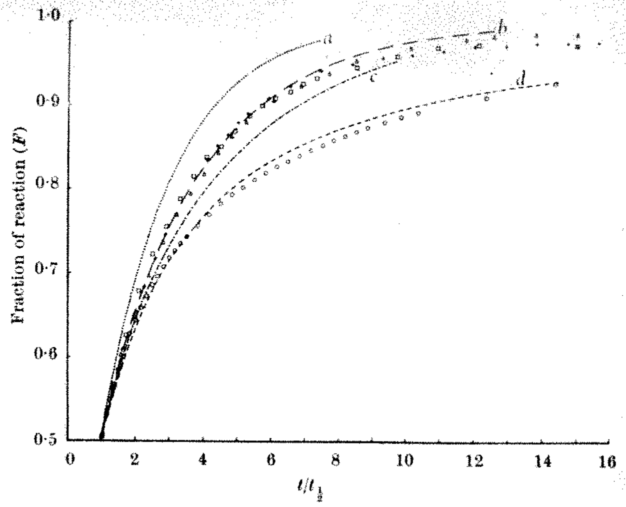

Fig. 5. Kinetics of oxidation as in Fig. 4 but plotted on a reduced time scale, $t/t_{1/2}$, along with theoretical curves for comparison. Below $F = 0·5$ all experimental points and theoretical curves almost coincide and so are omitted.

Experimental data:
○, 24·6
+, 9·5 (1)
△, 9·5 (2)
□, 4·0
Initial concentration (percentage of Fe^{2+}).

Theoretical curves for diffusion in a cube:
a, one
b, two
c, three
Dimensional diffusion, constant *D*. (See text for precise definition of boundary conditions.)

d. Three-dimensional isotropic diffusion computed for an initial composition of 24·6 per cent Fe^{2+} and for *D*, which varies with concentration according to Fig. 6.

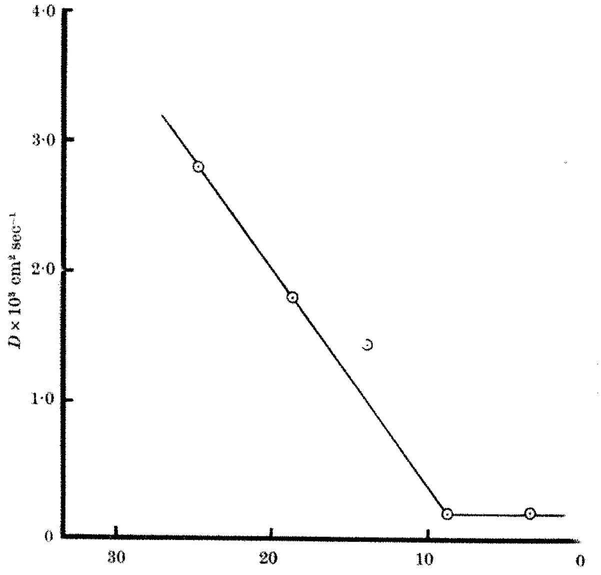

Fig. 6. Variation of diffusion coefficient of iron cations with composition at 190° C.

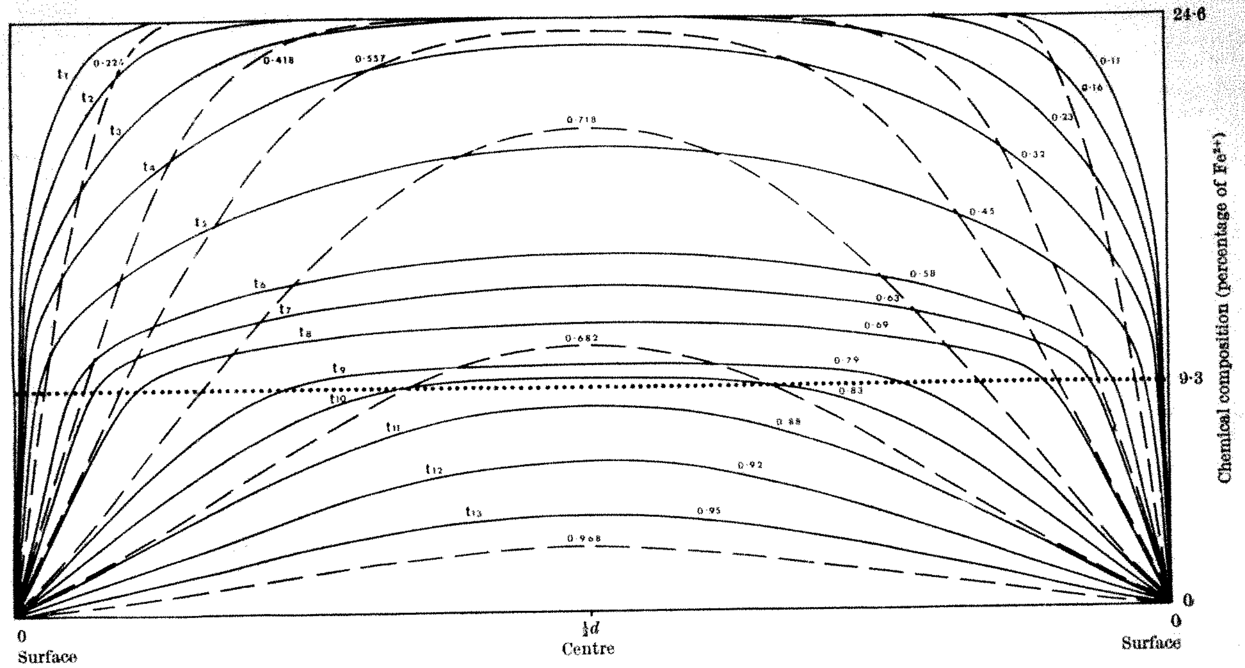

Fig. 7. Computed concentration profiles along a crystallographic axis of a cubic crystal of side d, at successive times during oxidation. - - - - Represents the solution of the equation for diffusion from a cube with a constant diffusion coefficient, —— represents the profiles for single crystal cubes of magnetite, calculated from the variable diffusion coefficient model. The other boundary conditions are the same in both cases and are defined in the text; they include isotropic diffusion throughout. Each curve is labelled with its corresponding F value.

in the constant diffusion case. The model thus explains the unexpectedly small amount of X-ray line broadening.

An inherent feature of the model proposed in this paper is a continuous change in non-stoichiometry from Fe_3O_4 to γ-Fe_2O_3. Discontinuities in composition in non-stoichiometry, however, are relatively common, but seem to be characteristic of compounds which change composition by an anion vacancy mechanism[10,11]. Compounds like magnetite which may suffer a change in composition through a cation vacancy mechanism are, however, thermodynamically more likely to give continuous changes in composition (that is, completely miscible solid solutions) because of the smaller size of the cation holes. We suggest that the success of this model in describing the kinetics over the whole composition range indicates that a continuous change in stoichiometry without composition discontinuities takes place during oxidation under these conditions. Whether this is also true under thermodynamically equilibrium conditions is another matter.

The model used for the computation of the theoretical curve for the kinetics of oxidation of the 24·6 per cent Fe^{2+} sample implies isotropic diffusion throughout the reaction whereas the experiment previously discussed indicated that when the composition falls below 9·3 per cent the diffusion is tetragonally symmetrical. The effect of not allowing for this feature in the model is likely to have only a small effect on the overall reaction kinetics when an initial composition as high as 24·6 per cent Fe^{2+} is used. It may, however, explain the small discrepancy between curve d and the experimental points for 24·6 per cent Fe^{2+}, and the gradual deterioration in fit between the experimental results of runs for decreasing initial compositions and curves computed with the isotropic concentration dependent model.

Colombo *et al.*[12] maintain that below 600° C magnetite never oxidizes to α-Fe_2O_3 unless α-Fe_2O_3 nuclei are already present. This is in contradiction to our observation. Even as low as 180° C particles larger than about 3000 Å have been observed to oxidize to α-Fe_2O_3, although according to the X-ray diagrams and the reducing conditions under which the magnetite was prepared α-Fe_2O_3 nuclei were absent. Furthermore, in a separate study of the transformation of γ-Fe_2O_3 to α-Fe_2O_3 Feitknecht and Mannweiler[5] have observed the transformation of γ-Fe_2O_3 to α-Fe_2O_3 at temperatures as low as 250° C. Colombo *et al.* have used natural magnetite in their experiments whereas we have used pure synthetic preparations. It has been observed[13] that some impurities, especially aluminium, stabilize the γ form of Fe_2O_3. Because natural magnetite may contain impurities in solid solution and in amounts which may vary from one locality to another, it is invalid to extrapolate its behaviour to that of pure Fe_3O_4.

Egger and Feitknecht[4] assumed that in the larger particles (over 3000 Å in diameter) spontaneous nucleation of α-Fe_2O_3 occurred because of greater lattice strain during the oxidation. The model proposed here for the mechanism of the oxidation to γ-Fe_2O_3 explains why this lattice strain should arise. The very low concentration gradient in the core of the magnetite particles during oxidation causes most of the change in the lattice parameter to be confined to a thin surface layer. The strain thus generated can be accommodated more readily in small particles, but above a certain critical particle size the unequal contraction within the surface layer causes the lattice strain to reach a level sufficient to activate the nucleation of the more stable α-form.

As far as we are aware, this is the first reaction of its kind which has been fully characterized and we would like to draw attention to the fact that this was made possible by a detailed analysis of the kinetic data in conjunction with the X-ray diffraction method.

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[1] Hägg, G., *Z. Physikal. Chem.*, **B29**, 95 (1935).
[2] Feitknecht, W., and Lehmann, H. W., *Helv. Chim. Acta*, **42**, 2035 (1959).
[3] Mackay, A. L., in *Reactivity of Solids* (edit. by de Boer, J. H.) (Elsevier, Amsterdam, 1960).
[4] Egger, K., and Feitknecht, W., *Helv. Chim. Acta*, **45**, 2042 (1962).
[5] Feitknecht, W., and Mannweiler, U., *Helv. Chim. Acta*, **50**, 570 (1967).
[6] Feitknecht, W., *Pure and Applied Chem.*, **9**, 423 (1964).
[7] Mannweiler, U., *Chimica*, **20**, 363 (1966).
[8] van Oosterhout, G. W., and Rooymans, C. J. M., *Nature*, **181**, 44 (1948).
[9] Gallagher, K. J., and Phillips, D. N., *Trans. Faraday Soc.*, **64**,785 (1968).
[10] Hyde, B. G., Bevan, D. J. M., and Eyring, L., *Phil. Trans. Roy. Soc.*, A, **259**, 583 (1966).
[11] Hoekstra, H. R., Santoro, A., and Siegel, S., *J. Inorg. Nucl. Chem.*, **18**, 166 (1961).
[12] Colombo, U., *Science*, **147**, 1033 (1965); *Nature*, **202**, 175 (1964).
[13] DeBoer, F. E., and Selwood, P. W., *J. Amer. Chem. Soc.*, **76**, 3365 (1954).
[14] Kochendörfer, A., *Z. Krist.*, **105**, 393 (1944).
[15] McKay, A. T., *Proc. Phys. Soc.*, **42**, 547 (1930).

Structural Units in Chromatin and their Orientation on Membranes

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A recent theory indicates that chromatin contains a structural unit, common to many vertebrate cells, arranged in a manner rather like the molecules in a liquid. More or less random in the interior, the unit was recognized because it forms ordered layers of definite geometry at the surface of the nucleus and monolayers confined by membranes in the cytoplasm.

AFTER cell division the DNA in the chromosomes of the interphase nucleus or the chromatin may undergo replication, or its genetic message may be transcribed into RNA. There is evidence[1-6], based on uptake of radioactive precursors and known physiological activity, which suggests that in cells or regions of cells where the concentration of DNA is relatively high, in chromocentres, condensed chromatin or heterochromatin[7], these metabolic activities are much less pronounced than in more diffuse or extended-state chromatin. Such variation in concentration may be accompanied by variation in structure, and hence repression and derepression of genes may have a structural basis. We therefore suppose that a knowledge of the structure of chromatin, together with biochemical studies (for example, refs. 4, 8 and 9), will eventually lead to a better understanding of the factors controlling nuclear activity in the cells of higher organisms. This article summarizes our earlier data and describes new electron microscope observations of the structure of condensed chromatin, identified by its electron-staining properties and by a comparison with images obtained from light microscopy of sections stained for DNA with the Feulgen reaction.

There have been several recent reports, based on electron microscopy of thin sections, of structures associated with the nuclei of certain mammalian leucocytes and thymocytes, variously referred to as nuclear blebs, pockets, loops or projections. These were found in lymphocytes[10,11], neutrophilic granulocytes[10,11] and monocytes[11] in peripheral blood of normal humans; in immature granulocytes[12-15], monoblasts[14] and cultured lymphoblasts[16] from human leukaemic patients, some undergoing chemotherapy; in neutrophilic granulocytes[17,18] from humans with an abnormal chromosome number; in thymocytes from normal guinea-pig[19] and human foetus[20]; also in lymphoblasts from Burkitt's tumour[21-23] and cells cultured from it. These nuclear structures are therefore a more or less common feature of both the lymphoid and myeloid[24] elements of the blood. Similar structures were also reported[25,26] in the myeloid cells of two amphibia, in the interphase nucleus and mitotic chromosomes of polychromatophil erythroblasts and in mature erythrocytes from the newt *Triturus cristatus*; also in a granulocyte from frog, *Rana esculenta*. The nuclear projections in cells from newt, because of similarity in staining properties to condensed chromatin, were thought to consist of sheets of chromatin limited on both sides by the nuclear envelope, and were therefore called the envelope-limited sheets of chromatin or, simply, the sheets. The variously termed structures associated with the nucleus, referred to here, seem to have a similar morphology. The remarkable feature[26] of these sheets of chromatin is their approximately constant width, between about 300 Å and 500 Å depending partly on methods of preparation. A second finding[27,28] in the nuclei of certain cells, including those from tissues other than blood, is that the condensed chromatin immediately adjacent to the nuclear envelope is organized into layers. In newt cells these layers have similar dimensions to the sheets. These numerical data have led to the theory[27,28] that condensed chromatin contains a structural unit common to many vertebrate cells. The unit has been recognized primarily because of this tendency to form layers of definite dimensions adjacent to the nuclear envelope. In certain blood cells the layers give rise to sheets with similar dimensions extending into the cytoplasm. In the interior condensed chromatin, that is in regions away from the nuclear envelope, the same structural unit is present and apparently more or less randomly oriented[28]. The concept of an orderly arrangement near the surface of the cell nucleus induced by the membrane closely resembles the concept of the crystalline layer at the free surface of a liquid, put forward by Bernal[29] in a new theory of the structure of liquids.

Electron Microscopy

We have now found envelope-limited sheets in myeloid cells from two further classes of vertebrates, namely, birds and cyclostomes, and the study of their fine structure which we report here supports the theory we have just outlined. Cyclostomes are particularly interesting because they are the most primitive of the vertebrates.

To aid in interpreting the electron micrographs, to explain terminology and make clear the relationship between sheets and layers, our findings are shown schematically in Fig. 1. It has to be appreciated that the appearances in electron micrographs depend on the way the section is oriented relative to the specimen. Tilting the section in the microscope has been used[27] to elucidate the three-dimensional structure in it. When the plane of the section is normal to the plane of the sheet, as, for example,

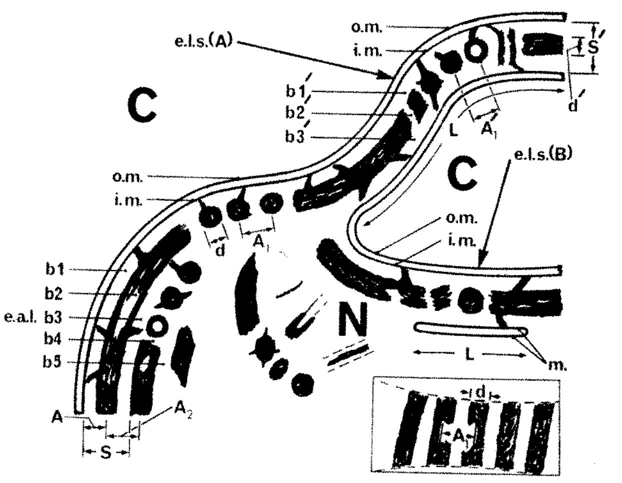
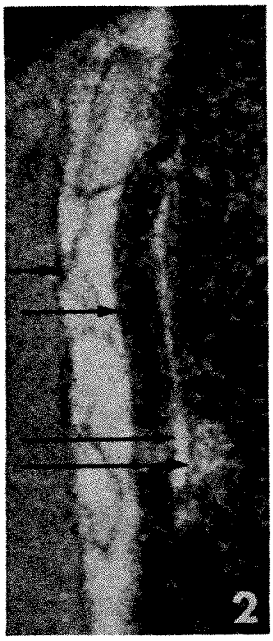


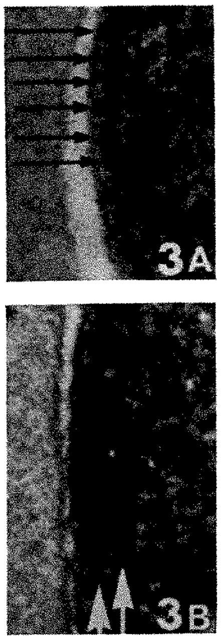
Fig. 1. Schematic diagram showing the envelope-limited sheets (e.l.s.) at (*A*) and (*B*), of length (*L*) with plane of section normal to sheet. i.m., o.m., The inner and outer membranes of the nuclear envelope. Bands $b1'$, $b2'$ (width d') and $b3'$ are seen in the sheet of total width S'. A_1' is the spacing of granules of diameter d' seen in $b2'$. The envelope-associated layers (e.a.l.) appear as bands $b1$, $b2$, and so on; S is the total width of bands $b1+b2+b3$; d is the width of the dark band or the diameter of the granules when well defined and A_1 is their separation; A_2 is the separation of bands $b2$, $b4$. A is the distance separating centre $b2$ from i.m., and $S \sim 2A$. N is nucleus, C is cytoplasm. Inset shows an envelope-limited sheet in face view, that is plane of section parallel to sheet.

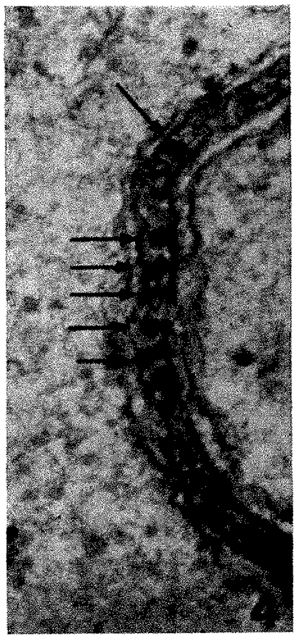
at e.l.s. (A) in Fig. 1, the membranes enclosing it appear as lines continuous with those representing the nuclear envelope. The sheet has a triple-banded appearance due to sectioning three layers. Band $b2'$ is relatively electron dense and when well defined it may appear as short segments or threads of width d', or as approximately equispaced granules of similar diameter. The appearance in electron micrographs was found to depend on orientation[28]. These elements, the band-segments and granules, are themselves composed of finer threads and similar threads cross the relatively less electron dense bands $b1'$, $b3'$, thereby joining $b2'$ with the inner membranes. Bands $b1'$ and $b3'$ are of approximately equal width. A second envelope-limited sheet is shown at e.l.s. (B). The sheets have a total width S'. Similar alternate dense and less dense bands $b1$, $b2$ and so on are found adjacent to the nuclear envelope[27], and by tilting, this appearance is found to result from sectioning envelope-associated layers, referred to simply as the layers (e.a.l. in Fig. 1). Frequently only $b1$, $b2$ and $b3$ are found, and this complex has been called the triple-structured band of width S (ref. 27).

Fig. 2 is an electron micrograph of an envelope-limited sheet of chromatin, with bands $b1'$, $b2'$, $b3'$, in a mature definitive erythrocyte from a chick embryo (17 day); it resembles that at (B) in Fig. 1. In a study of erythropoiesis in chick, where many thousands of cells have been observed, only six nuclei were encountered with such sheets. They occur infrequently and the length (L) is always small compared with the perimeter of the nuclear envelope seen in section. The average value of S' is 380 Å. In a previous study[28] of mature erythrocytes from adult chicken the total width S of the envelope-associated layers, $b1$, $b2$, $b3$, was 330 Å ± 18 Å after fixation in OsO_4 and embedding in methacrylate. Similar bands (Figs. 3A and B) have now been found in primitive and definitive erythrocytes from chick embryo, fixed in glutaraldehyde plus OsO_4 ('Araldite' embedding). In definitive erythrocytes from a 17 day embryo the values for parameters in Fig. 1 are: $S = 402 \pm 30$ Å (120 measured), $A = 208$ Å ± 25 Å, $d = 174$ Å, average (range 150 Å to 200 Å), $A_1 = 280$ Å $\pm$ 40 Å, $A_2 = 260$ Å $\pm$ 25 Å. These values, about 20 per cent higher than those found previously for chicken, indicate that the fixation procedure may affect the dimensions of the layers. Sheets have also been seen in the neutrophilic granulocytes (heterophils) in the spleen of embryonic chick.

In the neutrophilic granulocytes of the lamprey, envelope-limited sheets associated with the nucleus (Fig. 7) are very common, being found in up to about 15 per cent of nuclei in any section. Frequently, more than one sheet is attached to the nucleus giving it a bizarre appearance, very similar to that seen in human neutrophilic granulocytes and cells from the Burkitt tumour. The total length (L) of sectioned sheet is often comparable with the perimeter of the nucleus. In sections normal to the sheet there are bands similar to $b1'$, $b2'$, $b3'$ (Fig. 1) and in certain regions only (parts of Figs. 4 and 5) band $b2'$ is composed of well-defined granules. Measurements made on one animal give d' average = 170 Å (150 Å–210 Å), $A_1' = 300$ Å ± 23 Å. The granules have a variable appearance similar to that previously reported[28] for the granules in the envelope-associated layers and the interior condensed chromatin of mature erythrocytes from lamprey. Occasionally they appear hollow, that is annular, and projections may be seen in Figs. 4 and 5 radiating out connecting them with the inner membranes. The value of S' in the same animal is 365 Å ± 21 Å (thirty-six sheets). When the plane of the section is more nearly parallel to that of the sheet (Fig. 6 and inset to Fig. 1), well-defined short bands, straight or slightly curved, are seen lying in groups parallel to one another. These bands, about 170 Å in diameter, centre to centre separation about 300 Å, are themselves composed of finer threads and granules ranging in size down to 20 Å–40 Å. The arrangement of these finer threads, of molecular dimensions, has not yet been elucidated. They sometimes run at an angle to the 170 Å band making it resemble a coil. When the section is tilted ± 22° in the electron microscope the groups of 170 Å bands are still distinguishable but the finer details in each 170 Å band are altered. Envelope-associated layers (bands) lying adjacent to the nuclear envelope are also seen in lamprey neutrophilic granulocytes and resemble the bands already described ($b1'$,







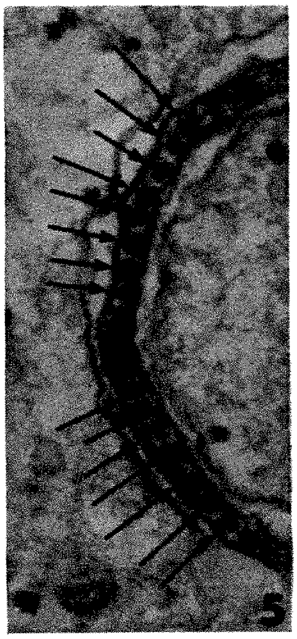


Fig. 2. Electron micrograph of mature erythrocyte from 17 day chick embryo showing an envelope-limited sheet cut normally: compare with e.l.s. (B) in Fig. 1. Membranes are arrowed, the cavity between the pair comprising the nuclear envelope being distended. (× 112,000.) All electron micrographs are of cells fixed in buffered glutaraldehyde followed by OsO_4, embedded in 'Araldite', stained in uranyl acetate and lead citrate, photographed in a Siemens 'Elmiskop 1'.

Fig. 3. Electron micrograph of mature erythrocyte from 17 day chick embryo showing the envelope-associated layers cut normally: compare e.a.l. ($b1$–$b5$) in Fig. 1. In A, arrows point to "granules"; in B, arrows indicate dense bands $b2$, $b4$. (× 112,000.)

Figs. 4 and 5. Electron micrographs of parts of two sheets from neutrophilic granulocytes of lamprey (*Petromyzon fluviatilis*); the plane of the section lies normal to the sheet; arrows indicate "granules"; see also Fig. 1. (× 105,000.)

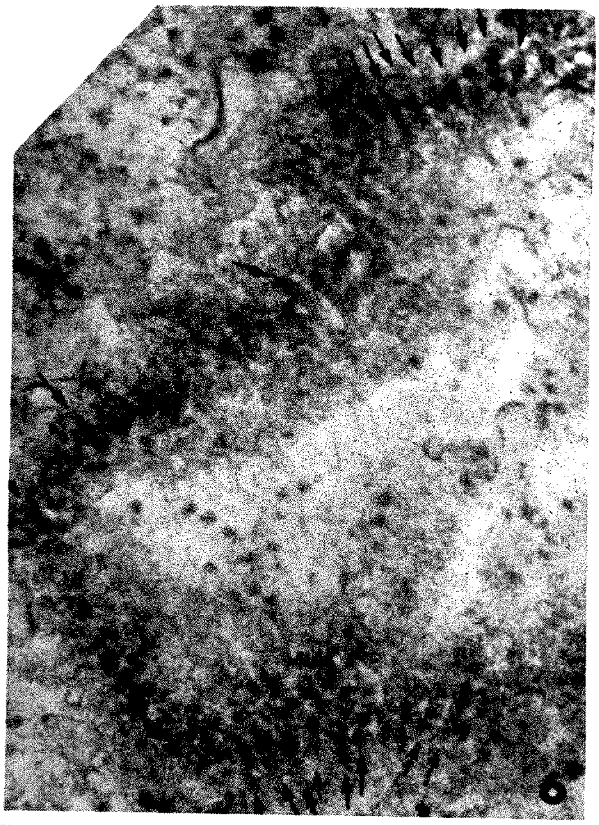
and so on in Fig. 1) in the sheets. In the material studied so far, however, they are not as well defined as those found previously[28] in mature erythrocytes from lamprey where the triple-structured band has a width $S = 346$ Å. We attribute this to difficulties in preservation. Envelope-limited sheets have also now been found in mature erythrocytes from lamprey.

Structural Unit

Summarizing certain of our measurements, in chicken erythrocytes S' is 380 Å and S is 402 Å, while in granulocytes from lamprey S' is 365 Å and in the erythrocytes S is 346 Å. This shows that the widths of the sheets (S') and triple-structured layers (S) are all very similar to one another and to the widths previously found in certain mammalian and amphibian cells. Consequently, the data support the theory that the chromatin in these cells contains a common structural unit and that the envelope-limited sheets are derived, by processes not yet fully understood, from the envelope-associated layers. The possible physiological role of the sheets is unclear, but where they extend into the cytoplasm they increase the area of chromatin in contact with it[11].

Consider now the nature of the structural unit which forms the sheets and layers and gives rise to the bands seen in the electron micrographs. It was at first suggested[26] that an envelope-limited sheet of chromatin was formed by folding a thread, about 400 Å in diameter, on the surface of a membrane, so as to form a sheet of equal width. When the sheets were found[27,28] to have a banded structure similar to that of the layers near the interior membrane of the nuclear envelope, this hypothesis had to be modified. Observations[28] of the envelope-associated layers and other more interior regions of the condensed chromatin in the nuclei of mature erythrocytes from lamprey and adult chicken, as well as in certain cells from newt spleen, suggested, as the structural unit in them, a thread of diameter between about 130 Å and 230 Å, the value depending partly on fixation. The dark bands in Fig. 3*B* arise from threads lying one above the other in planes parallel to the optic axis. When the axes of the threads are parallel to the optic axis the resulting end-on views give rise to "granules" (Fig. 3*A*). Sometimes end-on views were annular in shape suggesting that the thread is a hollow cylinder, that is a microtube. To account for the separation of the dark bands and granules both from one another and from the membranes, it had to be assumed that there were projections arranged along the threads or microtubes. Finer threads could indeed be seen in the micrographs crossing *b*1, *b*3 and so on.

These data on the fine structure of the sheets confirm and extend the hypotheses relating to the structural unit in condensed chromatin and its role in forming the sheets. Figs. 4, 5 and 6 show, respectively, end-on and face views of 170 Å threads about 300 Å apart. These values are similar to those found[28] for the threads (range 130 Å–230 Å diameter) thought to exist in the envelope-associated layers. In the embryonic chick erythrocytes measured here the thread-diameter (d) is also 170 Å and the separation (A_1) about 280 Å. The face views of the sheets in granulocytes from lamprey provide critical new information. Previously, because of the chance of overlapping structures in the section we could not rigorously exclude the possibility that the structural units in chromatin were not granules or spheres regularly arranged along axes. The appearance of the layers near the envelope is very similar to that seen in sections through certain virus crystals known to contain overlapping spherical units. In the sheet extending into the cytoplasm, however, there is only a single layer and we can be quite sure from the face views (for example,



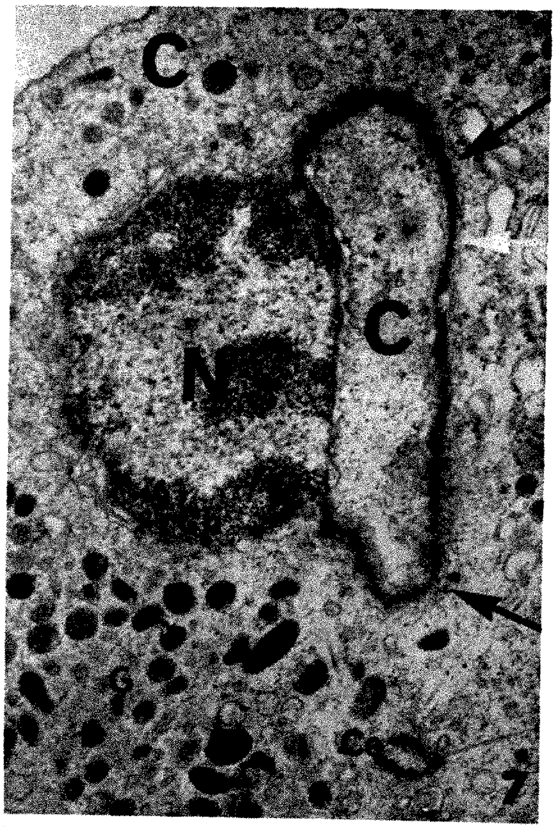


Fig. 6. Electron micrograph of that part of the sheet shown at lower arrow in Fig. 7. In this approximately face view, arrows point to the 170 Å bands (threads).

Fig. 7. Electron micrograph of a neutrophilic granulocyte from the lamprey; arrows point to an envelope-limited sheet; at the white arrow the plane of the section is approximately normal to that of the sheet, whereas elsewhere the sheet is cut at an angle giving approximately face views (see inset to Fig. 1 and Fig. 6). *N*, Nucleus; *Ch*, one piece of condensed chromatin; *C*, cytoplasm; *Ce*, centriole; *G*, granules. (×15,000.)

Fig 6) that the unit in it is threadlike We infer, as we had previously supposed, that the unit in the envelope-associated layers, being of similar dimensions, is also thread-like The projections which we have referred to may also be seen connecting 170 Å "granules" to the membranes in the end-on views (Figs 4, 5) and, as we noted, some end-on views are annular We have not shown that all the end-on views we observe are consistent with a tube and associated projections variously oriented with respect to the optic axis, and so we regard this model for the distribution of electron scattering material in the 170 Å thread and the finer threads which radiate from it as a working hypothesis

In the interior condensed chromatin, that is in regions away from the inner membrane, threads and granules, about 170 Å in diameter with internal fine structure, are common in both lamprey granulocytes and mature erythrocytes of embryonic chick They appear to be randomly arranged There are also still finer threads and granules All electron microscopists are aware of the fibrillar–granular elements of chromatin and have reported a range of diameters, about 20 Å to several hundred angstroms (see refs listed in ref 28) It has been shown[28] that some of these variations result from overlapping of the structures within the section thickness and there is possible variation due to distortion during preparation and to partial sectioning Because of these factors it is difficult to prove conclusively that condensed chromatin contains a structural unit of unique diameter, but there is no doubt that there is a very close similarity between the threads we are able to recognize clearly on the membranes and those found randomly arranged in the interior

Finally, we do not know what are the arrangements of the molecules in the structural units in condensed chromatin, although we can make certain suggestions There are various reasons[31] to be confident that in chromosomes and the nucleohistones derived from them the DNA is coiled or folded The problem is how this is effected, and various ideas have been put forward that in nucleohistone the DNA molecules are coiled around one another and linked by histone bridges[31] or from new X-ray diffraction data[30] on nucleohistone that the DNA forms a coil (super-helix) of diameter 100 Å, pitch 120 Å, that in the threads isolated from nuclei the DNA is coiled with histone to form a super-coil diameter 200 Å–300 Å (refs 32, 33) or that these same threads are formed from two parallel 100 Å threads each containing randomly folded DNA and histone[34] Our data suggest that the 170 Å threads in intact nuclei are probably tubular, which supports the concept of a superhelical configuration for the DNA We cannot, however, exclude the possibility that the molecular organization in the intact nucleus may be more complex For example, the DNA molecules may contribute to the formation of the projections from the 170 Å threads Quite likely it will be possible to define better the structural unit by further studies of the sheets which should be regarded as monolayers confined by membranes

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[1] Hsu, T C , *Exp Cell Res* ,**27**, 332 (1962)
[2] Cameron, I L , and Prescott, D M , *Exp Cell Res* , **30**, 609 (1963)
[3] Grasso, J A , Woodard, J W , and Swift, H , *Proc US Nat Acad Sci* , **50**, 134 (1963)
[4] Littau, V C , Allfrey, V G , Frenster, J H , and Mirsky, A E , *Proc US Nat Acad Sci* , **52**, 93 (1964)
[5] Harris, H , *J Cell Sci* , **2**, 23 (1967)
[6] Chapman, J A , Elves, M W , and Gough, J , *J Cell Sci* , **2**, 359 (1967)
[7] Heitz, E , *Z Indukt Abst U-Vererbungsl* , **70**, 402 (1935)
[8] Bonner, J , Dahmus M E , Fambrough, D , Huang, R C , Marushige, K , and Tuan, D Y H , *Science*, **159**, 47 (1968)
[9] Comings, D E , *J Cell Biol* , **35**, 699 (1967)
[10] Smith, G F , and O'Hara, P T , *Nature*, **215**, 773 (1967)
[11] Huhn, D , *Nature*, **216**, 1240 (1967)
[12] Stalzer, R C , Kiely, J M , Pease, G L , and Brown, A L , *Cancer*, **18**, 1071 (1965)
[13] Ahearn, M J , Lewis, C W , Campbell, L A , and Luce, J K , *Nature*, **215**, 196 (1967)
[14] McDuffie, N G , *Nature*, **214**, 1341 (1967)
[15] Anderson, D R , *J Ultrastruct Res* , Suppl 9, 1 (1966)
[16] Foley, G E , Lazarus, H , Farber, S , Uzman, B G , Boone, B A , and McCarthy, R E , *Cancer*, **18**, 522 (1965)
[17] Huehns, E R Lutzner, M A , and Hecht, F , *Lancet*, i, 589 (1964)
[18] Lutzner, M A , and Hecht, F , *Lab Invest* , **15**, 597 (1966)
[19] Torő, I , and Oláh, I , *Nature*, **212**, 315 (1966)
[20] Sebuwufu, P H , *Nature*, **212**, 1382 (1966)
[21] Epstein, M A , Barr, Y M , and Achong, B G , *Brit J Cancer*, **19**, 108 (1965)
[22] Epstein, M A , and Achong, B G , *J Nat Cancer Inst* , **34**, 241 (1965)
[23] Achong, B G , and Epstein, M A , *J Nat Cancer Inst* , **36**, 877 (1966)
[24] Bloom, W , and Fawcett, D W , *A Textbook of Histology* (W B Saunders, Philadelphia and London, 1962)
[25] Davies, H G , and Tooze, J , *Nature*, **203**, 990 (1964)
[26] Davies, H G , and Tooze, J , *J Cell Sci* , **1**, 331 (1966)
[27] Davies, H G , *Nature*, **214**, 208 (1967)
[28] Davies, H G , *J Cell Sci* (in the press)
[29] Bernal, J D , *Proc Roy Soc* , A, **280**, 299 (1964)
[30] Pardon, J F , Wilkins, M H F , and Richards, B M , *Nature*, **215**, 508 (1967)
[31] Wilkins, M H F , Zubay, G , and Wilson, H R , *J Mol Biol* , **1**, 179 (1959)
[32] DuPraw, E J , *Nature*, **209**, 577 (1966)
[33] Gall, J G , *Chromosoma*, **20**, 221 (1966)
[34] Ris, H , in *Regulation of Nucleic Acid and Protein Biosynthesis* (edit by Koningsberger, V V , and Bosch, L), 11 (Elsevier, Amsterdam, 1967)

Gene Duplication and the Evolution of Enzymes

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The relationship between structure and function of proteins makes it unlikely that a new enzyme activity can evolve unless the genome contains extra DNA with suitable coding properties such as is provided by the duplication of a gene for a functionally related enzyme. Evidence from the Annelida suggests that their diverse phosphagen kinases evolved in this way

SEVEN different phosphagens (Fig 1) have been found in the muscles of the Annelida, sometimes more than one occurring in a single species The group is thus unique in the animal kingdom, where all other phyla contain only arginine (in most invertebrates) or arginine and creatine (in the groups leading to and including the Chordata) It is nowadays realized that small metabolites such as the phosphagens have the disadvantage, for evolutionary studies, of being remote from the genome, their nature is determined by the interplay of enzyme systems within the organism. The enzymes themselves, on the other hand, are directly related to the genome and their comparative biochemistry is a true reflexion of genetic differences.

While the mutation of a gene could be described only in terms of gross results, such as changes in size or colour, it was possible to believe that evolution proceeded by the action of selection on organisms bearing different genes for such gross characters. In this view, some mutant genes were advantageous but most were disadvantageous. It was realized that one gene might modify another's action and hence its selective value, but the mechanism of these effects remained obscure[1]. In our present working concept, the unit of detectable mutation is a change in a single amino-acid of a particular polypeptide chain. (Behind this, not yet of great practical use, lies the change of a single nucleotide of a particular codon in the DNA.) At the same time, a protein is seen as a delicately balanced functional entity the integrity of which may be destroyed by one such change. It has thus become necessary to analyse the process of evolution in terms of protein structure and synthesis, the primary phenotypic effects which form the basis of the gross phenotype to be exposed to selection in the whole organism.

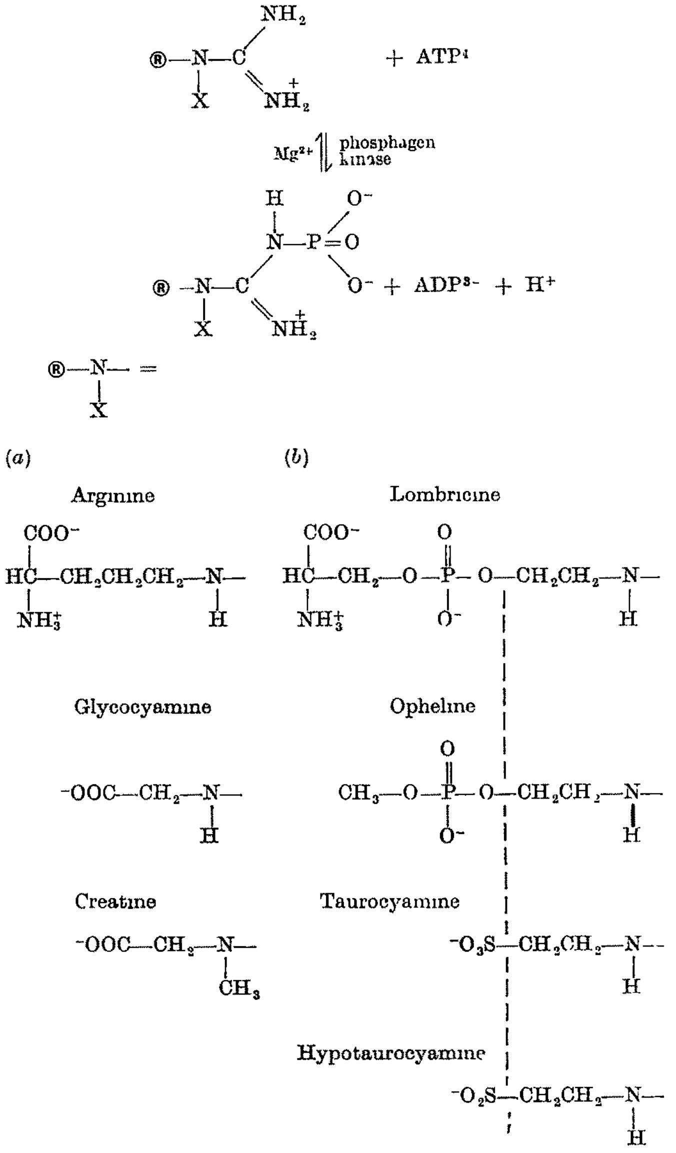

Fig. 1. General reaction for phosphagen kinases and formulae of the natural phosphagens. (a) Phosphagens whose kinases show high specificity, (b) phosphagens (all substituted guanidinoethanes) whose kinases show activity towards other members of the group.

Essential Enzyme Features

The establishment within a polypeptide chain of a small number of essential amino-acid residues orientated to form a catalytic entity must have been contemporaneous with the origin of life. A few types of catalytic sites can be recognized[2-4], corresponding to different classes of chemical change catalysed, in each of which specific amino-acid residues must occur in their correct place in the polypeptide chain and be maintained in their correct spatial arrangement through the tertiary structure of the molecule. All the basic mechanisms for catalysis in present day organisms probably already existed in the earliest forms of life, slight modifications in three-dimensional orientation of the catalytic amino-acids to permit the processing of new substrates appearing gradually during the evolutionary divergence of the various groups of organisms. A mutation resulting in change of a residue directly involved in catalysis would cause loss of enzyme function and, except in special cases, would always be lethal. A possible example is loss of one of the two essential thiols from the creatine kinase of mice with a fatal hereditary muscular dystrophy[5]. Changes in residues concerned in maintenance of the three-dimensional structure of the catalytic site might not always change the structure sufficiently to cause loss of function, so that selection against such changes would not be absolute.

The phosphagen kinases catalyse a phosphoryl transfer between ATP and a guanidino compound (Fig. 1) and enzymological evidence[6] supports the expectation that all have catalytic sites of one type. The differences between them involve other functions requiring particular amino-acids at particular positions in the enzyme molecule which can be listed as follows: (1) direct participation in the binding of substrates, (2) direct participation in specificity—the exclusion of unwanted substrate-like molecules to which the enzyme might be exposed in the cell, (3) direct participation in internal interactions permitting any conformational changes which might be associated with the catalytic process, (4) direct participation in interactions of a structural kind between the sub-units of the enzyme or between the enzyme and other macromolecules of the cell, (5) the maintenance of tertiary structure required in (1), (2), (3) and (4) and to adapt the catalytic site to the particular substrate, (6) the maintenance of tertiary structure required to confer stability on the enzyme in its particular cellular environment, which in turn may depend on the particular external environment of the organism.

Compensating Changes

A protein carrying out a particular function must be correctly constructed in many of its component parts in order to work at all. Moreover, residues may be critical for more than one function, a particular amino-acid having been selected because it satisfies all the functional needs. Adaptations of the protein molecule to encompass, for example, new substrate specificities would be likely to impair the correctness of the structure with respect to stability or in other ways. Thus even if a single mutation could bring about such an adaptive change, which is in itself unlikely, other changes in combination with the first change in the protein would almost certainly be necessary to restore its functional fitness. This phenomenon of compensating amino-acid changes has been shown in the *A* protein of the *E coli* tryptophan synthetase system[7] Here a glycine residue in one tryptic peptide and a tyrosine in another have been shown to be changed in certain mutants to glutamic acid and cysteine, respectively Both these changes result singly in loss of enzyme activity, but, when the two are combined, a partial revertant type is produced showing enzyme activity, although lower than that of the wild type Similar compensation has been shown in *E coli* alkaline phosphatase[8]

Comparison of the amino-acid sequences of a number of vertebrate haemoglobin and myoglobin polypeptides[9] showed that variation occurred in all but nine (about 6 per cent) of the residues, while the tertiary structure and function remained virtually the same Hence, 94 per cent of the residues have been non-essential One non-lethal change, however, could produce a cascade effect in the selection of counterbalancing changes elsewhere Thus it is particular combinations of residues which should be regarded as essential The idea of interdependent changes in residues underlies much of the discussion of globin variation by Zuckerkandl and Pauling[10]

To convert a protein to a new function or to adapt it to existence in a changed environment must in general require a number of mutations including both primary and compensatory ones Anfinsen[11] has suggested that, according to the metabolic importance, the selection pressure on enzymes will vary and hence their rate of evolutionary change A functional enzyme could, however, only acquire a new function as an extra by a slackening of specificity and changes in binding properties A new individual enzyme requires a gene of its own DNA coding for a once useful enzyme might have two immediate disadvantages its base sequence might be quite unlike that coding for any enzyme which could be of use to the organism and, at the point when its particular step in metabolism became redundant, mutations replacing amino-acids at the catalytic sites might have a strong selective advantage instead of being lethal This might not present a serious evolutionary problem Benzer[12] has shown, in phage, that variation in the ratio of forward back mutation rate at the same point is greater than can be explained by the possible nucleotide interconversions, selection may have established a "stable" sequence around the essential residues. giving them a low forward back mutation ratio A third disadvantage of "unused" DNA would, however, develop with time, its isolation from selective pressure would permit the accumulation of random changes in base sequence resulting in its inability to produce a protein resembling any enzyme It has been pointed out by Freese and Yoshida[13], however, that, because mutagenic substances are most active on single-stranded DNA, regions of the genome not involved in *m*RNA production would be to some degree protected, particularly in higher organisms where they may carry the additional barrier of a histone sheath This idea suggests that unused DNA might be kept in "cold storage", but furthermore that, before the most rapid evolution could occur, the probability of mutation would have to be increased by the process of producing a meaningless *m*RNA

Gene Duplication

The existence in the cell of "extra" DNA, as distinct from that "released" from its function because the protein for which it coded is redundant, is bound up with the concept of duplication of the whole or part of the genome This represents a departure from the essential processes of DNA replication and distribution to daughter nuclei whereby its quantity and nature are maintained in succeeding cell generations

Duplication of the whole genome has its own evolutionary significance In plants, vegetative reproduction allows the stabilization of polyploidy, and hermaphroditism eliminates the problem of interference with sex-determination, polyploidy is an essential feature of the evolution of this group[14] and has been widely studied Muldal[15] has compared chromosome counts for a number of oligochaete annelids and shown that those species which are parthenogenetic show various increases in ploidy above that in hermaphrodite species He also suggests that the latter are only secondarily diploid, having possibly arisen from the bisexual polychaetes[16] through tetraploid formation He points out that such events must lead to a freezing of the evolutionary change found in cross-fertilizing organisms Such a process may sometimes be reversible, as in the suggested origin from various ancestors among polyploid hermaphrodite fish of several vertebrate groups which subsequently adopted bisexual reproduction[17]

The theoretical advantages of the duplication of small parts of the chromosome was appreciated before the days of molecular biology Huxley wrote in 1942 that "small 'repeats' of this type constituted the chief method by which the number of genes is increased, thus providing duplicate factors and the opportunity for slight divergent specialization of homologous genes, giving great delicacy of adjustment"[18]

The occurrence of gene duplication was long ago recognized in *Drosophila* from gross genetic analysis of the *Bar eye* gene, which had the property of giving rise to normal and "*double Bar*" eyes in crosses where only *Bar eye* would have been expected and with a frequency far above that of mutation[19] The phenomenon of unequal crossing-over following occasional incorrect pairing in the *Bar* region was postulated to explain these results, and Sturtevant[19] believed *double Bar* to be a duplicated form of *Bar*, while the revertants had lost the gene altogether Bridges[20] found that numerous small duplicated regions could be detected in preparations of *Drosophila* salivary chromosomes, and discovered[21] that *Bar* was one of these, while *double Bar* showed the same region triplicated He realized that the no-gene concept for the wild type did not accord with the origin of *Bar* by a normal mutation Unequal crossing-over between two duplicated genes giving rise to a single and a triplicated gene, that is to normal and *double Bar* (Fig 2), is then achieved without the unlikely event of synapsis of lengthy non-homologous regions of the chromosomes[22] Such homologous unequal crossing-over does not explain the origin of a tandem duplication but can account for its development into multiple replicated regions The nature of primary duplication events, which may have occurred in the past, is a subject for conjecture based on cytogenetic evidence for possible modes of reorganization of the chromosomes Genetic investigations of the mechanism of tandem duplication[23,24] have dealt only with secondary duplications in regions of the chromosome consisting of pseudoalleles, arrays of genes with a fair degree of homology being the product, presumably, of earlier unexplained duplication events It was shown[25] that the occurrence of homologous unequal crossing-over within such a series did not induce non-homologous unequal crossing-over in adjacent regions of the chromosome

Usually, duplications must be detected a very long time after their arising, when their relative positions on the chromosome may have been altered by subsequent rearrangements and can thus provide small clues to the nature of the original events Possible mechanisms for the origin of duplicated genes, based on cytogenetic evidence for types of chromosome rearrangement, have been reviewed[26] These lead to the duplicate gene being sited in various parts of the genome relative to the original gene, and may be classified into four groups, with duplicate genes (1) adjacent, (2) close together in the same chromosome arm, (3) in different arms of the same chromosome, (4) in different chromosomes From the evolutionary point of view, the first two groups are probably the most important because they permit further (secondary) duplication to occur by homologous unequal crossing-over It must be concluded, however, that there is no evidence to suggest how any of the known gene duplications originated, although it may be inferred that the duplication by which the δ haemoglobin gene of primates arose from the β haemoglobin gene was of the adjacent type, on the basis of sequence analysis and phylogenetic evidence for its recent occurrence in conjunction with linkage data[27]

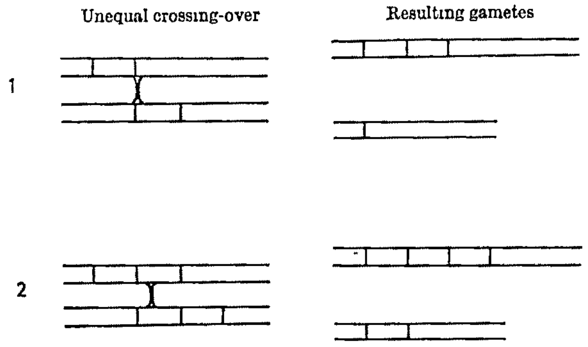

Fig 2 Unequal crossing-over at the *Bar* locus in *Drosophila melanogaster* (1) According to Sturtevant[20], non-homologous unequal crossing-over, (2) according to Bridges[21], homologous unequal crossing-over

Few of the mechanisms for gene duplication based on chromosome rearrangements directly yield tandem duplications This, together with a desire to explain partial duplication of the haptoglobin α-chain gene by a one step mechanism (as opposed to duplication followed by deletion, for example), may have prompted Smithies, Connell and Dixon[28] to discuss the implications of non-homologous unequal crossing-over as a source of primary duplications and partial duplications The mechanism is not new but this comprehensive treatment is probably responsible for its great popularity with biochemists interested in evolution We have found no evidence for its occurrence It is often confused with homologous unequal crossing-over, for which there is good evidence Until molecular genetics has thrown considerably more light upon the chemistry of crossing-over—in particular on how much homology is necessary for it to occur—non-homologous crossing-over should be treated with much greater scepticism

As a result of a survey of the properties of aldolase enzymes in a wide range of organisms, Rutter[29] proposed that the A and B isoenzymes in the rabbit, which can be distinguished by means of kinetic parameters, are the products of genes derived in the past by duplication of an ancestral gene Our scheme for the evolution of the annelid phosphokinases is based on what is probably still the only evidence for gene duplication giving rise to enzymes with different substrate specificity in different species—one essential feature of divergent evolution

Duplication of a gene can result in the release of one of the duplicate genes from selection while the other provides a viable phenotype The subsequent possibilities are the same as those for the DNA no longer used because the enzyme is no longer required by the organism, except that there would be no initial selective advantage in loss of enzyme activity or other aspects of the original "tailor made" structure These considerations make released DNA of this kind a very favourable material for the development of enzymatic capacities which are new but related to that of the original gene Related activities might include the further metabolism of the product of the original enzyme, or the parallel metabolism of related substrates prevented by the specificity of the original enzyme For a phosphagen kinase, mediating the reversible transfer of the terminal phosphoryl group of ATP to a compound which forms a dead-end storage product for chemical energy (Fig 1), only the latter can apply

Evolution of Phosphagen Kinases

In effect, a phosphagen kinase mediates a complete metabolic pathway, if its substrate is changed, new enzymes are not required to deal with the products of the reaction Adaptation of an organism to the use of a new phosphagen kinase would depend on the presence of the new enzyme and the availability of the new phosphagen The latter can most probably be provided by means of metabolic pathways commonly found in animals, arginine, the only guanidino amino-acid of protein, occurs universally and all other phosphagens are formed by transamidination from it (see formulae in Fig 1) The non-guanidino part of the molecule may most frequently be derived from general amino-acid metabolism Additional evidence for the presence of the appropriate kinase being the limiting factor in the use of a particular phosphagen is derived from the fact that in addition to all those animals which use any phosphagen other than arginine, many annelids contain appreciable quantities of more than one guanidine but only use one as a phosphagen[30]

Once duplicate genes have been formed, two other processes besides point mutation might be mentioned as potentially contributing to their divergence The first might occur in any DNA, whether duplicated or not, and has the effect of duplicating or deleting small groups of bases The mechanism is based on physical studies made by Lerman on DNA-acridine complexes[31] His results were compatible only with an intercalation model for the complex, he pointed out that in higher organisms local regions of the homologous chromosomes after synapsis could be out of register by one or several base pairs if intercalation of this kind had occurred randomly, and that crossing-over in such regions would result in deletion and reciprocally, duplication of the number of bases out of line Although there is little evidence in favour of whole genes undergoing non-homologous pairing, identical bases are not required at the actual point of crossing-over Two tryptophan synthetase mutants with an amino-acid difference can give recombinants having a third amino-acid at this point[7], indicating recombination within dissimilar codons When amino-acid sequences of related proteins are compared, amid regions of similar sequence, single extra or missing residues are frequently found[32,33], Lerman's mechanism would exactly account for this phenomenon

The second process for modification of the gene applies only in the case of adjacent duplicate genes, and has been suggested by Wagner and Mitchell[34], Horowitz[35] and recently by Comings[36] as a source of apparent multiple mutation It might sometimes have happened that recombination between large parts of two duplicate genes by unequal crossing-over gave rise to a functional new protein Such events would be necessarily linked to changes in the number of duplicate genes

If it is assumed that the ancestral annelid possessed arginine kinase, as is the case with most invertebrates, duplications resulting in two or three arginine kinase genes might be followed by various kinds of evolutionary divergence (Fig 3) A modern descendant may still possess arginine kinase either alone or with one or more homologous enzymes using other phosphagens as substrates Alternatively, after the evolution of a second kinase was completed, the ancestral arginine kinase activity might have been lost and replaced by one or more other kinase activities It seems unlikely that modern forms should continue to possess several genes coding for identical arginine kinases, for, making the reasonable assumption that one gene can provide all the enzyme required by the organism, the surplus genes would tend to be inactivated

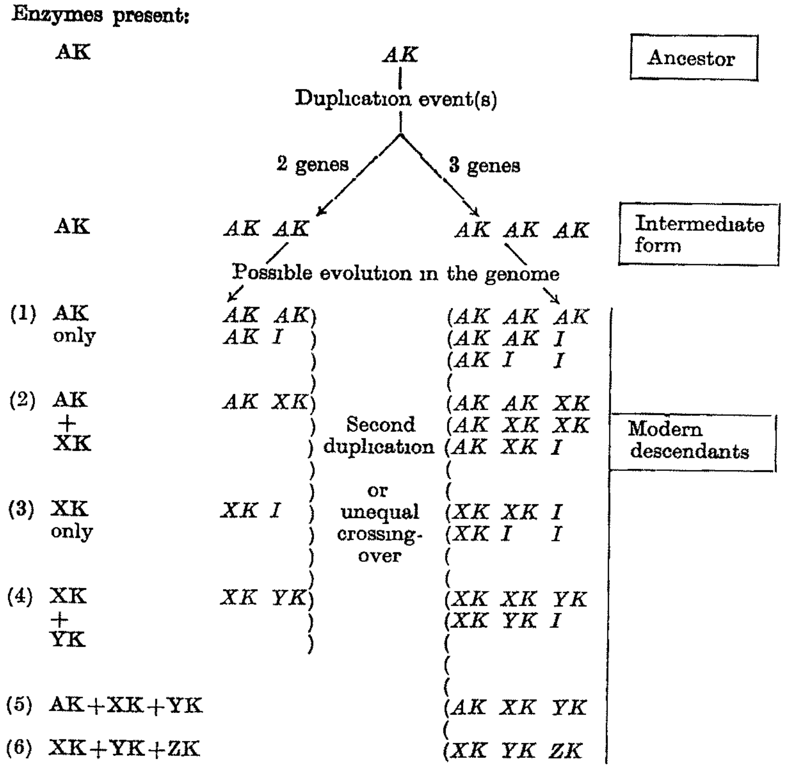

Fig 3 Theoretical scheme for the evolution of phosphagen kinase enzymes subsequent to the duplication of an ancestral arginine kinase gene Symbols AK, arginine kinase, *AK*, gene for AK, XK, YK, ZK, any other phosphagen kinases, *XK*, *YK*, *ZK*, genes for XK, YK, ZK, I, inactive protein, or none, *I*, gene for I

by mutation. On the other hand, genes for isoenzymes of arginine kinase, with slight structural differences of advantage in particular tissues or under the control of different control systems, might very well be maintained by selection It also seems unlikely that modern forms should possess two genes coding for identical *X*-kinases, because this would indeed be a freak of convergent evolution, unless in stepwise duplication the second step occurred very recently Once again, the possibility of isoenzymes of *X*-kinase exists

In summary, duplication events could give rise to a diversity of homologous enzymes both within and between species of the descendants These might be isoenzymes dealing with one substrate or enzymes dealing with related substrates Further predictions about evolving enzymes would be that such features as substrate specificity or structural rigidity might be less absolute

Annelid Phosphagen Kinases

Information on the phosphagen kinases in a range of polychaete annelids is set out in Table 1 The second column shows whether the species has been found to possess arginine kinase activity Other phosphagen kinases which have been demonstrated are also listed, and the third column shows the total of different activities known for each species The fourth column contains information on kinase specificity obtained from studies with substrate analogues (D-arginine, homoarginine, glycylglycocyamine, δ-guanidino-*n*-valerate, L-α-amino-γ-guanidinobutyrate and L-canavanine sulphate), and the fifth, in the cases where more than one activity has been shown, contains information about the additive nature of the reaction in the presence of mixtures of substrates The latter information is of use in reaching a decision as to whether two separate enzymes deal with two different substrates as opposed to one enzyme of lower specificity In the last column, where it has been possible to apply this technique, starch gel electrophoresis results for the enzymes are noted.

It can be seen that actual results with polychaete annelids completely parallel the theoretical expectations and can in fact be explained by the hypothesis illustrated in Fig 3, where three homologous sections of DNA were postulated The maximum number of different activities found in one species was three, including arginine kinase in *Myxicola* (as in Fig 3, possibility 5) and excluding it in *Nereis* (as in possibility 6) In both cases, additivity has been only partially checked, but was found to occur with the substrate combinations tested The possibility therefore remains that only two enzymes, coded for by two homologous sections of DNA, could possess the activities found As discussed by Virden and Watts[37], however, the data of Thoai[38], Thoai *et al* [39] and Gaffney *et al* [40] on the specificity of kinases the substrate of which contains the guanidino-ethane group (Fig 1) make it clear that although specificity is low as between the members of the group, activity with other natural guanidines is almost entirely absent This would make it extremely unlikely that the GK and TK activities in *Myxicola* are functions of the same enzyme or the CK and TK in *Nereis* For *Nereis*, all possible paired activities are thereby ruled out, while for *Myxicola* the AK and GK might still be functions of one enzyme The activity of the *Myxicola* extract with glycocyamine was very high, 45 per cent of the arginine kinase level of activity, although the specificity with substrate analogues was shown to be lower than for most arginine kinases, the highest unusual activity, with D-arginine, was only 5 per cent of the arginine kinase level It seems reasonable to conclude that there are three distinct enzymes in *Myxicola*

Aphrodite and *Hyalinoecia* both have arginine kinase plus creatine kinase (possibility 2) and *Sabellaria* has arginine kinase plus taurocyamine kinase Until the latter species was investigated, it seemed that there might have been some evolutionary bias towards the AK and CK combination, which occurs also in two other major groups, the Tunicata[37] and the Echinodermata[41,42], suggesting that evolution from AK to CK may be simpler or more advantageous than from AK to any other kinase In this respect, as in others, it now seems that the Annelida

Table 1

Animal	Presence of kinases AK	CK	GK	TK	Total No	Specificity	Additivity (where more than one kinase present)	Isoenzymes
Aphrodite aculeata[37]	AK	CK			2	Rather low	nt	
Arenicola marina[44]			GK	TK	2	nt	nt	
Branchiomma vesiculosum[37]				TK	1	Normal	—	
Hermione hystrix[44]		CK			1	nt	—	
Hyalinoecia tubicola[37]	AK	CK			2	Normal	nt	1 AK
Myxicola infundibulum[37]	AK		GK	TK	3	Rather low	AK+TK	
Nephthys caeca[44]		CK	GK		2	nt	nt	
Nereis fucata[37]		CK	GK	TK	3	Normal	GK+TK GK+CK	1 CK 1 GK (coincident)
Sabella pavonina[37]	AK				1	Low	—	2 AK
*Sabellaria alveolata**	AK			TK	2	nt	AK+TK	
Travisia forbesii[45]	AK	CK			2	nt	nt	

AK, Arginine kinase, CK, creatinine kinase, GK, glycocyamine kinase, TK, taurocyamine kinase, nt, not tested

* Unpublished results.

must be distinguished from other groups The fourth possibility, two kinases neither of them AK, is represented by *Arenicola* and *Nephthys*

Sabella affords an example of the situation where no kinase other than AK is found, the first possibility This enzyme, however, showed a particularly low specificity toward substrate analogues, suggesting that this species, like the others, had an ancestor in which duplications occurred, and that it is also an example of evolution in action The indication of two AK isoenzymes in starch gel electrophoresis supports the view that *Sabella* is not a "living fossil" with only one kinase gene Possibility 3 is found in the case of *Branchiomma*, which shows only taurocyamine kinase activity, and in *Hermione*, with only creatine kinase Some reservation may be appropriate with *Hermione*, since the related species, *Aphrodite aculeata*, also found by Hobson and Rees to show only creatine kinase, was subsequently found to possess a relatively labile arginine kinase[37] If results for for phosphagens[17] are considered, a long list of species with only one phosphagen, differing from phosphoarginine, could be made In those species where kinase activities have been measured, however, agreement with the phosphagen results is not good, the enzyme measurements being clearly more sensitive and occasionally in conflict with those for the phosphagens[37]

The group specificity of enzymes towards phosphagens containing the guanidinoethane group, the fact that *Sabella* (AK), *Aphrodite* (AK+CK) and *Myxicola* (AK+GK+TK) were all capable of phosphorylating substrate analogues, and that a number of arginine kinases had a lower than usual stability are all features to be expected if the enzymes were still undergoing evolution

Evidence for multiple forms of the activities studied is not abundant Only three of the species possessed kinase activities powerful enough to be revealed by the rather insensitive methods used for staining after starch gel electrophoresis In all these, the activities found had migrated with medium velocity at *p*H 8 5 In *Nereis* the CK and GK activities were located in the same band indicating (in conjunction with the additivity of these activities) that the two enzymes had the same mobility In *Hyalinoecia* only AK could be demonstrated on the gel, and in *Sabella*, where no other activity had been shown, two arginine kinase bands appeared, one in the same zone as the activities in other species, and one of slightly greater mobility Including isoenzymes, more than three activities have never been shown in one species As it seems that the properties of molecular dimensions and charge upon which electrophoretic mobility depends may be closely similar in most of the enzymes, isoenzymes may occur which cannot be demonstrated under the conditions of electrophoresis used

In contrast to the diversity of phosphagen kinases found among the polychaete annelids, only lombricine kinase could be detected[43] among sixteen species of oligochaete annelids As previously mentioned, polyploidy is common in the latter group, accompanied by parthenogenesis At first sight, it seems paradoxical that, in a group of species known to have at least a double complement of genetic material, only one enzyme is found, whilst in a related group several homologous enzymes occur There are two main possibilities for the evolution of the oligochaete situation, either this group diverged from the polychaetes after the primary duplication of the arginine kinase gene and followed the same pathway as a polychaete in group 3 (Fig 3) or, alternatively, the extra gene making possible the evolution of lombricine kinase arose in the polyploidization The fact that no diversity of kinases has been evolved is witness to the great importance of recombination in evolution The mutation rate in the oligochaete genes is unlikely to be greatly different from that in the polychaetes, but regrouping of portions of changed genetic material is virtually absent in a parthenogenetic organism In consideration of this, it seems most probable that the oligochaetes and polychaetes diverged after the primary gene duplication and that polyploidization in the former group did not occur until after the evolution of lombricine kinase was complete

The hypothesis presented here for the origin of diverse enzymes following duplication of the appropriate material in the genome is in itself no novelty Its theoretical development, originally undertaken to examine its feasibility for the annelid phosphagen kinases, should also prove useful in understanding the evolution of other proteins Perhaps the most remarkable feature of the somewhat sparse data available for the annelid kinases is that, in addition to being in no way in conflict with the hypothesis, they afford illustration of almost all the possibilities predicted by it

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[1] Huxley, J , *Evolution The Modern Synthesis*, 124 (Allen and Unwin, London, 1942)
[2] Koshland, jun , D E , *Adv Enzymol* ,**22**, 45 (1960)
[3] Sanger, F , *Proc Chem Soc* , **76** (1963)
[4] Watts, D C , in *Studies in Comparative Biochemistry* (edit by Munday K A), 159 (Pergamon Press, Oxford, 1965)
[5] Hooton, B T , and Watts, D C , *Biochem J* ,**100**, 637 (1966)
[6] Thoai, N V , Kassab, R , and Pradel, L A , *Biochim Biophys Acta*, **73**, 574 (1963)
[7] Yanofsky, C , in *Cytodifferentiation and Macromolecular Synthesis* (edit by Locke, M), 15 (Academic Press, New York and London, 1963)
[8] Garen, A , Levinthal, C , and Rothman, F , *J Chim Phys* , **58**, 1068 (1961)
[9] Perutz, M F , Kendrew, J C , and Watson, H C , *J Mol Biol* , **13**, 669 (1965)
[10] Zuckerkandl, E , and Pauling, L , in *Evolving Genes and Proteins* (edit by Bryson, V , and Vogel, H J), 97 (Academic Press, New York and London, 1965)
[11] Anfinsen, C B , *The Molecular Basis of Evolution* (John Wiley, New York, 1959)
[12] Benzer, S , *Proc US Nat Acad Sci* , **47**, 403 (1961)
[13] Freese, E , and Yoshida, A , in *Evolving Genes and Proteins* (edit by Bryson, V , and Vogel, H J), 341 (Academic Press, New York and London, 1965)
[14] Stebbins, G L , *Variation and Evolution in Plants* (Columbia University Press, New York, 1950)
[15] Muldal, S , *Heredity*, **6**, 55 (1952)
[16] Smith R I , *Systemat Zool* , **7**, 60 (1958)
[17] Ohno, S , and Atkin, N B , *Chromosoma*, **18**, 455 (1966)
[18] Huxley, J , *Evolution The Modern Synthesis*, 89 (Allen and Unwin, London, 1942)
[19] Sturtevant, A H , *Genetics*, **10**, 117 (1925)
[20] Bridges, C B , *J Hered* **26**, 60 (1935)
[21] Bridges, C B , *Science*, **83**, 210 (1936)
[22] Roberts, P A , *Genetics*, **54**, 969 (1966)
[23] Lewis, E B , *Proc US Nat Acad Sci* ,**27**, 31 (1941)
[24] Bender, H A *Genetics*, **55**, 249 (1967)
[25] Roberts, P A , *Genetics*, **52**, 1017 (1965)
[26] Watts, R L , and Watts, D C , *J Theoret Biol* (in the press)
[27] Ingram, V M , *Nature*, **189**, 704 (1961)
[28] Smithies, O , Connell, G E , and Dixon, G H , *Nature*, **196**, 232 (1962)
[29] Rutter, W J , *Fed Proc* ,**23**, 1248 (1964)
[30] Thoai, N V , and Roche, J , in *Taxonomic Biochemistry and Serology* (edit by Leone, C A), 347 (Ronald Press Co , New York, 1964)
[31] Lerman, L S , *Proc US Nat Acad Sci* , **49**, 94 (1963)
[32] Hartley, B S , Brown, J R , Kauffman, D L , and Smillie, L B , *Nature*, **207**, 1157 (1965)
[33] Smith, E L , Markland, F S , Kaspar, C B , Delange, R J , Landon, M , and Evans, W H , *J Biol Chem* ,**241**, 5974 (1966)
[34] Wagner, R P , and Mitchell, H K , *Genetics and Metabolism*, 355 (John Wiley, New York, 1964)
[35] Horowitz, N H , in *Evolving Genes and Proteins* (edit by Bryson, V , and Vogel, H J), 15 (Academic Press, New York and London, 1965)
[36] Comings, D E , *Nature*, **212**, 545 (1966)
[37] Virden, R , and Watts, D C , *Comp Biochem Physiol* , **13**, 161 (1964)
[38] Thoai, N V , *Bull Soc Chim Biol* , **39**, 197 (1957)
[39] Thoai, N V , Robin, Y , and Pradel, L , *Biochim Biophys Acta*, **73**, 437 (1963)
[40] Gaffney, T J Rosenberg, H , and Ennor, A H *Biochem J* , **90**, 170 (1964)
[41] Moreland, B , Watts, D C , and Virden, R , *Nature*, **214**, 458 (1967)
[42] Griffiths, D E , Morrison, J F and Ennor A H , *Biochem J* , **65**, 612 (1957)
[43] Thoai, N V , in *Colloque sur L'Evolution Biochimique*, Paris, 1967 (in the press)
[44] Hobson, G E , and Rees, K R , *Biochem J* , **65**, 305 (1957)
[45] Thoai N V , Thiem, N V , Lacombe, G , and Roche, J , *Biochim Biophys Acta*, **122**, 547 (1966)

LETTERS TO THE EDITOR

ASTRONOMY

Stimulation of Interstellar OH by Phonons

THE attempts which have so far been made to explain the observed features of the interstellar OH molecule cannot be regarded as entirely successful[1] The most interesting observations are the OH sources in emission, where it is clear that some form of maser action is involved Any theoretical model to explain these data must provide a mechanism for producing stimulated emission while also accounting for the formation and stability of the OH molecule under the conditions prevailing in the emitting region An optical pumping device involving absorption and re-emission at $\lambda = 3080$ Å in the $X^2\Pi \rightarrow A^2\Sigma^+$ bands was suggested by Perkins *et al* [2], but this process has recently been criticized by Solomon[3] It has been argued[3] that the OH would be dissociated faster than it would be pumped by ultraviolet absorption Solomon has in fact suggested that OH may form in the upper level of the Λ doublet by inverse pre-dissociation reactions taking place at a few thousand degrees Kelvin, but the possibility of other formation and stimulation mechanisms cannot be ruled out at the present moment

The possibility that OH may be closely associated with grains has so far not been seriously considered Recent observations by Ishida and Akabane[4] indicating a close coincidence between the isophotes of OH emission with those of optical absorption in *W*3 seem strongly to support such a view According to current ideas[5], interstellar grains consist of graphite flakes of radii $\sim 5 \times 10^{-6}$ cm from stellar sources which accrete mantles chiefly consisting of H_2O, in quiescent *HI* regions The outer ice mantles of the grains are quickly sputtered away[5,6] in shock fronts and *HII* regions where the gas temperature rises above a few thousand degrees. The end-products of the sputtering reaction cannot be specified definitely, but it is not unlikely that large quantities of OH might be liberated by this process It is also likely that free graphite surfaces will be very efficient in catalysing the recombination of OH in the manner discussed by Stecher and Williams[7] A considerable fraction of the OH formed in hot clouds may be able to diffuse into the graphite lattice and become trapped between the hexagonal layers The inclusion of OH as impurities would make them quite stable against dissociation by ultraviolet light It would also then be reasonable to expect that solid state effects would play a part in the stimulation of the OH molecule

The results of two recent experiments seem to have an important bearing on this issue

(1) It is found that acoustic microwaves may be greatly amplified in a semiconducting material provided currents involving supersonic electron drift velocities can be generated[8] In these conditions the electrons, which may be imagined as bunched together in the potential troughs of the wave, transfer their energy towards amplifying the acoustic wave

(2) Acoustic microwave phonons may be generated by electromagnetic microwaves and, conversely, acoustic microwave phonons may give rise to electromagnetic microwaves in conducting and semiconducting material[9]

The following sequence of events is proposed Thin graphite flakes of radii $\sim 10^{-6}$ cm containing OH impurities* are exposed to collisions with gas atoms in a shock front or an *HII* region where the kinetic temperature $T > 3{,}000°$ K Because of collisions the grains acquire angular speeds ω given by

$$\frac{1}{2} I\omega^2 \simeq \frac{3}{2} kT \qquad (1)$$

where I is the moment of inertia For a thin flake (thickness $\sim 10^{-7}$ cm) an angular speed $\sim 10^9$ Hz may be obtained quite easily in the regions we consider With respect to axes fixed in the grain the galactic magnetic field will have rapidly oscillating components and the process of diamagnetic relaxation will take place The details of this process have been discussed elsewhere[5] in relation to the problem of the alignment of grains The energy dissipated by such a process is expected to be largely in the form of low amplitude microwave phonons. It is also likely that a background microwave radiation at $\sim 10^9$ Hz would feed in low amplitude phonons at the same frequency (compare experimental result (2)) If the conditions necessary for experimental result (1) to hold are satisfied, these microwaves may be very greatly amplified in a maser-like fashion There is also some evidence to suggest that maximum amplification occurs for frequencies in the range 10^9–10^{10} Hz, very close to the frequency of the Λ doubling transition in OH (ref 8) The result will be a continuous pile-up of phonon states with energies close to that required for the excitation of the upper state of the Λ doublet Finally, from the experimental result (2) we would expect the microwave phonons to give rise to photons of the same wavelength which actually cause the population inversion in the OH impurities

The velocity of sound in graphite is $\sim 10^6$ cm s^{-1} Although this is close to the thermal velocities of conduction electrons in the grain, the production of systematic currents with drift velocities $v \sim 10^6$ cm s^{-1} would require external electric fields $E \sim v/\mu$, where μ denotes the electron mobility For ideal graphite $\mu \simeq 3 \times 10^6$ E S U (ref 10), but for impure samples it is considerably lower, for our case it may be reasonable to assume $\mu \simeq 10^5$ E S U (ref 11) The external electric field required is thus $E \sim 10$ E S U Such a field may be supplied by ~ 10 electrons residing at any time on a grain of radius $\sim 10^{-6}$ cm By considering the rates of arrival of electrons and protons at a grain surface, Spitzer[12] has shown that such a net charge is indeed likely in an *HII* region

The estimation of the energy output is difficult on account of several uncertain parameters involved The very small sizes, $\leqslant \sim 0\ 01$ pc, of the emitting regions would suggest that drastic compressions of the interstellar medium must be involved For our model it would suffice if this compression is chiefly restricted to the dust component The early work of Spitzer[13] suggests that such compressions of dust relative to gas may take place in the presence of intense radiation fields, which may be appropriate to clouds in *O* and *B* associations This is due to an attractive force which is set up between two dust particles, because each grain casts a "shadow" on the other and cuts off the radiation field in a small solid angle Assuming a compression of dust from an initial dimension of ~ 5 pc to $\sim 0\ 01$ pc, the dust density would increase from $\sim 10^{-11}$ cm^{-3} to $\sim 10^{-3}$ cm^{-3} The gas density may rise by a smaller factor, say, 10^2–10^3

According to the picture we have, the chief source of energy for the excitation of OH arises from the constant arrival of electrons at the grain surface It is the electrostatic force exerted by the impinging electrons which accelerates the conduction electrons to supersonic speeds, and these in turn give up their energy to amplify the 18 cm microwaves The arrival rate of electrons is

$$\sim n_e \pi r^2 v_e \qquad (2)$$

where n_e is the number density of ambient electrons, v_e is their thermal velocity and r is the radius of the grain

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* We may assume that graphite flakes with a high impurity content possess the semiconducting properties required for experimental result (1) to be valid The impurities have the effect of separating the valence and conduction bands of graphite, and reducing the free carrier concentration

The rate of supply of energy to the conduction electrons is therefore

$$E \simeq n_e \pi r^2 v_e kT \text{ erg s}^{-1} \quad (3)$$

assuming that the entire kinetic energy of impinging electrons is transferred to the solid. With plausible values, $n_e \simeq 10^2$ cm^{-3}, $r \simeq 3 \times 10^{-6}$ cm, $T \simeq 10^3$ °K, $v_e \simeq 10^7$ cm s^{-1}, we obtain $E \simeq 3 \times 10^{-16}$ erg s^{-1} If an appreciable fraction of this energy is converted into 18 cm wavelength photons, the photon production rate will be $\sim 10^2$ s^{-1} grain^{-1}. With an estimated grain density of $\sim 10^{-3}$ cm^{-3} this would give a production rate of ~ 0 1 photons cm^{-3} s^{-1}, similar to the minimum output (assumed isotropic) of the stronger sources

A further linear compression of the dust by a factor $\sim 10^2$–10^3 would raise the total output of radio photons to $\sim 10^6$–10^8 cm^{-3} s^{-1} We may then be able to obtain very high brightness temperatures, $T > 10^{13}$ °K, for OH sources without requiring a collimation of the microwave output

A slight degree of collimation is to be expected in the microwave output if the highly anisotropic graphite flakes are aligned by a magnetic field The harmonic motion of the radiating dipoles will be largely confined to the hexagonal planes, and the scattered light profile of an individual grain will be strongly directed along the crystal axis We may therefore expect the microwave output to be directed more or less along the magnetic field It may also be possible to explain some of the polarization effects which have been reported in terms of the alignment of spinning grains The narrow widths of the observed OH lines may be understood as being because of the very low thermal velocities of the grains

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[1] Robinson, B J, and McGee, R X, *Ann Rev Astron Astrophys*, **5**, 183 (1967)
[2] Perkins, F, Gold, T, and Salpeter, E E, *Astrophys J*, **145**, 361 (1966)
[3] Solomon, P M, *Nature*, **217**, 334 (1968)
[4] Ishida, K, and Akabane, K, *Nature*, **217**, 435 (1968)
[5] Wickramasinghe, N C, *Interstellar Grains* (Chapman and Hall, 1967)
[6] Wickramasinghe, N C, *Mon Not Roy Astron Soc*, **131**, 177 (1966)
[7] Stecher, T P, and Williams, D A, *Astrophys J*, **146**, 88 (1966)
[8] Paige, E G S, in *Phonons in Perfect Lattices and in Lattices with Point Imperfections* (edit by Stevenson, R W H), (Oliver and Boyd, 1966)
[9] Abeles, B, *Phys Rev Letters*, **19**, 1181 (1967)
[10] Soule, D E, *Phys Rev*, **112**, 698 (1958)
[11] Klein, C A, *Rev Mod Phys*, **34**, 56 (1962)
[12] Spitzer, L *Astrophys J*, **93**, 396 (1941)
[13] Spitzer, L *Astrophys J*, **94** 237 (1941)

Lunar Occultation of a Solar Active Region at 4 cm Wavelength

The solar eclipse of July 20, 1963, was observed from Ann Arbor, Michigan, using the 85 ft paraboloid telescope of the University of Michigan As observed from Michigan the solar eclipse was partial, however, the Moon completely occulted two discrete regions on the Sun—one near the east limb and the other near the west limb We observed the lunar occultation of the eastern region At the 4 cm wavelength at which this occultation was observed, the pencil beam of the radio telescope had a half-power width of 5′ 95 of arc Drift scans were taken across the region before the occultation in order to find the position of the peak These scans indicated an angular size of about 3′ of arc and a position which was in agreement with the position indicated by Stanford λ 9 1 cm maps Thus the beam almost completely covered the discrete region, and the observations consisted in tracking the region throughout the period of occultation with the beam pointed on the peak of the region The occultation was observed in both immersion and emersion.

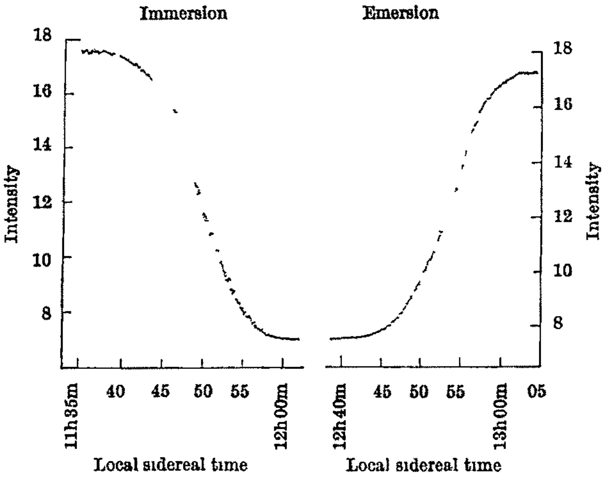

Fig 1 The curves of immersion and emersion of the eclipse of July 20, 1963 The radiometer intensity is in arbitrary units

Both analogue and digital records at intervals of 4 s were taken The occultation curves for immersion and emersion are shown in Fig 1 From the calculated positions of the Moon's limb at intervals of 4 s, we have determined the position angle and the velocity at which the Moon's limb moved across the source These calculations led to an independent determination of the position of the source It coincided well with the position of the region as given by the Stanford map at λ 9 1 cm

The strip-integrated brightness distribution was obtained by differentiating the occultation curve for both immersion and emersion The smoothed brightness distributions for immersion and emersion are shown in Fig 2 From the emersion distribution it seems that the source has a central component of angular size about 45″ of arc and a very weak outer component Furthermore, the distribution is not symmetrical, the intensity rises sharply on the east, and decreases rather slowly on the west side

From the derived brightness distributions, one can find the exact times of disappearance and reappearance of the peak of the active region These times in turn give the position of the peak with a precision of better than 5″ of arc Converted into heliographic co-ordinates the position thus obtained is N. 9°·5, E 52°·6 at 2200 hours UT This position is slightly displaced from the position of the associated sunspot as it appears on the Fraunhofer Institut map This is because the source at λ 4 cm is situated above the sunspot Indeed, assuming that the source is situated radially above the associated sunspot, the height estimated is found to be 14,000 km above the photosphere These results are in agreement with the observations[1] made earlier on λ 3 cm, and are consistent

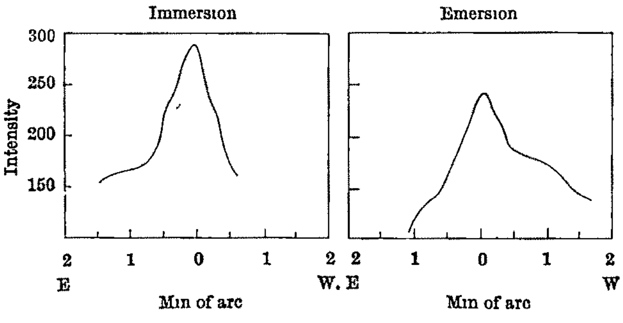

Fig 2 The derived strip-scan brightness distributions across the discrete region, zero of ordinate represents the position where the beam was pointed Directions of scanning for immersion and emersion are at angles of 23° and 4° 5, respectively, to the east–west diameter of the solar disk

with the theory of resonance absorption that has recently been proposed to explain the slowly varying component[2,3] According to this theory, resonance absorption at harmonics of the gyrofrequency in the strong sunspot magnetic field can explain the small size and high brightness temperature of the source at centimetre wavelengths This theory also indicates that the intense peak at centimetre wavelengths should lie above the associated sunspot

We thank Professor F T Haddock for permitting one of us (M R K) to use the 85 ft dish for these observations

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[1] Kundu, M R , *CR Acad Sci* , 246, 2740 (1958)
[2] Kakinuma, T , and Swarup, G , *Ap J* , 136, 975 (1962)
[3] Zheleznyakov, V V , *Soviet Astro* , 6, 3 (1962)

PLANETARY SCIENCE

Sunspot Cycle and the Quasi-biennial Stratospheric Oscillation

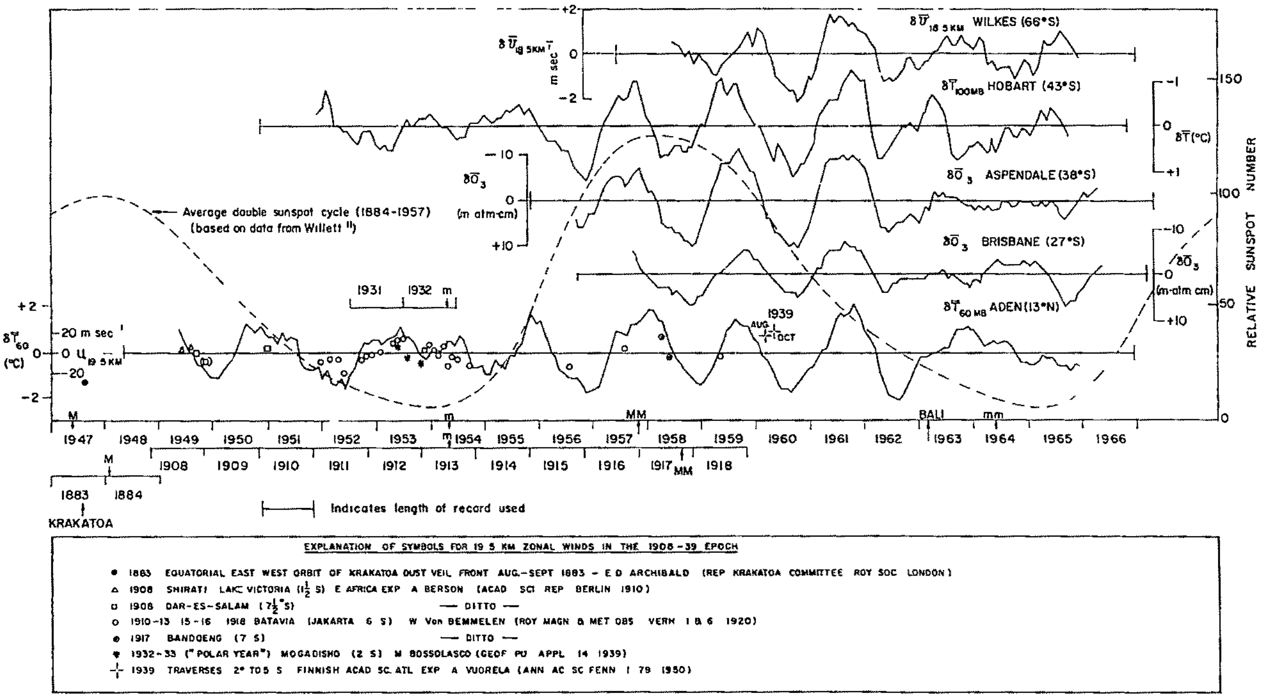
Previous communications[1,2] reported observations from the southern hemisphere indicating an attenuation of the quasi-biennial stratospheric oscillation in the years 1963–64 The 24 to 26 month cycles of wind and temperature in the lower stratosphere, and of total ozone, weakened or vanished after a series of pronounced cycles[2,3] in the years 1955 to 1962 (which were characterized by high sunspot activity)

During recent years, quasi-biennial modes reappeared with a reversed phase in observations from many places The breakdown of the oscillation in 1963 had been linked[1] with a reduction of direct solar radiation in the southern hemisphere, following the injection of volcanic dust into the lower stratosphere during the eruption of Mount Agung, Bali (8° S , 115° E), in March 1963 Ebden[4] has since shown that a weakening and subsequent phase reversal in the quasi-biennial modes in 30 and 50 mbar temperatures at Ascension Island (7° 58′ S , 14° 24′ W , about 14,000 km west of Bali) commenced with a relatively sudden and sustained temperature rise which was compatible with the eastward spread of the dust and its radiation effects

Ramanathan[5] had already noted that in the long series of observations of total ozone at Arosa (47° N) from 1939 to 1963 breaks and phase shifts occurred in the quasi-biennial mode, about half-way between sunspot maxima. Because 1964 was a year of sunspot minimum an extraterrestrial influence on the oscillation is suggested

We present here further evidence to support a connexion of the quasi-biennial oscillation with sunspot cycle, including observations in the tropics north of the equator The curves in Fig 1 summarizing this evidence are based on time series of monthly means to which a suitable filter[1] has been applied to eliminate trend and annual waves The recent break in the cycles of total ozone at Brisbane (27 5° S) and Aspendale (38° S) in the southern hemisphere, where the ozone amplitude has been generally larger than in the northern hemisphere[6], can be seen The reappearance of a quasi-biennial cycle with reversed phase in 1965 is indicated Early Australian ozone observations with the old type Dobson spectrograph at Mount Stromlo, Canberra (35° S), in the four years preceding a sunspot minimum in 1932 also showed a markedly weakening biennial mode

In the longer records of 60 mbar temperature at Aden (13° N) and Hobart (43° S) the attenuation prior to the sunspot minimum in 1964 stands out clearly There is no discernible quasi-biennial oscillation for several years preceding the sunspot minimum in 1954 At Aden this means a break in secular continuity because the earliest records, before 1951, reveal a marked biennial mode The curve for the meridional wind component at 18 5 km ($\simeq$ 60 mbar) over Wilkes (66° S) on the coast of Antarctica —a corrected and extended version of one previously published (ref 1)—bears out the global nature of the



EXPLANATION OF SYMBOLS FOR 19 5 KM ZONAL WINDS IN THE 1908–39 EPOCH

- ● 1883 EQUATORIAL EAST WEST ORBIT OF KRAKATOA DUST VEIL FRONT AUG.–SEPT 1883 – E D ARCHIBALD (REP KRAKATOA COMMITTEE ROY SOC LONDON)
- △ 1908 SHIRATI LAKE VICTORIA ($1\frac{1}{2}$ S) E AFRICA EXP A BERSON (ACAD SCI REP BERLIN 1910)
- □ 1908 DAR-ES-SALAM ($7\frac{1}{2}$°S) —— DITTO ——
- ○ 1910–13 15–16 1918 BATAVIA (JAKARTA 6 S) W Von BEMMELEN (ROY MAGN & MET OBS VERH 1 & 6 1920)
- ◉ 1917 BANDOENG (7 S) —— DITTO ——
- ✱ 1932–33 ("POLAR YEAR") MOGADISHO (2 S) M BOSSOLASCO (GEOF PU APPL 14 1939)
- ┼ 1939 TRAVERSES 2° TO 5 S FINNISH ACAD SC ATL EXP A VUORELA (ANN AC SC FENN I 79 1950)

Fig 1 Solar activity and breaks in the quasi-biennial oscillation of temperature, wind and total ozone The operator δ signifies 12 month minus 24 month moving means representing a band-pass filter Major and minor sunspot maxima are shown as *MM* and *M*, minima as *mm* and *m*, respectively

phenomenon, though with a reduction of amplitude At Macquarie Island (53° S) quasi-biennial cycles of the 100 mbar temperature continued after 1962 (ref 2)

In search of still earlier evidence, we collected all records (see legend to Fig 1) of sufficiently high pilot balloon ascents near the equator where, in the lower stratosphere annual and semi-annual waves in zonal wind are swamped by the quasi-biennial variations They can thus be recognized in scattered samples, each consisting of a few days of observations, as seen in Fig 1 in the plots of 19 5 km ($\simeq$ 60 mbar) zonal wind velocity in the 1908–33 epoch (on a level with the Aden temperature curve) The zonal velocity indicated by the dust veil from the Krakatoa eruption is also shown The connexion with sunspot cycle is brought out in these wind data in another way, by a broadly in-phase relation with the Aden curve, the time scales having been fixed relative to the sunspot cycle (the minimum *m*)

An association of amplitude fluctuation in the quasi-biennial oscillation of temperature and ozone with the single (about 11 yr) sunspot cycle suggests the influence of variable solar corpuscular emission This would, however, require a polar rather than an equatorial drive Lindzen[7] concluded that one can rule out an equatorial drive by radiative-photochemical response (that is, through absorption in the Hartley–Huggins bands of ozone) to small fluctuations in solar ultraviolet emission Variation in the absorption of reflected light from below in the Chappuis band of O_3 in the visible was, however, a feasible cause of the oscillation, and semi-annual (6 month) variations of cloudiness in the tropics have been suggested as a mechanism To explain a biennial (24 month) oscillation, one has to invoke a mechanism of sub-harmonic response which Staley[8] questions in regard to zonal momentum in the equatorial stratosphere In any case, as Staley points out, this would yield integral multiples of semi-annual or, in the extratropical region, annual forcing periods Subharmonic response would therefore fail to explain a period of about 26 months (A value of 26 2 months has been calculated from monthly means of zonal velocity based on 120 months of records at Balboa, at 9° S (ref 9))

We can show, however, that variations in solar emission associated with the double (22 yr) sunspot cycle[11] provide a possible mechanism by which the frequency of a biennial cycle can be shifted Assuming a sinusoidal carrier wave of orbital frequency $\nu_0 = \frac{1}{2}$ yr^{-1} with angular modulation by a single wave with frequency $\nu_M = 1/22$ yr^{-1}, one obtains principal side-band frequencies of $\nu_0 \pm \nu_M$, the corresponding periods being 22 0 and 26 4 months It is important to point out that angular modulation is a feasible mechanism whereas amplitude modulation yielding similar side bands would require additional energy presumably not available, for the oscillation We are indebted to Mr R R McGregor who drew our attention to this distinction

At this stage no plausible mechanism can be suggested whereby only the lower side band with an approximately 26 month period is observed, nor can we explain how the amplitude of the quasi-biennial oscillation varied with the single, 11 yr solar cycle But it is noteworthy that low-amplitude wind cycles with a 13 month period which have been detected in good data from the lower tropical stratosphere[10] can be interpreted in terms of modulation by this cycle, of a weak annual wave present in the tropical belt

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[1] Berson, F A , *Nature*, 210, 1243 (1966)
[2] Kulkarni, R N , *Nature*, 210 286 (1966)
[3] Berson, F A , *Proc Polar Meteorology Symposium, Geneva, 1966, WMO Tech Rep* (in the press)
[4] Ebden, R A , *Weather*, 22, 245 (1967)
[5] Ramanathan, K R , *Proc Ozone Symposium, Albuquerque, WMO publ P.1* (1964)
[6] Kulkarni, R N , *Quart J Roy Met Soc* , 92, 363 (1966)
[7] Lindzen, R S , *J Atmos Sci* , 22, 469 (1965)
[8] Staley, D O , *J Atmos Sci* , 20, 506 (1963)
[9] Reed, R J , *Met Abh* , 36, 245 (1963)
[10] Probert-Jones, J R , *Quart J Roy Met Soc* , 90, 16 (1964)
[11] Willett, H C , *J Atmos Sci* , 22, 120 (1965)

Longitudinal Spread of pc 1 Micropulsations in the Magnetosphere

One of the important problems in the field of pc 1 micropulsation (pearl) research is to determine what portion of the magnetosphere is involved in the propagation of an individual structured pc 1 event. It is generally recognized[1] that these micropulsations propagate back and forth in a dispersive medium[2,3] between conjugate hemispheres guided by the Earth's magnetic field, but it is not known whether the waves are constrained to propagate along a single field line or whether they can spread out along an appreciable portion of a magnetic shell Because energy is transferred back and forth between trapped particles and ultra-low frequency (ULF) waves in the cyclotron resonance interaction[4], and because trapped particles undergo longitudinal drift, it is reasonable to assume that a structured pc 1 micropulsation may spread out along a magnetic shell even if it started on a particular field line Dispersion measurements[5–8] which have been used to locate the latitude where the field lines or shells intercept the surface of the Earth cannot be used to determine longitude, and hence one must resort to interstation comparison of data

Unfortunately, measurements of differences in polarization and of the arrival time of the same event at spaced stations are not sufficiently accurate to allow an unambiguous measurement of the longitude or spread in longitude of the magnetospheric portion of the path Comparison of the signal strength or occurrence rates at different stations may also be impossible because the final portion of the propagation path involves an ionospheric duct with unknown attenuation parameters

Fig 1 illustrates the type of interaction that may be present at the interface between the magnetosphere and ionosphere In the reflexion region, part of the incident wave must be reflected back along the field line and part must enter the ionospheric waveguide and be ducted horizontally in all directions at the broad minimum of phase velocity located between 300 and 700 km[9,10] Both

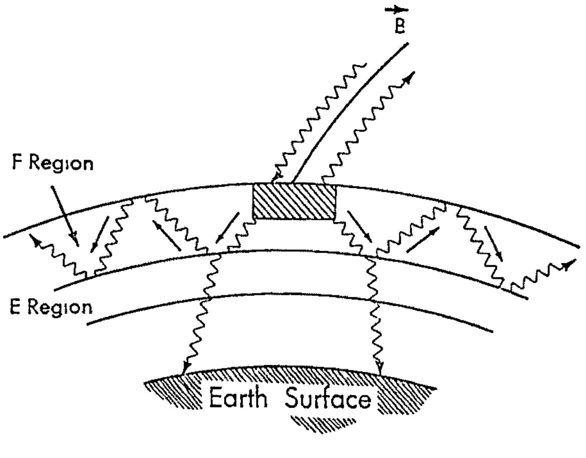

Fig 1 Possible ray paths of a structured pc 1 micropulsation The wavy lines represent the direction of propagation of the incident reflected and ducted waves as well as those which leak out of the *F*-region duct Mode conversion takes place in the cross-hatched area

the incident and reflected waves that are guided must be left-hand polarized whereas the unguided or ducted wave cannot have left-hand polarization Because waves are detected at the surface of the Earth, the waveguide must be leaky at the lower boundary and perhaps also at the upper boundary The spectral energy density of the received signal may be modified drastically during the short passage through the lower F and E region after leaking out of the duct because attenuation is orders of magnitude larger at these lower altitudes[9,10]

Some of the effects of this attenuation can be demonstrated by noting that stations located near 65° geomagnetic latitude rarely detect structured pc 1 at night[11], even though mid-latitude stations[8] detect many events at night which propagate on field lines or shells entering the duct at about 65° Because of this strong local absorption, it is difficult to determine the longitudinal extent of the magnetospheric path from interstation comparison of signal strengths and occurrence rates of pc 1 events

Further progress can, however, be made towards the solution of the longitude problem because of recent advances[12] in the interpretation of the pi 1 events known as "irregular pulsations of diminishing period" (IPDP)[13] These events usually last about an hour and consist of broad-band noise gradually increasing in frequency with structured elements frequently superimposed These structured elements arrive simultaneously in conjugate hemispheres and do not have a simple repetitive pattern[14] It has been shown[12,14] that these elements are guided along the magnetic field lines after having been initiated out in the magnetosphere near the equator Structured pc 1 and structured pi 1 events have the same frequency range, so that the effects of the ionosphere on these two different classes of guided micropulsations should be very similar

The results of the dispersion analysis of a large IPDP data sample[12] show that the structured elements propagate along field lines with equatorial geocentric distances between 7 and 13 Earth radii The diurnal variation of occurrence[1,12,15] and the rising frequency of IPDPs show that the event is characterized by a motion of some disturbance region being swept in and approaching the Earth by crossing field lines in the late evening hours

An interstation comparison of data presented in Fig 2 shows that the rate of detection of IPDP events at Seattle is approximately the same as that at College and is strongly associated with local magnetic disturbances as indicated by comparison with the Victoria K-index The College data were taken from the *High-Latitude Geophysical Data*[16] series without regard to emission frequency, and the Seattle data were scaled from original records in the same fashion Upper frequency limits of approximately 1 Hz are common at Seattle, whereas the IPDPs detected at College rarely exceed 0 5 Hz[15] Because all these emissions propagate on field lines which enter the ionosphere at higher latitudes than College[12], the loss of high frequency activity at College should not be caused by F-region duct attenuation but more likely by attenuation at lower altitudes after leaking out of the bottom edge of the duct Furthermore, the fact that the Seattle rate is essentially the same as the College rate, as shown in Fig 2, indicates that only a very small attenuation is incurred by the passage of the waves through an additional 1,500 km path in the F-region duct

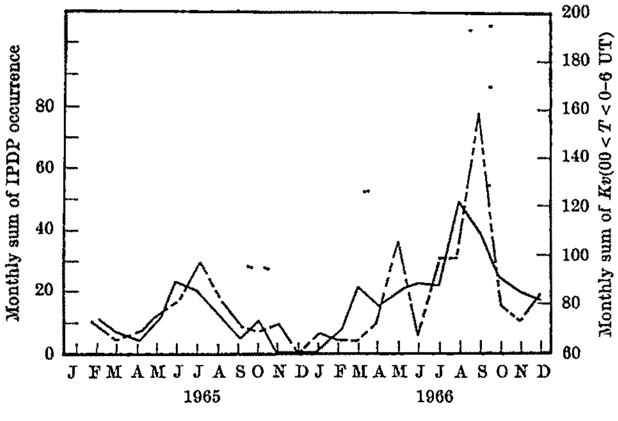

Fig 2 The variation of monthly occurrence of IPDP events for 1965 and 1966 The dashed line represents Seattle data and the solid line is for College data The dotted line forms a histogram showing the variation of the monthly sum of the local magnetic index Kv for Victoria, B C , during the hours (0–6 UT) , $\Sigma[Kv(0\text{–}3\ \text{UT} + Kv(3\text{–}6\ \text{UT})]$

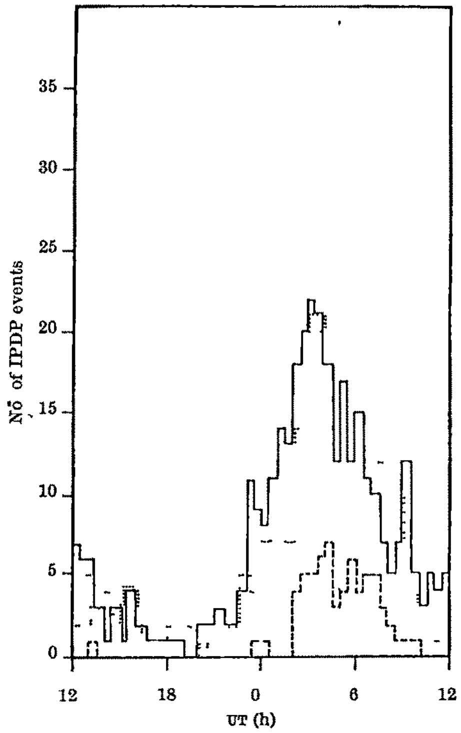

Fig 3 Three superimposed histograms showing the diurnal variation of occurrence of IPDP events from February 1965 to December 1966 The dotted histogram shows the diurnal variation of all IPDP events observed at Seattle, the solid one is for all IPDP events observed at College, and the dashed curve shows the diurnal variation of all IPDP events detected simultaneously at both sites

Similar occurrence rates can exist for these two stations if they always see the same event or if they merely see different events with the same probability Fig 3 is a plot of the diurnal variation of occurrence of all IPDP events detected either at Seattle or at College or simultaneously at both sites It is clear that the two stations do not normally see the same event even though they are separated by only 2 h in longitude An explanation that seems reasonable for the good north–south propagation of micropulsations (similar IPDP occurrence rates at Seattle and College) and the poor east–west propagation (low rate of simultaneous IPDP events at Seattle and College) is anisotropic coupling into the F region at the reflexion point shown schematically in Fig 1 Anisotropic coupling into the duct is more likely than anisotropic propagation after the wave is in the duct because of the peculiar boundary conditions that must be considered at the magnetosphere–ionosphere interface

On the other hand, there are many observations[1,17] of the detection of identical structured pc 1 events at stations widely separated in longitude A possible explanation for these observations is that a structured pc 1 event can have an appreciable spread in longitude along a shell in the magnetosphere rather than be constrained to propagate along a flux tube that is very restricted in longitude

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[1] Tepley, L R , *J Geomag Geoelect* , 18, 227 (1966)
[2] Jacobs, J A , and Watanabe, T , *J Atmos Terr Phys* , 26, 825 (1964)
[3] Obayashi, T , *J Geophys Res* , **70**, 1069 (1965)
[4] Liemohn, H B , *J Geophys Res* , 72, 39 (1967)
[5] Dowden, R L , and Emery, M W , *Planet Space Sci* , 13, 773 (1965)
[6] Wentworth, R C , *J Geomag Geoelec* , 18, 257 (1966)
[7] Watanabe, T , *J Geophys Res* , 70, 5839 (1965)
[8] Liemohn, H B , Kenney, J F , and Knaflich, H B , *Earth Plan Sci Lett* , 2, 360 (1967)
[9] Teplev, L R , and Landshoff, R K , *J Geophys Res* , 71, 1499 (1966)
[10] Manchester, R N , *J Geophys Res* , 71, 3749 (1966)
[11] Heacock, R R , and Hessler, V P , *J Geophys Res* , 67, 3985 (1962)
[12] Knaflich, H B , and Kenney, J F , *Earth Planet Sci Lett* , 2, 453 (1967)
[13] Troitskaya, V A , *J Geophys Res* , 66, 5 (1961)
[14] Gendrin, R , Gokhberg, M , Lacourly, S , and Troitskaya, V , *CR Acad Sci* , 262, 845 (1966)
[15] Heacock, R R , *J Geophys Res* , 72, 399 (1967)
[16] Hessler, V P , editor, *High Lat Geophys Data*, **39–46** (Geophysical Institute, College, Alaska, 1965–66)
[17] Kennev, J F , and Knaflich, H B , *J Geophys Res* , 72, 2859 (1967)

Heating of the Upper Atmosphere during Geomagnetic Disturbances

During geomagnetic disturbances the upper atmosphere may be heated[1] either by injected charged particles and electrojets[2] or by magnetohydrodynamic waves and plasma oscillations[3] Early studies of the heating were based on some peculiarities of auroras at large heights[2] The detection of the increase of artificial satellite drag during geomagnetic disturbances[4-6] led to more widely accepted data on the phenomenon

Investigations of ordinary auroras by an interferometer method[7] show that within an error of ± 5 per cent the temperature of the upper atmosphere does not differ from that given in the 1965 Cospar International Reference Atmosphere for undisturbed conditions[8] This is the case even during auroras of the IV IBC type during which an energy of up to 600 ergs cm^{-2} s^{-1} can be introduced[9] About 10 per cent of this energy inevitably turns into heat Nevertheless, there are no observations showing considerable heating Before these investigations it was assumed[10] that during auroras at low heights a considerable cooling of the upper atmosphere may take place due to emissions by vibrationally excited NO and NO$^+$ molecules Such molecules can be easily formed not only at a high temperature but also as a result of different atomic exchange reactions Such reactions can develop most effectively during vibrational excitation of O$_2$ and N$_2$ It was supposed that the vibrational excitation of N$_2$ is possible during collisions with electrons[11] This process seems to be confirmed by the fact that during geomagnetic disturbances at middle latitudes the electron temperature in the ionosphere decreases[12] After the great geomagnetic storm on February 11, 1958, a decrease of ordinary daytime radio wave absorption in the D layer was observed[13] This can be interpreted as a result of the accumulation of very large amounts of NO in the upper atmosphere, perhaps exceeding 10^{17} NO cm^{-2}. In such circumstances, hydrogen Lyman α emission, penetrating into the D layer and ionizing NO there, will be considerably weakened, and will not be able to produce an ionized layer ensuring considerable absorption of radio waves Truttse (unpublished work) has observed that the published material of the IGY and the IQSY[14] contains indications of a considerable increase of the continuum of the night sky emission at middle latitudes during large geomagnetic storms This continuum is a result of the appearance of considerable NO concentrations[15,16] in the upper atmosphere It should also be recalled that in the spectrum of the aurora on February 11, 1958, two unidentified emissions at 3780 Å and 3812 Å were found[17] which are near to the components of the most intense 0–11 band of the β-system of NO (ref 18) NO and NO$^+$ molecules can emit about 3×10^{-13} ergs in 0 1 s and nearly restore their vibrational excitation during each collision with surrounding atoms and molecules[10] Thus if the result of interferometric investigations of the temperature during auroras is reliable, there are serious grounds for believing that the heating of the upper atmosphere at low heights (below 200 km) because of the injection of charged particles and electrojets cannot be important.

During large geomagnetic storms, however, the metastable atomic oxygen emission at 6300 Å (refs 19 and 20) which appears above 300 km is the main and predominant auroral emission Even in the mid-latitude region its diffuse fields have enormous extents and intensity The Doppler contour of this emission is very wide This indicates that even temperatures averaged over several hours reach 3,500° K (ref 21) Thus instantaneous temperatures can be much higher At the most intense spots during the aurora on February 11, 1958, the emission at 6300 Å reached 10^8 R, which corresponded to an energy flow amounting to 300 ergs cm^{-2} s^{-1} The intensity of this emission exceeded by four orders of magnitude the intensity of the green emission of atomic oxygen at 5577 Å, while the excitation potentials amount to 1 96 eV for the former and 4 17 eV for the latter, that is, they do not differ considerably Neither energetic, nor low-energy electrons nor hot electron gas with a Maxwellian energy distribution can be a reason for the excitation of red emission at 6300 Å In high-latitude regions during the injection of low-energy protons, which do not penetrate deeply into the atmosphere, the intensity of the emission at 6300 Å increases considerably[22]. But on February 11, 1958, hydrogen emission in high-latitude regions was not intensive and at middle latitudes did not exceed the observability threshold at all Thus the heating of the upper atmosphere by some plasma oscillations or magnetohydrodynamic waves remains at present the only probable reason for the appearance of red atomic oxygen emission at 6300 Å

The top of the upper atmosphere should be heated most intensively The boundary of the heated region will be lowered to levels at which the removal of injected heat outside the atmosphere will become possible The greater the injected energy, the lower the boundary of the high-temperature region The removal of the heat downward, however, as a result of thermal conductivity, is insignificant even during very great heating despite a large surface which removes heat and which is secured by a rapid levelling of the temperature in the top of the upper atmosphere

In these circumstances, the most probable processes are collisions and the atomic exchange reaction NO($^2\Pi$) + O(^{3}P)→NO($^2\Pi$) + O(^{1}D), during which the Wiegner rule is observed At high temperatures when the average energy of atoms and molecules approaches 2 eV, the rate of this reaction can amount to 10^{-10} cm^3 s^{-1} In such circumstances, most oxygen atoms will be in the ^{1}D state Thus at about 10^9 O cm^{-3} it is necessary to have about 10^{15} NO cm^{-3} for an emission at 6300 Å at 10^8 R (the maximum intensity observed) At a lower temperature but with an equal number of collisions these NO molecules can be excited and emit infrared photons with energies of about 0 2 eV The total energy in this region of the spectrum, however, will be an order of magnitude lower than in the red emission at 6300 Å the photons of which have energies of 1 96 eV At the same time the afore-mentioned NO molecules will take part in the creation of the continuum in the reaction $NO+O \rightarrow NO_2+\text{continuum}$[15] This reaction has a rate[16] of about 10^{-16} cm^3 s^{-1}, giving a continuum of 10^2 in a very wide region of the spectrum Such additional emission cannot be observed against the general background of the ordinary continuum Thus the increase in the continuum may only be due to the accumulation of large quantities of NO in lower parts of the atmosphere with a larger atomic oxygen concentration Large amounts of NO will, however, considerably cool the atmosphere As a result of this, the temperature at low heights will not increase essentially, because the downward flow of heat from strongly heated parts of the atmosphere, where emission at 6300 Å is emitted, is considerably weakened

The increase of artificial satellite drag during geomagnetic disturbances was interpreted in terms of heating of the upper atmosphere during which the height distribution of heat inflows corresponded to that typical of daytime ordinary, undisturbed conditions Indirect measurements of the increments in the exospheric temperature ΔT obtained from the value of the drag were not great, being about 1° K per a_p unit Direct measurements of such temperatures have not yet been accomplished Jacchia[23] has noted that the maximal drags and ΔT take place when the perigee of a satellite is situated at a height of about 650 km Lower and higher perigees correspond to smaller drags and ΔT. A higher location of the main source of heating does not contradict the relative distribution of drag with height described by Jacchia

The actual data available at present do not permit us to establish all the details of the heating process Truttse (unpublished work) has shown that the drag and the logarithm of the intensity of emission at 6300 Å (ref 24) are in phase and proportional to D_{st} On one hand, this dependency can be interpreted as evidence of a sharp non-linear increase of the heating agent with an increase of D_{st} On the other hand, it can be regarded as an indication of a sharp conversion from vibrational excitation of NO molecules and their quenching by infrared photons to the generation of emission photons at 6300 Å in the atomic exchange reaction because of the increase in the temperature In the latter case, the relation between D_{st} and the total energy in the NO infrared emission and the atomic oxygen emission at 6300 Å will not be so non-linear and perhaps will even be linear The choice between these two alternatives can only be made on the basis of simultaneous investigation of these emissions

Twenty-seven day and semi-annual variations of artificial satellite drag can also be associated with the heating of the upper atmosphere by plasma oscillations or magnetohydrodynamic waves which accompany geomagnetic disturbances and auroras The latter are characterized by remarkably similar synphase variations Variations of the upper atmospheric density with the time of day and with solar activity are well described by the existing models of the atmosphere, in which only variation of the solar electromagnetic radiation is taken into account It is very likely, however, that with further, more detailed determination of the heating processes a small contribution from magnetohydrodynamic and plasma wave absorption will be detected This should be taken into account despite the fact that solar hard ultraviolet radiation, absorbed in the atmosphere, is reliably known The problem is that the process of the transformation of energy into heat and the removal of this heat outside the atmosphere has not yet been explored with sufficient comprehensiveness

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[1] Chamberlain, J W , *Physics of the Aurora and Airglow*, 98 (Academic Press, New York and London, 1961)

[2] Krassovsky, V I , *Planet Space Sci* , 1, 14 (1959)

[3] Dessler, A J , *J Geophys Res* , 64, 397 (1959)

[4] Jacchia, L G , Slowey, J , and Verniani, F , *J Geophys Res* , 72, 1423 (1967)

[5] Jacobs, R L , *J Geophys Res* , 72, 1571 (1967)

[6] Moe, K , *Planet Space Sci* , 15, 1821 (1967)

[7] Hilliard, R L , and Shepherd, G G , *Planet Space Sci* , 14, 383 (1966).

[8] COSPAR, *CIRA 1965* (North-Holland Pub Co , Amsterdam, 1965)

[9] Dalgarno, A , Latimer, I D , and McConkey, J W , *Planet Space Sci* , 13, 1008 (1965)

[10] Dalgarno, A , *Planet Space Sci* , 10, 19 (1963)

[11] Dalgarno, A , McElroy, M B , and Moffett, R J , *Planet Space Sci* , 11, 463 (1963)

[12] Reddy, B M , Brace, L H , and Findlay, J A , *J Geophys Res* , 72, 2709 (1967)

[13] Warwick, J W , *Science*, 127, 1047 (1958)

[14] Yao, I G , in *Ann I G Y* (edit bv Roach, F E), 24 (1962)

[15] Krassovsky, V I , *Dokl Akad Nauk SSSR*, 78, 669 (1951).

[16] Schiff, H I , *Ann Géophys* , 20, 86 (1964)

[17] Clark, K C , and Belon, A E , *J Atmos Terr Phys* , 16, 220 (1959)

[18] Pearse, R W B , and Gaydon, A E , *The Identification of Molecular Spectra*, 225 (Chapman and Hall, London, 1963)

[19] Krassovsky, V I , in *Solar-Terrestrial Physics* (edit by King, J W), 275 (Academic Press, London, 1966)

[20] Krassovsky, V I , *Planet Space Sci* , 16, 13 (1968)

[21] Mulyarchik, T M , and Shcheglov, P V , *Planet Space Sci* , 10, 215 (1963)

[22] Galperin, G I , *Planet Space Sci* , 1, 57 (1959)

[23] Jacchia, L G , *Space Res* , 2, 747 (1961)

[24] Truttse, Y L , *Planet Space Sci* , 16, 26 (1968)

Age of the Upper Boulder Clay Glaciation in the Midlands

THE radiocarbon dating of 30–36,000 yr provided by Shotton[1] for peaty samples obtained from gravels occurring beneath the Upper Boulder Clay of the Four Ashes area [*SJ*9208], situated about 5 miles north of Wolverhampton, is probably the most important advance in absolute Pleistocene chronology yet achieved by the method of age determination in the English Midlands It has proved that the Upper Boulder Clay is a viable time-stratigraphic unit within the Shropshire–Cheshire Basin[2,3] and disproved the hypothesis that the maximum limit of advance of the ice-sheet which laid down the Upper Boulder Clay in north Cheshire reached only as far as the Wrexham–Bar Hill morainic suite situated in the centre of the Shropshire–Cheshire Basin[4] By inference this dating must support the view that the complex Wrexham–Bar Hill end-moraine is of pre-Upper Boulder Clay (Middle Sands) age[2,3] and therefore cannot be correlated with the Brandenberg and Lesnow moraines as suggested by Boulton and Worsley[4] Furthermore, it also shows that the variations in carbonate leaching properties discovered by Boulton and Worsley in the Upper Boulder Clay to the north and south of this morainic suite are not necessarily indicative of age differences between these deposits as suggested by them[4]

The sequence of glacial deposits within the Shropshire–Cheshire Basin[2,3] comprises an Upper Boulder Clay (now shown to be of Wurm II age), Middle Sands (of radiocarbon age-determinations ranging from 28,000[4] to 57,000[5] yr old) and Lower Boulder Clay (by inference probably of Würm I age) The melt-phase of each glaciation produced separate and distinct glacial lake features within the Shropshire–Cheshire Basin[2] and from the Four Ashes age determination of 30–36,000 yr we must conclude that the Main Terrace of the River Severn (dated downstream from Ironbridge at 42,000 yr (ref. 6) correlates through the Ironbridge Gorge with a glacial lake-stage formed during the melt-phase of the Lower Boulder Clay glaciation (products of which probably include the Middle Sands Wrexham–Bar Hill morainic suite) rather than with the long accepted correlation with glacial Lake Lapworth[7], for the uppermost Lake Lapworth features are cut into and obviously postdate the Upper Boulder Clay of the type Shrewsbury (sheet 153) area[2,3,7]

While this new radiocarbon dating has helped to standardize the chronology of the Shropshire–Cheshire Basin, it accentuates the need for further such absolute chronology to be obtained in the critical Wolverhampton–Birmingham area A glance at the one-inch geological drift maps 153, 154 and 168 shows that the now-proved Wurm II Upper Boulder Clay of the Four Ashes area occurs on the north-western margin of an almost continuous till-sheet which extends eastwards over the Cannock Coalfield and south-eastwards into the West Bromwich and Edgbaston area of Birmingham The so-called "Wolverhampton Line"[1] is merely an approximate limit of large "Irish Sea" erratics in the Upper Boulder Clay and as such is not absolute evidence of the maximum limit reached by ice-sheets in the Wurm II period of glaciation The boulder clay to the south of the River Tame south of Walsall is chiefly composed of locally derived Coal Measures material in which "Irish Sea" erratics are occasionally found It is notable that Kelly has recorded "Irish Sea" erratics in the Upper Glacial deposits of the North Birmingham area[8] and it would seem at least possible that the uppermost boulder clay (Edgbaston Till) of his complex succession hereabouts may correlate with the Wurm II boulder clays of the Four Ashes area

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[1] Shotton, F W , *Nature*, **215**, 1366 (1967)

[2] Poole, E G , and Whiteman, A J , *Quart J Geol Soc* , **117**, 91 (1961)

[3] Poole, E G , and Whiteman, A J , *Geology of the Country around Nantwich and Whitchurch, Mem Geol Surv* (1966)

[4] Boulton, G S , and Worsley, P , *Nature*, **207**, 704 (1965)

[5] Simpson, I M , and West, R G , *New Phytol* , **57**, 239 (1958)

[6] Shotton, F W , and Strachan, I , *Quart J Geol Soc* , **115**, 1 (1959)

[7] Wills, L J , *Quart J Geol Soc* , **80**, 274 (1924)

[8] Kelly, M R , *Phil Trans Roy Soc* , B, **274**, 533 (1964)

Koyna Earthquake

THE Koyna earthquake of December 11, 1967, killed about 200 people and injured a few thousand The epicentre was within 5 km of the Koyna Dam (17° 23′ N , 73° 45′ E) A magnitude of the order of 7 5 on the Richter scale has been reported by the Indian Meteorological Department and the Central Water and Power Research Station The depth of focus of the earthquake has been estimated at about 30 km The earthquake was felt over distances up to 700 km from the epicentre Geologists, basing their opinion on this information, have suggested that the earthquake resulted from movements along a fault, possibly a satellite of the principal fault along the Malabar coast, and that it may be an isolated event unconnected with the reservoir and the earlier seismic activity

Koyna Dam and the Shivaji Sagar Lake formed by it are situated in the western part of the peninsular shield which is covered by the Deccan lava flows commonly known as Deccan Traps The dam was constructed on the Koyna river which originates in the Mahabaleshwar region and runs practically north–south up to Helwak, a point which is very close to the epicentre and where the river turns sharply to the east

The peninsular shield is one of the nearly non-seismic stable central shields of the continents Isolated shocks of low magnitude have been reported on the western and eastern ghats The Koyna reservoir was impounded in 1962 and reports of Earth tremors near the site of the dam began to be prevalent soon afterwards The frequency of tremors increased considerably from mid-1963 From September 1964 to March 1966 sixty-one tremors were recorded and their epicentres were determined using data from a network of four closely spaced seismological observatories at Mahabaleshwar, Satara, Govalkot and Koyna Nagar (Fig 1) During 1967, five tremors were recorded by a few other seismological observatories as well as the four situated in the neighbourhood of Koyna Dam Studies of these tremors showed that the epicentres of most of these were located within a small radius around the dam site The foci of these tremors were located at a depth of about 4 km, and they were attributed to crustal adjustments within the Deccan lava flows[1] Before the earthquake of December 11, there was a shock on September 13, 1967, in this region which was widely felt and caused minor local damage This was the first earthquake in this area to arouse public anxiety

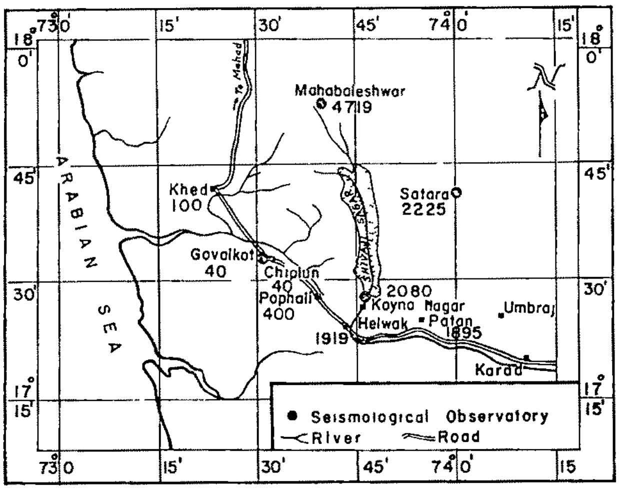

Fig. 1 Koyna Nagar and its surroundings

Field investigations carried out by us after the Koyna earthquake of December 11 showed very localized damage Koyna Nagar and some areas to the south are the most affected, where the intensity hardly exceeds 8 on the MM scale Poorly constructed brick and mud houses have withstood this earthquake at small distances of 8–10 km Field studies indicate a much smaller magnitude than 7 5 and a depth of focus shallower than 30 km The large distances of 700 km over which the earthquake was felt may be a consequence of the good transmission of energy in the shield area

We have obtained seismograms from Adelaide, Athens, Anpu, Kongsberg, Valentia and Kirkenes, where the seismological observatories are equipped with WWSSS. The surface wave magnitude has been determined for the Koyna earthquake from these seismograms and an average value of 6 2 is obtained (Table 1) The Uppsala observatory has reported a magnitude of 6 25 for this earthquake and the same value has been assigned by Adams (personal communication) It is therefore established that the magnitude of this earthquake is of the order of 6 2

Table 1

Recording station	Surface wave magnitude
Adelaide, Australia	6 2
Anpu, Taiwan	6 3
Athens, Greece	5 9
Kongsberg, Norway	6 3
Kirkenes, Norway	6 3
Valentia, Ireland	6 3

Seismic activity has been reported to be associated with the filling of the Boulder Dam by Carder[2] where several hundred small earthquakes were felt and several thousands were recorded by seismographs in the vicinity of the Boulder Dam reservoir after it was filled in 1935 These earthquakes were of low magnitude and did not cause any damage Adams (personal communication) has found a three-fold increase in seismic activity at Mangla Dam in Pakistan following its filling

Evans[3,4] has put forward the classic example of Rocky Mountain Arsenal near Denver where a deep well was used for disposal of waste fluids Otherwise a non-seismic area, Denver experienced about 700 tremors during 3 5 yr The frequency of these tremors was found to be high during the months of high injection and vice versa The Pre-Cambrian rocks of the Arsenal reservoir are highly fractured and rest on steep slopes The increase of fluid pressure within the reservoir decreases the frictional resistance along the fracture planes and the movement occurs The elastic wave energy thus released is recorded as earthquakes The largest recorded magnitude in this area is 4 8 on the Richter scale

Association of the enhanced seismic activity in the Koyna region with the impounding of the dam is established beyond doubt Guha *et al* [1] have shown an increase in the seismic energy release in this area with the increase of the reservoir level with a certain time lag The highest recorded magnitude for tremors in this region until March 1966 was of the order of 4 00 The earthquake of September 13 had a magnitude of the order of 5 0 and the earthquake of December 11 of the order of 6 0 These events all seem to be connected

The exact mechanism of the seismic activity associated with the loading of the reservoir is not known Without doubt the hydrostatic pressure of 50 lb /in ^{2} exerted by the Koyna reservoir could not be directly responsible for an earthquake of magnitude 6 0

There is the possibility that the Koyna river itself flows along a fault and that water from Shivaji Sagar Lake percolating through great depths could have created certain imbalances in hydrostatic pressures resulting in slippage along fracture planes This mechanism itself could also act as a triggering agency for some sort of chain reaction at depths of 5 to 10 or 15 km Detailed geological and geophysical investigations in this region and studies of the seismological records of the earthquake, its fore-shocks and after-shocks are essential if the cause of this earthquake in this part of the stable peninsula is to be understood

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[1] Guha, S K , Gosavi, P D , Padale, J G , and Marwadi, S C , *Third Symp Earthquake Engineering Roorkee*, 399 (1966)

[2] Carder, D S , *Seismol Soc Amer Bull* , 35, 175 (1945)

[3] Evans, M D , *Geotimes*, 10, 11 (1966)

[4] Evans, M D , *Geotimes*, 12, 19 (1967)

Chemical Composition of Dew and Dry Fallout in Jerusalem, Israel

THIS report describes the first results of an analysis of the chemical composition of dew collected in Jerusalem, Israel, during the dry summer season of 1967 The data are compared with analyses of precipitation and dry fallout carried out during previous years (unpublished results of Yaalon and Jacobs), which were made in an attempt to evaluate the contribution of airborne salts to the circulation of soluble salts[1,2]

Thirty-five samples of dew were collected during the rainless months July to October 1967 In twenty-one of these the amount exceeded 150 ml (equivalent to more than 0 15 mm of dew per night), which sufficed for a complete chemical analysis (including Na, K, Ca, Mg, Cl SO_4, HCO_3, *p*H and conductivity) according to the methods currently used by this laboratory for rain water analyses Only partial analyses of the remaining smaller samples were made.

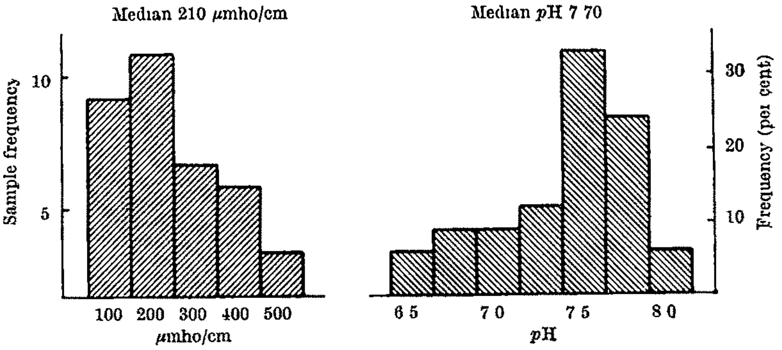

Fig 1 Frequency distribution of conductivity and *p*H values in 33 samples of dew, Jerusalem, summer 1967 Minimum and maximum values of conductivity are 100 and 500 μmhos cm^{-1} (25° C), the *p*H range is 6 55 to 8 10

Dry fallout during the rainy winter season of 1962–63 was collected by washing a large polyethylene funnel (area 700 cm^2) and the bottle which served as rain collector with exactly 1 1 of double distilled water

The concentration of soluble salts found in the dew is usually within the range of concentrations found previously in the precipitation (unpublished work of Yaalon and Jacobs) There is a clear inverse relationship between the amount of dew and salt concentration The average chloride concentration in dew on days with < 0 1 mm of dew is three times as large as in the rest of the samples In part the effect may be because of some evaporation of the dew from the glass collecting plate Because of occasional high concentrations the frequency distributions of the ionic concentrations are all positively skewed and

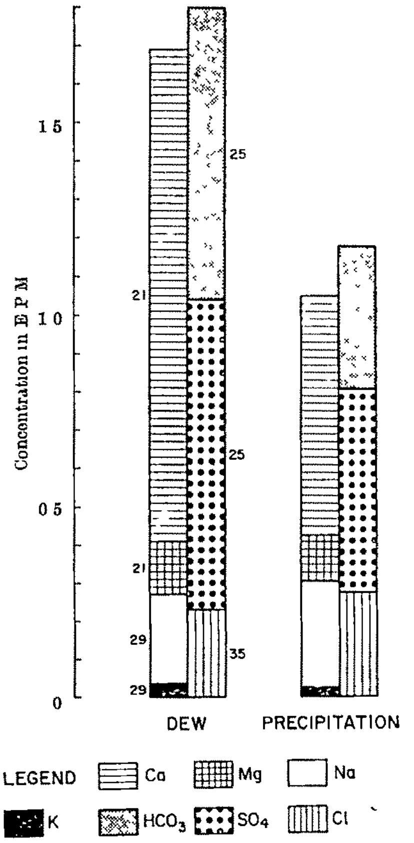

Fig 2 Average chemical composition of dew and bulk precipitation in Jerusalem The number of analyses available for the calculation of the median values of dew are indicated in the graph Values of bulk precipitation are the means of rainfall years 1960/61–1962/63

tend to be log-normal Medians rather than means were therefore calculated The frequency distributions of conductivity and pH values are shown in Fig 1, the medians being 210 μmhos cm^{-1} and pH 7 7, respectively Median values for all the analysed ions are plotted in Fig 2, and compared with the weighted mean concentrations of precipitation in Jerusalem The inequality between the sum of cations and anions is explained principally by the disregard of ammonia in the analyses[3] The median total amount of dissolved solids (calculated) in the dew is 120 mg l^{-1}, more than that in the precipitation Fog water has also recently been found to contain greater concentrations than rainfall[4]

From Fig 2 it seems that dew is richer than precipitation in Ca, HCO_3 and SO_4. This enrichment becomes still more evident from the ternary plots showing the relative amounts of cations and anions (Fig 3) In spite of the fairly large variations in the concentration of the salts, their relative proportions in the dew vary remarkably little In the precipitation the variations are usually much larger (unpublished work of Yaalon and Jacobs, and ref 6) The dominance of Ca and HCO_3 is easily explained as being due to calcareous dust from rocks and buildings in the Jerusalem environment[5] Possibly some of the fine calcareous dust particles act as condensation nuclei for the dew and are dissolved in the process Sulphate is doubtless derived from sulphur dioxide in the air It seems to dissolve the carbonate minerals in the dusty air, thereby producing some calcium sulphate

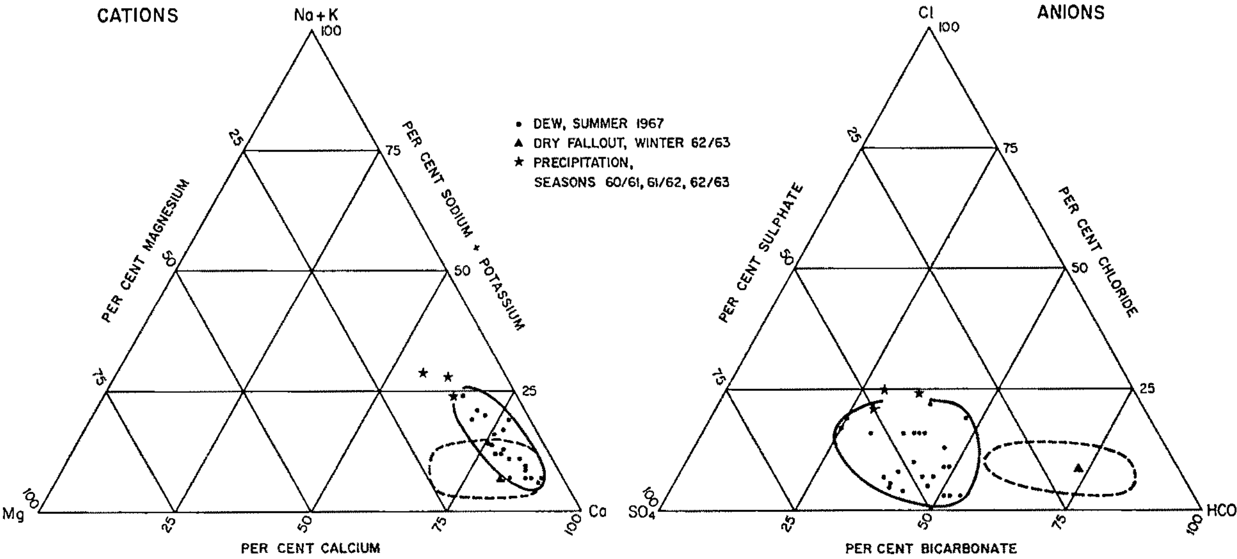

Fig 3 Diagram showing general chemical character of dew, bulk precipitation and dry fallout in Jerusalem, Israel Sum of equivalent reacting values for cations and anions equals 100 per cent

Both the average concentration and the relative ionic proportions of Cl, Na, K and Mg in dew are remarkably similar to those in the precipitation This seems to suggest the same maritime source for these salts Evidently the moisture which condenses as dew scavenges salts and aerosols from the air just like bulk precipitation Whitehead and Feth[6] reported that in California bulk precipitation, which includes dry fallout deposited on the collector between rains, is 4 to 10 times higher in mineral concentration than rainwater

Sodium and potassium have been combined in the ternary diagrams The low Na/K ratio, which in all of the twenty-eight analyses was less than 10 (median 6 5), is, however, noteworthy The Ca/Mg in the dew, varying from 7 to 25 (median 10), is relatively high, reflecting the effect of dissolution of $CaCO_3$ dust The influence of calcareous dust is even more evident in the composition of dry fallout (Fig 3) In the dry fallout (eighteen samples), the relative proportions of all constituents except Ca and HCO_3 are similar to those in the precipitation

Only very approximate calculations can be made of the total contribution of dew and dry fallout to total salt precipitation. Measurements of dew in Israel[7,8] indicate that in Jerusalem there are about 150 dew nights per year, depositing something like 0 15 mm per dew night or a total of about 25 mm per year, with maximum frequency and quantities in September Although the amount of dew depends greatly on the nature of the surface on which it collects, the present measurements generally confirm these averages Dew would thus contribute an amount of Cl and the other oceanographic salts equivalent to 25 mm of winter precipitation Dry fallout contributes several times more than this, principally because it is not confined to the few night hours of dew condensation The total amount of dry fallout in summer cannot yet be estimated because of inadequate data In the rainy winter season it is included with the precipitation Estimates from other countries that dry fallout contributes more than rainfall[6,9] may well be correct for Jerusalem

In conclusion, these data seem to suggest that dew condensation represents rather well the chemical composition and relative proportions of the oceanogenic part in aerosols and dry fallout It may be worthwhile to determine the relative concentrations of these and other salts at other places also, and to determine the concentration gradients with distance from the sea

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[1] Yaalon, D H , *Bull Res Counc of Israel*, 11G, 105 (1963)
[2] Eriksson, E , *Tellus*, 12, 63 (1960)
[3] Yaalon, D H , *Tellus*, 16, 200 (1964)
[4] Mrose, H , *Tellus*, 18, 266 (1966)
[5] Yaalon, D H , and Ginzbourg, D , *Sedimentology*, 6, 90 (1966)
[6] Whitehead, H C , and Feth, J H , *J Geophys Res* , 69, 3319 (1964)
[7] Shalem, N , *La Météorologie*, 182 (Paris, 1950)
[8] Gilead, N , and Rosenan, N , *Israel Exp J* , 4, 120 (1954)
[9] Eriksson, E , *Tellus*, 7, 243 (1955)

PHYSICS

Self-trapping of Radiation Produced Electrons

THE problem treated here is the trapping of free electrons produced by ionizing radiation in dielectric systems Detailed theoretical descriptions of the equilibrium properties of the solvated electron are available[1-3], but its formation is still under discussion Electrons are formed and thermalized in the coulomb field of the positive ions, but not fully recaptured by the ions Magee[4] considered the auto-ionization of the H_3O radicals as a possible secondary source of electrons, Freeman[5-7] assumed the electrons to be trapped in the defect sites of the medium at the instant of thermalization, while we thought[8,9] that in polar dielectrics the escape of the electrons from the ions can be a free-energy decreasing, and therefore spontaneous, process In our approach, the electrons which escape are treated as quasi-free, and the question of the kinetics and mechanism of electron trapping arises Diffusion kinetic calculations on the radiolysis of water[10,11] suggest very high initial hydrated electron densities, which require fast trapping processes

Our electron escape theory used a continuous dielectric medium to which the notion of any defect site is not applicable This theory has already been proved experimentally to some extent[12] Thus in keeping with Platzman's original conception[13], and with the picture given recently by Boag[14], we shall discuss the stabilization of the electron in the medium as a self-trapping process, that is, as the formation of an energy well in the medium under the influence of the electric field of the electron The question to be answered is how much time is needed for a free electron in a dielectric substance to form an energy well of suitable depth ?

An electric charge of density ρ induces in a dipolar medium some polarization of its environment The extent of this process can be characterized by a polarization charge density ρ^* In the case of equilibrium, $\rho_e^* = (1-\varepsilon_s^{-1})\rho$, where ε_s is the static dielectric constant of the substance (see appendix) This relationship is correct only as far as the absolute values are concerned, ρ^* and ρ being of opposite sign An electron becomes self-trapped if the polarization charge density is high enough to produce a strong positive field which is able to trap the electron The presence of solvated electrons in a system shows ρ_e^* to have met this requirement

An electron will become trapped at a place $\mathbf{r}$ and a time t, where the actual density of polarization charges equals or surpasses the equilibrium value, that is, where the following inequality holds

$$\rho^*(\mathbf{r},t) \geq (1-\varepsilon_s^{-1})\rho(\mathbf{r},t) \quad (1)$$

An inducing charge immediately influences non-polar dielectrics to form their polarization charges The polarization is much faster than the change in the field strength caused by the motion of a thermal electron Thus the condition of inequality (1) is met at the instant and site of thermalization, or not met at all Because it has been shown[9] that thermal free electrons occur with only a very low probability in non-polar systems, this point will not be discussed

Polar dielectrics need some time for the polarization charges to be formed For a large class of polar substances, including water, this formation can be described by the equation

$$\rho^*(\mathbf{r},t) = (1-\varepsilon_\infty^{-1})\,\rho(\mathbf{r},t) + \bar{\varepsilon}^{-1}\vartheta^{-1}\exp(-t/\vartheta)\int_0^t \rho(\mathbf{r},t')\exp(t'/\vartheta)\,dt',$$

$$\bar{\varepsilon}^{-1} = \varepsilon_\infty^{-1} - \varepsilon_s^{-1}, \quad \vartheta = \varepsilon_\infty\tau/\varepsilon_s \quad (2)$$

where ε_s and ε_∞ are the static and infrared dielectcir constants, respectively, and τ is the dielectric relaxation time (see appendix)

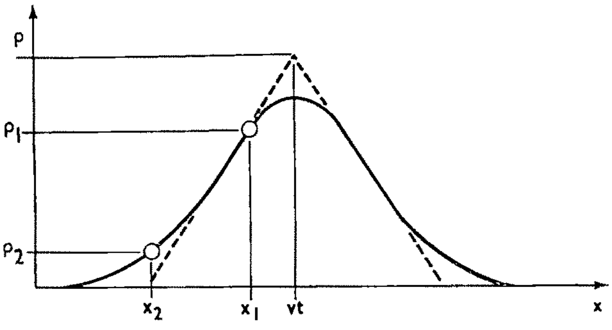

Fig 1 Schematic electron density distribution

The sluggishness of the polarization, reflected in equation (2), helps the electron to be trapped Thermal electrons spreading out over a number of water molecules can be characterized by some continuous charge density, which depends on space and time, and is qualitatively similar to the density distribution given in Fig 1 Let the electron move in the direction of the $+x$ axis The high charge density ρ_1 tends to induce a certain amount of polarization in the medium at x_1 The polarization charges form slowly and, although they continue to do so after ρ_1 has left site x_1, this process is far from complete by the time the low charge density ρ_2 reaches x_1 The polarization charges at x_1 are insufficient to trap the high density part of the electron, because their density is much lower than required by inequality (1) The polarization at x_1 as induced by ρ_1 might, however, trap the low density part of the electron, ρ_2 This is meaningful in statistical terms

This qualitative picture can be formulated by combining inequality (1) and equation (2) to give the expression

$$\rho(\mathbf{r},0) + \int_0^t [\partial\rho(\mathbf{r},t')/\partial t']\exp(t'/\vartheta)\,dt' \leq 0 \quad (3)$$

Inequality (3) defines for each moment t a volume $V(t)$ which contains all the points—that is, the space vectors $\mathbf{r}$, where this inequality is met.

The trapping probability $p(t)$ for the period of time $[0,t]$ is equal to the probability of finding the electron in the volume $V(t)$

$$p(t) = \int_{V(t)} \rho(\mathbf{r},t)\,dV \quad (4)$$

The quantum mechanical calculation of the time-dependent electron wave-function in a potential well formed by the electron itself is insoluble A very crude estimate can be made by describing the electron as a travelling wave packet of constant geometry; that is, the effect of elapsing time which tends to broaden the charge-distribution and the effect of the potential well being formed which tries to confine the electron are neglected If the electron moves with a constant velocity v in the $+x$ direction, the one-dimensional density function has the general form $\rho = \rho(x-vt)$, and inequality (3) assumes the form

$$\rho(x,0) - \vartheta v(\partial\rho/\partial x)[\exp(t/\vartheta) - 1] \leq 0 \quad (3')$$

The solvation probability is given as

$$p(t) = \int_{-\infty}^{x} \rho(x')\,dx' \quad (4')$$

Solvation takes place, according to equation (3′), only at those sites where $(\partial\rho/\partial x) > 0$, that is, along the ascending part of $\rho(x)$ Hence this model leads to a 50 per cent solvation as an upper limit.

A numerical guess can be made by using the "roof" dotted in Fig. 1 as a normalized electron density function This has the following analytical form

$$\rho(x-vt) = (-1)^\alpha(\sqrt{2}\lambda)^{-2}(x-vt) + (\sqrt{2}\lambda)^{-1}$$

$$\alpha = \begin{cases} 0 \text{ if } x-vt \leq 0 \\ 1 \text{ if } x-vt > 0 \end{cases} \quad (5)$$

whereas the continuous curve in Fig 1 is that of the Gaussian function $[(\lambda\sqrt{\pi})^{-1} \exp [(x-vt)/\lambda]^2$ According to equation (5), τ has a finite range and so a well defined period of time for the completion of trapping The de Broglie wavelength λ of an average thermal electron is 60 Å, and its average velocity $v = 1 \times 10^7$ cm s^{-1} Using these values and the dielectric characteristics of water[15] ($\varepsilon_s = 80\ 1$, $\varepsilon_\infty = 4\ 9$, $\tau = 1\ 01 \times 10^{-11}$ s), the self-trapping process takes about $t_m \approx 3 \times 10^{-13}$ s

If the travelling velocity of the polarization charges is known, the site of the self-trapping can be determined It is shown in the appendix that there is only a small difference between the velocity of the centre of the polarization charge cloud $\mathbf{v}^*$ and that of the centre of mass of the electron $\mathbf{v}$ Thus we simply compute with v and evaluate[13] the average distance travelled by the electron from the site of thermalization to the site of self-trapping as the geometrical mean of its maximum path length of vt_m and minimum displacement of $\sim 10^{-8}$ cm Here we get about 17 Å for the mean displacement This figure agrees with the radius of electron spurs used as parameters in diffusion kinetic calculations by Schwarz[10] and Kuppermann[11] This final result is almost independent of v

This agreement is thought to be somewhat fortuitous and cannot be considered as a heuristic proof of our treatment There are, however, semiquantitative results of the present calculation which we consider both valid and important

According to inequality (3), solvation can take place at those sites where the electron density decreases, that is, where $(\partial\rho/\partial t) < 0$ This proves our previous assumption[8,9] that the electrons of a spur or track which become solvated are those which are able to escape, moving outward from the positive ions and thus decreasing the density of the electron heap

Quasi-free electrons in a polar substance become self-trapped in much shorter periods than the dipolar relaxation time, self-trapping being about as fast as thermalization Thus thermalization and self-trapping take place almost simultaneously

Appendix Let us define ρ^* as the source of the polarization Using standard notation[16]

$$\rho^* = \mathrm{div}\,\mathbf{P} = \frac{1}{4\pi}\,\mathrm{div}\,(\mathbf{D}-\mathbf{E}) = \rho - \frac{1}{4\pi}\,\mathrm{div}\,\mathbf{E} \tag{6}$$

For a large class of polar substances **D** and **E** are interrelated by the differential equation[16]

$$\mathrm{d}\mathbf{E}/\mathrm{d}t + \mathbf{E}/\vartheta = \mathbf{D}/\varepsilon_\infty \tau + (1/\varepsilon_\infty)\mathrm{d}\mathbf{D}/\mathrm{d}t \tag{7}$$

By combining equations (6) and (7) one gets equation (2) In the case of equilibrium $\mathbf{D} = \varepsilon_s \mathbf{E}$ and, when this is inserted into equation (6), ρ_e^* is found

The centres of the electron cloud $\mathbf{r}_0$ and that of the polarization charge cloud $\mathbf{r}_0^*$ are defined as

$$\mathbf{r}_0 = \int_V \mathbf{r}\rho(\mathbf{r})\mathrm{d}V \quad \text{and} \quad \mathbf{r}_0^* = \int_V \mathbf{r}\rho^*(\mathbf{r})\mathrm{d}V \tag{8}$$

The corresponding velocities are

$$\mathbf{v} = \mathrm{d}\mathbf{r}_0/\mathrm{d}t \quad \text{and} \quad \mathbf{v}^* = \mathrm{d}\mathbf{r}_0^*/\mathrm{d}t \tag{9}$$

The combination of equations (6), (7) and (9) leads to the expression

$$\mathbf{v}^* = \frac{1-\varepsilon_\infty^{-1}}{1-\varepsilon_s^{-1}-\bar{\varepsilon}^{-1}\exp(-t/\vartheta)}\,\mathbf{v} + \frac{(1-\varepsilon_s^{-1})(\bar{\varepsilon}\vartheta)^{-1}\exp(-t/\vartheta)\int_0^t \mathbf{v}[\exp(t'/\vartheta)-1]\mathrm{d}t'}{[1-\varepsilon_s^{-1}-\bar{\varepsilon}^{-1}\exp(-t/\vartheta)]^2} \tag{10}$$

or, because the second term in equation (10) is never negative

$$\mathbf{v} \geq \mathbf{v}^* \geq [(1-\varepsilon_\infty^{-1})/(1-\varepsilon_s^{-1})]\mathbf{v} \tag{11}$$

The lower limit even for highly polar water is as high as 0 8 v Therefore the approximate identity

$$\mathbf{v} \approx \mathbf{v}^*$$

holds

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[1] Davydov, A S , *Zh Exp Teor Fiz* , **18**, 913 (1948)
[2] Deigen, M F , *Zh Exp Teor Fiz* , **26**, 293 (1954)
[3] Jortner, J , *Radiat Res Suppl* , **4**, 24 (1964)
[4] Magee, J L , *Radiat Res Suppl* , **4**, 21 (1964)
[5] Freeman, G. R , and Fayadh, J M , *J Chem Phys* , **43**, 86 (1965)
[6] Freeman, G R , *J Chem Phys* , **43**, 93 (1965)
[7] Freeman, G R , *J Chem Phys* , **46**, 2822 (1967)
[8] Schiller, R , *J Chem Phys* , **43**, 2760 (1965)
[9] Schiller, R , *J Chem Phys* , **47**, 2278 (1967)
[10] Schwarz, H A , *Rad Res Suppl* , **4**, 106 (1964)
[11] Kuppermann, A , in *Radiat Res* (edit by Silini, G), 212 (North-Holland Publ Co , Amsterdam, 1967)
[12] Schiller, R , *J Chem Phys* , **47**, 2281 (1967)
[13] Platzman, R L , *Physical and Chemical Aspects of Basic Mechanism in Radiobiology*, 22 (Nat Res Coun , Washington, 1953)
[14] Boag, J W , in *Rad Res* (edit by Silini, G), 43 (North-Holland Publ Co , Amsterdam, 1967)
[15] Auty, R P , and Cole, R H , *J Chem Phys* , **20**, 1309 (1952)
[16] Frohlich, H , *Theory of Dielectrics* (Oxford University Press, Oxford, 1950)

Vibration Analysis by Stroboscopic Holography

This communication describes a method for vibration analysis in which fringes are directly related to the amplitude of the vibrating object. This is achieved by combining the frozen fringes technique with stroboscopy

Wavefront reconstruction interferometry is, by now, a well established technique, and some applications of this technique, sometimes referred to as the "frozen fringes" method, have been described[1,2] Powell and Stetson[3,4] have extended the method to vibrating objects

When a hologram is made in the normal way from a vibrating object[3,4], interference fringes are seen on reconstruction These fringes are uniquely related to the probability density function of the motion of the object and not to the particular motion Thus the fringe pattern is not directly related to any physical parameter which could be otherwise measured In any vibration analysis the position of vibration nodes is of primary interest This method does not give the direct answer

With a conventional optical arrangement a hologram of the object was recorded on the photographic plate The exposure time, however, was half that which would be required to produce a good hologram The object was then acoustically excited using a signal generator and a 6 W loudspeaker At the same time, the laser light was chopped in synchronization with the vibrating object by a slot in a disk driven by a synchronous motor coupled to the same signal generator The same photographic plate, retained in precisely the same position, was exposed again The exposure time was adjusted so that the final density (about 0 5 log units) gave a bright good-quality reconstruction The object, a "T" piece clamped by its central shorter leg to the loudspeaker frame, was vibrating at its first resonance frequency, about 226 Hz The chopper produced a light pulse of approximately 40 μs The phase was adjusted to "freeze" the motion at approximately maximum displacement—that is, at the position where the velocity is zero. Fig 1 shows stroboscopic fringes obtained by double exposure

The object was vibrating about the horizontal axis, parallel to the plane of Fig. 1, so all fringes should have been horizontal and parallel. Because of the vibration of the whole mounting of the loudspeaker, however, the recorded resultant motion was far more complicated than simple harmonic oscillation. This changed the shape of the fringes. The motion of the frame was detected, of course, by the presence of stroboscopic fringes on it. For comparison Fig. 2 shows Powell and Stetson fringes[3] of the same object under exactly the same excitation.

This stroboscopic method also allows the analysis of objects vibrating with larger amplitudes than the method of Powell and Stetson. This is shown in Figs. 1 and 2; the fringes in Fig. 2 have smaller spacing than in Fig. 1 for the same amplitude. Furthermore, stroboscopic holography can be used to obtain fringes at any amplitude less than the maximum. In this case the light pulse would have to be reduced as the velocity would have a finite, and possibly, large value. This, in turn, would require an increase of the exposure time. The exposure times used were moderately short. With the illuminating pulse occurring every ninth cycle and an 80 mW c.w. He–Ne laser the exposure time was between 2–4 min. The hologram produced measured approximately 10× 10 cm.

The interpretation of fringes obtained stroboscopically is far easier than of those of Powell and Stetson. The fringes here, as in the frozen fringe method, are directly related to displacement and therefore to the amplitude of the vibrating object. The interpretation of such fringes was discussed by Haines[2] and his conclusions are directly applicable. Here, as in all interferograms, the sign ambiguity of the amplitude exists. The sign must be predicted from experience.

With this method, as with those previously described[1-4], live fringes can be obtained. The hologram is first made from the non-vibrating object and replaced in exactly the same position as it occupied during the exposure. Then if the vibrating object is illuminated stroboscopically and viewed through the hologram the reconstructed image from the hologram will interfere directly with the light scattered from the object and live fringes will be seen.

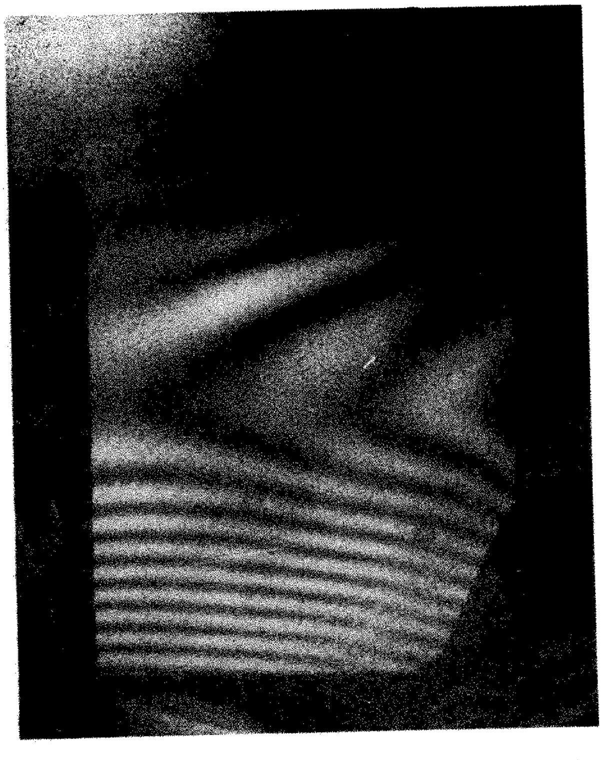

Fig. 1. Frozen fringes obtained stroboscopically from a vibrating object.

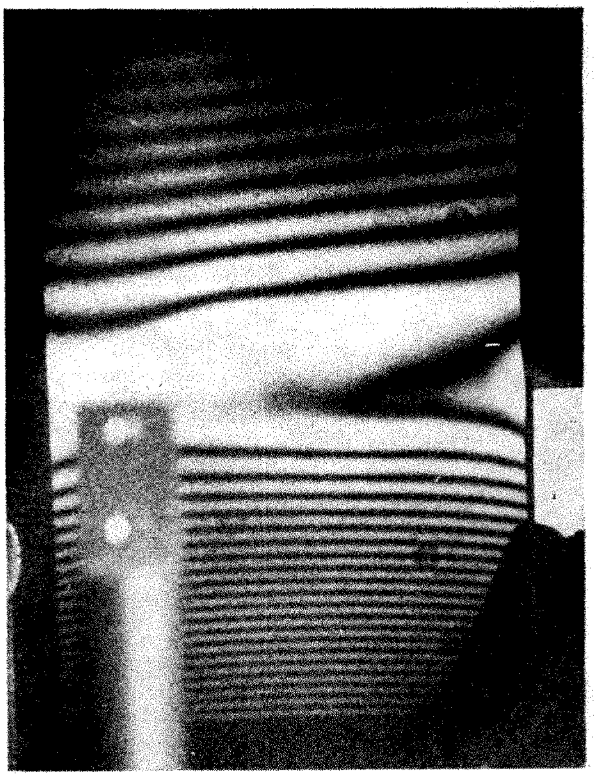

Fig. 2. Powell and Stetson fringes obtained with the same vibrating object. In the foreground a standard optical bench rod is seen.

Finally, if a Powell and Stetson hologram is made with stroboscopic instead of continuous illumination, the pulse width of the chopped light will determine the range of amplitude recorded on the hologram. Thus on reconstruction, the fringes will correspond to an object vibrating over a smaller amplitude range.

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[1] Burch, J. M., Ennos, A. E., and Wilton, R. J., *Nature*, **209**, 1015 (1965).
[2] Haines, A., and Hildebrand, B. P., *Appl. Optics*, **5**, 595 (1966).
[3] Powell, R. L., and Stetson, K. A., *J. Opt. Soc. Amer.*, **55**, 1593 (1965).
[4] Powell, R. L., and Stetson, K. A., *J. Opt. Soc. Amer.*, **55**, 1694 (1965).

Measurement of the Diffusion Coefficient of Radium A

THE potential hazard in mines from the inhalation of the progeny of radon gas has become increasingly interesting in recent years. This hazard involves radon daughters in two forms: (*a*) attached to aerosol particles or condensation nuclei; and (*b*) unattached atoms or ions. The form of the unattached daughters is not fully known, but one of their important and basic characteristics is their diffusion coefficient. Of special interest is the diffusion coefficient of the first radon daughter, ^{218}Po, called radium A. This parameter is very important not only in describing atmospheric behaviour[1] but also in considering the biological hazard of inhaling unattached daughters[2].

Radon decays by alpha emission, with a half-life of 3·825 days, to form radium A with a recoil energy of

120 KeV. It is positively charged from the stripping of orbital electrons by the departing alpha particle or by the recoil motion. Its recoil range in air is about 50μ. How long it remains charged and into what chemical reactions it enters have not been demonstrated. It is possible that the radium A ion is quickly neutralized and forms polonium oxide.

On contact, radium A becomes attached to surfaces. Because it has a high diffusion coefficient, and because the most available surfaces are the surfaces of aerosol particles, radium A rapidly becomes attached to aerosol particles at a rate dependent on the aerosol concentration and on the diffusion coefficient. Similar effects occur with other unattached radon progeny, the most important being ^{214}Pb, called radium B, and ^{214}Bi, called radium C. Curie[3] observed that the attachment seemed to be affected by water vapour, but this effect has not been elucidated.

If unattached radium A is inhaled by a human, it deposits by diffusion on the walls of the respiratory tract. How much is deposited during breathing and where in the respiratory tract this deposition occurs depend on the diffusion coefficient of radium A. The diffusion coefficient of radium A is reported to be approximately 0·05 cm^2 s^{-1} (refs. 2 and 4). This is smaller than would be expected for a single uncharged atom (0·1 cm^2 s^{-1}), implying either a charged atom or a small cluster of atoms. The importance of water molecule interactions has not been clearly demonstrated. In this study, the diffusion coefficient of radium A was measured in two different conditions of humidity (described by the temperature of the dew point). An apparent effect of the humidity of the atmosphere on the diffusion coefficient was found.

The technique introduced by Townsend[5] was used to make these measurements. This technique consists of drawing the radon daughters through cylindrical tubes of various lengths and observing the penetration fractions. For these experiments glass tubes of small diameter were used. A filter was placed at the end of each of the tubes to collect the penetrating radon daughters, and these filters were counted for alpha activity. The air concentrations of the radon daughters were calculated by mathematical study of the decay of each sample[6]. To correct for the production of radon daughters during passage of the radon gas through the tubes, similar measurements were made with filters also at the entrances of the tubes and the small concentrations of radon daughters found with these prefiltered samples were subtracted from the corresponding unfiltered penetration concentrations.

Samples were taken from the centre of a 1,000 l. chamber through which clean air containing radon gas was continually flowing. The supply of air for the chamber was doubly filtered. The state of humidity depended on the meteorological conditions. The radon gas was generated by bubbling a small quantity of air through a dilute hydrochloric acid solution containing radium chloride. The arrangement was basically that described by Wilson[7]. Before entering the radium chloride solution, the air was filtered and passed through a conditioning bubbler for humidification. The radon-laden air was passed through an overflow and droplet trap and then through a soda lime trap. It was doubly filtered at the entrance to the chamber, where it was mixed with the clean air. Independent measurements of the daughters in the chamber were made with filters inserted directly into the chamber. All flow rates for sampling were 1·08 l. min^{-1}. The humidity was measured with a thermistor psychrometer.

Two experiments were conducted: experiment 1 used an atmosphere with a dew point of 27° Fahrenheit, and experiment 2 used an atmosphere with a dew point of 47° Fahrenheit. For both experiments the chamber flow was 180 l. min^{-1} and the radon concentration was about $1·5 \times 10^{-8}$ Ci l.$^{-1}$ with the ratio of radium A atoms to radium B atoms at about 2·4 : 1. There was little radium C. In both cases, the air temperature was 78° Fahrenheit.

To study the electrical charge state of the radium A, samples were drawn through a condenser with an electrical field up to 50,000 Vm^{-1}. Radium A was undisturbed in passage through this condenser, indicating that the radium A ions were neutralized to uncharged atoms because of the high radon concentration.

Townsend[5] derived an equation describing the penetration of diffusing particles drawn through a cylindrical tube, and this equation was more accurately calculated by Gormley and Kennedy[8]. The relationship can be expressed as

$$F = 0{\cdot}819 \exp(-3{\cdot}66\pi D\eta) + 0{\cdot}0975 \exp(-22{\cdot}3\pi D\eta) + 0{\cdot}0325 \exp(-58{\cdot}6\pi D\eta)$$

with F the penetration fraction, D the diffusion coefficient of the diffusing material and η equal to the length of the tube divided by the volumetric flow rate through the tube. Because the last two exponential terms are unimportant except very close to the entrance of the tube, this relationship yields a straight line for penetration fractions less than about 0·8 when the logarithm of penetration is plotted against the parameter η. The slope of this line is proportional to the diffusion coefficient.

To analyse the data collected in these experiments, the concentration of radium A based on each sample was corrected for the radon decay in the tube and then normalized by dividing by the concentration of radium A measured with the chamber sample (zero diffusion length). The corresponding variances and standard deviations were also calculated. These experimental data were fitted by the method of weighted least squares to a simple exponential with the zero-length intercept equal to 0·819.

The calculated penetration fractions and their standard deviations for the two experiments are plotted in Fig. 1 as percentage penetration. Also shown are the experimental points reported by Chamberlain and Dyson[2] for the thoron daughter ^{212}Pb, called thorium B, which they used to confirm their calculation that the diffusion coefficient should be 0·054 cm^2 s^{-1}.

The diffusion coefficient calculated in experiment 1 was 0·047 cm^2 s^{-1}, which is similar to the value given by Wellisch[4]. The diffusion coefficient calculated from the

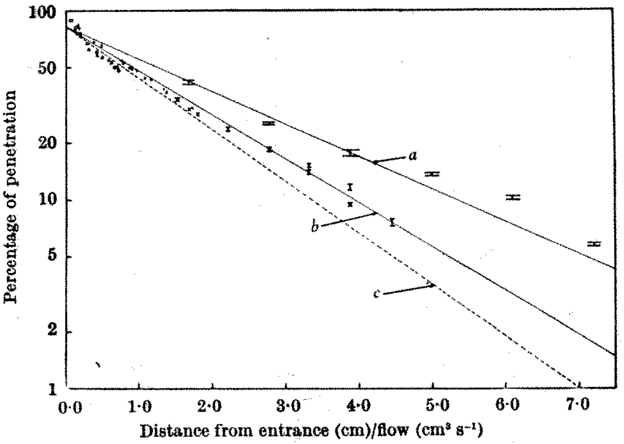

Fig. 1. Experimental data and standard deviations with the fitted theoretical lines for the percentage of penetration of radium A passing through a diffusion tube. The data points and theoretical line reported by Chamberlain and Dyson[2] (referred to here as $C+D$) are also given. The temperature of the dew point is indicated as DP. a, $D=0{\cdot}034$ cm^2 s^{-1} (DP = 47° Fahrenheit); b, $D=0{\cdot}047$ cm^2 s^{-1} (DP = 27° Fahrenheit); c, $D=0{\cdot}054$ cm^2 s^{-1} ($C+D$).

Symbol	Flow (cm^3 s^{-1})	Tube diameter (cm)	Source
●	16·6	0·4	$C+D$
×	33·3	1·8	$C+D$
△	83·3	0·4	$C+D$
○	83·3	1·8	$C+D$
I	18·0	0·7	Experiment 1
⌶	18·0	0·5	Experiment 2

data of experiment 2 was 0 034 cm^2 s^{-1} The principal difference between these two experiments was the absolute humidity, which, when expressed as the temperature of the dew point, was 27° Fahrenheit and 42° Fahrenheit, respectively

Tyndall[9] predicted that polar molecules, such as water, would tend to cluster around ions such as the initially charged radon daughters This type of clustering may explain the apparent diminution of the radon daughter diffusion coefficient with increased absolute humidity Possibly chemical reactions such as the formation of oxides are involved

These diffusion coefficient measurements indicate that there is a need for a more thorough study of the diffusion coefficient of radon daughters in various conditions, with special concern for the absolute humidity

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[1] Lassen, L , and Rau, G , *Z Physik*, **160**, 504 (1960)
[2] Chamberlain, A C , and Dyson, E D , *Brit J Radiol* , **29**, 317 (1956)
[3] Curie, M , *CR Acad Sci* , **145**, 477 (1907)
[4] Wellisch, E M , *Phil Mag* , **28**, 417 (1914)
[5] Townsend, J S , *Phil Trans Roy Soc* , A, **193**, 129 (1900).
[6] Raabe, O G , and Patterson, F S , *US Atomic Energy Comm Research and Development Rep UR-658* (1965)
[7] Wilson, R H., *Amer Ind Hyg Assoc. J* , **22**, 408 (1961).
[8] Gormley, P G , and Kennedy, M , *Proc Roy. Acad Dublin*, **52**, A, 163 (1949)
[9] Tyndall, A M , *The Mobility of Positive Ions in Gases* (Cambridge University Press, 1938)

THE SOLID STATE

Particle Coarsening in the Solid State

PARTICLE growth in supersaturated solutions has stimulated much theoretical and experimental work, but less attention has been paid to the subsequent reaction of particle coarsening in a matrix of solute content in local equilibrium with small particles The driving force for coarsening is the decrease in total energy accompanying a reduction of particle/matrix interfacial area Coarsening is controlled either by the diffusion of solute down the concentration gradient between neighbouring particles of differing size, or by the difficulty of assimilating solute atoms at the interface of a growing particle

It has been shown[1-3] that a particle dispersion with an initial mean radius $\bar{r}_0$ at the beginning of the coarsening process will usually have at time t a mean radius $\bar{r}$ given by an equation of the form

$$\bar{r}^n - \bar{r}^n_0 = At \tag{1}$$

where n and A are constants The value of n depends on the mechanism controlling the coarsening process The condition that $n = 2$ indicates an interface controlled reaction[1] while $n = 3$ indicates volume solute diffusion control[1,3] Speight[4] has recently proposed a model for coarsening of particles in grain boundaries, in which $n = 4$ In the latter case growth takes place by grain boundary rather than volume diffusion The constant A contains various parameters which depend on the precise mechanism

A difficulty with reactions following equation (1) is that of determining the index n It is common practice to plot the logarithm of the measured parameter versus the logarithm of the time, thus neglecting the constant $\bar{r}_0$ in equation (1) If $\bar{r}_0$ is small compared with $\bar{r}$ for the time interval of interest, then n is equal to the reciprocal of the slope If $\bar{r}_0$ is not negligible, however, the slope of a log $\bar{r}$ versus log t plot is no longer inversely proportional to n and the slope tends towards zero at small t as $\bar{r}$ tends towards $\bar{r}_0$ This difficulty is enhanced when such log–log plots show breaks or changes of slope which are usually interpreted as indicating changes in mechanism or rate

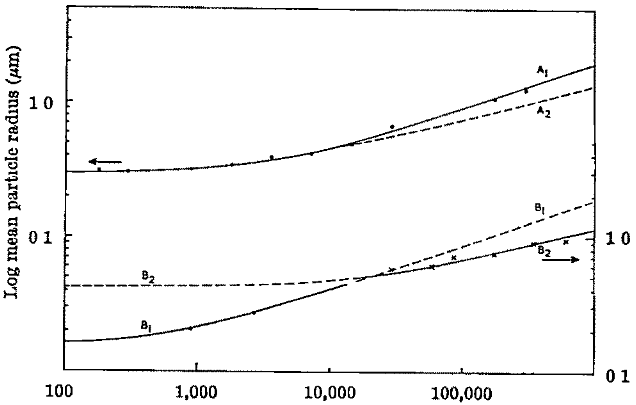

Fig 1 Coarsening of iron carbide particles in steel

Data for coarsening of iron carbide (Fe_3C) in a 0 3 per cent by weight carbon steel at 720° C and at 675° C are presented in Fig 1 as plots of log $\bar{r}$ versus log time The radii plotted were obtained from the procedure given by Fullman[5] for the sectioning of uniform spheres of radius r_u If the shape of the size distribution histogram of $r/\bar{r}$ is invariant with time, as it should be for a coarsening reaction, then no error is introduced in the determination of n by utilizing r_u from the uniform sphere formula rather than $\bar{r}$ values

Curve A_1, showing the data for coarsening at 720° C, has a slope near to zero at small t The slope gradually increases with t until it corresponds to an n value of 3 at $t \simeq 2 \times 10^4$ s, when $\bar{r}^3 \simeq 6\bar{r}_0^3$ The solid curve A_1 was not drawn as a best fit through the data points but was calculated from equation (1), using values of $\bar{r}_0$ and A derived from a plot of $\bar{r}^3$ versus t Curve A_2 was calculated from equation (1) using values of $\bar{r}_0$ and A derived from a plot of $\bar{r}^4$ versus t The two curves are indistinguishable for $t < 10^4$ s Clearly the data best fit equation (1) with $n = 3$ This n value indicates that a matrix diffusion process is the activated step controlling coarsening, possibly carbon diffusion, although co-operative diffusion of iron cannot be excluded[6]

Table 1 VALUES OF THE CONSTANTS GIVEN BY THE NON-LINEAR REGRESSION ANALYSIS

Temp (°C)	n	A cm^n s^{-1}	r_0 cm
720	3 1	0 73 × 10^{-17}	0 28 × 10^{-4}
675	3 09	0 29 × 10^{-17}	0 21 × 10^{-4}
675	4 11	0 58 × 10^{-22}	0 49 × 10^{-4}

To verify this interpretation of the log–log plot the data were analysed using a non-linear regression analysis on an IBM 760/350 computer This gave a value of $n = 3$ 1 for the whole of the time span investigated The other constants are given in Table 1, but their values as determined from a non-linear analysis are probably not as accurate as would be obtained using a linear analysis with the correct n value

The data for coarsening at 675° C—curves B_1 and B_2—show a break in the log–log plot Treating each section as a linear relation gives slopes of 0 34 and 0 22 It is possible that the lower slope for $t > 2 \times 10^4$ s is a transient effect caused by the neglect of a new (and greater) $\bar{r}_0$ term and that the time dependence is the same as in the early stages The data were split at the break in the plot, and application of a non-linear regression analysis for each half showed that the data for shorter times were best fitted by an n value of 3.09 and the data for longer times by $n = 4.11$. These results are reasonably close to possible ideal values, indicating a real change in reaction mechanism.

The constants $\bar{r}_0$ and A were obtained from plots of $\bar{r}^3$ and $\bar{r}^4$ versus t for each half of the data, and curves B_1 and B_2 were calculated from equation (1) with the constants appropriate to $n = 3$ for the shorter time data and $n = 4$ for the longer time data. Clearly all the data cannot be described by a single curve of the form of equation (1). The complementary curves using the values of $\bar{r}_0$ and A calculated from $n = 4$ for the shorter time data and $n = 3$ for the longer time are not shown, but do not provide a fit with the data. Many of the carbide particles were seen to lie at sub-grain boundaries in both steels at all ageing times, and, as already indicated[4], a value of $n = 4$ suggests that a grain boundary diffusion growth process becomes predominant at long ageing times.

We have found similar behaviour in other steels and we are investigating the factors affecting the transition from matrix to grain boundary diffusion control more closely as a function of temperature, chemical composition and microstructure.

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[1] Wagner, C., *Z. Electrochem.*, **65**, 581 (1961).
[2] Lifshitz, I. M., and Slyozov, V. V., *J. Phys. Chem. Solids*, **19**, 35 (1961).
[3] Greenwood, G. W., *Acta Met.*, **4**, 243 (1956).
[4] Speight, M. V., *Acta Met.* (in the press).
[5] Fullman, R. L., *Trans. Amer. Inst. Mining Eng.*, **197**, 447 (1953).
[6] Oriani, A., *Acta Met.*, **12**, 1399 (1964).

Optical Study of Particle Size of Montmorillonite with Various Adsorbed Cations

In sodium-montmorillonite suspensions the single clay plates are largely separated from each other, whereas in calcium-montmorillonite they form aggregates or tactoids containing from 5 to 15 parallel plates[1]. This property of calcium-montmorillonite may affect its swelling[2], negative adsorption[3] and ion exchange kinetics and equilibrium[4,5].

Because of this difference in particle size, dilute sodium-montmorillonite suspensions are less opaque—that is, they absorb and scatter less light in the visible range—than calcium-montmorillonite suspensions[2,5–7]. Differences in the opacity also exist between dilute suspensions of montmorillonite with other adsorbed cations. It is known that, in general, the absorption and scattering of light is largely dependent on the size of the colloidal particles, and can be used to estimate particle size. In fact, optical methods have been used to estimate the orientation and the shape of particles in pure sodium-montmorillonite suspensions[6,8]. This has not, however, been done for mono-ionic montmorillonite with other adsorbed ions. We therefore decided to study systematically the optical properties of a series of montmorillonite suspensions with different adsorbed cations, and to use the findings to estimate the particle size.

We found that the relative size of the average montmorillonite particle depends on the type of adsorbed ion. The size tends to increase systematically with the position of the ion in the lyotropic series, probably because of the formation of tactoids containing greater numbers of clay plates. Even the monovalent ions were observed to induce tactoid formation, so this process seems to be the rule for montmorillonite in any ionic form rather than an exception for calcium-montmorillonite, as previously assumed.

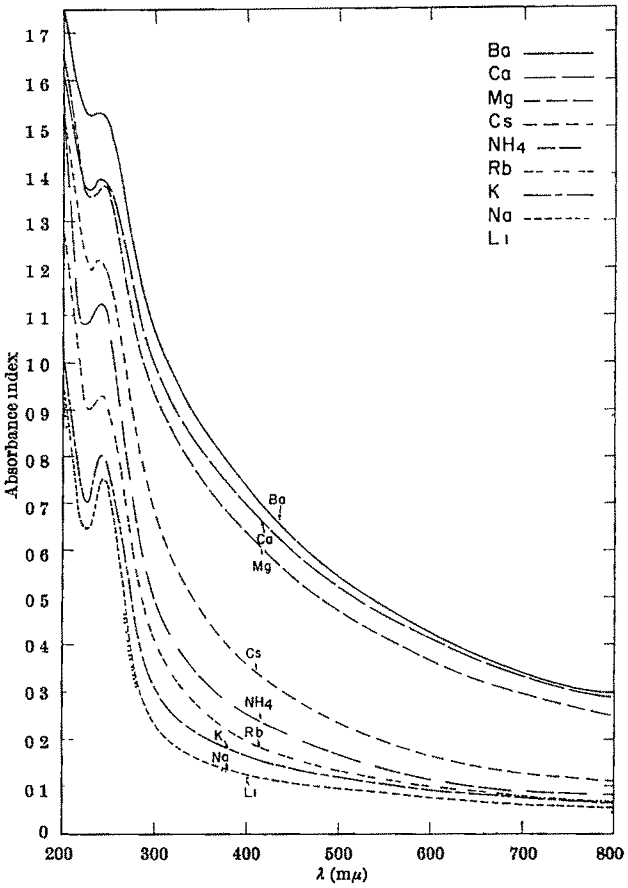

Fig. 1. The absorbance index (optical density for unit optical path length) of dilute, mono-ionic montmorillonite suspensions (250 mg clay l.$^{-1}$) in the wavelength range 200–800 mμ. Notice the selective absorption peak at 245 mμ, and the absence of such selective light absorption in the visible range.

Mono-ionic, essentially salt-free, montmorillonite suspensions of known clay concentration were prepared, as described in detail elsewhere[9], and their optical density was determined in the range 200–800 mμ with a Cary model 15 recording spectrophotometer. From the results, the absorbance index K, defined as the optical density for unit path length, was calculated.

Fig. 1 shows the spectra of montmorillonite saturated with Li^+, Na^+, K^+, NH_4^+, Rb^+, Cs^+, Mg^{++}, Ca^{++} and Ba^{++}. All the suspensions had the same clay concentration of 250 mg l.$^{-1}$. Similar spectra were obtained for other clay concentrations in the range up to 500 mg l.$^{-1}$. It can be seen that the adsorbed cation does not affect the general shape of the spectrum, but the higher the adsorbed ion in the so-called lyotropic series, the higher is the absorbance index of the suspension at a given wavelength in the visible range. Because the crystallographic composition and the concentration of the clay are the same in all the suspensions, and because the ions *per se* do not adsorb or scatter light in the visible range, it seems that changes in the average particle size of the clay can be the only possible cause for the observed differences in K.

We will now attempt to estimate the relative sizes of the clay particles in a more quantitative way. In the experimental system used, little of the scattered light entered the measured beam, so the absorbance index measured the total decrease in light intensity resulting from both light absorption by the particles and scattering from their surfaces. Thus[10]

$$K = t + d \qquad (1)$$

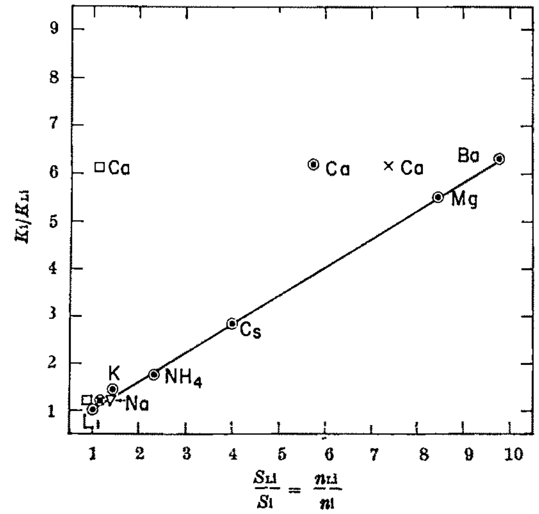

Fig 2 Correlation between absorbance index (K) ratios and specific surface area (S) ratios K values were taken from Fig 1 at 700 mμ S values, determined by the negative adsorption method, were taken from the literature and serve to estimate the relative number (n) of plates per tactoid for montmorillonite in various ionic forms Values for Li–montmorillonite were taken as the basis in all the calculations Sources for S values ○, Edwards, Posner and Quirk[13], ×, Edwards and Quirk[3], □, Bolt and Warkentin[14], ▽, Schofield[15]

where t and d are the scattering and absorption contributions respectively It is known, however, that clays do not absorb light in the visible range[11] We confirmed this for the montmorillonite suspensions we used by measuring separately the light absorbance component of several suspensions using a total diffuse reflectance accessory in the Cary spectrophotometer We found that absorbance was negligible compared with scattering As a first approximation, equation (1) thus reduces to

$$K = t \tag{2}$$

Furthermore, t is related to the turbidity τ by $t = 0\ 4343\tau$. The turbidity is proportional to the particle weight M and the concentration c as follows

$$\tau = HcM \tag{3}$$

where H is a constant depending on the optical properties of the particles and the solvent[12] This relationship can then be used to estimate the particle weight from the optical measurements

The montmorillonite tactoids consist of several parallel clay plates separated from each other by water layers of 9–10 Å thickness[3] For such an arrangement the composite particle weight is simply given by

$$M = mn \tag{4}$$

where m is the average weight of a single clay plate and n the average number of plates in a tactoid

Using equations (2), (3) and (4) we can calculate the ratio of the absorbance indices at a given wavelength of two suspensions having the same clay concentration but different adsorbed ions (1) and (2), and we get

$$\frac{K_1}{K_2} = \frac{H_1 n_1}{H_2 n_2} \tag{5}$$

We see that the absorbance index ratio is proportional to the relative size of the tactoids

Because the lithium–montmorillonite suspension gave the smallest K values, it apparently had the smallest n, assumed to be equal to one, and it was used as the reference (ion 2) for the calculations of the relative average tactoid sizes in other ionic forms by use of equation (5) and data from Fig 1 To check these calculations we estimated the relative tactoid sizes by another, independent, method We assumed, following Edwards and Quirk[3], that the existence of tactoids in the suspension decreases the volume of exclusion of chloride ions Thus the surface area S calculated from the measured volume of exclusion is also decreased, being in inverse proportion to the tactoid size More explicitly we write $S = S_t/n$ where S_t is the total surface area The relative average tactoid size in two different ionic forms can then be found from the relationship $n_1/n_2 = S_2/S_1$ For the calculations, we used surface area data reported in refs 3, 13, 14 and 15, again with Li as the reference form Finally we plotted the K ratios versus the n ratios, as shown in Fig 2 Apart from the deviations of calcium, a rather good correlation is found between the two sets of values This fact lends support to the hypothesis that the average particle size of montmorillonite in suspension is affected by the type of adsorbed ion This is a hitherto unknown effect of the exchangeable ion on the particle size of montmorillonite, and it should help in understanding the many other properties of clay suspensions that depend on the average particle size A possible cause of the great discrepancies between measured surface areas of calcium–montmorillonite reported in the literature, for example, refs 3, 13 and 14, and reflected in Fig 2, may be variations in the tactoid size of the clays studied, resulting from different materials or preparation methods

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[1] van Olphen, H , *Clays and Clay Minerals, Sixth Conference*, 196 (Pergamon, New York, 1959)
[2] Blackmore, A V , and Miller, R D , *Soil Sci Soc Amer Proc* , **25**, 169 (1961)
[3] Edwards, D G , and Quirk, J P , *J Colloid Sci* , **17**, 872 (1962)
[4] Banin, A , *Science*, **155**, 71 (1967)
[5] Banin, A , *Israel J Chem* , **6** (1968) (in the press)
[6] Kahn, A , in *Clays and Clay Minerals, Sixth Conference*, 220 (Pergamon, New York, 1959)
[7] Rebhun, M , and Sperber, H , *J Colloid Interface Sci* , **24**, 131 (1967)
[8] M'Ewen, M B , and Pratt, M I , *Trans Faraday Soc* , **53**, 535 (1957)
[9] Banin, A , and Lahav, N , *Israel J Chem* (in the press)
[10] Lothian, G F , *Absorption Spectrophotometry* (Hilger and Watts, London, 1958)
[11] Eitel, W , *The Physical Chemistry of the Silicates* (University of Chicago Press, 1954)
[12] Debye, P , *J Phys Chem* , **51**, 18 (1947)
[13] Edwards, D G , Posner, A M , and Quirk, J P , *Trans Faraday Soc* , **61**, 2816 (1965), ibid , **61**, 2820 (1965)
[14] Bolt, G H , and Warkentin, B P , *Trans Intern Congr Soil Sci Sixth Congr , Paris*, B, 33 (1956)
[15] Schofield, R K , *Nature*, **160**, 408 (1947)

CHEMISTRY

Co-ordination Compounds of Alkali Metals, with 8-Hydroxyquinoline and Isonitrosoacetophenone

As part of a study of the uptake and transport of alkali metals by plants, we are investigating their co-ordination chemistry in an attempt to obtain compounds in which the alkali metal is unequivocally co-ordinated Earlier reports[1] on compounds formed with salicylaldehyde (HL) suggested formulae of the form $ML n$HL In this system we have found n to be continuously variable, only the salt ML being definitely isolated But with the ligands 8-hydroxyquinoline and isonitrosoacetophenone we have succeeded in isolating crystalline complexes ML HL or ML 2HL, the product formed depends primarily on the radius of the metal and second on the reaction conditions

Addition of the ligand 8-hydroxyquinoline (HQ) to a suspension of metal hydroxide in absolute ethanol—it is essential that water be absent—gave *M*Q HQ for *M* = Li, Na and K but *M*Q 2HQ for *M* = Rb and Cs, irrespective of the ratios of reactants

For the ligand isonitroacetophenone (I), HL, the reactions took place in ethanol between the metal hydroxide and the ligand

Whatever the proportions of the reactants lithium and caesium gave only LiL and CsL HL, respectively For the alkali metals of intermediate radius *M*L or *M*L HL was formed according to the conditions For mole ratios 1 1, with potassium or rubidium in slight excess, the yellow ethanol solution deposits red flaky crystals of KL or RbL These crystals give red solutions in dimethyl formamide or *N*-methylpyrolidone If they are warmed in ethanol or benzene with excess HL, yellow needles of KL HL or RbL HL separate These give a yellow solution in *N*-methylpyrolidone In other organic solvents the red flakes *M*L are deposited, and in water the ligand separates leaving a yellow solution of the salt The sodium salt NaL is obtained as a cream coloured precipitate, when this is suspended in benzene and treated with HL a gelatinous white substance is formed which when dried was analysed as NaL HL

The new compounds are summarized in Table 1 All gave satisfactory chemical analyses Entries for the crystal system indicate that the molecular weights have been determined by X-ray studies on single crystals, KL HL is isomorphous with RbL HL, RbQ 2HQ and CsQ 2HQ are isomorphous No information about molecular symmetry can be deduced from any of the space groups

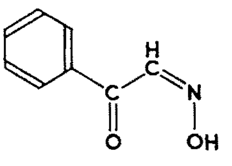
Table 1 PROPERTIES OF THE COMPOUNDS

Compound	Colour	m.p (dec)	Crystal system
LiL	Yellow	180	
NaL	Cream	150	
KL	Red	95	
RbL	Red	108	
NaL HL	White	105	
KL HL	Orange-yellow	87	Triclinic }
RbL HL	Yellow	110	Triclinic }
CsL HL	Yellow	85	Monoclinic
LiQ HQ	Pale yellow	130	Hexagonal
NaQ HQ	Pale yellow	115	
KQ HQ	Pale yellow	150	Monoclinic
RbQ 2HQ	Pale yellow	120	Triclinic }
CsQ 2HQ	Pale yellow	110	Triclinic }

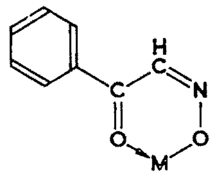
The infrared spectra of all the compounds have been measured in Nujol mulls, as have those of HL and HQ under the same conditions All the alkali metal HQ compounds in Table 1 gave similar spectra They differed from that of the ligand molecule by the absence of a weak broad band at 3,200 cm^{-1} (probably O–H stretching) and by an increase in the number of bands in the 1,570–1,610 cm^{-1} region from one in the ligand to four in caesium compound The spectra were similar to those of the magnesium chelate MgQ$_2$ (ref 2)

The infrared spectra of the *M*L compounds are similar to one another and differ from that of the ligand by the absence of the hydroxyl band at 3,260 cm^{-1} and a shift in the carbonyl peak from 1,680 cm^{-1} to 1,640, 1,635, 1,625 and 1,610 cm^{-1} for Li, Na, K and Rb, respectively This suggests a chelate form (II) for the *M*L compounds.

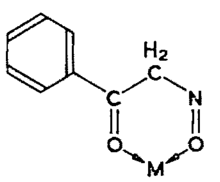
The infrared spectrum of the compound NaL HL closely resembles that of HL, in particular in having a hydroxyl band at 3,200 cm^{-1} and a carbonyl stretch at 1,675 cm^{-1} This evidence is equally consistent either with a second molecule being chelated in form I or with the product being a mixture of NaL and HL Potassium, rubidium and caesium compounds, *M*L HL, have spectra distinct from those of *M*L and of the ligand, no hydroxyl frequency appears and all three show a sharp band at 1,635 cm^{-1} Another sharp band appears at 1,660, 1,655 and 1,650 cm^{-1} in the K, Rb and Cs compounds, respectively These spectra are consistent either with the alkali metal being chelated by one anionic ligand as in (II) and a second neutral one as in (III), or with the hydrogen bonded structure (IV) The latter would make both ligands equivalent and might more satisfactorily explain the colour changes on co-ordination by a second ligand.



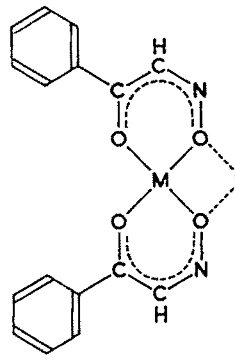
(I)



(II)



(III)



(IV)

Preliminary experiments with mixed ligands, giving, for example, KL HQ, have yielded promising results and will be reported when the compounds are fully characterized

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[1] Brewer, F M , *J Chem Soc* , 361 (1931)

[2] Stone, K Q , *J Amer Chem Soc* , 76, 4997 (1954).

Molecular Conformation of Thymidine and a Comparison with some Electron Spin Resonance Results

The determination of the structure of thymidine (C10 N2 O5 H14, Fig 1) was undertaken as part of a series of structure determinations of nucleic acid components Thymidine is the only nucleoside which has been studied[1] by electron spin resonance of irradiated single crystals and, in particular, the orientation of the pyrimidine ring has been predicted from these studies Radiation effects on thymine make an important contribution to the spectra obtained in electron spin resonance experiments on irradiated deoxyribose nucleic acid[2]

Crystals were obtained from aqueous solution The unit cell is orthorhombic with dimensions $a = 4\ 86$ Å, $b = 13\ 91$ Å and $c = 16\ 32$ Å The space group is $P2_12_12_1$ and there are four molecules in each cell These results are in good agreement with those found by Furberg[3] In all, 1,203 reflexions were observed using molybdenum $K\alpha$ radiation The data were collected using the Hilger and Watts linear diffractometer

The structure was determined by the method described by Tollin and Cochran[4]. The function $I(\Theta\ \varphi)$ (ref 4) was calculated using the 100 largest sharpened $|F|^2$ values and is shown in Fig 2. This map indicated that the plane of the pyrimidine ring has a normal defined by $\Theta = 90°$, $\varphi = 43.5°$, where Θ and φ are the spherical polar angles with respect to the axial set a, b, c. The orientation of the pyrimidine ring within this plane was obtained from the section in this orientation through the origin of the Patterson function. The position of the ring in the unit cell was determined using the Q-functions defined by Tollin[5].

A three-dimensional electron density map, using the magnitudes of the observed structure factors and phases calculated from the atoms of the pyrimidine ring alone, revealed the positions of the remaining atoms. Fourier refinement on the a-axis projection and least-squares refinement using the three-dimensional data led to a structure giving an R factor, where

$$R = \frac{\Sigma ||F_0| - |F_c||}{\Sigma |F_0|},$$

of 0.13 using isotropic temperature parameters. The determination of the positions of the hydrogen atoms from a three-dimensional difference Fourier and subsequent refinement with anisotropic temperature parameters gave a final structure which led to an R factor of 0.08 for the observed reflexions. These results together with the details of the structure determination will be published later, but a number of observations can be made at present.

The orientation of the pyrimidine ring is given by $\Theta = 89°$, $\varphi = 45°$, the maximum deviation from this plane being 0.015 Å, which confirms the electron spin resonance results which predict angles of approximately $\Theta = 90°$, $\varphi = 45°$. The conformation of the sugar residue relative to the pyrimidine ring is best described by the torsion angle φ_{CN} defined by Donohue and Trueblood[6] which has a value in this case of $-39°$, the sugar being in the *anti* conformation with respect to the pyrimidine ring. This value agrees with previous results for pyrimidine nucleosides and nucleotides[7]. Moreover, in all nucleosides and nucleotides the sugar ring has one atom, either C2′ or C3′, displaced from the mean plane of the other four ring atoms. In thymidine the atom C3′ is displaced 0.5 Å on the opposite side of the plane from the atom C5′ (the *exo* conformation). Only one other nucleoside or nucleotide—deoxyadenosine[8]—has this displaced atom in the *exo* position. The bond C5′–O5′ is *gauche* with respect to the C4′–O1′ bond and *trans* with respect to the C4′–C3′ bond[9].

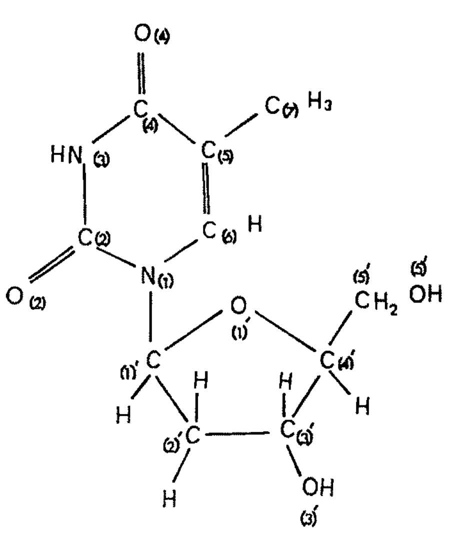

Fig. 1. The molecular formula of thymidine and the conventional numbering system.

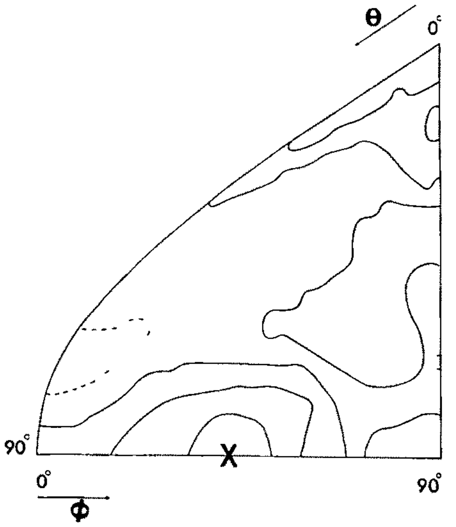

Fig 2. The $I(\Theta,\varphi)$ function. Contours are drawn at equal intervals. The lowest contour is dashed. The cross (×) marks the position of largest peak.

These results can be compared with the conformation of the molecule in calcium thymidilate[10] in which $\varphi_{CN} = -48°$, the displaced atom is C3′ in the *endo* position and the conformation of the bond C5′–O5′ is *gauche-gauche*.

Pruden *et al*[1] state in their conclusions from the electron spin resonance studies of irradiated thymidine that "The equivalence of the coupling of the $C_{(5)}-CH_3$ protons proves that the methyl group is rotating about the C—C bond". The hydrogen atoms of the methyl group are well resolved in the three-dimensional difference map, so we conclude that in the undamaged crystals the methyl group is not rotating.

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[1] Pruden, B., Snipes, W., and Gordy, W., *Proc US Nat Acad Sci*, **53**, 917 (1965).
[2] Gordy, W., Pruden, B., and Snipes, W., *Proc US Nat Acad Sci*, **53**, 751 (1965).
[3] Furberg, S., and Hordvik, A., *Acta Chem Scand*, **10**, 135 (1956).
[4] Tollin, P., and Cochran, W., *Acta Cryst*, **17**, 1322 (1964).
[5] Tollin, P., *Acta Cryst*, **21**, 613 (1966).
[6] Donohue, J., and Trueblood, K. N., *J. Mol Biol*, **2**, 363 (1960).
[7] Haschemeyer, A. E. V., and Rich, A., *J Mol Biol*, **27**, 369 (1967).
[8] Watson, D. G., Sutor, D. J., and Tollin, P., *Acta Cryst*, **19**, 111 (1965).
[9] Shefter, E., and Trueblood, K. N., *Acta Cryst*, **18**, 1067 (1965).
[10] Trueblood, K. N., Horn, P., and Luzzati, V., *Acta Cryst*, **14**, 965 (1961).

BIOCHEMISTRY

Growth Hormone in Human Development

Many investigators have established that changes occur in the structure of enzymes and other proteins during development. For example, Cahn *et al* have shown changes in the isoenzymes of lactic dehydrogenase (LDH)[1], and Korber was the first to recognize that foetal haemoglobin is more resistant to alkali than maternal haemoglobin[2]

There is thus a precedent for the possibility that human peptide hormones undergo changes during foetal development To pursue this question, complement fixation, a technique extremely sensitive to slight changes in antigenic structure[3-5], was employed to compare growth hormone in the human foetus and human adult

Adult human growth hormone (*HS*-496 *A*), prepared by the method of Wilhelmi[6], was purified by column chromatography on diethyl amino-ethyl cellulose in 0 02 M borate–NaOH buffer, *p*H 8 0 Three protein peaks were obtained Peak *A*, the purified antigen in this study contained 2 IU/mg of growth activity[7] and 6 8 IU/mg of crop gland stimulating activity[8]

Thirteen pituitaries, and a small piece of foetal fibrous connective tissue from the area around the sella turcica, used as a control, were obtained at autopsy from foetuses ranging from 12 weeks (crown–rump, 8 cm) to 40 weeks old, and were frozen at − 20° C Before use they were thawed, weighed to the nearest mg, and individually extracted in 1 ml of ice cold isotonic *tris* buffer, *p*H 7 4, by homogenization in a glass tissue grinder[9]

After standing for 1–2 h at 2° C the homogenates were centrifuged at 10,000*g* for 10 min at 4° C The supernatant solutions were decanted and used for immunological studies without further purification

A single anti-adult human growth hormone A rabbit serum (*RA* 452*B*-7) was used It was prepared as previously described and diluted 1 1,500 before use[10] No evidence of immunochemical heterogeneity of the antiserum was shown by double diffusion in agar In addition, only one complement fixation peak was observed with the antiserum even when there was a great excess of antigen against human growth hormone or a crude extract of adult human pituitary glands

Quantitative micro-complement (*C′*) fixation was performed according to Wasserman and Levine[11] The *C′* fixation curves (Fig 1) obtained with antiserum *RA* 452*B*-7 (diluted 1 1,500) and *HS*-496 *A* and thirteen crude extracts of human foetal pituitary glands from 12 to 40 weeks of gestation are all superimposable These curves show that 85–88 per cent of complement is fixed at the zone of antibody–antigen equivalence (0 08 µg of antigen) No complement was fixed when foetal fibrous tissue was used as the antigen

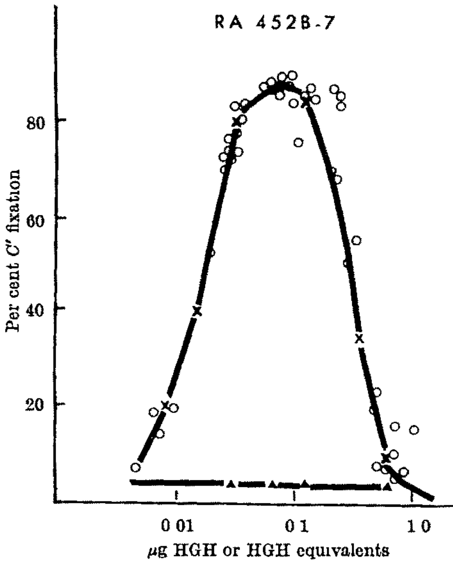

Fig 1 *C′* fixation curves obtained with adult human growth hormone *A* (HGH-*A*) (×) or with a crude extract of any one of thirteen foetal pituitaries (○) and antiserum 452*B*-7, diluted 1 1,500 The curves are drawn with their points of equivalence at the same antigen concentration The maximum *C′* fixations obtained with purified HGH-*A* and the crude foetal pituitary extracts are identical No *C′* fixation was obtained when a crude extract of foetal fibrous tissue was used as an antigen (▲)

The question of whether or not there are specific foetal hormones may have important implications for medicine and biology If they do exist, there may be abnormalities of their regulation, analogous to some of the haemoglobinopathies, which have not yet been recognized Also, the persistence of the varying levels of the foetal hormone in the adult may account for some of the hormone-like substances which have been detected in adult serum[12,13] On the other hand, the immutability of growth hormone during development may be equally significant

This experiment does not conclusively exclude the possibility that a specific foetal hormone exists It is possible that (*a*) areas of the peptide hormone other than those containing the antigenic determinants are structurally different, (*b*) growth hormones exist in the foetus which are so different antigenically that they do not react with the antiserum used in this experiment, and (*c*) earlier stages of development than those investigated have different growth hormone(s)

Further studies, including investigation of still younger foetuses and study of the primary structure of the peptide hormones, are necessary to elucidate the problem The present studies indicate that pituitaries of human foetuses contain a substance which is antigenically indistinguishable from purified adult growth hormone by quantitative complement

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[1] Cahn, R , Kaplan, N O , Levine, L , and Zwilling, E , *Science*, **136**, 962 (1962)

[2] Körber, E (1866), as cited by Solomon, J B , in *The Biochemistry of Animal Development* (edit by Weber, R), 418 (Academic Press, New York, 1965)

[3] Van Vunakis, H , Lehrer, H , Allison, W S , and Levine, L , *J Gen Physiol* , **46**, 589 (1963)

[4] Gerstein, J F , Levine, L , and Van Vunakis, H , *Immunochemistry*, **1**, 3 (1964)

[5] Reichlin, M , Hay, M , and Levine, L *Immunochemistry*, **1**, 21 (1964)

[6] Wilhelmi, A E , *Canad J Biochem Physiol* , **39**, 1659 (1961)

[7] Marx, W , Simpson, M E , and Evans, H M , *Endocrinology*, **30**, 1 (1942)

[8] Riddle, O , Bates, R W , and Dykshorn, W , *Amer J Physiol* , **105**, 191 (1933)

[9] Tashjian, jun , A H , Levine, L , and Wilhelmi, A E , *Endocrinology*, **77**, 563 (1965)

[10] Tashjian, jun , A H , Levine, L , and Wilhelmi, A E , *Endocrinology*, **77**, 1023 (1965)

[11] Wasserman, E , and Levine, L , *J Immunol* , **87**, 290 (1961)

[12] Samaan, N A , Fraser, R , and Damster, J , *Diabetes*, **12**, 339 (1963)

[13] Bürgi, H , Müller, W A , Hummel, R E , Labhart, A , and Froesch, E R , *Biochim Biophys Acta*, **121**, 349 (1966)

Pink Spot as a Diagnostic Test in Schizophrenia

THE first report of the presence of DMPEA in the urine of schizophrenic patients[1] was followed by other confirmatory[2-4] and conflicting[5-9] results We have used different methods to give a better resolution of the substance Recently a new technique for the isolation and identification of the pink spot was described[10] This seems to be a specific method by which at least seven constituents of the pink spot (DMPEA being only a minor one) have been discovered

Only one of the previous investigations[4] involved the chromatographic procedure first described by Friedhoff and van Winkle, and the pink spot has regularly been found to be absent from normal individuals, while it was present in varying proportions in a group of schizophrenic patients We wished to verify whether the pink spot—identified by a special method and consisting of many other compounds as well as DMPEA—was specific for schizophrenia If it was, this would signify that, as well as the production of DMPEA, a greater disturbance of amine metabolism could be involved in schizophrenia[11], this would prove the validity of our method as a diagnostic test

We treated the urine of 221 individuals according to the extraction and chromatographic method of Friedhoff and van Winkle Of these, 106 cases had psychic disorders, eighty-eight were physically ill and twenty-seven were normal subjects Twenty hour urine samples were collected in the first 24 h after admission to hospital, before the beginning of any treatment The patients had received no drugs for periods of from 3 weeks to several months Of the 106 patients with psychic disorders, fifty-one were diagnosed as schizophrenics by three psychiatrists The remaining fifty-five patients were divided as follows sixteen had neurotic disorders, fifteen had psychopathic disorders—seven mania and nine endogenous depression—and eight had involutional or artheriosclerotic dementia Of the patients with physical disorders, nine were suffering from Parkinson's disease, and the rest were cardiac, rheumatic or digestive cases

The pink spot was present in the urine of twenty-three out of fifty-one schizophrenics (45 per cent), and three cases were impossible to assess We could not find any significant correlation between the form of schizophrenia and the frequency of the pink spot[4] As for the urine of the other seventeen subjects, only in one case of Parkinsonism did we obtain a positive reaction and in another case with the same diagnosis the reaction was impossible to assess The presence of DMPEA in the urine of patients with Parkinson's disease has been reported before[12]

We consider these results to confirm the chromatographic technique of Friedhoff and van Winkle as a useful diagnostic method If the pink spot consists of at least seven amines, and if it is specific for schizophrenia and possibly for a few restrained disturbances of the basal ganglia as well, then a certain common derangement of amine metabolism, including the production of DMPEA, occurs in such cases These data agree with the high concentration of catecholamines and dopamine in subcortical structures and with the Parkinson-like symptoms induced by psychoactive drugs which have a depressant action on these structures[13]

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[1] Friedhoff, A J , and van Winkle, E , *Nature*, **194**, 897 (1962)
[2] Sen, N P , and McGeer, P H , *Biochem Biophys Res Commun* , **14**, 227 (1964)
[3] Kuehl F A , Hichens, M , Ormond, R E , Meisinger, M A P , Gale, P H , Cirillo, V J , and Brink, N G , *Nature*, **203**, 154 (1964)
[4] Bourdillon, R E , Clarke, C A , Ridges, A P , Sheppard, P M , Harper, P , and Leslie, S A , *Nature*, **208**, 453 (1965)
[5] Perry, T L , Hansen, S , and MacIntyre, L , *Nature*, **202**, 519 (1964)
[6] Faurbye, A , and Pind, K , *Acta Psychiat Scand* , **40**, 540 (1964)
[7] Studnitz, W V , and Nyman, G E , *Acta Psychiat Scand* , **41**, 117 (1965)
[8] Boulton, A A , and Felton, C A , *Nature*, **211**, 1404 (1966)
[9] Bell, C E , and Somerville, A R , *Nature*, **211**, 1406 (1966)
[10] Creveling, C R , and Daly, J W , *Nature*, **216**, 190 (1967)
[11] Talesada, M , Kakimoto, Y , Sano, I , and Kaneko, Z , *Nature*, **199**, 203 (1963)
[12] Barbeau, A , DeGroot, J A , Joly, J G , Raymond-Trembley, D , and Donaldson, J , *Rev Canad Biol* , **22**, 469 (1963)
[13] Steiner, W G , and Himwich, H E , *Science*, **136**, 873 (1962)

Effects of Enzymes on the Pharmacology of Isolated Organs

A NUMBER of reports[1-5] have described the effects of enzymes on the mechanical responses of isolated muscles obtained in response to drugs and other stimuli Enzyme treatment, however, does not always provide a way of examining the nature of drug receptors because the drug receptor interaction is only the first of a chain of events leading to contraction If the predominant enzyme effect is on a stage of the process following the drug receptor interaction, no information about the nature of the receptor can be adduced. In such cases a proportional reduction in the response to several different stimuli might be expected We wish to present our own findings as a case in point

The effects of tryptic digestion on the sensitivity of frog rectus abdominis muscle to acetylcholine (stimulus via drug receptors) and to direct current (stimulus bypassing drug receptors) were studied Both the aggregate (contracture) and the discrete (single muscle cell membrane potentials) responses were monitored by conventional means The results show that although classical stimulus-effect relationships are maintained after enzyme treatment (Fig 1), the responses to both acetylcholine and d c stimulus deteriorate at comparable rates (Fig 2) with continued exposure to trypsin

Furthermore, this relationship holds for all concentrations of trypsin which produce a measurable desensitization of the muscle There is concomitant obliteration of muscle cell membrane potentials successively deeper within the preparation (Table 1) Histological sections confirm this invasive deterioration of cellular structures

Our findings indicate that, for the rectus muscle and trypsin, the penultimate process of contracture is affected

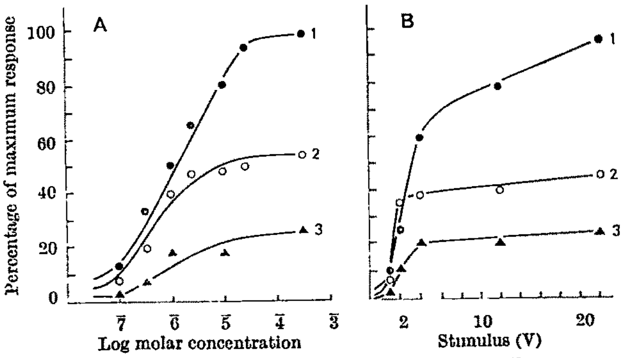

Fig 1 Effect of digestion by 0 1 mg/ml of trypsin on the response of frog rectus abdominis to acetylcholine (*A*) and to electrical stimulation (*B*) 1, Control, 2, after 10 min digestion, 3, after 20 min digestion

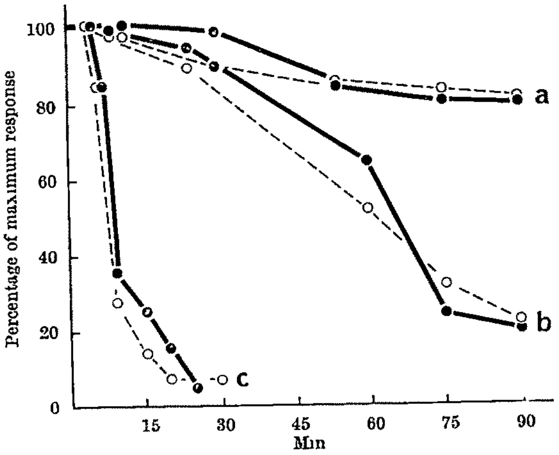

Fig 2 Change in sensitivity of frog rectus abdominis muscle to acetylcholine (——) and to electrical stimulus (– – – –) as a function of time and of enzyme concentration *a*, 0 01 mg/ml of trypsin, *b*, 0 1 mg/ml of trypsin, *c*, 1 mg/ml of trypsin

Table 1 LATENT PERIOD FOR REDUCTION OF MUSCLE CELL MEMBRANE POTENTIALS FROM INITIAL LEVELS (−65 TO −78 mV) TO −10 TO −20 mV AT VARIOUS LOCATIONS WITHIN A RECTUS MUSCLE TREATED WITH 0 1 mg/ml OF TRYPSIN

Depth (μ)	No of observations	Time of depolarization (min)
100	5	2 5–3 5
100–400	5	7 0–14
400–600	3	24–45

to the extent that precludes an assessment of specific effects of the enzyme on drug receptors We suggest that a similar examination of other systems may show that this is a common limitation to such studies

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[1] Cuthbert, A W, *J Pharm Pharmacol*, 18, 561 (1966)
[2] Edery, H, *J Pharmacol*, 22, 371 (1964)
[3] Graham, J D P, and Alkatib, H, *Brit J Pharmacol*, 28, 1 (1966)
[4] Lu, F C, *Brit J Pharmacol*, 7, 637 (1952)
[5] Wooley, D W, and Gommi, B. W, *Nature*, 202, 1074 (1964)

Production of Renin by *in vitro* Cultures of Human Chorion and Uterine Muscle

Renin-like enzymes have been extracted from a variety of tissues other than the kidney, including cat placenta[1], rabbit uterine muscle and placenta[2–4], hog aorta[5] and mouse submaxillary gland[6,7]

In situ synthesis has been claimed in pregnant rabbit uteri on the basis of the finding that bilateral nephrectomy does not lead to depletion of renin from this tissue[8] Proof of synthesis by *in vitro* culture techniques has, however, only been shown in renal cortical cells[9,10], and even in these studies renin production was poor, with supernatant concentrations below those normal in plasma In addition, evidence of renin production through sub-culture was not provided

The description by Brown *et al* [11] of high concentrations of renin in human amniotic fluid has led us to measure the renin content of maternal and foetal tissue, and to reveal that chorion laeve had a higher content per wet weight than myometrium, decidua, amnion and placenta Concentration in chorion was four times that of either amnion or liquor amnii, and 100 times that of placenta and uterine muscle These findings suggested that chorion could be an important site of renin synthesis We report here confirmation of this suggestion, together with evidence that cells in human uterine muscle are capable of producing renin during *in vitro* culture

Tissues were collected from five normal pregnant subjects at the end of gestation Four of the five subjects were delivered by caesarean section before the onset of labour, and one subject was delivered vaginally Peripheral chorion (chorion laeve) was collected by stripping amnion from chorion, and chorion was also dissected from the foetal surface of the placenta (chorion plate) Placenta itself was sampled from the maternal surface, and uterine muscle and decidua were dissected at caesarean section

Tissues were washed five times in an antibiotic solution of 10^4 μg/ml of kanamycin and 500 μg/ml of fungizone in 'Dulbecco' phosphate buffer (personal communication from Von der Borch) Tissues were minced into small fragments (approximately 1 mm^3) and explanted into chick plasma clots in Leighton tubes The culture medium consisted of 6 per cent chick embryo extract, 40 per cent human AB serum and 54 per cent tissue culture medium 199 (Commonwealth Serum Laboratories) The cultures were maintained at 37° C and the medium was changed every 48 or 72 h and replaced with 2 ml of fresh medium After each change, the culture medium was frozen and stored, together with a similar volume of control culture medium not exposed to the tissue culture When confluent growth had occurred—usually after 7–10 days—sub-cultures were made by trypsinization with 0 25 per cent trypsin and resuspension of the cells in the culture medium Placenta was taken from two subjects, chorion laeve from four subjects, chorion plate from two subjects, amnion from three subjects and uterine muscle from two subjects. All tissues were cultured at least in duplicate.

The growth of the tissue cultures is summarized in Table 1 In ten out of thirty-five primary cultures, no growth occurred In the remaining twenty-five cultures, some primary growth occurred, and eleven were suitable for sub-culture

Supernatants collected at each medium change were frozen for storage and control medium was sampled at the same time. The concentration of renin in selected samples was then assayed quantitatively by the technique described for plasma[12] In this method, renin is prepared free of endogenous substrate and then incubated in standard conditions with a constant amount of added substrate in an angiotensinase-free environment The velocity of angiotensin formation in the incubate was assayed using rat blood pressure, and specificity for renin was tested by the method used before[12], which is based on (*a*) the peptide nature of the end product, (*b*) consumption of substrate, and (*c*) antibody inactivation. Non-specific pressor and depressor responses were not encountered Renin was present in control media in approximately half the normal concentration in plasma[12], and this was subtracted accordingly in deriving renin production

Fig 1 shows the concentration of renin in supernatants of all cultures which displayed some growth. The six primary cultures of chorion consistently produced large amounts of renin into the media The time course of this production was similar for all cultures, with peak concentrations on days 5–9 The highest concentration achieved was 1,000 U/ml, approximately 100 times the usual concentration of renin[12] In five cultures a fall in renin production after day 9 was paralleled by a decline in the rate of growth. Renin production, nevertheless, continued at a lower level for 24 days Only one primary culture of chorion had a consistent growth rate throughout (closed squares, Fig 1), and this was successfully sub-cultured three times during a 55 day period, at which time supernatant concentrations were still reaching the concentrations displayed by the primary growth

Myometrium also produced large amounts of renin, but for an entirely different time course (Fig 1) Three of four cultures displayed grew well throughout, but each produced renin only after an initial interval of 10–15

Table 1

Tissue	No of cultures	Explant growth*		No of cultures sub-cultured	No of sub-cultures	Total time of culture (days)
Chorion laeve	10	No growth	4	—	—	—
		Fair growth	3	—	—	All 15 days
		Good growth	2	—	—	27, 27
		Excellent growth	1	1	3	55
Uterine muscle	5	No growth	1	—	—	—
		Fair growth	1	1	1	30
		Good growth	3	3	1, 1, 3	30, 27, 27
Placenta	4	Poor growth	2	—	—	20, 20
		Fair growth	2	—	—	24, 24
Amnion	6	No growth	1	—	—	—
		Good growth	2	2	1, 1	27, 27
		Excellent growth	3	3	2, 1, 1	27, 25, 25
Decidua	4	Poor growth	2	—	—	20, 20
		Fair growth	2	—	—	18, 18
Chorion plate	4	No growth	2	—	—	—
		Poor growth	1	—	—	—
		Good growth	1	1	2	57

* The terms poor, fair, good and excellent growth apply to the primary culture

These terms refer to the period of time in culture before outgrowth from the explant occurred Thus, with poor growth, outgrowth occurred after 10–14 days, fair growth occurred after 7–10 days, good growth after 3–7 days and excellent growth within 3 days

days, during which none was detected After the tenth day, renin concentration increased progressively, reaching a level 120 times that of normal plasma before sub-culture The fourth culture, which showed fair growth, is not shown in Fig 1, and produced no renin during 30 days of incubation Myometrium was successfully sub-cultured on two occasions and in each instance renin production continued

Placenta did not grow well, but two of the four cultures showed fair growth and produced some renin into supernatant The time course was similar to that for chorion, but at a much lower level No growth was adequate for sub-culture

Although amnion grew consistently well and was readily sub-cultured, no renin was produced into the supernatant (Fig 1) Decidua neither produced renin nor grew well, and was not sub-cultured. One culture of chorion plate grew well and was sub-cultured, but produced no renin, while a second grew poorly and produced low but significant amounts

These experiments provide the first proof that other tissues as well as the kidney can synthesize renin Both chorion and myometrium growing *in vitro* produced renin at remarkable rates In 2 days the amount of renin released into the 2 ml of culture medium totalled more than that normally present in 200 ml of plasma That this renin was synthesized and not simply a release of stored enzyme is shown by its continued production through sub-culture, and by the fact that the total amount produced was far in excess of that shown previously to be present in these tissues[12]

The different time course for the production of renin in chorion and myometrium cannot be related to different growth rates, for both grew well from the start Nor is it likely that the particular cell producing renin took longer to become established in cultures of myometrium than chorion, because both cultures seemed to produce only fibroblast-like cells The possibility that myometrium contains an enzyme inhibitor which is progressively lost during culture has been excluded (unpublished work of Skinner, Lumbers and Symonds) by finding that added renin displays the expected activity in myometrial extracts We have previously shown that human myometrium contains little renin, and so it seems more likely that the synthesis of this enzyme is weakly repressed in human myometrial cells, and that, after passage through several *in vitro* generations, this repression is lost It follows from this hypothesis that production of renin by human myometrium *in vivo* probably does not usually occur Rabbit myometrium is notably different, however, with a high renin concentration[2] and presumably in this species synthesis is not repressed[8] Such extreme differences between species are likely to be of functional significance, but the reasons are not apparent at present

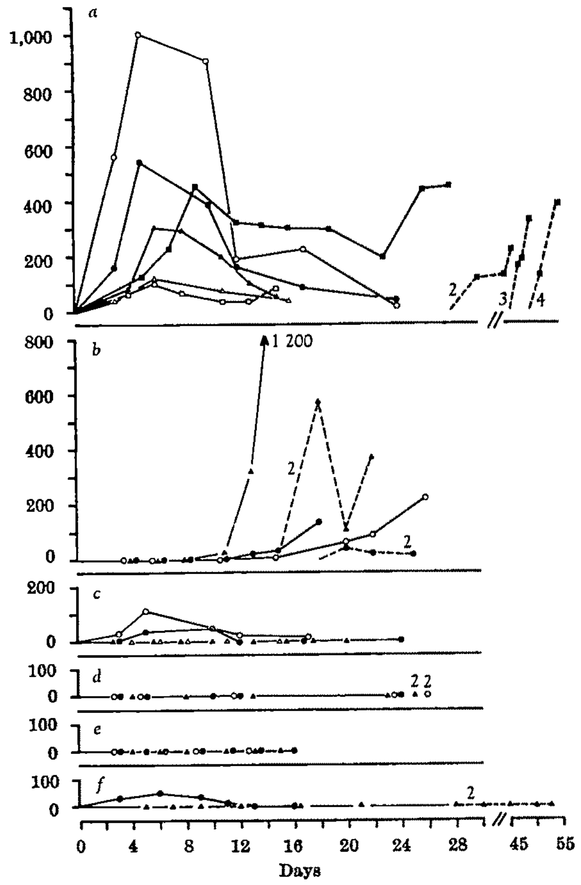

Fig 1 Renin concentration (U/ml) in selected culture media changed every second or third day Primary cultures are shown in solid lines Secondary, tertiary and quaternary cultures are shown as broken lines between symbols corresponding to the appropriate primary culture, and are numbered 2, 3 and 4, respectively *a*, Chorion, *b*, myometrium, *c*, placenta, *d*, amnion, *e*, decidua, *f*, chorion plate

No conclusion can be reached at this stage as to the specific cells involved in renin synthesis Although the growing cells looked like fibroblasts, histological investigation may reveal some heterogeneity We cannot exclude the possibility that the cells growing in culture may be decidual This seems unlikely, in view of our earlier finding that decidua contains much less renin per wet weight than chorion (unpublished) The close proximity to the amniotic sac of cells capable of synthesizing renin provides a logical explanation for the high concentrations of renin in liquor amnii

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[1] Stakemann, G , *Acta Pathol Microbiol Scand* , **50**, 350 (1960)
[2] Gross, F , Schaechtelin, G , Ziegler, M , and Berger, M , *Lancet*, i, 914 (1964)
[3] Bing, J , and Faarup, P , *Acta Pathol Microbiol Scand* , **67**, 169 (1966)
[4] Ferris, T F , Gorden, P , and Mulrow, P J , *Amer J Physiol* , **212**, 698 (1967)
[5] Gould, A B , Skeggs, L T , and Kahn, J R , *J Exp Med* , **119**, 389 (1964)
[6] Werle, E , Vogel, R , and Goldel, F L , *Naunyn Schmiedeberg Arch Exp Pathol* , **230**, 236 (1957)
[7] Bing, J , and Faarup, P , *Acta Pathol Microbiol Scand* , **64**, 203 (1965)
[8] Gorden, P , Ferris, T F , and Mulrow, P J , *Amer J Physiol* , **212**, 703 (1967)
[9] Robertson, A L , Smeby, R R , Bumpus, F M , and Page, I H , *Science*, **149**, 650 (1965)
[10] Robertson, A L , Smeby, R R , Bumpus, F M , and Page, I H , *Hypertension*, **14**, 131 (1966)
[11] Brown, J J , Davies, D L , Doak, P B , Lever, A F , and Robertson, J I S , *Lancet*, ii, 64 (1964)
[12] Skinner, S L , *Circulat Res* , **20**, 391 (1967)

Ratios between Contents of DNA, RNA and Protein in Different Micro-organisms as a Function of Maximal Growth Rate

THE growth medium determines the macromolecular composition as well as the growth rate (number of doublings per hour) of micro-organisms in the steady state of growth For a given micro-organism the ratios of RNA protein and RNA DNA are linear functions of the growth rate, whereas the ratio of DNA protein is virtually independent of the rate of growth[1–4] All these studies involved one micro-organism studied at different growth rates

We have looked for a correlation between the maximal growth rates of different micro-organisms and their contents of DNA, RNA and protein. The organisms tested included twelve bacteria, a haploid and a diploid strain of yeast and a ciliate protozoon, selected to cover a range of generation times for growth at 30° C in complex media which create optimal growth conditions for many organotrophic micro-organisms[5]

All organisms were grown in batch cultures The bacteria, except *Lactobacillus bulgaricus* and *Clostridium perfringens,* were grown aerobically in 0 8 per cent Difco broth, 0 1 per cent NH_4Cl, 0 05 per cent $MgSO_4$ $7H_2O$ and 0 5 per cent glucose *Cl perfringens* was grown anaerobically in this medium and *Lactobacillus bulgaricus* was grown aerobically in 4 per cent Elliker broth (Difco) *Saccharomyces cerevisiae* was grown aerobically in 2 per cent Bacto peptone, 0 1 per cent yeast extract and 2 per cent glucose *Tetrahymena pyriformis* was grown aerobically at 28° C in 2 per cent 'Proteose' peptone (Difco), 0 1 per cent liver extract (Wilson Laboratories) and inorganic salts as described earlier[2] Generation times of the bacteria and of the yeast were determined by measuring the increase in turbidity at 450 mμ (bacteria) and at 420 mμ (yeast). The cells were collected in the steady state of growth at an optical density (*O D*) of approximately 0 5 Growth of *Tetrahymena pyriformis* was followed by particle counts in a Coulter electronic cell counter, model *F* *Tetrahymena* was grown at 28° C, and so corrections were made for the generation time assuming a doubling of the growth rate for a 10° C increase in temperature All growth rates were calculated as the number of doublings per hour at 30° C

The chemical analysis of the cellular content of DNA, RNA and protein was carried out as follows Nucleic acids were extracted from about 100 mg wet weight of cells by treatment with 0 5 N perchloric acid at 70° C for 20 min Deoxyribose was determined by the diphenylamine procedure using dAMP as standard[6] Ribose was determined by the orcinol procedure using AMP as standard[7] The protein content in the residue was determined by the method of Lowry using bovine serum albumin (Armour) as standard[8]

The rate of protein synthesis per unit of RNA has previously been determined according to the expression protein/RNA × μ, where μ = doublings per hour (refs 1, 2 and 4) Table 1 shows the rates of protein synthesis per unit of RNA for different organisms collected from steady state growing cultures The values for the rate of protein synthesis per unit of RNA vary from 2 0 to 3 1 h^{-1} This suggests a similar efficiency of the ribosomes in protein synthesis in the organisms which we studied

Table 1 RATIOS BETWEEN DNA, RNA AND PROTEIN OF DIFFERENT MICRO-ORGANISMS GROWN IN COMPLEX MEDIA AT 30° C

Micro-organism	Generation time(h)	μ = Growth rate (doublings/h)	$\frac{\text{Protein}}{\text{RNA}}$	$\frac{\text{Protein}}{\text{RNA}} \times \mu$ (h^{-1})	$\frac{\text{RNA}}{\text{DNA}}$	$\frac{\text{RNA}}{\text{DNA}} \times \mu$ (h^{-1})
Aerobacter aerogenes	0 43	2 33	1 18	2 7	12 0	28
E coli	0 50	2 00	1 47	2 9	10 9	22
Salmonella typhimurium	0 50	2 00	1 57	3 1	9 8	20
Citrobacter freundii	0 50	2 00	1 35	2 7	10 6	21
E coli B	0 52	1 92	1 54	3 0	10 4	20
Serratia marcescens	0 52	1 92	1 35	2 6	10 2	20
Bacillus subtilis	0 58	1 72	1 41	2 4	15 0	26
Erwinia carotovora	0 68	1 47	1 67	2 5	10 9	16
Micrococcus anhaemolyticus	0 72	1 39	1 85	2 6	11 2	16
Cl perfringens	0 77	1 30	1 83	2 4	12 0	16
Pseudomonas aeruginosa	0 68	1 47	1 84	2 7	5 1	7
Lactobacillus bulgaricus	0 92	1 09	2 81	3 1	9 4	10
Saccharomyces cerevisiae (diploid) No 66	1 25	0 80	2 44	2 0	82 0	66
Saccharomyces cerevisiae (haploid) 1507–7*A*	1 50	0 67	2 98	2 0	105 0	70
Tetrahymena pyriformis GL	2 00	0 50	4 34	2 2	24 0	12

The determination of the growth rates of the micro-organisms and the chemical analysis of DNA, RNA and protein were carried out as described in the text Each value represents the mean of triplicate determinations of DNA, RNA and protein in two independently grown cultures

The ratio of DNA protein for *Escherichia coli, S typhimurium, A aerogenes* and *T pyriformis* in the steady state of growth has been shown to be almost independent of the growth rate of these organisms[1–4] The relationship is almost linear when the ratios of DNA protein for each of the organisms studied are plotted against their maximal growth rate (Fig 1) The ratio of DNA protein for *S cerevisiae* is lower than would be expected from the growth rate When, however, a haploid strain of *S cerevisiae* was compared with a diploid strain, the general trend shown in Fig 1 was confirmed The diploid strain shows a significantly higher ratio of DNA protein as well as showing faster growth

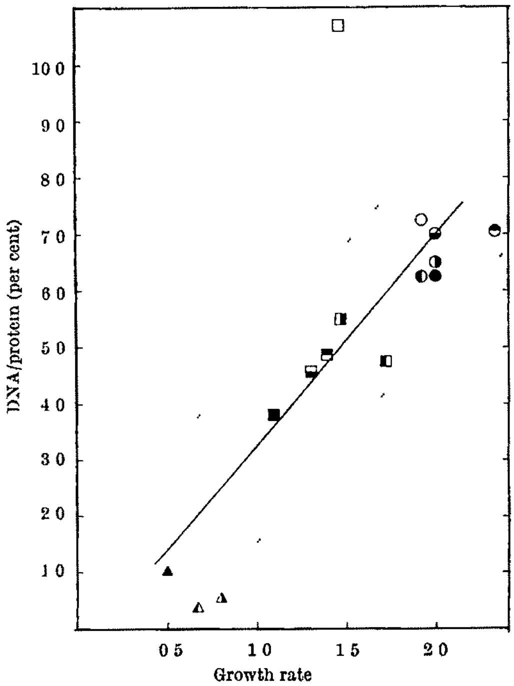

Fig 1 Ratio of DNA protein as a function of maximal growth rate for a number of micro-organisms growing at 30° C Each ratio of DNA protein represents the mean of triplicate determinations of DNA and protein contents in two independently grown cultures DNA and protein were determined as described in the text The solid line is drawn as calculated by the "estimate (regression) equation" The zone ± 1 $s_{Y\,X}$ (standard error of estimate) is shown by the dotted lines ◓, *Aerobacter aerogenes*, ●, *Escherichia coli*, ◐, *E coli B*, ◑, *Salmonella typhimurium*, ◧, *Erwinia carotovora*, ○, *Serratia marcescens*, ◒, *Citrobacter freundii*, ◧, *Bacillus subtilis*, □, *Pseudomonas aeruginosa*, ■, *Lactobacillus bulgaricus*, ⬒, *Micrococcus anhaemolyticus*, ⬓, *Cl perfringens*, ◭, *Saccharomyces cerevisiae* (haploid) 1507–7*A*, ◮, *Saccharomyces cerevisiae* (diploid) No 66, ▲, *Tetrahymena pyriformis* GL

The correlation shown in Fig 1 suggests that the amount of the DNA template can be a rate limiting factor for the flow of information from DNA to protein, that is, that the amount of DNA can be limiting for *m*RNA synthesis A high ratio of DNA protein could favour a high overall production of *m*RNA in fast growing organisms This explanation is consistent with the hypothesis that the degradation of *m*RNA is possibly correlated with the translation of *m*RNAs on the ribosomes[9–11] A fast growing organism with a high ratio of DNA protein might thus have a higher turnover of *m*RNA than a slow growing organism because of its higher content of DNA and ribosomes

A second explanation could involve the fact that ribosomes are active in the regulation of *m*RNA synthesis in bacteria[11–14] This mode of regulation, which suggests that ribosomes are needed for the detachment of *m*RNA from the DNA template, could depend on the appropriate amount of DNA per ribosome Thus the ratio of RNA DNA as well as the ratio of RNA protein could be important for a micro-organism to grow at the maximal rate

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[1] Kjeldgaard, N O , and Kurland, C G , *J Mol Biol* , **6**, 341 (1963)
[2] Leick, V , *CR Trav Lab Carlsberg*, **36**, No 7 (1967)
[3] Neidhardt, F C , and Magasanik, B , *Biochim Biophys Acta*, **42**, 99 (1960)
[4] Rosset, R , Julien, J , and Monier, R , *J Mol Biol* , **18**, 308 (1966)
[5] Mason, M M , *J Bact* , **29**, 103 (1935)
[6] Burton, K , *Biochem J* , **62**, 315 (1956)
[7] Mejbaum, W , *Z Physiol Chem* , **258**, 117 (1939)
[8] Lowry, O H , Rosebrough, N J , Farr, A L , and Randall, R J , *J Biol Chem* , **193**, 265 (1951)
[9] Fan, K , Higa, A , and Levinthal, C , *J Mol Biol* , **8**, 210 (1964)
[10] Gros, F , Naono, S , Woese, C , Wilson, G , and Attardi, G , *Informational Macromolecules* (edit by Vogel, H J , Bryson, V , and Lampen, O J), 387 (Academic Press, New York, 1963)
[11] Naono, S , Rouviere, J , and Gros, F , *Biochim Biophys Acta*, **129**, 271 (1966)
[12] Revel, M , and Gros, F , *Biochem Biophys Res Commun* , **27**, 12 (1967)
[13] Shin, D H , and Moldave, K , *J Mol Biol* , **21**, 231 (1966)
[14] Stent, G S , *Science*, **144**, 816 (1964)

Evidence for an Extragranular Localization of Tyrosine Hydroxylase

GROWING interest in the intracellular localization of enzymes has led to the standardization of techniques generally used in tissue fractionation The scope and limitations of these methods have been defined already, in particular the use of marker enzymes in profiling the distribution of different particles[1]

The data concerning the intracellular localization of tyrosine hydroxylase are rather conflicting Nagatsu *et al* [2], who first isolated the enzyme, described it as particle-bound, while Udenfriend[3] has proposed that all enzymes involved in biosynthesis of catecholamine are organized into granules containing catecholamine In the brain, tyrosine hydroxylase was found to be linked to a particulate fraction[4] Stjarne and Lishajko[5], however, have shown, by incubating *in vitro* various fractions of a bovine splenic nerve homogenate, that this enzyme occurs outside the granules

This communication describes experiments dealing with the activity of tyrosine hydroxylase in various fractions of bovine adrenal medulla and in different segments of the splenic nerves of dogs

Bovine adrenal medullae were homogenized in five volumes of either 0 25 M sucrose or of 0 16 M potassium chloride with a 'Dual' homogenizer The sediment obtained after low speed (7,000g min) centrifugation was resuspended in the same volume and centrifuged again at the same speed to provide a pellet of the nuclear fraction A sample of the pooled supernatants was kept and the rest was further fractionated by centrifuging at $6 \times 10^6 g$ to obtain a large particulate fraction and a final supernatant The whole fractionation procedure was carefully carried out at 0° C

Splenic nerves were ligatured as previously described[6] Two segments of nerves were removed 48 h after operation, one above (proximal to) and another below (distal to) the ligature As with the coeliac ganglion or normal splenic nerves, they were homogenized in twenty volumes of 0 25 M sucrose

The total activity of dopamine-β-hydroxylase was assayed using tyramine as the substrate and in the presence of 'Triton X-100', as previously described[7] Tyrosine hydroxylase was assayed according to Nagatsu *et al* [2] using labelled tyrosine (New England Nuclear Corp , ^{14}C-L-Tyrosine, specific activity 300 mCi/mmole) The dopamine-β-hydroxylase was separated on Al_2O_3 columns and the radioactivity was counted in a Packard liquid scintillation spectrometer In order to eliminate interfering of large amounts of catecholamines, acid-insoluble proteins were determined according to Lowry's method[8]

Fig 1 shows the distribution patterns of tyrosine hydroxylase and dopamine-β-hydroxylase when the fractionation of adrenal medulla was carried out either in a sucrose or in a KCl medium Dopamine-β-hydroxylase provides a valuable marker to fix the position of the catecholamine granules on the distribution diagrams[10] This enzyme behaves similarly in both experimental conditions, as shown by its distribution patterns, in that more than 60 per cent of the activity is found in the large particulate fraction On the other hand, the distribution of tyrosine hydroxylase is quite different from that of dopamine-β-hydroxylase In sucrose solutions 34 per cent of the activity is found in the nuclear fraction and 41 per cent in the supernatant This bimodal distribution becomes nearly unimodal in potassium chloride solutions with values of 16 per cent and 70 per cent

The different distribution of tyrosine hydroxylase could be a result of higher adsorption of soluble proteins in sugar solutions than in ionic media Nevertheless, these experiments show clearly that, by comparing the distribution patterns of these two enzymes, it cannot be suggested that tyrosine hydroxylase is contained in the catecholamine granules Furthermore, other data concerning the latency of all the enzymes of noradrenaline biosynthesis and dealing with the uptake of various amines[11] support the view that tyrosine hydroxylase is located in an extragranular site, probably the cell sap The results listed in Table 1 lead to a similar conclusion

Thus the activity of tyrosine hydroxylase was found to decrease slightly above the proximal segment 48 h after ligation, although, for the same period, the activity of dopamine-β-hydroxylase was twenty times greater than that of the control values In contrast, both enzymatic activities decreased similarly in the distal segments It should also be noted that these activities are slightly

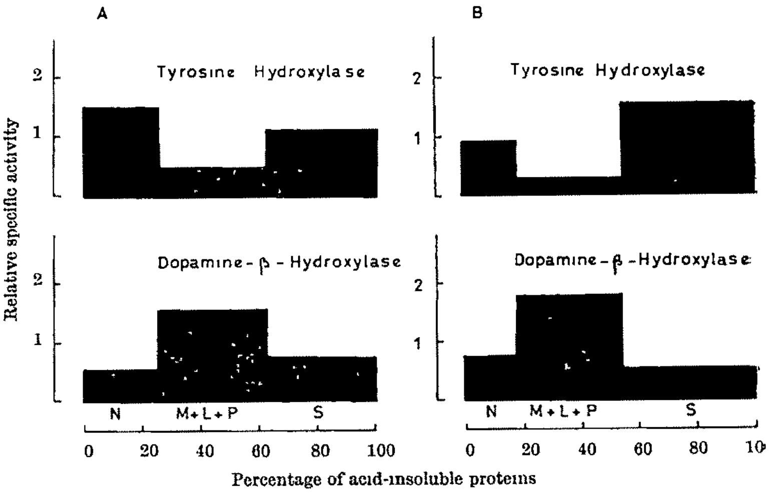

Fig 1 Distribution pattern of enzymes *A*, fractionation was performed in 0 25 M sucrose and, *B*, in 0 16 M potassium chloride Each graph represents the mean of three experiments N, Nuclear fraction, M+L+P, large particulate fraction, S, supernatant. The results are presented in the manner used by de Duve *et al* [9]

Table 1. ENZYMATIC ACTIVITY IN SPLENIC NERVES AND COELIAC GANGLIA OF DOGS

	Tyrosine hydroxylase (μmoles/g/h ± S D)	Dopamine-β-hydroxylase (μmoles/g/h ± S D)
Splenic nerve		
Control	0 39 ± 0 09 ($n=5$)	0 79 ± 0 56 ($n=5$)
Proximal part 48 h after ligation	0 21 ± 0 09 ($n=12$)	15 1 ± 6 8 ($n=5$)
Distal part 48 h after ligation	0 12 ± 0 07 ($n=12$)	0 39 ± 0 35 ($n=5$)
Coeliac ganglion		
Control	0 56 ± 0 24 ($n=16$)	1 49 ± 0 36 ($n=11$)

higher for the coeliac ganglion than for the splenic nerves

If tyrosine hydroxylase were associated with granules containing catecholamine and dopamine-β-hydroxylase, a similar accumulation above a constriction of the sympathetic nerves, as proved for dopamine-β-hydroxylase and previously for noradrenaline[6], should be expected for tyrosine hydroxylase The fact that tyrosine hydroxylase does not increase in the proximal part after ligation points to an extragranular localization of the enzyme This new approach confirms the previous data of Stjarne and Lishajko[5]

Because these observations show that tyrosine hydroxylase in the adrenal medulla and sympathetic nerves is independent from the granules containing catecholamine, the first step of catecholamine biosynthesis, as well as the second one[11], occurs outside these granules

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[1] de Duve, C , *J Theoret Biol* , **6**, 33 (1964)
[2] Nagatsu, T Levitt, M , and Udenfriend, S , *J Biol Chem* , **239**, 2910 (1964)
[3] Udenfriend, S , in *Mechanisms of Release of Biogenic Amines* (edit by von Euler, U S , Rosell, S , and Uvnas, B), 103 (Pergamon Press, Oxford, 1966)
[4] McGeer, P L , Bagchi, S P , and McGeer, E G , *Life Sci* , **4**, 1859 (1965)
[5] Stjarne, L , and Lishajko, F , *Biochem Pharmacol* , **16**, 1719 (1967)
[6] Laduron, P , and Belpaire, F , *Life Sci* , **7**, 1 (1968)
[7] Belpaire, F , and Laduron, P , *Biochem Pharmacol* (in the press)
[8] Lowry, H , Rosebrough, N J , Far, A L , and Randall, J , *J Biol Chem* , **193**, 265 (1951)
[9] de Duve, C , Pressman, B C , Gianetto, R , Wattiaux, R , and Appelmans, F , *Biochem J* , **60**, 604 (1955)
[10] Belpaire, F , and Laduron, P , *Arch Intern Physiol. Biochim* , **75**, 550 (1967)
[11] Laduron, P , and Belpaire, F , *Biochem Pharmacol* (in the press)

BIOLOGY

Germination of Caryophyllaceae at Low Temperatures in relation to Geographical Distribution

SEED germination is one of many factors which determine whether a particular species is able to maintain itself in a given locality This must occur at a season which will allow the subsequent development of the plant and the production of flowers and viable seed There have been many reports of the mechanisms which regulate the time of germination based on requirements for a period of after-ripening, temperature conditions, light or mechanical injury Yet few studies have been made on the relation between germination patterns and natural distributions of different species This work was done with a group of species of Caryophyllaceae distributed in different localities throughout Europe, in an attempt to determine features of the germination response which could be correlated with climatic conditions

Ripe seed was collected from plants growing in the Royal Botanic Gardens, Kew, and stored over calcium chloride at 18° to 22° C, until required for tests. Tests were done in incubators at 2° C, or on a specially designed thermogradient bar set to provide continuously variable temperatures from about 5° to about 18° C Temperatures on the bar were monitored through thermo-couples and could be defined at any point throughout the bar's length to within ±0 5° C The seeds were sown on a basic medium consisting of the standard Long Ashton nutrient solution[1], used at half-strength and stabilized with 1 per cent agar They were taken as having germinated as soon as the radicle was visible Records were made either at convenient intervals in the incubators at 2° C, or daily on the thermo-gradient bars

Fig 1 illustrates the way in which the rate of germination at 2° C varied between different species grouped according to their broad geographical distributions These groupings are approximate, for the occurrence of some of these species as weeds makes their natural distribution hard to define The geographical definitions used, however, are so broad and the difference between the groups so distinct that they overwhelm any differences of opinion on the precise ranges of the species concerned

There were wide variations in the rates of germination and the limits for reaching the 50 per cent level lay between 19 days for *Agrostemma githago* and nearly 190 days for *Silene alba* All the species from the Mediterranean germinated rapidly at this low temperature, whereas none of the species from northern Europe reached the 50 per cent level in less than 100 days; central European species occupied intermediate positions

Germination rates of a selection of species were recorded at temperatures between 5° C and 18° C on the thermo-gradient bar, and graphs were prepared from which the number of days required for each species to reach 50 per cent germination at different temperatures could be calculated. The results (Fig 2) showed that the rates of germination varied from one species to another, and that the speed of germination could be related to the geographical range over the whole temperature range tested There was no straight line relationship between germination rate and temperature, and the exponential curves obtained showed a rapidly increasing steepness as the temperatures fell. There was evidence that the temperature range over which the curves tended to the vertical was correlated with geographical range, occurring at increasingly higher temperatures as the distribution of the species moved north *Kohlrauschia prolifera*, classed as a central European species, was a possible exception to these generalizations appearing in Fig 2 in association with the Mediterranean species *Agrostemma githago* and *Silene nocturna*

Supplementary experiments on these species have shown that most of them have a short period of relative dormancy, after the fully mature seed is shed, during which they fail to germinate at temperatures above about 20° C Germination at lower temperatures is normally unaffected by after-ripening requirements, and, because the seed in these tests had been kept in store for several months and germinated freely to 90 per cent or more at temperatures between 10° C and 18° C, it is unlikely that the results obtained were distorted by any unsatisfied after-ripening requirements of the seed It seems probable that the measurements represent the capacity of each species to grow at low temperatures, and reflect a phenomenon similar to that reported by Cooper[2] for ecotypes of *Lolium perenne* collected from varying geographical locations Cooper found that *Lolium perenne* from northern or colder areas stopped growth at higher temperatures than those from regions with milder winters such as the Mediterranean

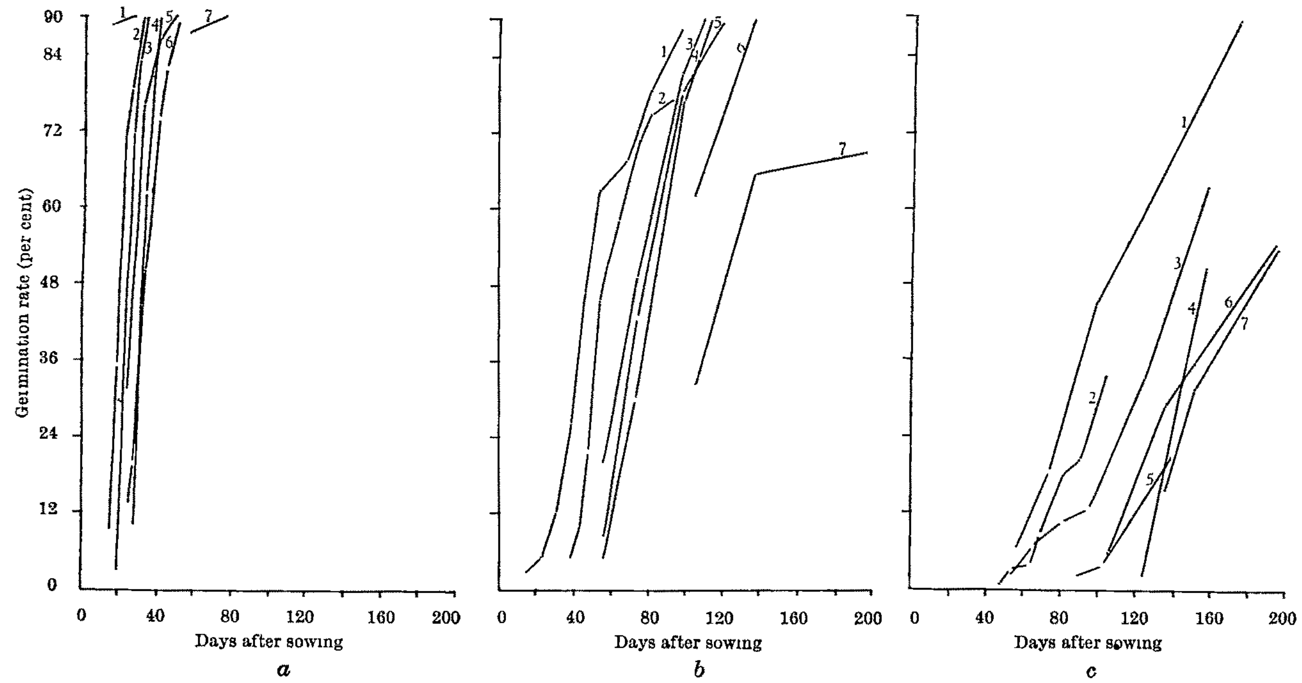

Fig 1 Rate of germination at 2° C of species of Caryophyllaceae from *a*, Mediterranean 1, *Agrostemma githago* L (1966), 2, *Agrostemma githago* L , 3, *Silene chloraefolia* Sm , 4, *S nocturna* L , 5 *S colorata Poir*, 6, *S rubella* L , 7, *S nocturna* (1966) *b*, Southern and central Europe 1, *Vaccaria pyramidata* Medicus, 2, *Silene gallica* L , 3, *Silene italica* (L) Pers , 4, *Lychnis flos-jovis* (L) Desr 5, *Silene compacta* Fischer, 6, *S italica* (L) Pers (1966), 7, *Kohlrauschia prolifera* (L) Kunth *c*, Northern and north-west Europe 1 *Silene dioica* (L) Clairu, 2, *Silene alba* (Mill) E H L Krause, 3, *Silene maritima* With , 4, *Dianthus deltoides* L , 5, *Silene cucubalus* Wibel, 6, *Lychnis viscaria* L , 7, *Silene alba* (1966) All seed from 1967 harvest unless otherwise marked

The exponential relationship between germination rate and temperature shows that, with these species, straight line extrapolations of the kind made by Went[3] to determine minimum temperatures for germination would be quite inadmissible, and that in fact no determinable minimum (above 2° C) exists Each of these species was capable of germination at all temperatures down to 2° C, although germination was very slow in some of the species from northern Europe at the low temperatures

No work has yet been done to relate the results to climatic situations in the localities from which the species come They may be similar in some respects to the results reported by Went and co-workers[4,5], on temperature effects on the germination of winter and summer annuals in California, to the extent that Mediterranean species with their capacity for germination at low temperatures seem to be adapted for germination in late autumn and winter whereas the species from northern Europe in which germination is very slow in soil temperatures around 9° C would not usually germinate to any extent in Britain after early September

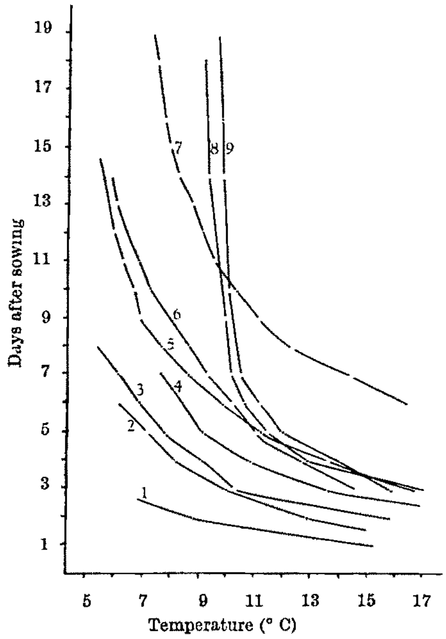

Fig 2 Temperatures at which various species of Caryophyllaceae attained 50 per cent germination rate on successive days after sowing 1, *Agrostemma githago* L , 2, *Kohlrauschia prolifera* (L) Kunth, 3, *Silene nocturna* L , 4, *Silene italica* (L) Pers , 5, *Lychnis flos-jovis* (L) Desr , 6, *Silene noctiflora* L , 7 *Lychnis viscaria* L , 8, *Silene cucubalis* Wibel, 9, *Silene dioica* (L) Clairu

The results reported here are based on relatively few species, but the correlations between distribution and germination are marked They provide no indication of the amount of variability within a particular species, however, and experiments are now in progress to determine to what extent germination behaviour of a species may show modifications according to the location of individual populations within its general range

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[1] Hewitt, E J , in *Sand and Water Culture Methods used in the Study of Plant Nutrition, Tech Commun* No 22, 2nd ed , 431 (Commonwealth Agricultural Bureaux, 1966)

[2] Cooper, J P , in *Climatic Response in Environmental Control of Plant Growth* (edit by Evans, L T), 381 (Academic Press, New York and London, 1963)

[3] Went, F W , in *The Experimental Control of Plant Growth* (Chronica Botanica Co , Waltham, Mass , 1957)

[4] Went, F W , *Ecology*, **29**, 242 (1948)

[5] Went, F W , and Westergaard, N , *Ecology*, **30**, 26 (1949)

Saprolegnia parasitica Coker in Estuaries

DICK's interesting comments[1] on our communication[2] point to the need for further work on this complex topic—a fact of which we are not unaware Indeed, our communication was intended solely as an interim report on work in progress in order to show that too facile dismissal of fungal involvement in previously reported work was premature

We did not set out to answer "whether *S parasitica* is a fresh-water fungus or a fungus capable of growing and sporing in both fresh-water and estuarine environments" We merely recorded two facts (*a*) that *Saprolegnia* has been isolated in a number of cases from fish that we can prove to be freshly arrived in an estuary, and (*b*) that *S parasitica* can grow in saline conditions

At this stage of the investigation, these are facts important enough to warrant recording and are not vitiated by Dick's comments For *Saprolegnia* to be involved at all in estuarine conditions it is not essential, as Dick states, to find out whether the fungus is capable of sporulating and so on equally well in both fresh-water and estuarine conditions (Q1–6), however desirable it may be to have further evidence along these lines We do not imply that the estuary is the sole locus for infection, and indeed believe that it is much more likely that *Saprolegnia* is more active in fresh water

Finally, we state that "the apparent correlation between the spread of the fungus in ulcerative dermal necrosis and water temperature merits investigation" This is a general observation and nowhere do we quote "temperature data for asexual sporulation" as Dick states

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[1] Dick, M W, *Nature*, 217, 875 (1968).
[2] Stuart, M and Fuller, H, *Nature*, 217, 90 (1968)

Resistance of Rainbow Trout to Ulcerative Dermal Necrosis

THE susceptibility of rainbow trout to ulcerative dermal necrosis[1], an infectious disease of some salmonids, has become a matter of importance to fish farmers We have shown that the aetiological agent of ulcerative dermal necrosis is filterable[2] although some workers have suggested that specific bacteria[3,4] or fungi[5] may be the primary pathogens Preliminary experiments indicated that rainbow trout exposed to a salmon affected by ulcerative dermal necrosis did not show symptoms of the disease whereas a healthy salmon in the same tank as the trout did The siphoning technique successfully used to reproduce ulcerative dermal necrosis in salmon[2] was adapted to the experiments described here, which were designed to ascertain the susceptibility of rainbow trout to the disease Further experiments, also described, were carried out to show whether rainbow trout can act as symptomless carriers

In experiment *A*, ten mature hatchery-reared rainbow trout obtained from the Inland Fisheries Trust farm at Roscrea were divided into two equal groups One group was kept as a control and the other was placed in a tank with a salmon obtained from the Boyne, a river free from ulcerative dermal necrosis Water was siphoned into the tank containing the trout and salmon from a tank containing a salmon obtained during a natural outbreak, and showing clinical signs of ulcerative dermal necrosis[1] Both tanks (and all tanks used in these experiments) were disinfected with a chlorine solution before use

The mean water temperature was 10 8° C, range 7 5–13 0° C On the fifteenth day of the experiment, the Boyne salmon died showing lesions of ulcerative dermal necrosis The five trout in the tank with the salmon showed no lesions, and they and the controls remained clinically healthy until the end of the series of experiments, more than 4 months later

In experiment *B*, the five rainbow trout exposed to infection in experiment *A* were placed in one disinfected tank and a healthy salmon from the Boyne was placed in another Water was siphoned from the tank containing the trout to that containing the salmon The mean water temperature was 7 2° C, range 5 0–8 0° C On the forty-seventh day of the experiment the salmon was in excellent health and showed no signs of ulcerative dermal necrosis The usual incubation period of the disease is 7–17 days[2]

In experiment *C*, the salmon used in experiment *B* was placed in a disinfected tank, and water was siphoned to it from a tank containing a salmon affected in a natural outbreak of ulcerative dermal necrosis The mean water temperature was 6 3° C, range 3 0–10 0° C The recipient salmon died showing lesions of ulcerative dermal necrosis

The results of experiment *A* confirmed previous findings that rainbow trout do not show clinical signs of ulcerative dermal necrosis Trout do not act as symptomless carriers of the disease, for the salmon which had been exposed to possible infection from the trout in experiment *B* was in fact susceptible, as shown in experiment *C*, so the result of experiment *B* was obtained because the trout were not infective

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[1] Carbery, J T, and Strickland, K L (in the press)
[2] Strickland, K L, and Carbery, J T (in the press)
[3] Brown, Margaret E, *Atlantic Salmon Journal* (summer, 1966)
[4] Brown, M E, and Collins, V G, *Salmon Trout Mag*, No 178, 180 (1966)
[5] Stuart, Mary R, and Fuller, Hubert T, *Nature*, 217, 90 (1968)

Imprinting: Drug-produced Isolation and the Sensitive Period

IMPRINTING as a phenomenon in early behavioural development owes much of its importance to the fact that it occurs only during a limited period of an animal's life Although there is some disagreement as to the exact nature and duration of this sensitive period, there is general agreement that such a period exists

Because of its importance in distinguishing imprinting from other forms of stimulus-response association, the sensitive period has been the subject of considerable theoretical interest This interest in turn has centred on the factors which influence the length and offset of the period Bateson[1] has recently reviewed empirical studies of the role of these factors and has concluded that the imprinting response will usually continue to occur to a particular stimulus unless the bird concerned has had an opportunity to develop a preference for something else This is, of course, equivalent to saying that imprinting as a process is inevitable and it will happen to either a class of stimuli in the bird's regular environment, or to a stimulus presented in an experimental imprinting situation.

I have developed this argument by assuming that the tendency to imprint on experimental objects declines as a result of the bird imprinting on some stimuli in its housing environment If this imprinting on the general environment is made less effective by procedures which are known to make experimental imprinting less effective, then the time during which the bird will remain responsive to test objects will be prolonged While it would be desirable to test directly the strength of attachment to aspects

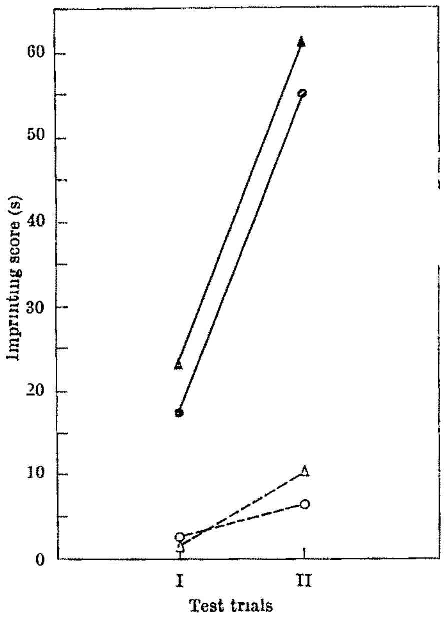

Fig 1 Imprinting scores for two drug groups and two control groups on the fourth day (test trial I) and the fifth day (test trial II) of life The imprinting score is the mean time spent within 12 in of the imprinting stimulus during 5 min Drug ●, 10 mg/kg, and ▲, 15 mg/kg Control ○, 10 mg/kg, and △, 15 mg/kg

of the housing environment, it is not possible to do so because of the presence of confounding factors such as food and warmth

A method of making imprinting on the general environment less effective without confounding stimulus characteristics and experience is suggested by an experimental study reported by John Bradford and myself to the Eastern Psychological Association in April 1966 The results of that study—on the effects of sodium pentobarbital on the development of the approach response in the domestic chick—showed that birds injected with a small dose of sodium pentobarbital (7 mg/kg) 15 min before imprinting training trials did not develop the associations necessary to produce approach behaviour when the imprinting stimulus was presented later It follows from this result, and from the foregoing line of argument, that newly hatched chicks reared under the influence of small doses of sodium pentobarbital would not imprint strongly on their environments and would therefore continue to be more responsive to experimentally introduced stimuli when the drug is withdrawn

This proposal was tested using forty-eight White Rock × Cornish chicks, which were removed from the incubator 2 h after hatching and placed in individual compartments in a warm air brooder Immediately after this the chicks were weighed and injected intraperitoneally with either 10 mg/kg or 15 mg/kg of sodium pentobarbital or equivalent volumes of control solution Thus there were two drug groups and two control groups The chicks were injected again 4 h later and again at approximately 10 a m , 2 p m and 6 p m on the following 2 days On the fourth day the subjects were injected at 10 a m and given imprinting test procedures at 4 p m On the fifth day the subjects were again tested in the imprinting situation

The imprinting procedure was a modification of that used by James[2] and consisted of placing the bird in a 1 ft × 1 ft × 6 ft alley with the stimulus, consisting of a flashing light and intermittent sound, on at one end When the bird approached within 12 in of the stimulus and when 60 s had elapsed, the stimulus was switched to the other end These 60 s trials were repeated five times and resulted in a procedure functionally equivalent to the bird following a stimulus The imprinting score was time spent within 1 ft of the stimulus during the 5 min period

As can be seen in Fig 1, the results were clear Both groups which were kept partially isolated and treated with the drug showed much higher responsiveness to the imprinting test stimuli than their corresponding control groups The experimental groups also showed a greater increase in imprinting scores from the first test day to the second test day than did the controls The differences were shown to be significant by analysis of variance ($P<0\ 01$) There were no significant differences between the two treated groups or between the two control groups

These results lend strong support to the notion that the offset of the critical period for experimental imprinting is a result of the birds imprinting on or forming associations with stimuli in their general environment Furthermore, because the birds were pharmacologically isolated for only a part of each 24 h period, there is the implication that imprinting on general environmental stimuli is not set in absolute opposition to imprinting on a test stimulus Rather, a partial reduction in imprinting on the general environment will result in a corresponding increase in the time that the bird remains sensitive to experimentally produced imprinting test stimuli

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[1] Bateson, P P G , *Biol Rev* , 41, 183 (1966)
[2] James, P H R *Canad J Psychol* , 13, 59 (1959)

Dispersal of Larvae by Equatorial Ocean Currents and its Importance to the Zoogeography of Shoal-water Tropical Species

PLANKTONIC larvae are dispersal stages which make it possible for marine species to breach faunal barriers and to colonize new regions The occurrence of trans-oceanic dispersal by larvae of the shoal-water tropical benthos can be deduced if the actual distribution of such larvae is known relative to the principal ocean currents likely to transport them

Taken as a whole, the North Atlantic surface circulation may be regarded as an enormous clockwise gyre The Gulf Stream continuing as the North Atlantic Drift passes eastward toward the European continent The Canary Current joins the North Equatorial Current which passes westward from the coast of Africa to the shores of South America In the southern hemisphere, the Atlantic forms a similar gyre, but rotates counterclockwise Circulation in the North and South Atlantic is actually much more complex than this simplification, which is adequate for the purpose here

Within the equatorial region the North Equatorial Current is separated from the South Equatorial Current by a rather weakly developed Equatorial Countercurrent moving eastward Unlike the currents on either side of it, the countercurrent moves a relatively small volume of water, and usually extends only part of the way across the western Atlantic Monthly charts of the North and South Atlantic surface circulation constructed by Schumacher and the US Hydrographic Office[1-3] show the Equatorial Countercurrent to be diffuse and seasonal in occurrence The surface currents of the Atlantic described here are common knowledge, and may be found illustrated in any good atlas

Consider now the distribution on the ocean surface of larvae that belong to tropical benthic species The biological data are from more than 600 plankton samples taken throughout the North and Equatorial Atlantic Larvae from the chief invertebrate phyla, including the Coelenterata, Echinodermata, Arthropoda, Annelida and Mollusca[4], are found in the surface waters of the open sea A diversity of taxa are known from each of these phyla For example, among the gastropods alone, at least nine families are represented, including the Cymatiidae, Triphoridae, Architectonicidae, Ovulidae, Cypraeidae, Tonnidae, Thaidae, Magilidae and Naticidae[5] About 79 per cent of all plankton samples taken from the North Atlantic contain veliger larvae of benthic gastropods, and it is certain that many additional families will be represented when the material is further studied

One example among the gastropods can be examined in more detail Veliger larvae of the genus *Philippia*, belonging to the prosobranch gastropod family Architectonicidae, are frequently found in the surface waters of the tropical North and South Atlantic Several species are represented, but one common form is believed to be *Philippia krebsii* Morch[6]

As a reproducing adult, this species is restricted entirely to tropical and subtropical regions Its vertical distribution, however, extends from 7 to 205 m (ref 6) Such a range in depth means that the species frequently must live at temperatures well below 20° C, for although tropical surface waters often exceed 27° C, at 150 to 200 m the water temperature at the bottom may be as low as 12°–15° C It is concluded from this that *Philippia krebsii* must be eurythermal or at least that the adult of the species lives at wider ranges of temperatures than might at first be expected from its tropical distribution

The larvae of *Philippia krebsii* are characteristic of tropical gastropod veligers found in the open sea The protoconch or larval shell is thin and not heavily calcified, the velum has large lobes used in swimming and feeding The vivid colours of the larvae can only be seen in living specimens at sea When the distribution of Architectonicid larvae is examined, particularly that of *Philippia krebsii*, the veligers are seen to be distributed along the entire periphery of the North Atlantic gyre The larvae are clearly also eurythermal, sometimes occurring in water much colder than 20° C in the North Atlantic Drift while more commonly at 27° C in the surface waters at the equator (Fig 1)

If the larvae can delay metamorphosis long enough, then the North Atlantic can be regarded as a giant roundabout, and the riders, that is the larvae, if lucky, can jump off at any point along the way There is already evidence, from holding larvae captured at sea, to suggest that a considerable number of tropical gastropod species have veligers that remain planktonic for a long time (for example, *Cymatium parthenopeum*[7]) Furthermore, it is known from experiments that settlement of some gastropod veligers can be delayed in the absence of an environment where post-larval survival is possible[8–10]

There are many tropical species that are shoal stenothermal warm-water forms, and yet are amphi-Atlantic in their distribution Such species will not readily survive the colder temperatures of 20° C and less that are

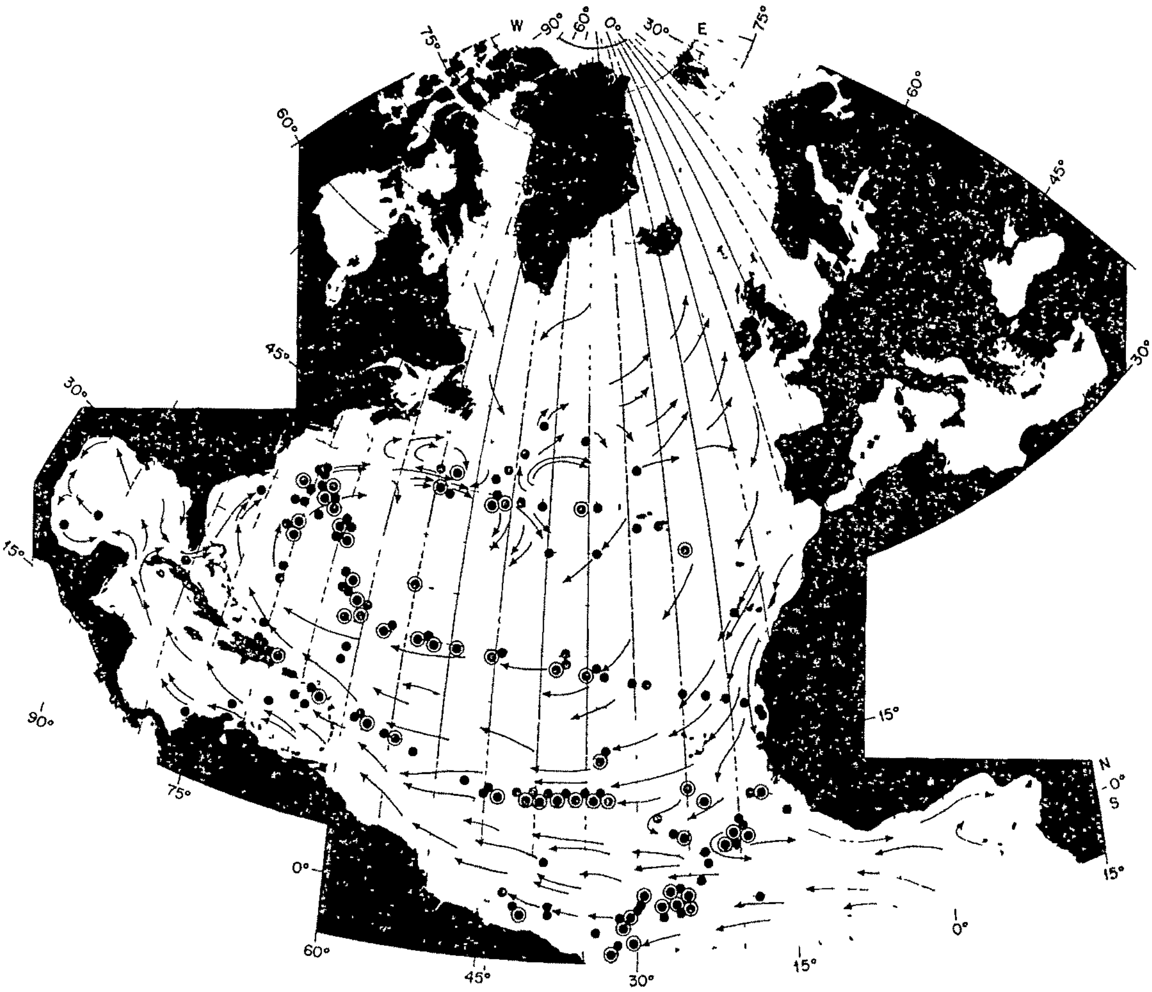

Fig. 1 Distribution of veliger larvae of the gastropod family Architectonicidae Adults belonging to this family are distributed throughout the tropics Circled stations include *Philippia krebsii* (Morch, 1875)

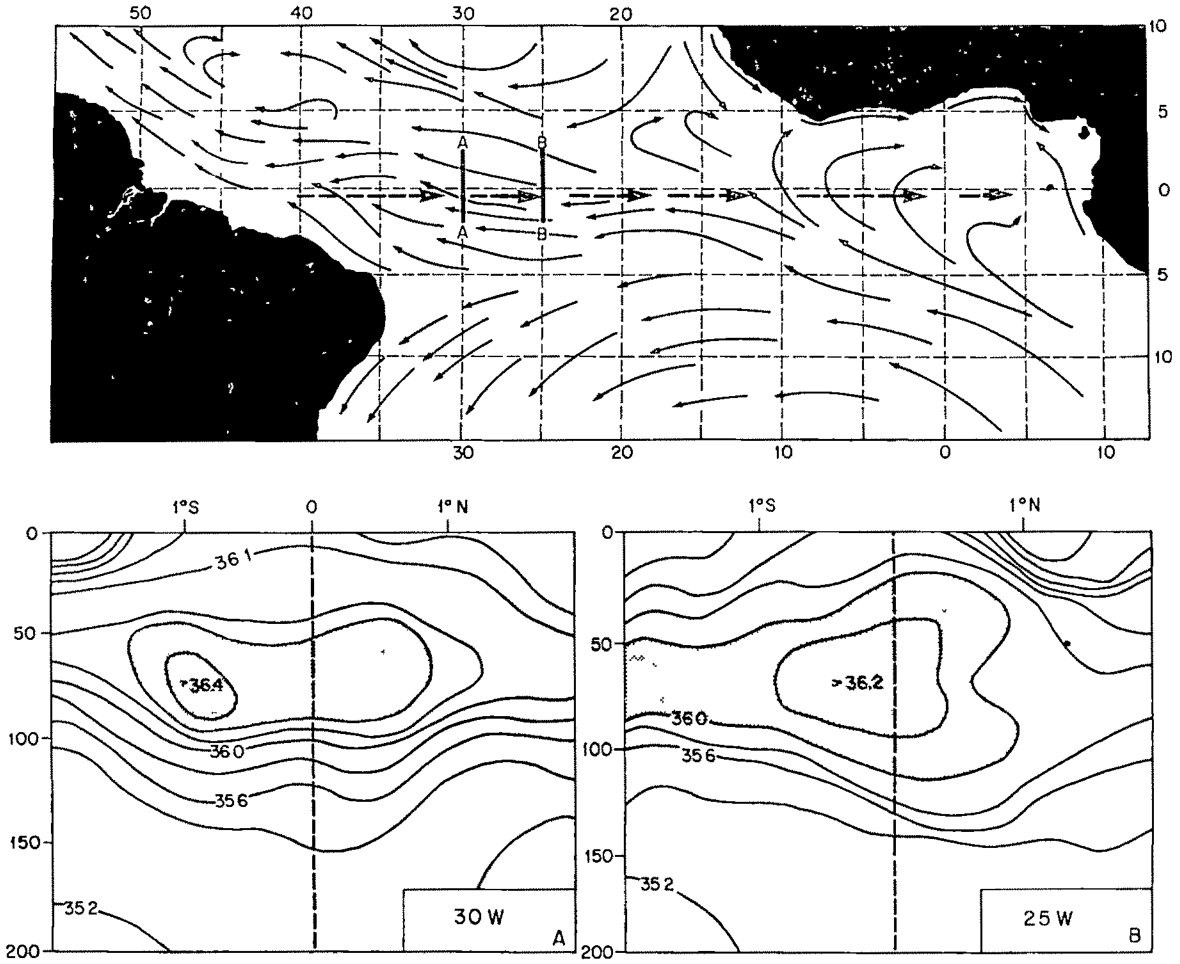

Fig 2 The Equatorial Undercurrent is a sub-surface core of saline water moving in an easterly direction at a depth of from approximately 60 to 90 m It flows directly under the equator between Brazil and the island of São Tomé in the Gulf of Guinea[12], and is shown in vertical cross-sections The more saline water of the undercurrent is stippled in the cross-sections Plankton samples were taken from this portion of the water column Vertical salinity sections modified after Khanaychenko *et al* [13]

frequently encountered in the North Atlantic Drift Examples of stenothermal forms are found among the Zoanthid corals and a wide variety of other organisms The question now arises how Zoanthid corals, restricted entirely to the tropics, can possibly be dispersed from the western to the eastern Atlantic, for although the transport of Semper's larvae from the coast of Africa to South America is readily explained by drift along the North or South Equatorial Current, it is not immediately obvious how transport in the opposite direction can occur Certainly the Equatorial Countercurrent does not provide a very likely means for easterly dispersal

The answer to this problem was unwittingly provided in 1886 by the hydrographer Buchanan when he described to the Royal Geographical Society "the very remarkable undercurrent setting in a south-easterly direction with a velocity of over a mile per hour"[11] This current was found directly under the equator as far as Buchanan was able to trace it It is remarkable that this information has been rediscovered only recently in an international oceanographic expedition of the tropical Atlantic commonly referred to as "Equalant" The current described by Buchanan, now generally known as the Equatorial Undercurrent, is an easterly subsurface flow at a depth of between 60 and 90 m It is evident in salinity profiles as a saline core of water (Fig 2) The maximum velocity of the current varies from 1 to 5 km/h and averages perhaps 2 km/h (ref 13) Most important is the fact that the water temperature in its core is warm, exceeding 25° C at its centre (Fig 3) Thus it might readily be supposed that the Equatorial Undercurrent could serve as an important agent for zoogeographical dispersal

Semper's larvae of the Zoanthid coral are not particularly striking, and their coelenterate affinities are not

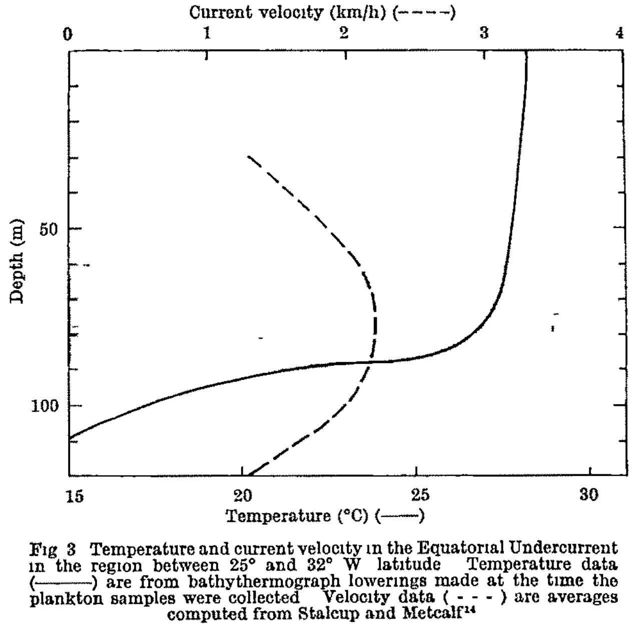

Fig 3 Temperature and current velocity in the Equatorial Undercurrent in the region between 25° and 32° W latitude Temperature data (——) are from bathythermograph lowerings made at the time the plankton samples were collected Velocity data (- - -) are averages computed from Stalcup and Metcalf[14]

immediately obvious When such larvae are sectioned, however, this affinity becomes evident from the presence of septa The distribution of the Semper's larvae from one species will serve to demonstrate the importance of the undercurrent

Throughout the tropical Atlantic there is a larval form, probably belonging to the genus *Palythoa*, which is entirely restricted to water at 23° C or more The species has rarely been found in the Gulf Stream at 36° north latitude but it is very prevalent throughout the equatorial Atlantic (Fig 4) But does it occur in the undercurrent, and can this account for its dispersal from South America to Africa ? Closing nets can be used to take samples at any given depth interval Net tows taken in the Equatorial Undercurrent, that is, between 50 and 100 m depth, show that Semper's larvae of this species regularly occur in the eastwardly moving undercurrent Hence the larvae of *Palythoa* can drift westward on the North or South Equatorial Current and eastward in the Equatorial Undercurrent While there are a great variety of other shoal water benthic larvae found in the undercurrent, interestingly enough *Philippia krebsii* is seldom among these, for it is almost entirely restricted to surface waters (0–50 m)

It is now apparent that there are two means by which larvae can be transported from east to west across the Atlantic The first is by way of the North Atlantic Drift and can only be accomplished by eurythermal forms, the second is by means of the Equatorial Undercurrent and is the only means available to stenothermal tropical species

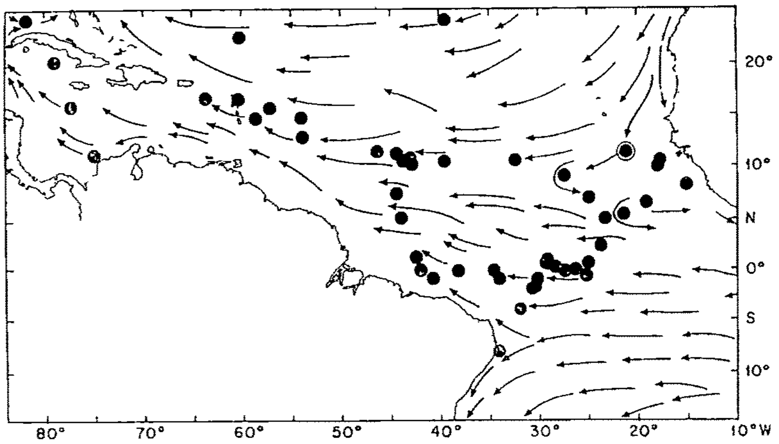

Fig 4 Distribution of Semper's larvae from a Zoanthid coral taken in plankton tows throughout the equatorial Atlantic

Direct evidence from their distribution in the open sea shows that larvae are commonly dispersed over long distances by ocean currents The examples that I have given are only illustrative of a large volume of unpublished data There is no doubt that the exchange of species can occur across the Atlantic in either direction between Africa and South America The dispersal of larvae over long distances by ocean currents offers one important explanation for the amphi-Atlantic distribution of tropical shoal-water benthic species

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[1] Schumacher, A , *Ann d Hydr* , 68, 109 (1940)
[2] Schumacher, A , *Ann d Hydr* , 71, 209 (1943)
[3] *Pilot Charts* (US Naval Hydrographic Office, 1967)
[4] Scheltema, R S , *Amer Zool* , 4, 299 (1964)
[5] Scheltema, R S , *Second Intern Oceanog Cong* (Moscow), Abstract No 375, 320 (1966)
[6] Robertson, R , *Proc Acad Nat Sci Philadelphia*, 116, 1 (1964)
[7] Scheltema, R S , *Deep Sea Res* , 13, 83 (1966)
[8] Scheltema, R S , *Biol Bull* (Woods Hole), 111, 312 (1956)
[9] Scheltema, R S , *Biol Bull* (Woods Hole), 120, 92 (1962)
[10] Kiseleva, G A , *Donnyye Biotsenosy i Biologiya Bentosnykh Organismov Chernogo Morya*, 71 (Respublikanskiy Mezhedomstvennyy Sbornik, ser "Biologiya Morya", Kiev, 1967)
[11] Buchanan, J Y , *Proc Roy Geog Soc* , 8, 753 (1886)
[12] Rinkel, M O , Sund, S , and Newman, G , *J Geophys Res* , 71, 3893 (1966)
[13] Khanaychenko, H K , Khlytov, N Z , and Zhidkov, V G , *Oceanology* (Moscow), 5, 222 (1965)
[14] Stalcup, M C , and Metcalf, W G , *J Mar Res* , 24, 44 (1966)

Relation between DDT in Diets of Laying Birds and Viability of their Eggs

Concern at the presence of organo-chlorine insecticides in birds' eggs has stimulated attempts to relate concentrations of toxicants in eggs to hatchability[1] We have tried to relate the ingestion of DDT by adult birds to the viability of their eggs Initially we carried out a pilot study similar to that of Cross *et al* [2] and followed this with the study described here, in which diets containing 200 p p m of DDT were fed after the start of laying to caged Japanese quail (*Coturnix c japonica*) Eggs were set at weekly intervals before and after the start of feeding of DDT and the hatchability of the eggs and survival of chicks were compared with those of a control group The concentration of DDT in the treated diet is expressed as the amount of the (*pp*) isomer contained in a commercial wettable powder containing 35 per cent (*pp*) and 10 per cent (*op*) isomers On this basis, the mean weight of *pp* DDT consumed per bird per week was estimated at 28 mg for the first week and 22 mg for the second.

Table 1 shows numbers of chicks hatched from fertile eggs, set at weekly intervals, from both groups Chi square tests show no significant changes in hatchability in either the treated or control group during the test, moreover, the hatchability of the batch of eggs collected during the second week after the start of feeding DDT showed no significant difference from the control batch We have, however, been aware of the desirability of measuring mortality in chicks for several days after hatching[3] In the present experiment, deaths among all chicks were recorded for 7 days after hatching of the first chick Approximately 79 per cent of all deaths of chicks occurred within 3 days of hatching Clear signs of DDT poisoning—strong tremors and loss of balance followed by collapse—were observed in both batches of chicks from treated parents Table 2 shows the total deaths among chicks from weekly settings A chi square test shows highly significant heterogeneity ($P = 0\ 001$) in mortality among batches of chicks from the treated group and this is largely the result of the high mortality among chicks obtained from eggs laid in the second week of consumption of food to which DDT had been added Heterogeneity in mortality among batches of chicks from the control group is not significant at the level $P = 0\ 05$

Table 1 NUMBERS OF CHICKS HATCHED FROM FERTILE EGGS

	Treated group			Control group		
Period (days)	Diet of parents	No of eggs set	No of chicks hatched	Diet of parents	No of eggs set	No of chicks hatched
1–7	Normal food only	125	74 (59%)	Normal food only	119	72 (61%)
8–14	200 p p m DDT	177	116 (66%)	,, ,, ,,	148	107 (72%)
14–21	200 ,, ,,	156	107 (69%)	,, ,, ,,	139	107 (77%)

Table 2 MORTALITY OF CHICKS DURING THE FIRST WEEK AFTER HATCHING

	Treated group			Control group		
Period (days)	Diets of parents	Nos of chicks* Hatched	Nos of chicks* Died	Diets of parents	Nos of chicks Hatched	Nos of chicks Died
1–7	Normal food only	58	10 (17%)	Normal food only	62	16 (26%)
8–14	200 p p m DDT	100	19 (19%)	,, ,, ,,	105	22 (21%)
14–21	200 ,, ,,	103	64 (62%)	,, ,, ,,	107	13 (12%)

* Chicks hatched later than 2 days after the start of hatching were discarded, as were all those which were deformed or died in the incubator before drying

We conclude that it is possible for eggs to carry amounts of DDT, or its metabolites, large enough to cause high mortality among chicks hatched from them without substantially affecting their hatchability It is also clear that the situation in which newly hatched chicks rely for some days on stored yolk for nutrition, which Koeman *et al* [3] suggested to represent a hazard to birds contaminated with dieldrin, also applies potentially to DDT

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[1] Dunachie, J F, and Fletcher, W W, *Nature*, 212, 1062 (1966)
[2] Cross, D L, King, H L, and Haynes, Dean L, *Quart Bull*, *Michigan State Univ*, 44, 4 (1962)
[3] Koeman, J H, Oudejan, R C H M, and Huisman, E A, *Nature*, 215, 1094 (1967)

Relationships among Functional Properties of Californian Grassland

THE communication by McNaughton[1] provides an example of the problems which arise when a quantitative approach is made to a subject such as ecology

McNaughton attaches significance to a correlation between a dominance index and number of species but, as defined, his parameters are not independent. If a sample contains only four species, the two species contributing the greatest (most important) proportion to the standing crop must exceed 50 per cent—otherwise all species contribute equally For the range of species number studied, this minimum value varies from 33 3 per cent for six species to 12 5 per cent for sixteen species Percentage contribution is not logically independent of species number

Species number (richness)[1] is not usually equated with diversity as normally defined; McIntosh[2] has examined the parameters species number, dominance and number of individuals in relation to indices of diversity These indices take into account both species number and "overall dominance" or equitability—that is, the distribution of individuals among the species The questions to be asked about the relationship between species number and dominance should perhaps be rephrased as three interrelated questions (*a*) What combinations of species number and abundance occur in the field ? (*b*) Do all possible combinations of diversity for a given species number and abundance (standing crop or number of individuals) occur in the field ? (*c*) Does the range of single species dominance observed in the field correspond with the possible range for the same number of species and the same abundance ?

Definitions of the terms "diversity" and "dominance" are needed before these questions can have a precise meaning McIntosh[2] has reviewed the definition of diversity, dominance can be defined as the abundance of the species which has the greatest observed abundance The limits of this value when abundance is measured as number of individuals are

$$\text{Dominance maximum} = N - S + 1$$

$$\text{minimum} = \frac{N}{S} \text{ when } \frac{N}{S} \text{ is an integer}$$

$$\text{or} = \frac{N}{S} + 1$$

where N is the number of individuals and S is the number of species.

When standing crop is used as a measure of abundance, it is not possible to define the maximum possible contribution of a species The contribution of the most abundant single species can, however, be compared with that expected with maximum diversity (minimal dominance), that is, when all species contribute equally

A dominance index D can be used

$$D = \frac{\dfrac{X\,S}{C} - 1}{S - 1} \text{ for } S \geqslant 2$$

where C is the standing crop and X is the contribution of species X to the standing crop $D = 0$ at maximum diversity, $D \rightarrow 1\ 0$ as dominance increases D is not independent of S, as $S \rightarrow \infty$, $D \rightarrow \frac{X}{C}$ (compare McNaughton[1]), when S is small, the percentage contribution must be greater to obtain an equivalent value of D

D cannot equal 1 0, but it would be of considerable interest to know how closely observed values approach this figure The size of individuals of other species present in the community will influence this, which suggests that for a given species number the community with the greatest range in size of species will have the larger possible dominance All hypotheses of this type, however, depend on an adequate definition of the term "community", which is at present lacking

McIntosh[2] quotes Gerard[3] " Before measurements can be meaningful they must be directed to the right things and even in science, finding these right things is the major achievement, entitation is more important than quantitation", and adds "Herein lies a major difficulty of ecology". More adequate definitions are necessary before McNaughton's suggestions regarding the relationships between the functional properties, species number and dominance, and between productivity and dominance can profitably be examined

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[1] McNaughton, S J, *Nature*, 216, 168 (1967)
[2] McIntosh, R P, *Ecology*, 48, 392 (1967)
[3] Gerard, R W, *Science*, 148, 762 (1965)

Artificial Reconstruction of a Species of Fern, *Asplenium adulterinum*

AMONG the flowering plants there is a limited number of records of artificial synthesis of a natural polyploid species Of these, the first and most familiar example is *Galeopsis tetrahit*[1] The example described here, however, is the first complete synthesis of a natural species of pteridophyte

The fern *Asplenium adulterinum* Milde has a wide but extremely intermittent distribution in Central Europe and Scandinavia It is virtually restricted to serpentine and other ultrabasic rocks In morphology, it is intermediate between *A trichomanes* L and *A viride* Hudson As its name suggests, its status has been widely disputed in the past, but it has been generally accepted for many years as a genuine and distinct species All examples of *A adulterinum* which I have investigated cytogenetically, whether from localities in Austria, Norway, Switzerland or elsewhere, have proved to be tetraploid, with $n=72$ chromosomes, and to reproduce sexually All chromosome counts of this species recorded by others[2-4] are likewise tetraploid

Hybrids have been synthesized between *Asplenium adulterinum* and the two diploids which are believed to be its parents, *A viride* and *A trichomanes* ssp *trichomanes* These hybrids are triploid, and include thirty-six bivalents and thirty-six univalents at meiosis[5] I have synthesized hybrids between *A adulterinum* and two other tetraploid European species of *Asplenium*, namely, *A majoricum* Litard and *A lepidum* C Presl These hybrids, themselves tetraploid, both show uniformly complete failure of chromosome pairing in meiosis, 144 univalents being present Such a result indicates an origin by allopolyploidy for *A adulterinum* (and, incidentally, also for both *A majoricum* and *A lepidum*) Moreover, it makes possible interpretation of the chromosome pairing seen in the two triploid hybrids obtained earlier, and already mentioned The thirty-six bivalents found in both of these triploid hybrids must arise from pairing between chromosome sets common to both diploid and tetraploid parents The triploid hybrids are backcross hybrids between an allotetraploid species and its two diploid parents The relationships can be symbolized as follows, where letters represent the chromosome sets present, and the underlining bars indicate the composition of the bivalents formed in meiosis *A viride* = $\underline{VV}$, *A trichomanes* ssp *trichomanes* = $\underline{TT}$, *A adulterinum* = $\underline{VV}\,\underline{TT}$, *A adulterinum* × *trichomanes* ssp *trichomanes* = $V\underline{TT}$, and *A viride* × *adulterinum* = $\underline{VV}T$

It proved possible to synthesize the diploid hybrid *Asplenium viride* × *trichomanes* ssp *trichomanes* (= VT) This hybrid shows complete failure of chromosome pairing with seventy-two univalents present at meiosis (Fig 1) It should be possible to induce tetraploid sectors in this diploid hybrid by treatment with an agent inducing polyploidy, for example colchicine This procedure has, however, been unnecessary, because the fronds of the diploid hybrid have been found to produce spontaneously an appreciable number of viable spores which have in turn produced normal sexual gametophytes More than 1,000 prothalli have thus been obtained, and random fertilization of these prothalli has resulted in the production of more than 120 sporophytes which are morphologically inseparable from characteristic examples of natural *A adulterinum* So far, ten of these sporophytes have been cytologically examined To all intents and purposes, all these plants are tetraploid, with a normal meiosis showing $n=72$ bivalents (Fig 2), although one or more plants can be aneuploid for a single chromosome (In this genus, it is technically difficult to obtain complete certainty in observations of small irregularities in chromosome behaviour) Sowings of spores from two of the cytologically tested plants in both cases resulted in copious and uniform growths of prothalli, showing that the spores produced by these plants have a high degree of viability The prothalli have now produced an abundant crop of sporophytes, indicating in turn that these gametophytes are highly fertile

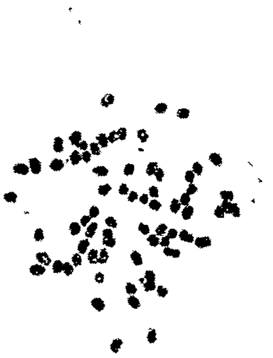

Fig 1 Permanent aceto-carmine squash preparation of meiosis in a synthetic diploid hybrid, *Asplenium viride* × *trichomanes* ssp *trichomanes*, showing seventy-two univalents (×1 000)

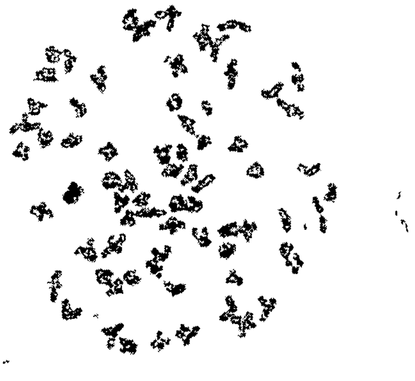

Fig 2 Permanent aceto-carmine squash preparation of meiosis in synthetic *Asplenium adulterinum*, showing seventy-two bivalents (×1,000)

The tetraploid nature of the sporophytes derived from the spores produced by the diploid hybrid clearly indicates that these spores must themselves be diploid; in other words, they contain an unreduced chromosome complement Their origin has not yet been critically studied, but it seems likely from observations already made[6] on a closely related hybrid of wild origin, *A trichomanes* ssp *inexpectans* × *viride*, that these spores arise by restitution of the spore mother cell nucleus after failure of meiosis to progress beyond first metaphase In this respect, it is significant that the spores produced by this wild hybrid have in a similar way given rise to fertile tetraploid sporophytes[7] Furthermore, this ready production of tetraploid sporophytes by the spores formed by a diploid hybrid has also been shown by myself and Reichstein[7] for a natural example of the hybrid *A trichomanes* ssp *trichomanes* × *viride* originally found wild in Switzerland by Dr W Gatzi (although not on serpentine rock) The morphology of this hybrid is virtually identical to that of the synthesized examples of this hybrid combination, while the chromosome behaviour observed in both of the two wild diploid hybrids and their tetraploid progeny is indistinguishable from that seen in the synthetic hybrids[6,7] The phenomenon of the production of spontaneous tetraploid progeny from a diploid hybrid has also been observed elsewhere in *Asplenium*. Wagner and Whitmore[8] obtained tetraploid plants of *A. ebenoides* from spores produced by a wild example of the diploid hybrid *A. platyneuron* × *rhizophyllum*, although in this case only a few prothalli were obtained.

In the case of a plant such as *Asplenium adulterinum* where the propagules are both very numerous and light, and are readily dispersed by air currents, the possibility of contamination of the experimental cultures by the very species one is attempting to reconstruct must be considered. The conditions in which these experiments have been carried out do not entirely exclude the possibility of the introduction of spores of adventive origin. Moreover, some plants of *A. adulterinum* of wild origin were in cultivation on our site at the time the experiments were in progress. All sporophytes produced by the prothallial cultures obtained from the synthetic *A. viride* × *trichomanes* ssp. *trichomanes* hybrid were retained. In spite of the presence of very numerous different sources of fern spores in our greenhouses, only 4 per cent of the sporophytes obtained are of evidently contaminant origin. Another more positive control has been possible. An exactly comparable experiment has been conducted in an immediately adjacent series of cultures using the spores produced by another diploid *Asplenium* hybrid of synthetic origin, *A. viride* × *fontanum* (L.) Bernh. This hybrid also shows complete failure of chromosome pairing. Tetraploid sporophytes have again been produced, showing a regular meiosis with $n = 72$ bivalents. In this case, the total progeny obtained again exceeds 120 sporophytes, more than 95 per cent of which are of the expected morphology. A fertile tetraploid form of this hybrid has never yet been found in the wild, so contamination of the prothallial cultures by the actual species one is attempting to synthesize is in this instance totally impossible.

Returning to the newly obtained synthetic examples of *Asplenium adulterinum*, a critical test of the degree of identity existing between the natural species and its synthetic simulant is possible. Comparison in uniform conditions of cultivation has shown that certain strains of *A. adulterinum* from different localities show small but constant morphological differences with respect to such characters as the shape of the pinna and the position on the rachis of the point of change in its colour from brown to green (unpublished results of Lovis and Reichstein). Use of these strains as one parent has made it possible to distinguish hybrids from self-fertilized plants in the progeny resulting from crosses made between natural and synthetic examples of *A. adulterinum*. (Because the prothalli are hermaphrodite and self-compatible, it is not possible entirely to prevent some self-fertilized progeny as the outcome of a hybridization experiment.) Cytological examination of these hybrids shows a regular meiosis, indicating that close homology exists between the chromosomes of natural examples of *A. adulterinum* and those of its synthesized facsimile.

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[1] Muntzing, A., *Hereditas*, **13**, 185 (1930); ibid., **14**, 153 (1930).
[2] Meyer, D. E., *Bibl. Bot.*, **123** (1952).
[3] Meyer, D. E., *Ber. Deutsch. Bot. Gesell.*, **70**, 57 (1957).
[4] Manton, I., *Bot. Soc. Brit. Isl. Conf. Rep.*, **6**, 105 (1961).
[5] Lovis, J. D., *Proc. Bot. Soc. Brit. Isl.*, **1**, 388 (1955).
[6] Lovis, J. D., Melzer, H., and Reichstein, T., *Bauhinia*, **2**, 231 (1965).
[7] Lovis, J. D., and Reichstein, T., *Die Naturwissenschaften* (in the press).
[8] Wagner, jun., W. H., and Whitmore, R. S., *Bull. Torrey Bot. Club*, **84**, 79 (1957).

IMMUNOLOGY

Immune Haemolysis: Reaction of the Terminal Complement Component

THE lytic step of the complement reaction sequence is biologically very important. An attempt to determine its nature by indirect methods was previously made in this laboratory[1,2]. Another direct approach to the problem was conceived when it was shown that the "classical" third component of haemolytic complement (C') comprises at least six distinct factors[3,4].

Sensitized sheep erythrocytes (EA) which had been reacted with the first eight components of complement (C'1–C'8, for nomenclature see ref. 5) were prepared and we have investigated the reaction of these EAC'1–8 cells with the last component of complement (C'9). This communication summarizes our findings, which will be published in detail later.

EAC'1–8 cells were prepared by a two-step procedure. First, sensitized sheep erythrocytes were incubated at 32° C with diluted guinea-pig serum adsorbed on zymosan, prepared by the method of Lepow and Leon[6]. After 10 min the cells were collected and washed twice. These cells were readily lysed by guinea-pig or pig serum in the presence of 0.010 M ethylenediaminetetraacetic acid (EDTA), which indicated that they had at least been brought to the state EAC'1a,4,2a. In a second step, these erythrocytes were incubated for 20 min at 37° C with a serum fraction containing C'3, C'5, C'6, C'7 and C'8. This fraction was obtained by gel filtration on 'Sephadex *G*-200' of the euglobulins of pig serum. EAC'1–8 cells were washed and stored at 4° C in veronal buffered saline containing Ca^{++} and Mg^{++} (VBS) which was used as a diluent throughout[7].

Our procedure for the preparation of EAC'1–8 is based on findings of Wellensiek and Klein. They fractionated guinea-pig serum on a 'Sephadex *G*-200' column and reported that of the C' components reacting subsequent to C'2 only the terminal one is eluted together with the albumin ("4*S* peak") while the others are associated with the 7 *S* gamma globulins[8]. When tested with reagents prepared according to Wellensiek and Klein[8,9], we observed that after gel filtration of the pig serum the distribution of the late acting C' components in the column eluate was similar to that described for guinea-pig serum.

EAC'1–8 are haemolysed by pig C'9 as well as by functionally pure preparations of guinea-pig and human C'9. They are characterized by a slow, spontaneous lysis when stored in VBS as well as by a decreased resistance against hypotonic solutions of NaCl, lysolecithin or sodium-deoxycholate[10]. Similar observations were reported with cells which had reacted with the first eight components of guinea-pig complement[11]. EAC'1–8 showed no detectable loss of reactivity with C'9 when stored in VBS at 4° C for more than 100 h or at 37° C for 48 h. They were, however, no longer reactive to C'9 after treatment with pronase, a proteolytic enzyme from *Streptomyces griseus* (Calbiochem, Lucerne, Switzerland). A sample of 10^8 EAC'1–8 was incubated with 125 µg of pronase in 1.0 ml for 60 min at 37° C.

We prepared C'9 from pig serum. The euglobulin fraction was precipitated by 2-ethoxy-6,9-diamino-acridine lactate ('Rivanol', Farbwerke Hoechst) and subsequently by ammonium sulphate (35–50 per cent saturation). Further purification was achieved by column chromatography on '*CM*-Sephadex *C*-50', gel filtration on 'Sephadex *G*-200' and by preparative zone electrophoresis. Functionally pure human C'9 was prepared similarly. Pig C'9 showed a single band in analytical agarose-polyacrylamide electrophoresis[12] with the migration characteristics of a fast β-globulin. Its activity is rapidly destroyed at 56° C with a half-life time of 7.5 min. Human C'9 (C'T) was reported to have a half-life of 6 min at 56° C (ref 13)

The activity of C′9 was completely abolished by dialysis against 0 10 M mercaptoethanol which suggests that there are disulphide bonds in the native molecule Di-isopropyl-fluorophosphate (DFP), *p*-chloromercuribenzoate (*p*CMB), $HgCl_2$ or iodoacetamide had no effect on the C′9 activity which would seem to indicate that seryl or SH-groups are not essential for the haemolytic activity of the component (Table 1)

Antibodies against pig C′9 were prepared by immunizing rabbits with functionally pure preparations incorporated into complete Freund adjuvant The antiserum was heat inactivated to destroy C′9 and absorbed with EAC′1–8 It strongly inhibited the activity of pig C′9 and, to a lesser extent, that of human C′9 (Table 2)

The dose-response curve of the reaction between EAC′1–8 and C′9 suggested a one-step mechanism The curve was concave to the abscissa and gave a straight line after transformation to the $Z = -\ln(1-y)$ plot according to Mayer[14] which indicated a linear relationship between the number of hits per cell and the C′9 concentration The reaction was temperature dependent and had its optimum at 42° C. Na-Cu-chlorophyllin (Merck, Darmstadt) was found to be a potent inhibitor which stopped the reaction within 10 s With the aid of this compound we were able to show that at 37° C the reaction between EAC′1–8 and C′9 started without a lag phase and was completed after 20 min A typical experiment is shown in Fig 1

In addition to Na-Cu-chlorophyllin a number of other inhibitors were detected (Table 3) While the inhibition by Cu^{++} ($CuSO_4$ or $Cu(NO_3)_2$) and Zn^{++} ($ZnSO_4$) was completely reversible by dialysis against cold VBS, the addition of Fe^{++} ($FeSO_4$) or Fe^{+++} ($FeCl_3$) to C′9 led to an irreversible inactivation of the component Its activity was not restored by dialysis against VBS or by the addition of the chelating agent 1,10-phenanthroline hydrate (Merck, Darmstadt) These findings are not in accord with recent observations of Hadding and Muller-Eberhard[15], who reported that 1,10-phenanthroline induces lysis of EAC′1a,4,2a,3,5,6,7,8 prepared with human C′ components Iron ions in the divalent form inhibited the lysis of the cells by both phenanthroline and human C′9, whereas trivalent iron had no such effect The authors inferred from their results that the capacity of C′9 and of phenanthroline to lyse EAC′1a,4,2a,3,5,6,7,8 might be caused by a reaction with Fe^{++} and that activation of C′8 sites on the cell might be brought about by withdrawal of Fe^{++}

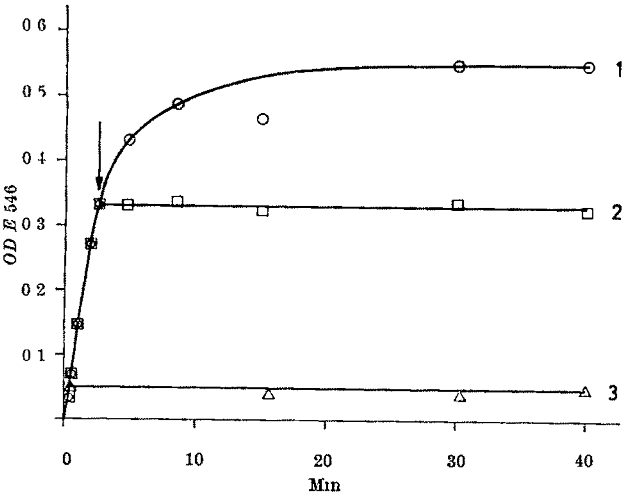

Fig 1 Kinetics of the reaction between EAC′1–8 cells and C′9 at 37° C Three flasks, 1, 2 and 3, each containing 10 ml of EAC′1–8 (2 × 10^8 cells/ml) were set up in parallel Flasks 1 and 2 received 10 ml of C′9, and flask 3, 10 ml of VBS At the times indicated 1 0 ml samples from flask 1 were pipetted into 0 5 ml of VBS containing 20 μg of Na-Cu-chlorophyllin (curve 1) To flask 2, 10 ml of VBS containing 40 μg of Na-Cu-chlorophyllin/ml was added at $t = 2$ 5 min as indicated by the arrow Samples of 1 5 ml were withdrawn at the times indicated (curve 2) To flask 3, 10 ml of VBS containing Na-Cu-chlorophyllin was added at $t = 0$ min Samples of 1 5 ml were withdrawn at the times indicated (curve 3) After sampling, each tube was left for a further 60 min at 37° C before the degree of haemolysis was determined

Table 1. EFFECT OF VARIOUS SUBSTANCES ON THE ACTIVITY OF C′9

Substance	Concentration (M)	Percentage inhibition
Mercaptoethanol*	1 0 × 10^{-1}	100
DFP†	1 0 × 10^{-3}	0
*p*CMB‡	1 0 × 10^{-3}	0
$HgCl_2$§	1 0 × 10^{-6}	0
Iodoacetamide ‖	2 0 × 10^{-2}	0

* C′9 was first dialysed against mercaptoethanol for 60 min at room temperature followed by dialysis against VBS or 2 × 10^{-2} M iodoacetamide and then VBS

† 0 9 ml C′9 and 0 1 ml 10^{-2} M DFP in anhydrous isopropanol were incubated at 37° C After 30 min EAC′1–8 were added and incubation was continued for 60 min

‡ C′9 was incubated with *p*CMB at 37° C After 15 min EAC′1–8 were added and incubation was continued for an additional 60 min Lysis in tubes containing *p*CMB and C′9 was 20 per cent higher than in tubes containing only C′9 EAC′1–8 treated only with *p*CMB showed no haemolysis within 60 min

§ $HgCl_2$ was incubated with C′9 at 37° C After 15 min EAC′1–8 were added and incubation was continued for an additional 60 min EAC′1–8 treated with $HgCl_2$ for 60 min at 37° C showed no haemolysis

‖ C′9 was dialysed against iodoacetamide for 4 h in the cold The iodoacetamide was then removed by dialysis against VBS

Table 2 INHIBITION OF C′9 BY ANTI-C′9 RABBIT ANTISERUM

C′9 preparation	Reciprocal dilution of antiserum	Percentage inhibition
Pig	1,200	100
Pig	5,000	50
Human	20	50

A sample of 0 5 ml of C′9 was incubated with 0 5 ml of antiserum dilution at 37° C After 20 min EAC′1–8 cells were added and incubation was continued for an additional 60 min

Table 3 INHIBITION OF HAEMOLYSIS OF EAC′1–8 BY C′9

Inhibitor	μg	Percentage inhibition*
Na-Cu-chlorophyllin	10	100
Na-chlorophyllin	50	100
Germanin (Bayer 205)	200	100
Heparin-Na (Nordmark, Hamburg)	740 (=100 international units)	34
	Concentration (M)	
$FeSO_4$	1 2 10^{-4}	86
$FeCl_3$	1 2 10^{-4}	97
$CuSO_4$	1 5 10^{-5}	97
$Cu(NO_3)_2$	1 5 10^{-4}	100
$ZnSO_4$	1 0 10^{-4}	100

A sample of 10^8 EAC′1–8 cells was incubated for 60 min at 37° C in the presence of the inhibitor with an amount of C′9 sufficient to give 70 per cent lysis in the controls Total reaction volume was 1 5 ml

* No inhibition was observed when EAC′1–8 cells were pretreated with the inhibitor and were washed before the addition of C′9

Table 4 EFFECT OF 1,10-PHENANTHROLINE HYDRATE AND 2,2-BIPYRIDINE ON EA AND EAC′1–8

Concentration (× 10^{-3} M)	Percentage haemolysis*	
Phenanthroline hydrate	EA	EAC′1–8
2	0	0
4	0	0
6	0 9	0
8	1 0	0
10	1 0	0
12	2 3	1 8
13	5 0	5 3
14	9 9	10 5
15	14 9	15 9
Bipyridine		
5	0	0
10	0	0 4
20	0	2 1
24	1 3	2 3
30	4 7	5 9

A sample of 10^8 cells was incubated for 60 min in the presence of the Fe^{++}-chelating agents in a total reaction volume of 1 5 ml

* Values were corrected for lysis of the controls not containing the chelating compounds

In view of these discrepancies, the effect of Fe^{++}-chelating agents on EA and EAC′1–8 was re-examined using 1,10-phenanthroline hydrate and 2,2-bipyridine (Merck, Darmstadt) As a result, EA and EAC′1–8 were slightly haemolysed to the same extent, but only by the highest concentrations used (Table 4) Thus in our system neither Fe^{++} ions nor phenanthroline seem to affect

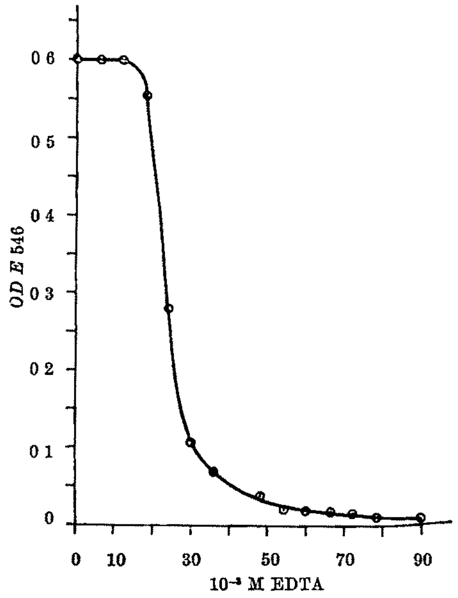

Fig 2 Effect of EDTA on the haemolysis of EAC′1–8 cells by C′9 Isotonic solutions of different concentrations of EDTA were prepared by mixing 0 090 M EDTA, *p*H 7 6, with appropriate volumes of 0 15 M NaCl EAC′1–8 cells were incubated with C′9 for 60 min at 37° C

C′9 or EAC′1–8 specifically. Most likely the results are caused by differences in the preparation of the cells and of C′9 They may, however, be caused by the different species origin of the complement components A detailed comparative study of the two systems may resolve these questions

Lysis of EAC′1–8 after reaction with C′9 was not observed in isotonic 0 090 M EDTA, *p*H 7 6 Lysis occurred, however, when these cells were washed and reincubated in VBS This type of inhibition was found by Frank *et al*[16] who studied the transformation of E^+ to ghost and haemoglobin It can be seen in Fig 2 that the effect of EDTA is already clear at concentrations much lower than 0 090 M Moreover, the degree of the spontaneous lysis of E, EA and EAC′1–8 is markedly reduced when these cells are stored in 0 090 M EDTA as compared with their lysis on storage in VBS (unpublished observations) Thus EDTA seems to exert an unspecific stabilizing effect upon the erythrocyte membrane which might be strong enough to inhibit the release of haemoglobin from EAC′1–8 treated with C′9

The described experiments do not yet allow any conclusions to be made concerning the molecular events of the terminal step in immune haemolysis We hope that with the aid of the established system more precise studies will be possible

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[1] Fischer, H , and Haupt, I , *Z Naturforsch* , 16b, 321 (1961)
[2] Fischer, H , and Haupt, I , in *Immunchemie* (edit by Westphal, O), 284 (Springer, Heidelberg, 1965)
[3] Nelson, R A , in *The Inflammatory Process* (edit by Zweifach, B W , Grant, L , and McCluskey, R J) (Academic Press, New York, 1965)
[4] Klein, P G , in *Complement* (edit by Wolstenholme, G E W), 34 (Churchill, London, 1965)
[5] Complement Workshop, *Immunochemistry*, 3, 495 (1966)
[6] Lepow, I H , and Leon, M A , *Immunology*, 5, 222 (1962)
[7] Mayer, M M , in *Experimental Immunochemistry*, by Kabat, E , and Mayer, M M , second ed (Thomas, Springfield, 1961)
[8] Wellensiek, H J , and Klein, P G , *Immunology*, 8, 604 (1965)
[9] Klein, P G , and Wellensiek, H J , *Immunology*, 8, 590 (1965)
[10] Götze, O , *Zbl Bakt I Ref* , 206, 551 (1967)
[11] Stolfi, R , *Fed Proc* , 26, 362 (1967)
[12] Uriel, U , *Bull Soc Chim Biol* , 48, 969 (1966)
[13] Hadding, U , Müller-Eberhard, H J , and Dalmasso, A P , *Fed Proc* , 25, 485 (1966)
[14] Mayer, M M , in *Immunochemical Approaches to Problems in Microbiology* (edit by Heidelberger, M , and Plescia, O J) (Rutgers University Press, New Brunswick, New Jersey, 1961)
[15] Hadding, U , and Muller-Eberhard, H J , *Science*, 157, 442 (1967)
[16] Frank, M M , Rapp, H J , and Borsos, T , *J Immunol* , 93, 409 (1965)

PHYSIOLOGY

Pheromones from the Abdominal Brushes of Male Noctuid Lepidoptera

Recent investigations have shown that benzaldehyde is the chief component of the pheromone extracted from the abdominal scent-brushes of *Leucania impura* (Huebner), *Leucania conigera* (Schiffermueller) and *Phlogophora meticulosa* (Linnaeus)

Many types of male eversible organs occur within the Lepidoptera, and these have been assumed to function as scent producing organs with some role in courtship The extreme variation in both their structure and location implies that these organs have evolved independently many times in response to a powerful selection pressure Their role has recently been described in detail for a large diurnal species, the Queen butterfly (*Danaus gilippus*)[1] In the related species *Lycorea ceres*, the components of the scent have been chemically identified[2] The courtship display of nocturnal moths is more difficult to observe, because moths do not readily produce natural behavioural responses in captivity

The brushes of the species we investigated are of the type restricted to noctuid moths and described by Eltringham[3] The brush is supported on a lever attached to the base of the first apparent abdominal sternite (Fig 1), and usually lies in a lateral pocket stretching from sternites three to five The lips of the pocket are tightly pressed together to restrain the hairs and to limit evaporation of the scent Eltringham suggested that the scales lining the pocket might produce the scent, but, unknown to him, Stobbe[4] had already described a pair of small glands giving rise to a thread of intertwined hairs which lead into the brush Excision of these glands from living moths, with appropriate controls, showed that they secrete the characteristic scent The brush is everted by specific muscles acting on the lever to pull it from the pocket and away from the abdomen A muscle across the back of the basal plate of the brush contracts to spread the apically expanded hairs After use, the brush returns to lie alongside the abdomen, and from there it is slowly pulled back into the pocket by hairs lining the lips, to be reloaded with scent

Laboratory observations of the courtship of *Phlogophora meticulosa* showed that mating took place at about dawn (4 a m in June). The males remained at rest until the female exposed her sex gland and released the attractant pheromone As this dispersed, the males became active and flew upwind towards the calling female In several cases, a male was observed to evert the brushes in the immediate vicinity of the female, followed at once by an attempt to copulate The brushes remained expanded for only 1 or 2 s On no occasion was copulation attempted without prior eversion of the brushes The scent thus naturally liberated by one moth could be easily discerned by the human nose This is apparently the first time that the use of these brushes in the courtship display of a noctuid moth has been confirmed

To extract the volatile material, the lever was cut and the brush lifted out and dropped straight into solvent (methylene dichloride) As long as the hairs are prevented

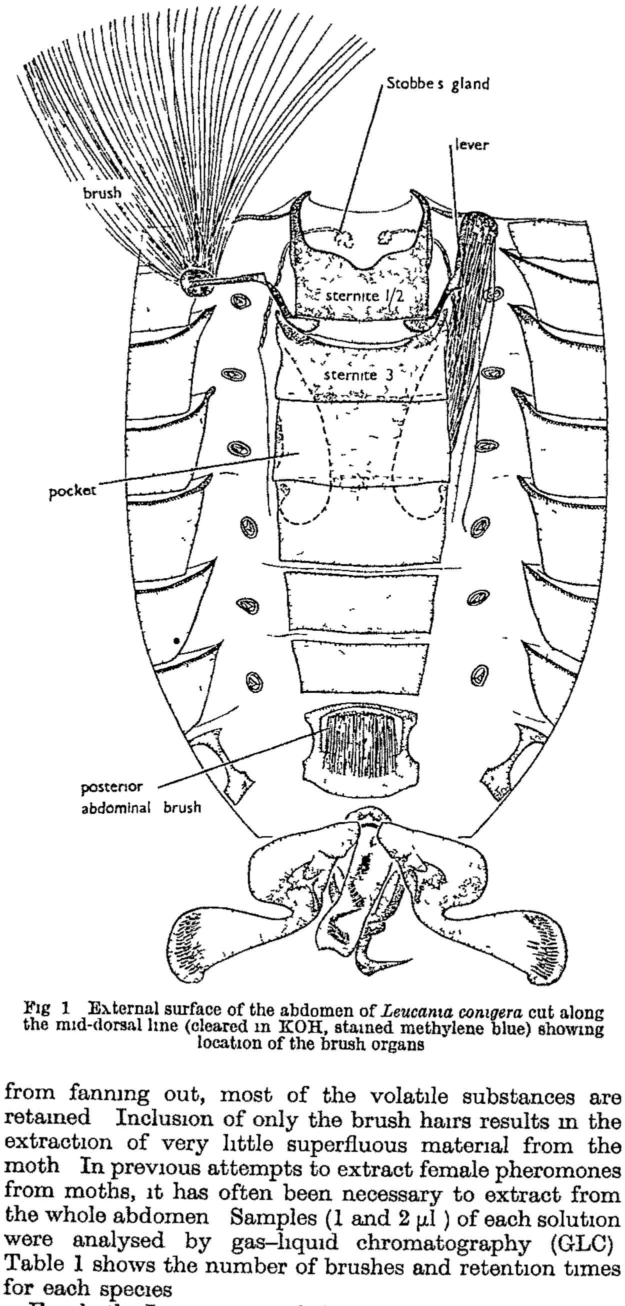

Fig 1 External surface of the abdomen of *Leucania conigera* cut along the mid-dorsal line (cleared in KOH, stained methylene blue) showing location of the brush organs

from fanning out, most of the volatile substances are retained Inclusion of only the brush hairs results in the extraction of very little superfluous material from the moth In previous attempts to extract female pheromones from moths, it has often been necessary to extract from the whole abdomen Samples (1 and 2 µl) of each solution were analysed by gas–liquid chromatography (GLC) Table 1 shows the number of brushes and retention times for each species

For both *L impura* and *L conigera* the single GLC peak had the same retention time as benzaldehyde, as shown by an enlargement of the peak area on addition of a benzaldehyde marker Analysis of both solutions on a mass spectrograph showed the presence of a major component with a molecular weight of 106 which exhibited the characteristic fragmentation pattern of benzaldehyde[5] These results confirm that benzaldehyde is a chief component of the scent The characteristic almond smell of the two *Leucania* species seems to be the main component of the scent (*Leucania pallens* (Linnaeus) also smells strongly of almonds) For *Lycorea*, Meinwald was forced to assume the presence of a scent component in trace amounts, in addition to the three components identified to account for the characteristic odour

Table 1

Species	No of brushes	Quantity of solvent (ml)	GLC retention time* (min)
Leucania impura	214	5	20
Leucania conigera	54	5	20
Phlogophora meticulosa	40	1	20+24
Apamea monoglypha	142	1	63

* On a Pye 104–14 gas chromatograph equipped with a flame ionization detector and 30 per cent 'Apiezon *L*', on an 80–100 mesh 'Embacil' column, operating at 100° C with an argon carrier gas flow of 40 ml /min

An estimate of the quantity of scent extracted was obtained by running a quantitative control of benzaldehyde on the chromatograph The sample of 214 brushes from *L impura* contained about 10^{-3} g, which gives an average of 5 µg for each brush This figure is certainly an underestimate because losses must occur through evaporation and oxidation to benzoic acid The amount must vary from moth to moth, depending on the length of time from the previous discharge

Of the two peaks recorded from *P meticulosa*, the first was shown to be benzaldehyde, the second has not yet been identified The longer retention time peak from *Apamea monoglypha* (Hufnagel) implies a less volatile compound with higher molecular weight A component with a molecular ion weight of 284 was observed on mass spectrographic analysis

Three biological functions can be suggested for this scent (1) It may act on other males as a deterrent Aldehydes are well known as major components of defensive secretions and benzaldehyde is secreted by several millipedes (*Diplopoda*) It is possible that other males lose interest in a mated female more quickly through distribution of a male scent than by cessation of a female one, although the rapid disappearance of the scent makes this doubtful (2) It may act on the female as a species specific signal to prevent cross-breeding This seems to be ruled out because *L impura*, *L conigera* and probably *L pallens* all have the same scent Because all three species are common grass-feeders and are active at the same time of year, the mechanism to prevent cross-breeding may lie in the time of mating The three species also possess additional posterior abdominal brushes, however, the role of which is not yet known The male scent may act as an isolating mechanism between some species Varley[7] has described several noctuid genera where closely related species have very different types of scent-organ or none at all (3) It may act on the female as an aphrodisiac to induce mating A combination of (2) and (3) seems to be most likely

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[1] Brower, L P , and Jones, M A , *Proc Roy Entomol Soc Lond* , A, 40, 147 (1965)
[2] Meinwald, J , and Meinwald, Y C , *J Amer Chem Soc* , 88, 1305 (1966)
[3] Eltringham, H , *Trans Entomol Soc Lond* , 73, 1 (1925)
[4] Stobbe, R , *Zool Jb* , 32, 493 (1912)
[5] McCollum, J D , and Meyerson, S , *J Amer Chem Soc* , 85, 1739 (1963)
[6] Eisner, T , and Meinwald, Y C , *Science*, 150, 1733 (1965)
[7] Varley, G C , *Trans Soc Brit Entomol* , 15, 29 (1962)

Effect of Humming on the Visibility of a Stabilized Retinal Image

Rushton[1] has recently described a series of ingenious experiments which demonstrate the effect of humming on the induction of stroboscopic patterns in non-fluctuating illumination By introducing a number of controls and by studying the perception of presumably non-peripheral phenomena (such as the Pulfrich pendulum), he concludes that the strobing is induced because the humming sets up a mechanical vibration of the retina, and not, as might be suggested, by inducing "central strobing" through driving the rate of neuronal firing.

These conclusions are supported when one considers the effect of humming on the perception of a perfectly stabilized image—which, of course, cannot be displaced with respect to the retina by mechanical vibration. We have recently performed two simple experimental studies, employing this technique, which are relevant to Rushton's work. In the first we examined the effect of humming on the perception of the pattern in Fig. 1 when viewed as a stabilized image (the method of stabilization involves the production of an intense, clear after-image by illuminating the target with a 1,250 J flash 1 ms in duration[2,3]). It has been suggested that the fringes or shadow patterns observed when Fig. 1 is fixated in normal illumination are the result of some central scanning process, but they are more probably caused by eye-movement scanning, for the phenomena are not observed in stabilized vision[4]. Humming at 100 Hz might possibly induce the fringe patterns in the after-image if the principal effect of humming were to drive neural input at some point in the visual system, but it would be expected to have no effect if it were merely causing retinal vibration, because both retina and after-image vibrate in phase. In fact, a series of simple observations reveals that humming does not induce any significant change in the perception of Fig. 1 as an after-image.

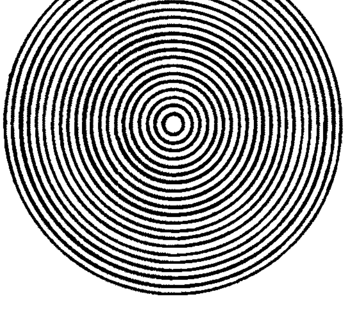

Fig. 1

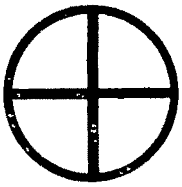

Fig. 2

In a second, quantitative, study, an after-image of the line drawing shown in Fig. 2 was viewed in four different conditions, and in each case the time was recorded from the first appearance of the perfect image to the first change of any kind. This is known as "time to first disappearance". The after-images were viewed with eyes closed, about 10 in. from a screen illuminated from behind to a brightness of 0.9 log ft.-lamberts in an otherwise dark room. In the first three conditions, the illumination was steady—provided by a d.c. lamp—and in the fourth it was strobed at 100 Hz. The first two (control) conditions were identical except that in one case a 100 Hz tone was heard through earphones while viewing, and in the other no tone was used. In the third (experimental) condition, the subject was required to hum in tune with the tone. Thus the four conditions and times were as in Table 1.

Table 1

	Mean time (s) (25 trials)	*S.D.*
(*a*) d.c. illumination, no sound	14.8	9.1
(*b*) d.c. illumination, 100 Hz tone	7.2	6.7
(*c*) d.c. illumination, 100 Hz tone hum	7.0	6.1
(*d*) Illumination strobed at 100 Hz, no sound	3.8	2.4

There is a highly significant difference ($P < 0.005$) between conditions (*a*), ($b + c$), and (*d*), but no difference between (*b*) and (*c*) themselves ($P > 0.3$). This makes it clear that the onset of fragmentation of the after-image is facilitated by the auditory input or by strobing the background, but that any change in the behaviour of an after-image which might be ascribed to the effect of humming really results from the sound input itself and not from the method of generating it. The effect of strobing the background is so much greater that it would not seem that the humming was operating by "strobing" the visual system central to the retina. This therefore provides interesting support for Rushton's conclusions.

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[1] Rushton, W. A. H., *Nature*, **216**, 1173 (1967).
[2] Bennet-Clark, H. C., and Evans, C. R., *Nature*, **199**, 1215 (1963).
[3] Evans, C. R., *Brit. J. Psychol.*, **58**, 333 (1967).
[4] Evans, C. R., and Marsden, R. P., *Brit. J. Physiol. Opt.*, **23**, 242 (1966).

Effect of Light and Dark on Secondary Induction of Cornea in Tadpoles of *Rana temporaria*

WE have found a close connexion between the functional state of the retina and certain inductive processes in the developing vertebrate eye. Such studies, carried out during functional ontogenesis, were based on theoretical concepts of the unity of the evolutionary origin of functional and formative phenomena and their close connexion in animals[1,2].

First, we repeated previous experiments on the secondary induction of cornea in skin grafted onto the eye[3,4], but subjected the tadpoles to different light conditions. A blinking pattern, highly active for retina, obviously accelerated induction, which was retarded in the dark[5]. Light of different quality (sunlight, colour), according to its physiological effect on the retina, either accelerated or slowed the induction of Wolffian regeneration in the larvae of *Pleurodeles waltlii*, and this was reflected in the structure of regenerating lenses[6].

Different light conditions also influenced temporarily the development of the more stable intact eye. Thus flashing light accelerated the differentiation of the retina photoreceptors and, consequently, primary induction of the cornea in young tadpoles, however, constant light (8 h/day) caused increased accumulation of ganglionic cells and enhanced the development[7] of the lens. It is interesting that the growth of eyes and optical brain lobes in chick embryos was accelerated under the influence of flashing light[8].

The influence of adequate external stimuli on the ability of the retina to induce the production of cornea by altering the specific and spontaneous function of the retina needs to be explained. It is possible that alternating light and dark influences not so much the inductive ability of the retina as the reactivity of the skin graft covering the eye, which in tadpoles[9,10] is very sensitive to light. We have investigated this important problem.

Experiments were carried out on tadpoles of *Rana temporaria* with three-membered hind limbs without digits In such tadpoles the electroretinogram is already formed[11], showing that their visual apparatus is functional In 390 experiments, skin was successfully grafted from the host's back onto the outer cornea of the recipient's right eye There were two series of experiments (1) the retina was kept intact on the operated eye, and (2) at the same time as grafting the optic nerve was cut so as to induce a limited retro-degeneration and destruction of the inner layers of the retina which it does in this species[12,13] About half the specimens in each series were exposed to diffused sunlight and natural days and nights (control) while the other half were kept in a dark chamber which was opened only in red light All other conditions (such as temperature and food) were identical for both groups All tadpoles developed normally

The dynamics of the transformation of the pigmented skin into hyaline cornea under the influence of the eye was first examined on flat preparations The stages of transformation were evaluated with a four-mark system —, $\pm$, ± and + Many of these eyes were also studied histologically

On the ninth day of the experiment, one out of ten controls (of series 1 and 2) showed signs of depigmentation of the skin grafts ($\pm$) We then chose daily at random ten "light" and ten "dark" tadpoles from both series and watched the changes which were visible in the grafted skin until the new cornea formed (+) in the control groups This allowed us to store data using two phase variance analysis (first the symbols were replaced by the figures 0, 1/3, 2/3, 1) and draw graphs which showed the difference in the rate of formation in light and darkness It is important that this difference is insignificant in series 1 but strongly increases in series 2 The influence of light and time on the secondary induction of cornea in the grafted skin in series 1 ($P > 0\ 95$) and even more so in series 2 ($P > 0\ 999$) is unquestionable

Our results undoubtedly depended on the different state of the retina in the two series of tadpoles In series 1 the retina was normal and in series 2 the ganglionic and inner nuclear layer opposite the pupil always degenerated under the influence of the optic nerve intersection Any alteration of formative properties of the light-sensitive skin graft could not influence results, because control and experimental tadpoles of both series were kept in the same conditions of light and darkness

These and our previous data on the effect of adequate external stimuli on very sensitive formative connexions in the vertebrate eye agree with what is known about the physiology of vision Thus when excited by light, the normal retina induces in good time new cornea in the skin graft, but in tadpoles in the dark the specific retina activity is replaced by its weaker spontaneous activity and the formation of a new retina is retarded After the optic nerve had been cut the photoreceptors, closely connected with cornea formation, do not lose their electrophysiological[14] and formative activity[15] They seem to increase the rate of the physiological reaction, probably because of acceleration of the inductive action of the denervated eye in the light and slowing down in darkness The latter phenomenon is also explained by the partial destruction of the inner layers of the retina, indicative of the decrease in the spontaneous production of impulses by the darkened retina

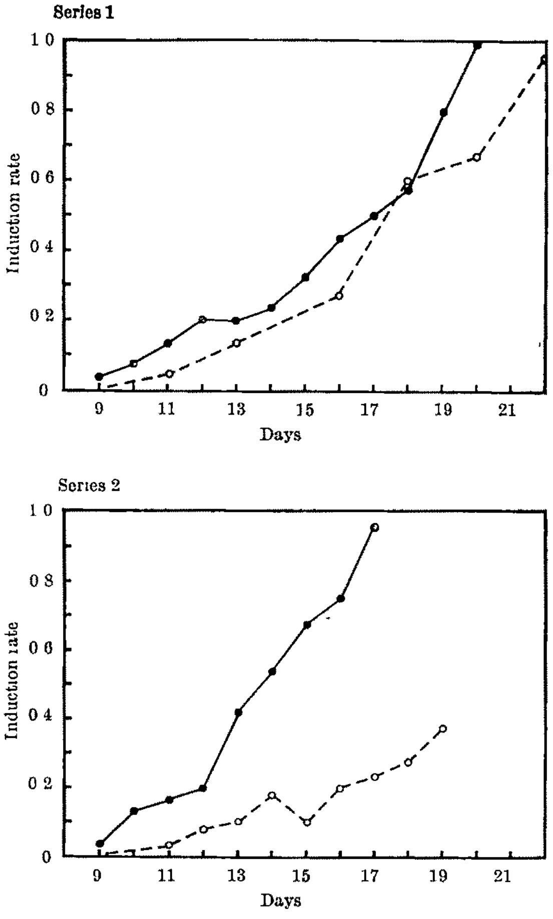

Fig 1 Dynamics of the secondary cornea induction under the influence of normal (series 1) and denervated (series 2) eye in light and darkness ●, Control (light), ○, experimental (dark)

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[1] Popov, V V , *Bull Soc Naturalists (Dep Biol* , in Russian), 62 (3), 103 (1957)
[2] Popov, V V , *J Gen Biol* (in Russian), 21, 393 (1960)
[3] Groll, O , *Arch Mikr Anat u Entw-Mech* , **100**, 385 (1923)
[4] Popov, V V , *Sci Rec of the Gorky State Univ* (in Russian), 8, 22 (1938)
[5] Popov, V V , and Farberov, A I , *Nauchnie Dokl Visshei Shkoli*, **3**, 48 (1958)
[6] Popov, V V , and Borsuk, R A , *J Gen Biol* (in Russian), **25**, 287 (1964)
[7] Popov, V V , and Farberov, A I , *Nature*, **202**, 1120 (1964)
[8] Manuilova, N A , Popov, V V , and Timoshuk, N B , *J Gen Biol* (in Russian), **24**, 98 (1963)
[9] Obreshkove, V , *J Exp Zool* , **34**, 235 (1921)
[10] Cole, W , and Dean, C , *J Exp Zool* , **23**, 361 (1917)
[11] Popov, V V , Golichenkov, V A , and Vsevolodov, E B , *Folia Biologica*, Krakow, **14**, 159 (1966)
[12] Popov, V V , and Al'Sakini, A V , *Dokl Akad Nauk USSR*, **142**, 1420 (1962)
[13] Popov, V V , and Pantellev, E I , *Dokl Akad Nauk USSR*, **150**, 1164 (1963)
[14] Popov, V V , *Dokl Akad Nauk USSR*, **131**, 1202 (1960)
[15] Popov, V V , and Tun Yun-Siui, *Dokl Akad Nauk USSR*, **132**, 481 (1960)

Some Aspects of Heart Function in *Patella vulgata* L.

WITHIN the pericardium of *Patella vulgata* L , in addition to the auricle and ventricle, there is a third chamber of the heart, the bulbus aortae or pseudo-chamber (Fig 1, *Ba*). This chamber is situated laterally to the ventricle (*V*) and all blood leaving the ventricle passes through a valve into the bulbus aortae before leaving the pericardium by the anterior or posterior aorta (*Aa* and *Pa*) The function of the bulbus aortae is of considerable doubt, for although it is histologically indistinguishable from the ventricle it has not previously been observed to contract rhythmically Wegmann[1] considered the bulbus aortae to be a sub-division of the ventricle, but all later workers argue that it is the fused, swollen bases of the anterior and posterior aortae[2]

The effect of tidal exposure on the heart rate of limpets while on the shore at Millport, Isle of Cumbrae, was recorded by impedance pneumograph This method involved placing two silver electrodes through the shell so that they touched the pericardium, and connecting these to an impedance pneumograph designed to detect pulsatile changes in volume, which was a c coupled to a multi-channel pen recorder Similar work on bivalves[3] has shown a retardation of heart rate during littoral exposure (to about 15 beats/min) and an increase during tidal coverage (to about 30 beats/min) The recordings obtained from *Patella*, however, indicate that the heart rate is about 33 beats/min when the tide is out (Fig 2*A*), but the recordings taken when the tide was in show a much slower rhythm of about 18 beats/min although the beats were of greater amplitude (Fig 2*B*), the precise rates varying from individual to individual This is not the original heart beat because the latter is in places superimposed on the slower rhythm (Fig 2*B*, *X*) The slower rhythm occasionally ceased when the tide was in and did not start at all when the tide was in and a storm was blowing The heart rate, when distinguishable, displayed a slight, but significant, increase when the tide was in, to about 38 beats/min

The slower rhythm was at first thought to be the result of the effect of the rasping movements of the radula because these are of a similar frequency Laboratory tests, however (Fig 2*C*), have shown that radula movements have no effect on heart recordings made in a similar manner to those of Fig 2*A* and *B*, and therefore this explanation seems unlikely to be true The effects of temperature were also excluded as the air and sea temperatures were similar at the time of the field experiments

The other most probable source of the slower rhythm is the bulbus aortae, but it proved impossible to take pneumograph recordings of heart beat while at the same time observing the bulbus aortae In an attempt to verify this hypothesis, a number of experiments were carried out First, the left half of the roof of the pericardium was removed, leaving intact the fibres joining the ventricle to the roof of the pericardium (Fig 1, *Pvf*), and a thread was attached round the auricular–ventricular junction to an isometric myograph Contractions of the bulbus aortae, which was visible through the pericardial wall, were marked manually onto the recorder (Fig 2*D*) Second, the whole roof of the pericardium was removed and threads were attached round the auricle and round both the ventricle and bulbus aortae to isometric myographs The bulbus aortae is attached at both ends to the pericardium and only contractions of the bulbus aortae were recorded in this manner, not those of the ventricle (Fig 2*E*)

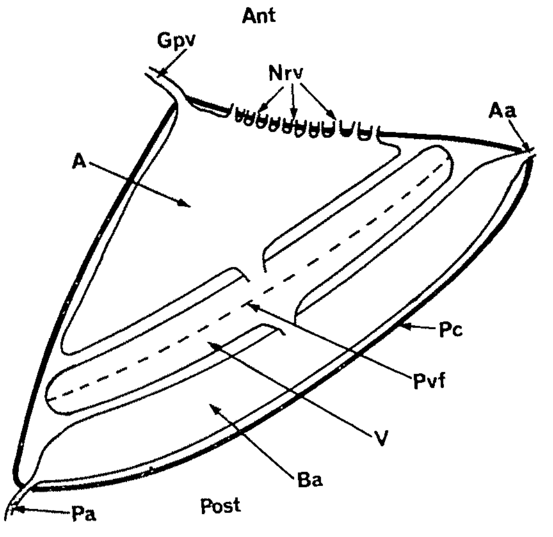

Fig 1 *Patella vulgata* L Dorsal aspect of the pericardium and heart *A*, Auricle, *Aa*, anterior aorta, *Ant*, anterior, *Ba*, bulbus aortae, *Gpv*, great pallial vein, *Nrv*, veinlets from the roof of the nuchal cavity, *Pa*, posterior aorta, *Pc*, wall of the pericardium, *Post*, posterior, *Pvf*, fibres joining the dorsal wall of the pericardium to the ventricle, *V*, ventricle

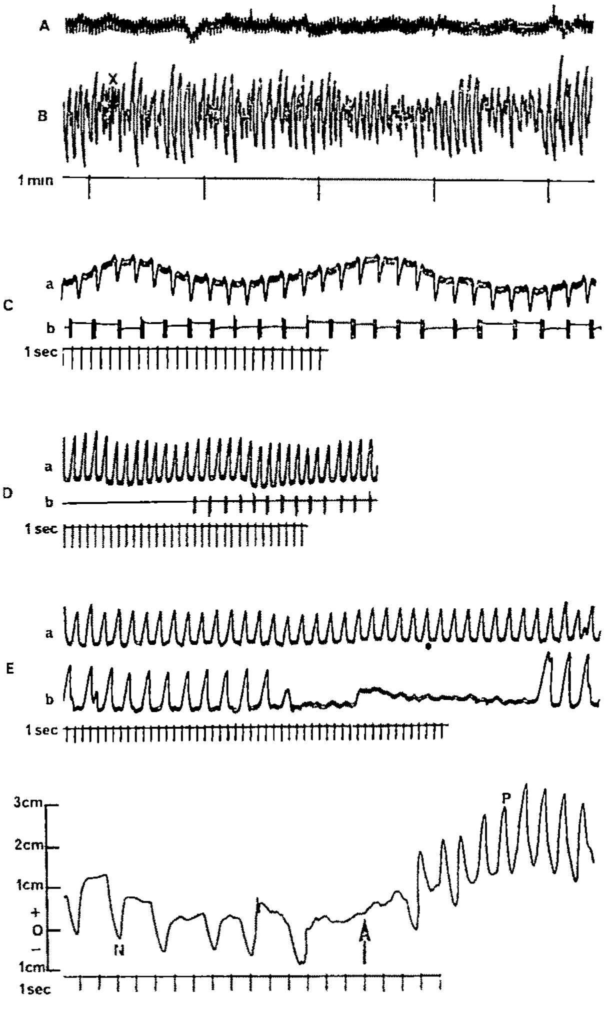

Fig 2 *Patella vulgata* L Recordings of the heart beat (*A*) Pneumograph recording during tidal exposure, (*B*) pneumograph recording when covered by the tide *X* is superimposed ventricular contractions, (*C*) pneumograph recording (*a*) while moving and feeding on glass with (*b*) simultaneous manual record of the rasping movements of the radula, (*D*) isometric recording of the contractions of the auricle and ventricle (*a*) with contractions of the bulbus aortae manually marked (*b*), (*E*) isometric records of auricular contractions (*a*) and contractions of the bulbus aortae (*b*), (*F*) pressure recording from within the pericardium and, after the arrow, from within the ventricle while the bulbus aortae was beating Pressure is reduced in the pericardium as the bulbus aortae contacts *N*, but the pressure increases in the ventricle during ventricular systole *P* Pressure in cm of water

Unsuccessful attempts were made to obtain pressure recordings from within the bulbus aortae and simultaneously from within the pericardium Fig 2*F*, however, shows a record from within the pericardium adjacent to the bulbus aortae, followed by a record of ventricular contractions (which were continually occurring), obtained when the cannula was manipulated into the ventricle (at arrow). The low frequency of the bulbus aortae appears as a negative pulse (*N*) in the pericardium, and ventricular contractions as positive peaks (*P*) in the ventricle

In each case the faster rhythm/slower rhythm ratio is as follows (letters refer to those of Fig 2), $A/B=1\cdot84$, $Da/Db=1\cdot35$, $Ea/Eb=1\cdot33$ and $F=1\cdot98$. The pressure recording caused the least disturbance to the animal and thus gives the best correlation This indicates that the contractions of the bulbus aortae bear a similar relation to the frequency of auricular/ventricular contractions as do the recordings taken when the tide is in to those obtained when the tide is out The records also show that the control of the bulbus aortae is independent of the auricle (and presumably the ventricle), for the contractions are capable of being arrested in spite of the continuing auricular beat (Fig 2*E*)

These results suggest that the recordings obtained when the tide was in are caused by the rhythmic contraction of the bulbus aortae The recordings illustrated (Fig 2) are almost the only successful laboratory observations of the contractions When the animals from which the original records were obtained were moved on their own substrate into the mobile laboratory housing the recording apparatus, only a recording similar to Fig 2*A* was obtained, even after synchronous tides were produced experimentally On replacing the limpets on the shore the recordings were similar to those originally obtained

The bulbus aortae of *Patella* may have either an active or a passive function The enlargement of the aorta of *Busycon canaliculatum* and the aortic bulb on the posterior aorta of the clam *Tridacna* would seem to play only a passive part, both acting simply to even out the pressure surges produced by the ventricle[4,5] That of *Tridacna* may also act as a reservoir for arterial blood squeezed back during adduction[6] The aortic ampulla of *Pila globosa* is, however, contractile, probably performing a more active role[7] The bulbus aortae of *Patella*, it is suggested, acts as an accessory pump, for it is clearly capable of strong contractions independently of the ventricle It may also act to synchronize blood flow with movements of the odontophore, though Fig 2*C* would seem to contradict this in that the bulbus was not contracting during feeding

The initiation of beating of the bulbus aortae may be concerned with the oxygen demand produced as a result of locomotion and feeding The oxygen consumption of *Patella* is at its highest when the tide is in[8], and the buccal muscles of *Buccinum* have a very high oxygen consumption[9] Most littoral animals are inactive when the tide is out and it may require periods of sustained activity for the beat of the bulbus aortae to be initiated Specimens of *Patella* kept in the laboratory feed only intermittently and possibly there is not a consistent enough high demand for oxygen for the beat to be initiated

It would therefore seem that the bulbus aortae of *Patella vulgata* chiefly acts as an accessory pump, providing increased blood flow to satisfy the higher oxygen demand produced as a result of the increased activity when the animal is covered by the tide

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[1] Wegmann, H , *Rec Zool Suisse*, **4**, 269 (1887)

[2] Fretter, V , and Graham, A , *British Prosobranch Molluscs* (Ray Society, London, 1962)

[3] Helm, M M , and Trueman, E R , *Comp Biochem Physiol* ,**21** 171 (1967)

[4] Hill, R B , and Welsh, J G , in *Physiology of Mollusca*, **2** (edit by Wilbur, K M , and Yonge C M) (Academic Press, New York and London, 1966)

[5] Purchon, R D , *Proc Malac Soc Lond* , **31**, 95 (1955)

[6] Pelseneer, P , *Mollusca*, in *A Treatise on Zoology*, **5** (by Lankester, E R) (A and C Black, London, 1906)

[7] Prashad, B , *Mem Indian Mus* , **8** (1925)

[8] Gompel, M M , *CR Acad Sci , Paris*, **205**, 816 (1937)

[9] Fange, R , and Mattison, A , *Acta Zool Stockh* , **39**, 53 (1958)

Observations on the Presence of Titanium and Zinc in Human Leucocytes

THE electron probe microanalyser makes possible rapid, precise localization and quantitation of elements in thin biological tissue during visual scanning of specimens This communication describes a study of its use in analysis of the metal content of normal and malignant blood cells The instrument used (Cameca, Paris) had an optical magnification of 400 diameters with a resolving power of about 0 5μ The X-ray spectrographs use quartz crystals which are bent and ground to a focusing circle of 250 mm radius Detectors are proportional counters, one with a beryllium window and another of gas-flow type with a thin plastic window

Of the numerous substrates possible for such work, single crystal disk wafers of highly polished silicon were found most suitable because such substrates (1) facilitate optical examination of specimens by reflected light in the electron probe instrument, (2) provide sufficient electrical conductivity to discharge specimens, (3) ensure the absence of particulate impurities, and (4) yield a low background of continuous X-rays

Under a 30 kV beam of electrons of 10^{-7} A, focused on a spot about 1μ across, single cells give useful X-ray spectra in which intensities of some lines are as high as 1,000 c p s with a peak-to-background ratio of at least 10-fold and sometimes as high as 100-fold

Specimens of whole blood, bone marrow and lymph nodes were obtained from human sources The blood smears were produced by the standard technique on opposing surfaces of silicon disk and glass cover slips, taking care to avoid contamination by dust, finger contact, stains, fixatives or substances containing metal The bone marrow specimens were similarly prepared by direct application and smearing on the substrate surface without anticoagulants, fixatives or dilutants The leucocytes from lymph node sources were isolated by gentle agitation of the node with 'Teflon' tweezers in physiological sodium chloride solution without buffering additives, immediately after surgical lymph node biopsy Smears were made of the resulting undiluted cell suspension and of separately prepared centrifugal concentrates

During a study of the intracellular cation content of human erythrocytes subject to dialysis after removal from the frozen state, the metal content of leucocytes which contaminated the specimens was also investigated Unexpectedly large amounts of titanium and zinc were occasionally found A repeated search was then made of the leucocytes of normal peripheral blood, with only rare similar findings Because of the difficulty in identifying the morphological subtypes of leucocytes in unstained specimens with reflected illumination at 400 diameters, attention was directed to relatively pure strains of cells to learn whether granulocytes or lymphocytes were the source of the signals Initial tests on blood of a subject with predominantly lymphoblastic forms in the circulating blood gave clear evidence of the presence of high concentrations of titanium and zinc in many cells This was in the range of 10^6 to 10^8 metallic atoms per cell This was substantiated by similar findings in three additional cases Control specimens from normal sources were strikingly different

Fresh bone marrow aspirates rich in leucocytes were then examined Again, cells containing titanium and zinc were found, but apparently much more frequently in cases of lymphoblastic lymphoma involving the marrow as shown in Table 1 In an effort to refine the technique further, fresh lymph nodes removed during surgical biopsies were next examined Again, cells containing titanium and zinc were found, most frequently in cases of lymphoblastic lymphoma Table 2 shows the results of a randomized white cell count and simultaneous

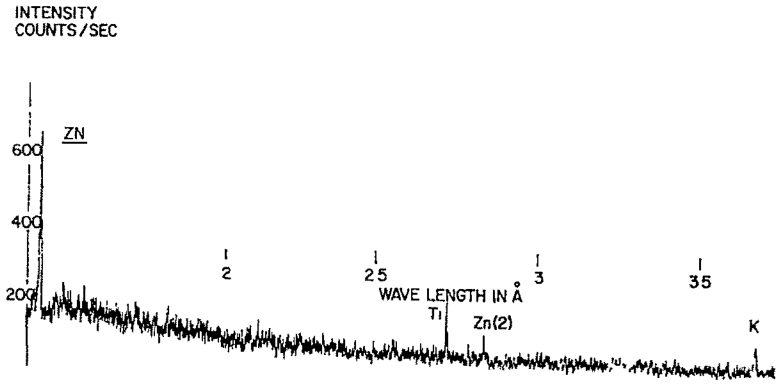

Fig 1 X-ray emission spectrum from a single lymph node cell, *Kα* lines ofpotassium, titanium and zinc Specimen from patient with Hodgkin's lymphoma.

recording of titanium and zinc emission X-rays on a strip chart recorder of the counting rates. A total of 300 cells were scanned in all cases but one, in which only 200 cells were available Randomization was accomplished by selecting two peripheral and one central field in a 300 μ^2 area The recorder settings were adjusted to reveal clearly on the chart zinc and titanium X-ray signals of intensity as low as 10 c p s Such an intensity of X-ray signal may be compared with an intensity of some 60,000 c p s on solid metal specimens of zinc or titanium under identical conditions of the electron beam Thus the sensitivity of the technique can be estimated to be below 0 1 per cent of a pure metal standard or, alternatively, a detectable limit of about 10^{-14} g of metal atoms per cell. This sensitivity was entirely adequate for our purpose and no attempts were made to increase sensitivity Fig 1 shows a typical X-ray spectrum of a single cell in which zinc, potassium and titanium are seen

Table 1 FREQUENCY OF CELLS CONTAINING TITANIUM AND ZINC IN WHITE CELLS FROM BONE MARROW

Specimen	Diagnosis	Frequency of occurrence of cells containing titanium and zinc
1	Normal marrow, toxic goitre	0 out of 100
2	Normal marrow, secondary anaemia	3 out of 200
3	Normal marrow, histiocytosis	0 out of 200
4	Myeloblastic leukaemia	1 out of 100
5	Lymphoblastic leukaemia	15 out of 85
6	Multiple myeloma	9 out of 300

Table 2 NUMBER OF CELLS CONTAINING TITANIUM AND ZINC IN 300 CELL RANDOMIZED COUNTS ON LEUCOCYTES EXTRACTED FROM LYMPH NODES

Specimen	Diagnosis	Number of titanium and zinc cells
1	Normal	1
2	Normal	1
3*a*	Hodgkin's lymphoma	25
3*b*	Hodgkin's lymphoma, centrifuged	16
4	Normal	1*
5	Lymphosarcoma and Hodgkin's lymphoma	15
6	Non-lymphomatous, undifferentiated carcinoma	1

* 200 cell count

Among samples from lymph nodes, blood and bone marrow from some thirty-six persons with malignant and non-malignant haematological diseases, variations in the ratio of zinc to titanium were observed In cases of lymphoblastic lymphoma, multiple myeloma and Hodgkin's disease, however, the atomic ratio of zinc to titanium maintained a constant value estimated at 3 or 4 to 1 In a single cell the two metal atoms, if present, were at precisely the same location Additional cells were observed in which titanium or zinc occurred alone, and often in a region smaller than the electron probe beam Occasional material containing zinc and titanium larger in physical size than a single cell was, however, observed This could represent either an extracellular deposit or an agglomerated mass of denatured cells Such extracellular material was not included in the results reported here.

The intracellular role of these metallic elements in the structure or function of leucocytes is unknown, but we considered that the differences in frequency of their presence in leucocytes from normal as opposed to immature (malignant) sources were sufficient to justify this preliminary report Multiple biochemical and physiological studies will be necessary to establish the function, if any, of these metals in cells

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[1] Castaing, R thesis, Univ of Paris (1951) *Advances in Electronics and Electron Physics* (edit by Marton, L), 13, 317 (Academic Press, NY, 1960)

[2] Cosslett, V E , *J Roy Microsc Soc* , Ser III, 83, 13 (1964)

[3] Mellors, Robert C , and Carroll, Kenneth G , *Nature*, 192, 1090 (1961)

[4] Carroll, Kenneth G , in *In Vivo Techniques in Histology* (edit by Bourne, Geoffrey H), 69 (Williams and Wilkins Company, Baltimore, 1967)

[5] Birks, L S , *Electron Probe Microanalysis* (Interscience, New York, 1963)

Efficacy of β-Adrenergic Blocking Agents in inducing Histamine Sensitivity in Mice

THE usually high resistance of mice to the lethal effects of histamine can be greatly reduced in certain strains by some substances The most potent of these histamine sensitizing substances is probably the so-called histamine sensitizing factor (HSF) of *Bordetella pertussis*[1]. Recently, substances with β-adrenergic blocking activity, such as dichloroisoproterenol (DCI), pronethalol ('Alderlin'®) and 4-(2-isopropyl-amino-1-hydroxyethyl) methane sulphonanilide HCl (*MJ*-1999), have also been shown to increase the sensitivity of mice to histamine[2,3] Some of these agents, such as DCI, have been found highly effective in reducing histamine tolerance[2] Preliminary work with DCI and the *CFW* strain of mice, reared in our laboratory, confirmed that treating mice with DCI does induce histamine sensitivity, but we did not find that DCI was effective in the small doses (0 005 mg/kg) reported by Fishel *et al* [2] With the advent of other β-adrenergic blocking agents, we felt it would be of value to test the histamine sensitizing effects of these agents and to determine if some were more efficient in this respect than others In order to duplicate more closely the experimental conditions used by Fishel *et al* [2], we used female *CFW* mice obtained from Carworth Farms, New City, New York

The mice, weighing 20–24 g, were treated intravenously with various doses of β-adrenergic blocking agents given in 0 2 ml of physiological saline and were challenged

Table 1 COMPARISON OF THE EFFICACY OF VARIOUS β-ADRENERGIC BLOCKING AGENTS IN INDUCING HISTAMINE SENSITIVITY IN MICE

Dose (µg/mouse i.v.)	β-Adrenergic blocking agent DCI†	Pronethalol	Butoxamine HCl	Propranolol
240	7/10*	8/10	8/10	10/10
80	6/10	7/10	5/10	10/10
27	1/10	1/10	5/10	10/10
9	2/10	2/10	2/10	9/10
3	2/10	0/10	1/10	10/10
2	1/10	2/10	3/10	8/10
1	1/10	1/10	1/10	3/10
0.5	0/10	2/10	1/10	4/10
0.25	3/10	0/10	1/10	2/10
0 (saline)		1/10		

* Deaths/number of mice tested (all mice challenged intraperitoneally with 0.5 mg histamine base, 20 min after receiving the blocking agent).

† DCI, Dichloroisoproterenol.

Table 2 SD_{50}s* OF β-ADRENERGIC BLOCKING AGENTS TO INDUCE SENSITIVITY TO A CHALLENGE WITH 0.5 MG OF HISTAMINE

β-Adrenergic blocking agent	Trial First	Second	Third
DCI	77.3 µg†	12.7 µg	68.6 µg
Pronethalol	71.3 µg	—	55.3 µg
Butoxamine HCl	125.0 µg	—	46.6 µg
Propranolol	0.22 µg	2.0 µg	1.0 µg

* Calculated by the method of Reed and Muench[8].

† Calculated quantity of blocking agent required to sensitize half of the mice to the lethal effects of the histamine challenge.

15–20 min later with an intraperitoneal injection of 0.5 mg histamine base in 0.2 ml of saline. Pronethalol, DCI, butoxamine-HCl and propranolol ('Inderal'®) were compared for their ability to increase the sensitivity of mice to histamine. Propranolol in doses of 2–240 µg/mouse gave consistent sensitization to histamine (Table 1). The other three drugs also sensitized mice to histamine, but greater doses were required to produce a consistent and high degree of sensitivity. These results were obtained in other experiments in which propranolol was always found to be more potent than DCI, pronethalol or butoxamine-HCl.

Table 2 summarizes the result obtained in three experiments with respect to calculated SD_{50}s for the β-adrenergic blocking agents to induce sensitivity to a standard challenge with 0.5 mg histamine. Some differences in degree of sensitization were noted in the various experiments and could only be explained by differences in the lots of mice employed. Even more striking differences had been observed when *CFW* mice raised in our laboratory were employed in this type of experiment. DCI was much less effective in sensitizing *CFW* mice raised in our laboratory than *CFW* mice purchased directly from Carworth Farms. This variation in lots of mice, or in mice raised in slightly different conditions, is at present unexplained, but it is a difference that makes it essential to use proper controls in this type of experiment.

Of the compounds we have tested, propranolol is the most potent on a weight basis of the β-adrenergic blocking agents in its histamine-sensitizing activity, and approaches in potency the soluble preparations of *B. pertussis*. Like other β-blocking agents, it produces histamine sensitivity within 15–20 min after injection and this sensitivity begins to disappear 1 h later. As with DCI and pronethalol[4], mice treated with propranolol could be protected from death by introducing about 7.5 µg of *l*-epinephrine intravenously 30 s after the histamine challenge.

Fishel *et al.*[5] have advanced a hypothesis which attempts to explain histamine sensitivity in mice after treatment with either β-adrenergic blocking agents or HSF from *B. pertussis*. They proposed their hypothesis as follows: "... the *B. pertussis* organism either possesses a component or causes the host to elaborate a substance with a steric configuration complementary to the β-adrenergic receptors. Because of natural affinity, this substance combines with the β-receptors and occupies the site normally available for the adrenergic transmitter, i.e., adrenaline and/or noradrenaline. When such an animal is challenged with histamine or serotonin, the antagonistic action of the excessively released catecholamines is blocked at the β-receptor level, leaving the α-adrenergic activities unopposed. The resultant imbalance produces a combination of unfavourable metabolic, and, consequently, smooth muscle as well as neural adjustments, culminating in a reduced resistance to histamine and serotonin."

An impressive amount of data has been reported which indicates that some sort of β-adrenergic blockade may indeed operate in pertussis-treated mice[6,7]. There are observations, however, which do not support some aspects of this hypothesis. There is little doubt that β-adrenergic blocking agents produce histamine sensitization, but proof is lacking that an imbalance between the α and β receptor responses is responsible for death after challenge with histamine. If an imbalance between the α and β receptor responses to endogenously released catecholamines were indeed the underlying cause of sensitization to histamine, one would not expect animals treated with β-blocking agents or with HSF from *B. pertussis* to be protected by injection of epinephrine. This type of protection has, however, been reported[3,4]. In addition, animals treated with β-blocking agents or HSF would be expected to be more sensitive than normal animals to injection of epinephrine, but this is not the case. Fishel *et al.*[2] reported very little difference in LD_{50} values when epinephrine was administered intraperitoneally to normal and pertussis-treated mice. We obtained similar results after giving *l*-epinephrine intravenously. The LD_{50}s in normal and HSF-treated mice were 8.0 and 6.4 µg, respectively, in an experiment in which epinephrine doses started at 0.0156 µg/mouse and increased to 16.0 µg/mouse in two-fold increments.

With the available data, it is quite tempting to suggest that histamine sensitivity in mice is caused by a blockade of the β-adrenergic receptors. As suggested by Nickerson[9], however, it cannot be assumed that all the effects of β-adrenergic blocking agents are caused by blockade of the β-adrenergic receptors. It does seem significant, however, that histamine sensitivity has been obtained with all β-adrenergic blocking agents tested (DCI, butoxamine-HCl, pronethalol, propranolol and *MJ*-1999), thus indicating that the β-adrenergic receptors may be involved in histamine sensitization in mice.

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[1] Munoz, J., and Bergman, R. K., *J. Immunol.*, **97**, 120 (1966).
[2] Fishel, C. W., Szentivanyi, A., and Talmage, D. W., *J. Immunol.*, **89**, 8 (1962).
[3] Townley, R. G., Trapani, I. L., and Szentivanyi, A., *J. Allergy*, **39**, 177 (1967).
[4] Bergman, R. K., and Munoz, J., *Proc. Soc. Exp. Biol. and Med.*, **122**, 428 (1966).
[5] Fishel, C. W., Szentivanyi, A., and Talmage, D. W., in *Bacterial Endotoxins* (edit. by Landy, M., and Braun, W.), 474 (Quinn and Boden Co., Rahway, New Jersey, 1964).
[6] Szentivanyi, A., Fishel, C. W., and Talmage, D. W., *J. Infect. Dis.*, **113**, 86 (1963).
[7] Fishel, C. W., and Szentivanyi, A., *J. Allergy*, **34**, 439 (1963).
[8] Reed, L. J., and Muench, H., *Amer. J. Hygiene*, **27**, 493 (1938).
[9] Nickerson, M., as quoted by Wolfson, S., and Gorlin, R., in *Ann. NY Acad. Sci.*, **139**, 1003 (1967).

Skeletal Abnormalities in the Hindlimbs of Young Cats as a result of Hypervitaminosis A

HYPERVITAMINOSIS A causes extensive confluent exostosis formation of the cervical spine in adult cats[1,2]. It was initially reported that no juvenile osteodystrophy occurred in this disease[1], but an abnormal hindquarter gait was described later in a group of young cats receiving large daily doses of vitamin A (ref. 2). No explanation of this abnormal gait was given and the condition was therefore studied further.

A litter of six healthy 8 week old kittens was divided randomly into two groups of three, whose diet consisted of raw meat and milk. The first group received no vitamin A supplementation and each cat in the second group was supplemented for 4 weeks with a daily oral dose of 100,000 I.U. of vitamin A acetate in polyethylene glycol and isopropanol. To prevent rickets due to a possible interaction between vitamin A and vitamin D (ref. 3), all cats received calcium and vitamin D supplementation throughout the experiment.

From the beginning of vitamin A supplementation the cats showed a marked disinclination to walk and depression of appetite. Immediately after cessation of vitamin A dosing, appetite returned, but reluctance to walk remained. Two weeks later the cats had a distinctly abnormal hindlimb gait which became more severe with time and was not a result of weakness. Their limbs appeared disproportionately short, particularly the hindlimbs. During walking, extension of the stifle joints was restricted and the hocks projected laterally. One cat from each group was killed after a further 4 weeks. After another 6 weeks some toes of the remaining test cats were observed to be shortened (Fig. 1). These animals and the two remaining controls were killed in the seventeenth week of the experiment—13 weeks after the cessation of vitamin A supplementation.

The outstanding pathological findings in the test cats included the following. (1) A dramatic shortening of the long bones of the limbs. (2) Alteration in structure of the stifle joint. The tibial crest was prolonged dorsally so that the tubercle lay above the remainder of the proximal epiphysis and rested against the distal femoral articular surface (Fig. 2). The femoral condyles were enlarged and displaced proximally so that the trochlea was the most distal part of the femur. (3) Irregular shortening of the metatarsal bones.

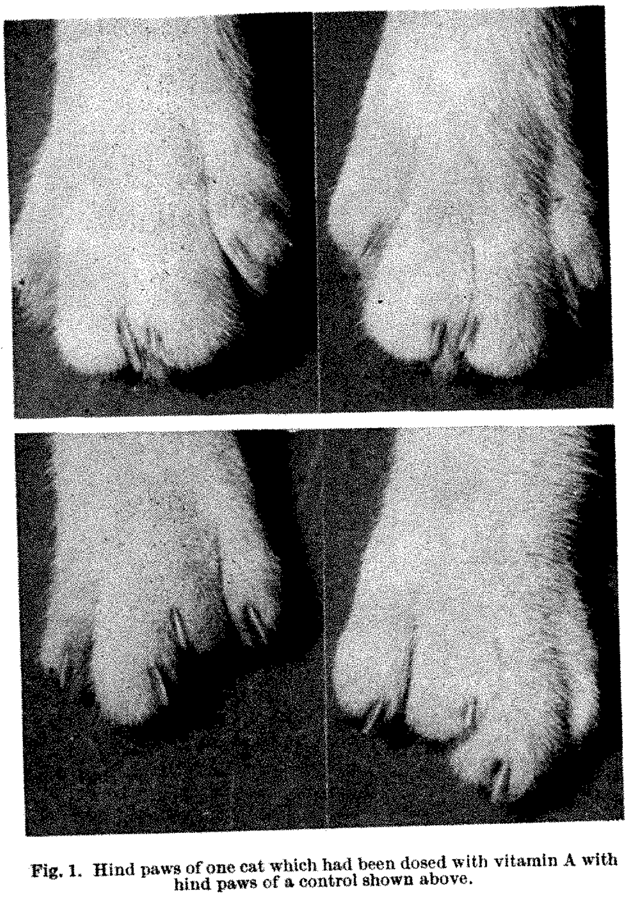

Fig. 1. Hind paws of one cat which had been dosed with vitamin A with hind paws of a control shown above.

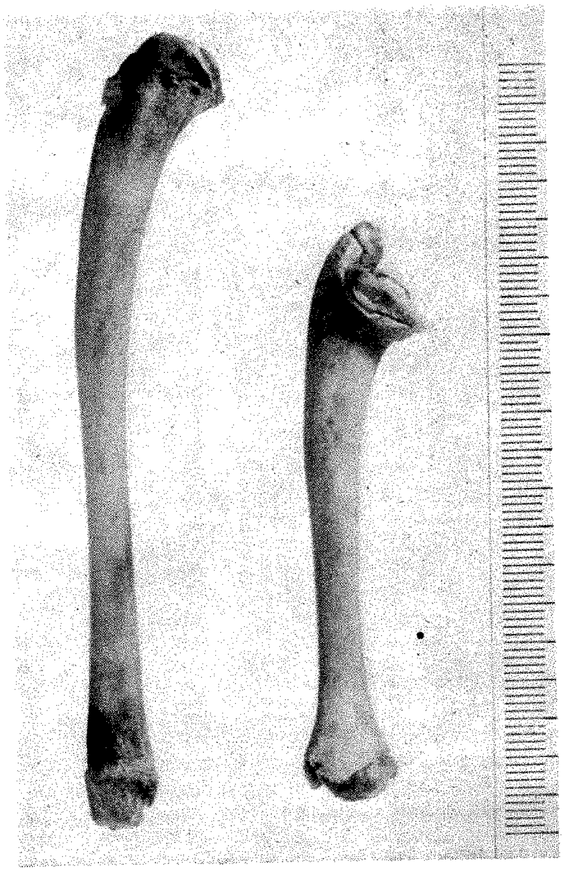

Fig. 2. The right tibia from a control (left) and hypervitaminosis A cat. (Normal size.)

The first two changes were bilaterally symmetrical in all three cats. The projection of the anterior proximal tibial diaphysis was longer and curved in a caudal direction in the cats killed at the later date. The third change was observed only in the latter two cats and was bilaterally symmetrical in one, affecting the third metatarsal bones. All four metatarsal bones were shortened in the right hind paw of the other cat, but only the fourth one was affected in the left. Histopathological examination of the distal ends of the metatarsal bones showed varying degrees of reduction of epiphyseal plates, sometimes with complete disappearance. In some bones, the medullary cavities of the epiphysis and diaphysis were continuous and a small piece of residual cartilage appeared free in the extended cavity, thus playing no part in the endochondral bone formation needed for longitudinal growth (Fig. 3).

It seems that after exposure to vitamin A for 4 weeks the growth of specific epiphyseal cartilages was arrested and as the bones continued to increase in diameter growth took place around the retarded epiphyseal cartilages and these were completely or partially resorbed.

These findings differ from those of Wolbach[4], using rats and guinea-pigs, who concluded that bone shortening in hypervitaminosis A was simply a result of premature closure of epiphyses caused by accelerated growth sequences. Our observations seem rather to support those of Fell and Thomas[5] and Thomas *et al.*[6], who found actual depletion of cartilage caused by dissolution of its matrix and loss of chondroitin sulphate in foetal mouse bones *in vitro* and in growing rats, respectively.

Fairbank[7] stated that the skeletal changes due to hypervitaminosis A in children resolved with no per-

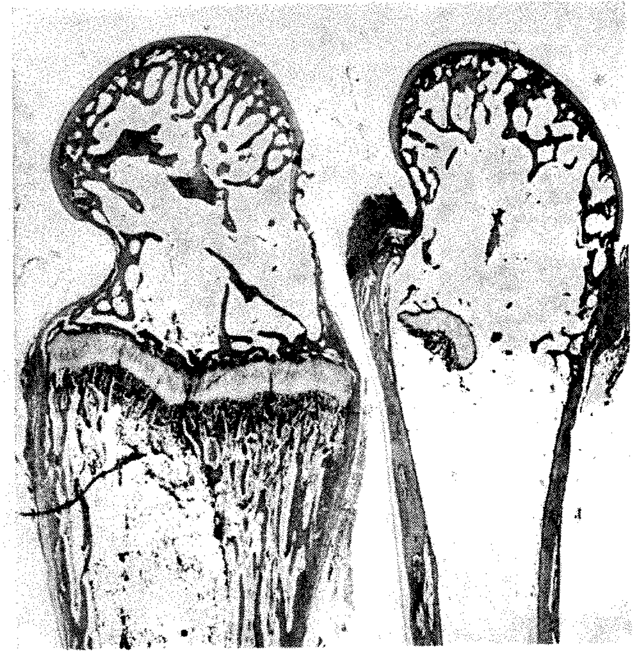

Fig. 3. Distal end of the right fourth metatarsal bone in a control (left) and hypervitaminosis A cat. (×8.)

sistence of bone lesions. Pease[8], however, reported permanent bone shortening in some children who suffered from hypervitaminosis A. The shortening of limbs was asymmetric and this was simply attributed to difference in weight bearing. The irregular nature of the shortening of metatarsal bones in young cats in this experiment is unlikely to have this explanation. The chief cause of the permanent gait abnormality in these cats was the bilaterally symmetrical prolongation of the anterior proximal tibia, making the extension of their stifle joints beyond ninety degrees mechanically impossible. Growth at the proximal end of the tibia takes place both at the cartilaginous epiphysis and also at an anterior fibro-osseous epiphysis[9,10]. The bone deformation observed in this study seems to be a result of restricted growth at the cartilaginous epiphyseal plate, while that at the anterior fibro-osseous region remained unrestricted, although probably modified by new stresses imposed by the changed stifle joint action.

High levels of vitamin A intake comparable with those in this experiment are possible for cats in natural conditions in Australia, where liver which is high in vitamin A content is readily available as pet food[1,2]. The osteodystrophy described here probably occurs in some kittens fed intensively for a short period on livers which are unusually high in vitamin A content.

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[1] Seawright, A. A., English, P. B., and Gartner, R. J. W., *Nature*, **206**, 1171 (1965).
[2] Seawright, A. A., English, P. B., and Gartner, R. J. W., *J. Comp. Pathol.*, **77**, 29 (1967).
[3] Nieman, C., and Klein Obbink, H. J., *Vitamins and Hormones*, **12**, 69 (1954).
[4] Wolbach, S. B., *J. Bone Joint Surg.*, **29**, 171 (1947).
[5] Fell, H. B., and Thomas, L., *J. Exp. Med.*, **111**, 719 (1960).
[6] Thomas, L., McCluskey, R. T., Potter, J. L., and Weissmann, G., *J. Exp. Med.*, **111**, 705 (1960).
[7] Fairbank, T., *An Atlas of General Affections of the Skeleton*, first ed., 354 (E. and S. Livingstone Ltd., Edinburgh and London, 1951).
[8] Pease, C. N., *J. Amer. Med. Assoc.*, **182**, 980 (1962).
[9] Lewis, O. J., *J. Anat.*, **92**, 587 (1958).
[10] Smith, J. W., *J. Anat.*, **96**, 209 (1962).

Frequency, Conduction Velocity and Rate of Depolarization in Rabbit Auricle

THERE is evidence that an increase in heart rate is accompanied by a decrease in the velocity of impulse conduction in heart muscle[1-2]. We have therefore looked for interdependence between the frequency of the isolated rabbit atrium, the conduction velocity in the atrium and the maximal rate of rise of the atrial action potential.

In our experiments, the crista terminalis was isolated together with the S.A. node and a small part of the right atrium. The muscle strip was put in a tissue chamber, which was perfused with oxygenated McEwen's solution[3] at 37° ± 0·1° C. The muscle strip was continuously stimulated with rectangular voltage pulses (1V above threshold lasting 0·5 ms), administered with a bipolar silver electrode on the proximal part of the crista terminalis, near the head of the S.A. node.

Stepwise changes in the rate of stimulation were imposed on the muscle strip. In each programmed series of changes the intervals between the stimuli varied from 150 to 330 ms. Intervals were always increased or decreased in steps of 20 ms, and each new frequency was maintained for 15 s. A reference interval of 250 ms was used between two series of interval changes.

Transmembrane action potentials were recorded with microelectrodes at two sites on the endocardial side of the crista terminalis. All the electrodes, one stimulating and two recording, were placed in a straight line. Each interval between two successive action potentials, the intrinsic conduction time, was measured with a digital counter.

When the stimulus interval was abruptly shortened, the intrinsic conduction time was increased gradually, in about 8 s, to a new steady state. After a sudden increase of the interval the conduction time fell gradually to a steady state, also in 8 s.

When the usual interval change (20 ms) was augmented, the time required to reach a new steady state increased proportionally. The effect was the opposite when the interval change was diminished.

The average value of the steady state conduction time and its standard deviation were calculated from the twenty values of the intrinsic conduction time, which

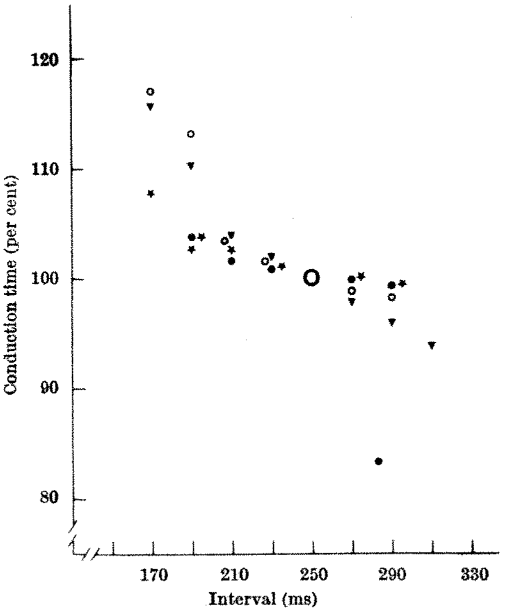

Fig. 1. Relation between steady state stimulus interval and intrinsic conduction time. Intrinsic conduction times are plotted as percentages of their value at the reference stimulus interval (250 ms).

were measured at the end of each frequency period ($\overline{S.D} = 0\ 4$ per cent, $S\ D_{\overline{S\ D}} = 0\ 2$ per cent).

Fig. 1 shows the results of four series of interval changes in one experiment Before we started a new series of changes the location of both microelectrodes was altered Almost the same relationship was found when two extracellular recording electrodes were used The maximal rate of rise of the depolarization phase of the atrial action potential was measured in each series

After a sudden change in frequency the rate of rise gradually adapted to a new steady state, which required the same time as the adaptation of conduction time During every increase in frequency we observed a significant decrease in the maximal rate of rise, whereas the opposite obtained during a decrease in frequency.

The average value of the maximal rate of rise together with its standard deviation were again calculated from the twenty values measured at the end of each frequency period ($\overline{S\ D} = 1{\cdot}9$ per cent, $S\ D_{\overline{S\ D}} = 0\ 5$ per cent)

After each series of interval changes the maximal rate of rise attained its previous reference value Between individual cells, however, this value varied from 50 to 110 V/s The relation between the stimulus interval and the maximal rate of rise as measured in six series of changes during one experiment is shown in Fig 2

Fig 3 shows superimposed action potentials recorded from the same cell at steady state levels during one series of interval changes The smallest potential with the slowest rate of rise corresponded to the shortest stimulus interval and the longest conduction time

During the frequency changes the level of the membrane potential did not change significantly, and repolarization was complete at all frequencies This observation cannot therefore be explained by the relation which was found earlier[4] between the level of the membrane potential and the rate of rise of the action potential We think that the relationship between frequencies exceeding 3 c p s. and the rate of rise might be explained by (a) incomplete reactivation of the Na-carrying system in crista terminalis fibres and (or) (b) insufficiency of the Na–K pump. The decrease in the rate of rise might be responsible for the decrease in conduction velocity[5]

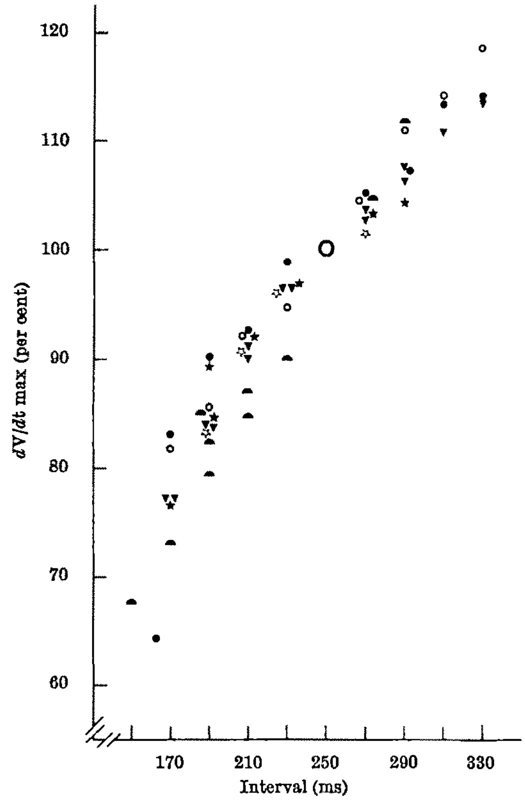

Fig 2 Relation between steady state stimulus interval and maximal rate of depolarization The maximal rates of rise are plotted as percentages of their value at the reference interval (250 ms)

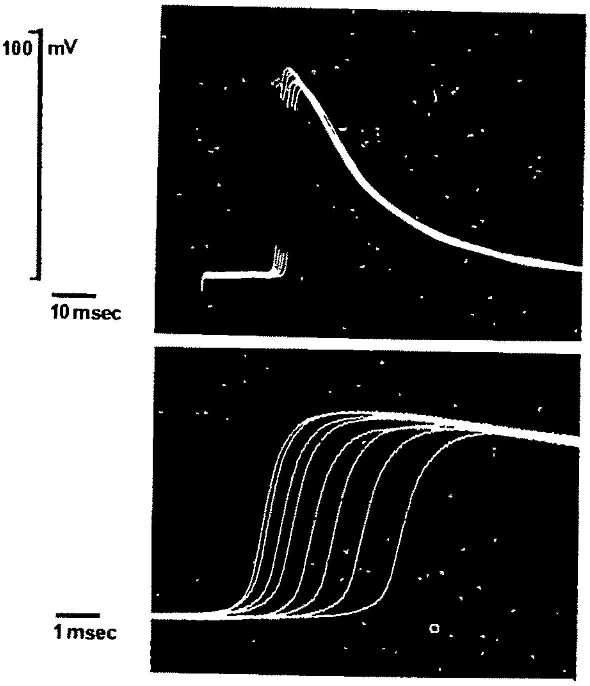

Fig 3 Superimposed action potentials at different stimulus intervals (170–330 ms) during one series of frequency changes The time base was triggered by the stimulus artefact

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[1] Bammer, H , *Z Ges Exp Med* ,121, 488 (1953)
[2] Yamagishi, S , and Sano, T , *Amer J Physiol* ,212, 829 (1967)
[3] McEwen, L M , *J Physiol* ,131, 678 (1956)
[4] Weidmann, S , *J Physiol* ,127, 213 (1955)
[5] Trautwein, W , *Pharmacol Rev* ,15, 277 (1963)

Immediate Circulatory Response to High Altitude Hypoxia in Man

Acute mountain sickness and high altitude pulmonary oedema are two acute medical problems for troops in the Himalayan terrain who frequently shuttle between sea level and high altitudes (10,000–16,000 ft) These two acute illnesses have some features in common time lag (they rarely occur within 12 h of arrival at the altitude or 96 h after arrival), throbbing headache, muscular cramps and pulmonary congestion. We have studied the acute circulatory response to high altitude hypoxia and investigated the extent to which shifts in the circulating blood volumes, if any, could be related to the time lag and clinical features of these acute illnesses

Thirty healthy volunteers were selected from the Indian Army. They were between 17 and 32 yr old (average 21 5 yr) and had never been exposed to high altitudes Sea level data were obtained at Delhi (height 650 ft) Right heart catheterization was performed by positioning a number 7 cardiac catheter in the pulmonary artery just below the valve The left atrium was entered by the percutaneous trans-septal method[1]. The right brachial artery was cannulated to obtain arterial blood pressures and dye curves. Consecutive dye dilution curves were obtained directly on

Table 1. CIRCULATORY RESPONSE TO ACUTE HIGH ALTITUDE HYPOXIA

Parameters			Total No	12–36 h		48–72 h		84–130 h	
Total blood volume ml /m^3		C	27	2,960	(11)	3,110	(9)	2,760	(7)
		HA		2,880		3,440		2,930	
Peripheral blood flow ml /100 ml /min tissue		C	12	4 4	(12)	4 4	(12)	4 4	(12)
		HA		3 6		3 4		3 8	
Cerebral blood flow ml /100 g brain/min		C	13	55	(5)	57	(3)	55	(5)
		HA		77		70		57	
Central blood volume ml /m^2		C	20	726	(8)	690	(6)	678	(6)
		HA		759		881		595	
Pulmonary blood volume ml /m^2		C	12	251	(5)	270	(4)	226	(3)
		HA		340		493		237	
Arterial blood mm Hg	*p*H	C→HA	27	7 41→ 7 47	(9)	7 41→ 7 46	(9)	7 41→ 7 45	(9)
	P_{CO_2}			39 0 →30 8		39 0 →31 0		39 0 →30 8	
	P_{O_2}			87 0 →53 0		87 →52		87 →52	

C, Control data at 650 ft. HA, Data at 12,000 ft. Numbers in parentheses indicate the number of subjects observed during the period

a 'Polyviso' channel through a continuous recording densitometer (Colson) by injecting 5 mg of indocyanine green dye into the main pulmonary artery and then into the left atrium The difference in the mean transit times of the two resulting curves was taken as the pulmonary transit time The cardiac output was measured from the dilution curves by the formula of Hamilton *et al* [2] The volume of blood between the pulmonary artery and the left atrium was obtained by multiplying the pulmonary mean transit time by the average cardiac output and this represented the pulmonary blood volume Similarly, the volume of blood between the pulmonary and brachial arteries was estimated by multiplying the pulmonary artery to brachial artery mean transit time by the average cardiac output and this gave the central blood volume[3] The total blood volume was determined by the Evans blue dye method Cerebral blood flow was estimated by the nitrous oxide technique of Kety and Schmidt[4] Peripheral blood flow was measured in the right forearm by venous occlusion plethysmography[5] All pressures were recorded through Statham *P23AA* strain gauge manometers on a four-channel single gun photographic system[6] Arterial blood *p*H, P_{CO_2} and P_{O_2} were determined using electrodes (Beckman) Cerebral blood flow and pulmonary blood volume were estimated in alternate subjects, other parameters were studied in every subject. For high altitude study, the team of investigators (nine doctors and four technicians) with equipment were airlifted a week earlier for acclimatization to an Army general hospital at 12,000 ft The subjects were subsequently flown in three batches at intervals of 5 days so that it was possible to observe the acute response in five to six subjects each day from day 1 to day 5 after arrival at the altitude Peripheral blood flow, however, was estimated in four subjects from each batch (total twelve subjects) daily from day 1 to 10 Physiological studies at Delhi and at the altitude were performed in identical conditions with regard to room temperature, equipment and observers

While there was no significant change in the total blood volume, peripheral blood flow diminished from the beginning, and remained so for the next 7 days On giving 100 per cent oxygen the diminished peripheral flow was somewhat increased. The cerebral flow increased by 40 per cent within 12–36 h and approached normal values by the fifth day Between 48 and 72 h the pulmonary blood volume increased by 80 per cent, and the central blood volume increased by only 28 per cent The arterial blood *p*H increased and P_{CO_2} and P_{O_2} fell from the beginning

Severinghaus *et al* [7] also found an increase of 33 per cent in the cerebral blood flow estimated in six men within 6–12 h of arrival at 12,500 ft Furthermore, we could relate the intensity of the headache of the sufferer to the greater increase in their cerebral blood flow Blood acid base changes observed by Severinghaus[7] showed similar patterns in the rise in arterial blood *p*H and fall in P_{CO_2} and P_{O_2}. Reduction in the peripheral blood flow has been observed in acute hypoxia by breathing 7 5 per cent oxygen[8] and also in congestive heart failure[9] The disproportionate increase in the pulmonary blood volume compared with the central blood volume (and no increase in the total blood volume) may contribute to the pulmonary congestion. Yu *et al* [10], on the other hand, by giving 12 per cent oxygen to sixteen patients with heart disease, found that the average central and pulmonary blood volume values decreased by 36 and 48 ml /m^2 The two groups are not comparable because our subjects were healthy young soldiers and we did not study induced hypoxia of only 10–15 min duration.

These observations cannot be critically reviewed, because comparable data on shifts in the circulating blood volumes caused by high altitude hypoxia are not available Decrease in the peripheral and increase in the cerebral blood flows, fall in the arterial blood P_{O_2} and P_{CO_2} with concomitant rise in *p*H can be related to the early onset of hyperventilation and respiratory alkalosis, throbbing headache and muscular cramps Marked increase in the pulmonary blood volume between 48–72 h may in part account for the maximum incidence of high altitude pulmonary oedema during this period It is concluded that time lag for the onset of the illnesses and such features as headache, muscular cramps and pulmonary congestion as seen in acute mountain sickness and high altitude pulmonary oedema may be related to the shifts in the circulating blood volumes and the fall in the arterial P_{O_2} and P_{CO_2} with rise in *p*H

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[1] Roy, S B , Bhatia, M L , and Guleria, J S , *Indian Heart J* , 15, 255 (1963)

[2] Hamilton, W F , Moore, J W , Kinsman, J M , and Spurling, G G *Amer J Physiol* , 99, 534 (1932)

[3] Roy, S B , Bhardwaj, P , and Bhatia, M L , *Brit Med J* , 2, 1466 (1965)

[4] Ketv, S S , and Schmidt, C F , *J Clin Invest* , 27, 376 (1948)

[5] Wood, J E , and Eckstein, J W , *J Clin Invest* , 37, 41 (1958)

[6] Rov, S B , Gadboys, H L , and Dow, J W , *Amer Heart J* , 54, 753 (1957)

[7] Severinghaus, J W , Chiodi, H , Eger, E I , Brandstater, B , and Hornbein, T F , *Circulation Res* , 19, 274 (1966)

[8] Eckstein, J W , and Horsley, A W , *J Lab Clin Med* , 56, 847 (1960)

[9] Sharpey-Schafer, E P , *Brit Med J* , 2, 1589 (1961)

[10] Yu, P N , Glick, G , Schreiner, B F , and Murphy, G W , *Circulation*, 27, 541 (1963)

Influence of Temperature on Isometric Contractions of Rat Skeletal Muscles

THE influence of temperature on isometric contractions of rat skeletal muscles *in vitro* has been determined for fast extensor digitorum longus muscles (EDL) and slow soleus muscles (SOL) from 4 week old female Wistar rats. Isometric contractions were recorded with the long axis of the muscle vertical, with one tendon tied to the frame below and the other tendon tied to a short steel wire connexion which linked it with the tension transducer above. The compliance of the transducer (Statham, *Gl*-80-350) and the steel wire connexion to the muscle was $4·5 \times 10^{-5}$ cm/g and the natural frequency of vibration was 2 kHz. The muscle was immersed in about 100 ml of fluid (137 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 2 g/l of $NaHCO_3$ and 2 g/l of glucose) which was bubbled continuously with 95 per cent O_2 and 5 per cent CO_2. Neuromuscular transmission was blocked by adding $2·0 \times 10^{-5}$ g of tubocurarine chloride/ml of bath fluid. The muscles were stimulated directly by a transverse electrical field of 15 V/cm for 0·2 or 0·3 ms applied to the bath fluid by "massive" bright platinum electrodes set about 1 cm apart[1]. All recordings were made with the muscle set at the optimal length determined for twitch contractions at 20° C.

Fig. 1 shows representative records of isometric twitch and tetanic contractions of an EDL muscle and a SOL muscle from one animal. Three twitches were recorded (1/10 s) about 10 min after each change in temperature for a series in which the temperature was altered in 5° C steps from 35° C to 20° C and from 20° C to 35° C. For both muscles the maximum twitch tension (P_t) at a given temperature did not differ by more than 2 per cent for the ascending and descending series of measurements. The isometric tetanic contractions were recorded 10 min after each change in temperature in a separate series of measurements at 35° C, 20° C, 25° C, 30° C and 35° C in that order. The muscles remained in good condition throughout the experiment and the ratio of the maximum isometric tetanic tension (P_o) for the final contraction at 35° C to P_o for the initial contraction at 35° C was 1·05 for SOL and 0·985 for EDL. The results show that decrease in temperature from 35° C to 20° C decreased P_t and P_o of SOL and P_o of EDL, whereas P_t of EDL was increased 1·7 times. The decrease in temperature from 35° C to 20° C increased the isometric twitch contraction time (T_c) in both muscles from 10·75 ms to 37·5 ms for EDL and from 28 ms to 122 ms for SOL and also increased the time for half relaxation from 9·25 ms to 42 ms for EDL and from 36 ms to 170 ms for SOL.

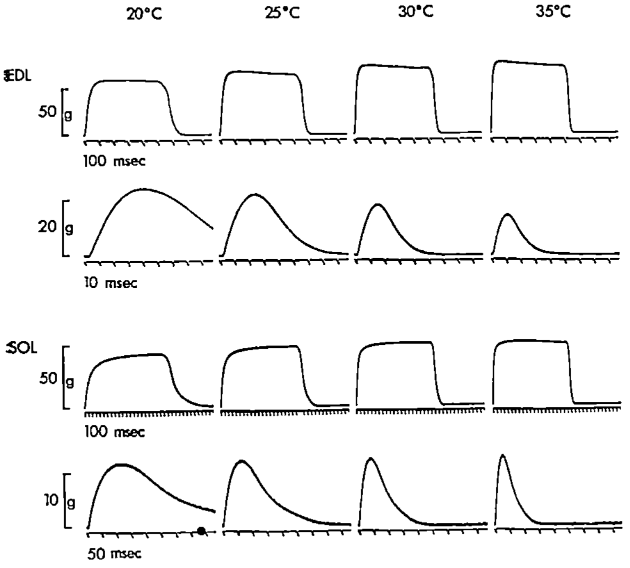

Fig. 1. Records of isometric twitch (10 ms and 50 ms time marks) and tetanic (100 ms time marks) contractions of extensor digitorum longus (EDL) and soleus (SOL) muscles at different temperatures. The frequencies of repetitive stimulation for tetanic contractions of EDL were 300 Hz at 35° C, 210 Hz at 30° C, 150 Hz at 25° C and 105 Hz at 20° C and the frequencies of stimulation of SOL were 250 Hz at 35° C, 180 Hz at 30° C, 125 Hz at 25° C and 90 Hz at 20° C. The rat was 4 weeks old and weighed 70 g. Muscle weights: EDL = 32 mg, SOL = 27·5 mg. Average muscle fibre length: EDL = 9·5 mm, SOL = 10·5 mm.

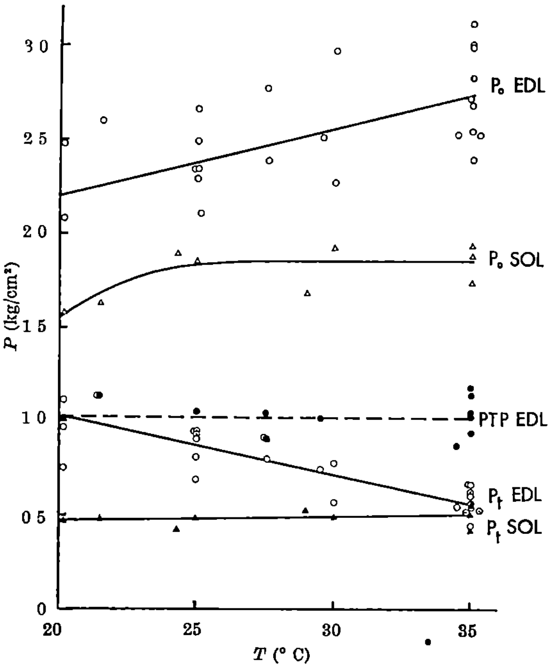

Fig. 2. The relation between maximum tension, P (ordinate in kg/cm² cross sectional area of muscle) and temperature (abscissa in ° C) for isometric twitch (P_t) and tetanic (P_o) contractions of fourteen extensor digitorum longus (EDL) and three soleus (SOL) muscles from four-week old rats (P_t of EDL = ○, P_t of SOL = ▲, P_o of EDL = ○, P_o of SOL = △). Responses of individual muscles at different temperatures were recorded for three SOL and five EDL muscles. Of the other nine EDL muscles, two were examined at 25° C only, two at 27·5° C only and 5 at 35° C only. Post-tetanic potentiation (PTP) was recorded at various temperatures in ten of the fourteen EDL muscles. The peak tensions of potentiated twitches recorded 10 s after the end of repetitive stimulation which produced maximum PTP have been plotted as filled circles (●) for comparison with the control pretetanic P_t. The average value for peak twitch tension in the potentiated twitches was 1·05 kg/cm² and is indicated in the diagram by the interrupted line.

The effects of temperature on P_t and P_o of fourteen EDL muscles and three SOL muscles are shown in Fig. 2. The linear changes in P_t and P_o of EDL are almost the same as those described before for rat triceps surae[2]. A similar influence of temperature has been described for amphibian muscle[3]. Recent measurements on the sartorius muscle of *Rana temporaria* which were stored at 3° C in October, using "massive" direct stimulation *in vitro*, show that P_t increases from 0·7 kg/cm² (0·5–0·96, $n = 5$) at 20°–22° C to 1·42 kg/cm² (1·21, 1·74, $n = 2$) at 2° C and that P_o decreases from 3·5 kg/cm² (3·37–3·63, $n = 5$) to 2·38 kg/cm² (2·06, 2·69, $n = 2$) for the same range of temperature (unpublished results of Close). The effect of temperature on contractions of SOL is similar to that described for neonatal rat EDL[4].

The differences in temperature dependence of contractions of EDL and SOL muscles may be related to differences in the effects of repetitive stimulation on these muscles. Post-tetanic potentiation (PTP) is usually absent in neonatal EDL and SOL and adult SOL, but appears in EDL during the first few weeks after birth. The peak twitch tension of EDL during maximum PTP at different temperatures has been plotted in Fig. 2 for comparison with the pretetanic P_t. The results show that there is little or no post-tetanic potentiation in EDL at 20° C and P_t at that temperature is about the same as the peak tension of the maximally potentiated twitch at other temperatures between 20° C and 35° C. In other words, the influence of temperature on contractions of EDL in the fully potentiated state is similar to the effect of temperature on contractions of SOL and neonatal EDL and involves a change in time course of the twitch response with little or no change in P_t At 35° C maximum post-tetanic potentiation leads to a two-fold increase in the peak twitch tension with little or no change in the time course of the response A similar degree of maximum post-tetanic potentiation has been observed in cat fast muscles at 37° C and frog twitch muscle at about 20° C and has been shown to occur in single muscle fibres[6-7]. A possible explanation for the differences in temperature dependence of frog twitch muscle and rat EDL muscle on one hand and rat SOL on the other is that a decrease in temperature decreases the intrinsic speed of shortening and the rate of removal of activator in all these muscles, thereby causing an inversely proportional increase in T_c with little or no change in P_t (ref. 4), but also increases P_t of frog twitch muscle and adult rat EDL, in much the same way that repetitive stimulation increases it, by increasing the degree of activation of individual muscle fibres with little or no change in the time course of the response. This interpretation of the results follows an earlier suggestion[4] that differences in isometric twitch contraction times of different muscles may be due largely to differences in intrinsic speed of shortening and that differences in P_t/P_o, or P_t as kg/cm^2, may result mainly from differences in the degree of activation of single muscle fibres

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[1] Gutmann, E , and Sandow, A *Life Sci* , 4, 1149 (1965)
[2] Truong, X T , Wall, B J , and Walker, S M , *Amer J Physiol* , 207, 393 (1964)
[3] Hill, A V , *Proc Roy Soc* , B, 138, 349 (1951)
[4] Close, R , *J Physiol* , 180, 542 (1965)
[5] Brown, G L , and von Euler, U S , *J Physiol* , 93, 39 (1938)
[6] Ramsey, R W , and Street, S F , *Biol Symp* , 3, 9 (1941)
[7] Colomo, F , and Rocchi, P , *Arch Fisiol* , 64, 189 (1965)

PATHOLOGY

Solubilization of Human Leucocyte Membrane Isoantigens

Isoantigens, thought to be important as histocompatibility antigens, have been identified on membranes of human cells by serological techniques[1] In order to interpret serological studies of histocompatibility antigens in terms of an understanding of the molecular representation of gene information, the isoantigens must be obtained in a suitable state for detailed chemical analysis Preparation of soluble isoantigen in relatively low molecular weight form would be an important step in this direction

Such an advance would also be relevant to efforts to achieve specific immunological tolerance of human tissue grafts Tolerance in animal systems has been difficult to induce across major histocompatibility loci when crude tissue extracts were used as antigens Previous studies, however, have indicated that tolerance can be induced to relatively pure, soluble protein antigens[2] During continuous work on transplantation immunology, we have investigated techniques that might yield human isoantigen in a soluble form

Methods for preparation of quantities of soluble human isoantigens have not been reported previously Mouse H-2 isoantigens, however, have been solubilized from cellular membranes in various ways[3-7]. In a very efficient proteolytic procedure, the enzyme papain was used to solubilize murine H-2 isoantigens When further purified (about 200-fold) by a combination of column chromatographic and electrophoretic techniques, the murine H-2 isoantigens were found to have the chemical characteristics of glycoproteins (85 per cent protein, 10–12 per cent neutral and amino sugar) and a molecular weight of the order of 70,000 (ref 8) In the present work, the method of papain digestion has been extended to the preparation of soluble, membrane-free human leucocyte isoantigens

Continuous cultures derived from human lymphoid tissue have been shown, in our laboratory, to possess a wide range of human leucocyte isoantigens Cell lines selected for preparation of isoantigens were *RAJI* and *RPMI*-4265 These two cell lines show different patterns of reactivity with a panel of human leucocyte isoantisera

Isoantigens were identified and quantified in the crude membrane and soluble preparations by a radioisotope release cytotoxicity technique modified to an inhibition assay[9] Quantitation was expressed as the reciprocal of the dilution of the various preparations necessary to inhibit 50 per cent of the cytotoxic reaction Antisera used to detect these isoantigens include a broadly specific antiserum, Iochum, which reacts with lymphocytes of 96 per cent of a normal donor panel and eight isoantisera of more limited specificities (Pinquette, Storm, Anderson, Kessler, T-155, T-53, Cutten, T-106) known to detect different genetically determined leucocyte isoantigens (Serum Bank, Transplantation Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health)

Crude membrane preparations were made by freezing and thawing 10^9 tissue culture cells in 12 ml of 0 14 M NaCl The cells and cellular debris were spun out at 1,500g (International centrifuge, *PR*-2) for 20 min at 4° C and the supernatant was collected The cells and cellular debris were washed for 10 min at 4° C in a stepwise fashion with solutions of decreasing concentration of NaCl (0 14 M–0 01 M NaCl). Cells and debris were removed after each wash by centrifugation Washing was discontinued when the supernatants were no longer turbid (usually about 0 01 M NaCl) The pooled supernatant solutions were centrifuged (Spinco *L*-2, 40 rotor) at 80,000g for 90 min and the resulting pellet suspended in distilled water at a concentration of 10–15 mg of protein/ml All protein determinations were by the method of Lowry[10] Crude papain II (Sigma Chem Co , St Louis, Mo) was dissolved in 0 05 M *tris* Cl–0 14 M NaCl buffer (pH 8 4) and stabilized with cysteine HCl at a final concentration of 0·01 M The papain solution was added to the crude membrane preparation to give several ratios of concentration. The ratio of 2 mg enzyme to 1 mg membrane protein was found to be particularly useful The release of protein and isoantigenic activity from the membrane was time dependent (Fig 1) Recovery of

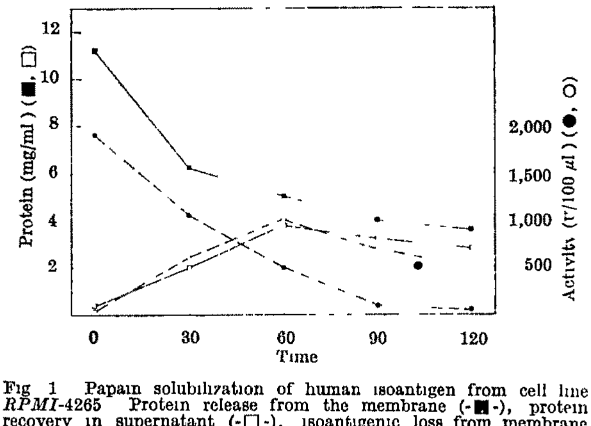

Fig 1 Papain solubilization of human isoantigen from cell line *RPMI*-4265 Protein release from the membrane (-■-), protein recovery in supernatant (-□-), isoantigenic loss from membrane (- - ● - -), isoantigenic recovery in the supernatant (- - ○ - -) Isoantigenic activity determined by the polyspecific antiserum, Iochum activity in the supernatant solution reached a maximum after 60 min of incubation at 37° C Isoantigenic activity in the supernatant decreased thereafter, indicating probable degradation by the enzyme

Recovery of isoantigenic activity in the soluble form was found to be 45–50 per cent of the total membrane activity in experiments with four preparations from two cell lines Inactivation of papain by iodoacetic acid (0 01 M final concentration) at time zero or incubation without enzyme resulted in release of less than 2 per cent of the activity This was not increased by adding 0 01 M cysteine alone These findings indicate that the antigen release shown in Fig 1 is a result of activity of the added enzyme preparation

The solubilized material was precipitated and concentrated by the addition of saturated ammonium sulphate solution (pH 7 6) to a final concentration of 75 per cent (ammonium sulphate at concentrations of 25 per cent and 50 per cent caused no visibly detectable precipitate) The solution was incubated for 30 min at 37° C and 18 h at 4° C The resulting precipitate was separated by centrifugation at 40,000g (Spinco L-2, 40 rotor) for 30 min at 4° C, dissolved in *tris* saline buffer (pH 8 4) and dialysed against this buffer at 4° C for 18 h An average of 17 per cent of the total protein and 67 per cent of the isoantigenic activity was recovered by ammonium sulphate precipitation of three enzyme-solubilized isoantigen preparations from each cell line

The solubilized material was further fractionated by chromatography on a column of 'Sephadex G-150' (Fig 2) Although at least four major protein peaks are evident, isoantigenic activity was found mostly in tubes 23–26 The isoantigenic activity was thirty times greater than the protein content in tubes 23–26 when compared with the starting material

The pattern of elution indicates that most isoantigenic activity is associated with molecules of 60,000–70,000 molecular weight if the membrane proteins separate chromatographically in a similar fashion to three proteins (ovalbumin, chymotrypsin and gamma globulin) which were used to calibrate the columns Some isoantigenic activity was evident in the excluded fraction of the first peak. It has not yet been determined whether this isoantigenic activity is associated with a different substance than the smaller molecular weight fraction, or whether it represents a larger material from which the smaller molecular isoantigens arise by enzyme action Two solubilized preparations from each cell line were fractionated on the same column of 'Sephadex G-150' Identical elution patterns were found for each preparation

Fractions were screened for isoantigen content by the polyvalent antiserum, Iochum Subsequently, six additional antisera, reacting with different isoantigens, were used to determine the presence of specific isoantigens in the various fractions These studies (not illustrated) indicated that the isoantigen molecules have similar but not necessarily identical distribution on gel-filtration chromatography.

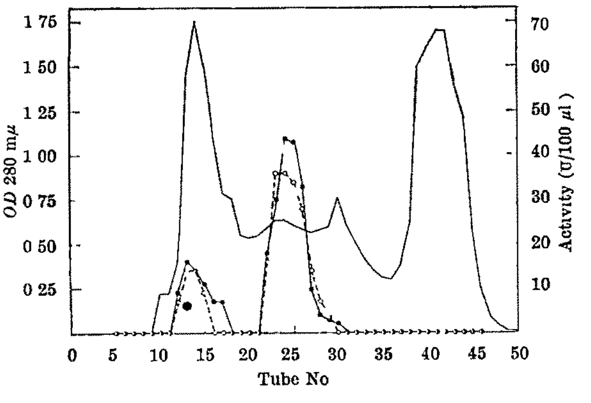

Fig 2 Gel-filtration ('Sephadex G-150') of papain solubilized human isoantigen Starting material was the $RAJI$ cell line Protein OD 280 mμ (—) Isoantigenic activity in units per 100 μl as detected by a polyspecific antiserum, Iochum (—●—), and an antiserum specific for LA-3 (Storm) (- - O - -)

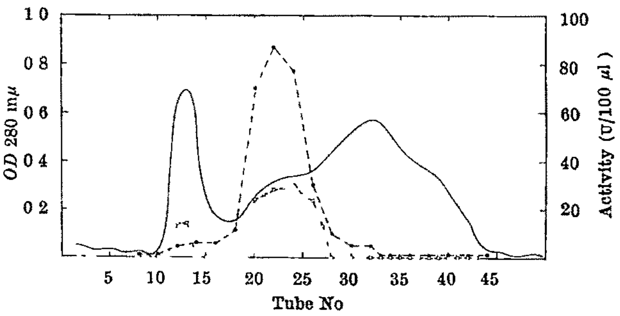

Fig 3 Chromatographic comparison of solubilized human and mouse isoantigens 'Sephadex G-150' gel-filtration of combined human ($RAJI$) and mouse ($C57Bl/6$) isoantigens obtained after papain treatment and 75 per cent sulphate precipitation Protein OD 280 mμ (—) Isoantigenic activity in units per 100 μl as detected by mouse antisera (H-2 5) (- - ● - -) and human isoantisera Storm (LA-3) (- - □ - -)

The elution pattern of human isoantigenic activity is similar to that found with papain solubilized mouse H-2 antigen[8] Human and mouse isoantigens solubilized by papain were directly compared by applying a mixture of soluble isoantigenic material from $C57Bl/6$ (H-2^b) mouse spleen cells and from human lymphoid cells on a column of 'Sephadex G-150' The elution pattern (Fig 3) demonstrates that human isoantigenic activity and mouse H-2 activity have similar chromatographic properties on 'Sephadex G-150' The observation that this enzyme releases molecules of similar molecular weight having known histocompatibility determinants (H-2) in the mouse and inferred histocompatibility determinants in man indicates that these components may have a similar structure in both species These results show that soluble human leucocyte isoantigens can be obtained in quantity both for biological studies (immunogenicity, immune tolerance) and for detailed chemical investigations.

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[1] Amos, D B, *Transplantation*, 5, 1015 (1967)
[2] Leskowitz, S, *Ann Rev Microbiol*, 21, 157 (1967)
[3] Kandutsch, A A, and Stimpfling, J H, *Transplantation*, 1, 201 (1963)
[4] Kahan, B D, *Proc US Nat Acad Sci*, 55, 153 (1965)
[5] Nathenson, S G, and Davies, D A L, *Proc US Nat Acad Sci*, 56, 476 (1966)
[6] Nathenson, S G, and Davies, D A L, *Ann NY Acad Sci*, 126, 6 (1966)
[7] Edidin, M, *Proc US Nat Acad Sci*, 57, 1226 (1967)
[8] Shimada, A, and Nathenson, S G, *Biochim Biophys Res Commun.* (in the press)
[9] Rogentine, G N, and Plocinik, B, *Transplantation*, 5, 1323 (1967)
[10] Lowry, O H, Rosebrough, N J, Farr, A L, and Randall, R J, *J Biol Chem*, 193, 265 (1951)

RADIOBIOLOGY

Low *p*H of Irradiated Sucrose in Induction of Chromosome Aberrations

SEVERAL reports have dealt with the deleterious effects on biological objects of irradiated sugars, or foods containing them Some people have even implied possible harmful consequences as a result of consuming these irradiated products Because current research programmes involve the use of radiation as an agent for preserving food, some of the reports should be further investigated to establish clearly the incidence, nature and conditions in which deleterious effects are induced We are therefore studying damage to chromosomes in the root tip of *Vicia faba* caused by treatment with irradiated sucrose solutions, one of the subjects included in a report by Holsten, Sugii and Steward[1]

Moutschen and Matagne[2] found that the low *p*H of irradiated glucose solutions caused "sticky" bridges and chromosome clumping, and therefore adjusted such solutions to *p*H 6 before treating root tips for chromosome studies In our early experiments, we also noted the influence of *p*H on the extent of chromosome aberration Some reports of chromosome aberrations caused by irradiated sugars and other products have not provided information concerning the *p*H of the treatment substances, and the reported results may therefore be misleading For this reason we report here the results of two experiments on the influence of *p*H on the incidence of chromosome abnormalities caused by treatment with irradiated and non-irradiated sucrose solutions

Primary roots of seedlings of *V. faba* cultivar 'Bell' were used In both experiments, root tips were treated with 2 per cent sucrose solutions irradiated with 2 Mrad of gamma radiation from cobalt-60 at a rate of 5 5 Krad/min Controls were non-irradiated 2 per cent sucrose solutions, which, at their normal *p*H of 6 0, induced fewer abnormal anaphases than did water controls and no more than appeared in untreated root tips De-ionized, glass-distilled, filter-sterilized water was used for all solutions Common buffers were tested for possible use in *p*H adjustment they were harmful to root growth and did not counteract the sticky chromosome effect, as reported also by Moutschen and Matagne[2] The *p*H of all solutions was therefore adjusted with either KOH or HCl Root tips of the twelve seedlings/treatment were fixed immediately after treatment, and the terminal 1 mm of each was subsequently squashed in propionocarmine For each point on the curves, several hundred anaphases were scored.

In the first experiment, the period of treatment was 4 h Test solutions were adjusted to form a series of *p*H from 3 0 to 7 5, progressively increasing by 0 2 units between *p*H 3 0 and 4 0 and by 0 5 units between *p*H 4 5 and 7 5 Because atmospheric conditions can cause the *p*H of unbuffered solutions to drift, each irradiated sucrose solution and its control were adjusted consecutively to the same *p*H and seedling roots were immersed immediately

The *p*H of the solutions changed during treatment, evidently a result of root metabolism The root hairs seem to be particularly active in the *p*H changes When only the regions below the root hairs were immersed in solutions of low *p*H, the increase in *p*H after 4 h was considerably less than when the solutions covered the root hair zone Similar changes of *p*H occur in plant tissue or organ culture (for example, Gautheret[3]), the cells shifting the *p*H toward the most suitable value for growth Thus in irradiated solutions adjusted before treatment to a *p*H below 5 0 the *p*H increased during treatment The *p*H of similar solutions adjusted above 5 0 decreased The same applied to control solutions, but the point below which increase and above which decrease in *p*H occurred was *p*H 6 The difference in reactions of the irradiated and control solutions presumably resulted from strong buffering action of one or more acid components of irradiated sucrose This was confirmed in duplicated titrations of the two types of solution. A 2 per cent solution of sucrose which had a *p*H of 3 3 after irradiation (2 Mrad) was titrated against a control solution adjusted to *p*H 3 3 with HCl Five times as much 0 06 N NaOH was required to neutralize the irradiated solution

Because of changes in *p*H of the solutions during treatment, the mean of the readings taken before and after treatment was used in plotting curves The simple mean may not reflect precisely the conditions during treatment, but for the purpose of these studies it has been found adequate Small differences in *p*H changes in replicate treatments together with the greater change in *p*H of the control solutions account for the irregular spacing of points on the curves in Fig 1, and also for failure of most points on the irradiated sucrose curve to coincide in *p*H with points on the control curve Three complete replicate experiments were performed, and for certain initial *p*H values more than three replicates were run in order to obtain identical *p*H means for statistical analysis by the *t* test

Results of the first experiment show that when the *p*H of the solutions was between 4 6 and 7 2, no more chromosome aberration was caused by irradiated than by control solutions (Fig 1) At *p*H values lower than 4 6, percentages of abnormal anaphases in root tips exposed to either control or irradiated solutions increased with decreasing *p*H. The degree of increase of aberrant anaphases was, however, higher in irradiated solutions The value of *p*H 4 6 is not an absolute, but only the value in the specific experimental conditions above which response to irradiated and non-irradiated solutions was the same The maximum percentage of abnormal anaphases attributable to irradiated sucrose occurred at *p*H 3 5 and amounted to 32 5, as determined by comparison of the curves as plotted on graph paper

In the second experiment, the *p*H of all sucrose solutions, both irradiated and control, was adjusted to 6 5 before treatment The only variable was duration of treatment, that is, 0, 4, 8, 12, 18 and 24 h Otherwise, the experimental conditions were the same as before The experiment was repeated three times The results (Fig 2) show that increased duration of treatment up to 24 h

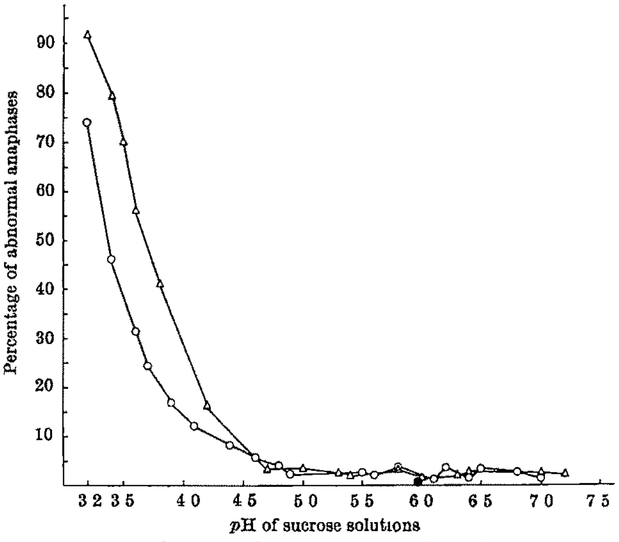

Fig 1 Effects of various values of *p*H of irradiated and control (2 per cent) sucrose solutions on the percentage of abnormal anaphases. Radiation dosage, 2 Mrads of gamma radiation from cobalt-60 Treatment period, 4 h △, Irradiated sucrose, ○, control sucrose The differences between percentages of abnormal anaphases in treated and control roots when solutions were at *p*H 3 4 were significant at the 1 per cent level, and at *p*H 7 0 were not significant at the 10 per cent level

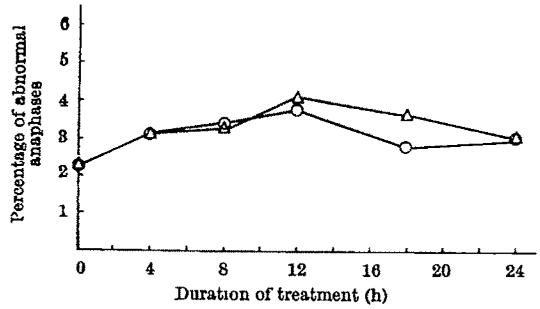

Fig 2. Effect of duration of treatment (0, 4, 8, 12, 18 and 24 h) on the percentages of abnormal anaphases when all irradiated and control solutions were adjusted to pH 6 5 before treatment Radiation dose and symbols for curves are the same as for Fig 1 Differences between percentages of abnormal anaphases in treated and control root tips at 12 and 18 h, respectively, were not significant at the 10 per cent level

when all solutions were adjusted to pH 6 5 caused no differences in response to irradiated sucrose.

The same types of chromosome aberrations appeared after treatments (24 h) with irradiated and control solutions Also, the proportions of specific types of aberration (spindle defects, fragments and bridges) were almost identical in the two samples of about two hundred anaphases each (unpublished data) The evidence from these studies thus points to low pH alone as the cause of mutagenic effects

The strong buffering in irradiated sucrose solutions may account for the higher incidence of abnormal anaphases from exposure to irradiated than control solutions The inability of root tips to raise the pH as much in irradiated as in control solutions of low pH means that root tips in irradiated solutions remain longer at pH values near the initial adjusted values than do those in control solutions The means of initial and final pH values therefore adequately express the differences in exposures of root tips to irradiated (or control) solutions adjusted to different pH values, but they are inadequate for comparing exposures to irradiated and control solutions having the same mean pH values This implies that the curves in Fig 1 should approximate each other more closely than they do

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[1] Holsten, R D , Sugii, M , and Steward, F C , *Nature*, 208, 850 (1965)
[2] Moutschen, J , and Matagne, R , *Radiat Bot* , 5, 23 (1965)
[3] Gautheret, R J , *La Culture des Tissus Végétaux* (Masson and Co , Paris, 1959)

MICROBIOLOGY

Degradation of 'Diazinon' by Synergistic Microbial Action

THE mechanisms and pathways involved in the degradation of pesticides have received more attention as efforts to define the extent of environmental contamination by these chemicals have increased. Complex interrelationships have been found between the soil microflora and organophosphate insecticides, such as 'Diazinon'[1-3] In the case of 'Diazinon', utilization of the chemical by an *Arthrobacter* sp. and a *Streptomyces* sp has been shown to alter the existing microbial population balance by stimulating a selective enrichment of these species[3] Labelled carbon dioxide was evolved when 'Diazinon' labelled on the C1 of the ethyl ester was incubated with a species of *Arthrobacter* for 24 h. Similarly, labelled carbon dioxide was given off when the same labelled insecticide was injected into soil which contained an unidentified microbial population We had been concerned with the pyrimidinyl ring of the 'Diazinon' molecule, and the possible differentiation of the degradative pathways associated with microbial metabolism by which the *Arthrobacter* and the *Streptomyces* that emerged as the predominant successive microfloral forms in soil treated with 'Diazinon' could attack the ring moiety of this compound

Both organisms were cultured in a mineral salts medium in which they could grow with 'Diazinon' as the primary source of carbon when the 'Diazinon' was solubilized in ethyl alcohol before being introduced into the growth medium Ring-labelled ^{14}C-'Diazinon' (specific activity 4 0 μCi/mg) was diluted in ethanol to give a net c p m of 200–300 in final dilution Cultures of the *Arthrobacter* and *Streptomyces* were collected after 18 and 96 h, respectively, to give comparable cell weights (40–50 mg wet weight) Cells were washed twice in sterile, distilled water and a third time in 1/15 M phosphate buffer, pH 6 5 The reaction mixture consisted of 20 ml of phosphate buffer, pH 6 5, 5 ml of washed cells in the same buffer, and 0 1 ml of ^{14}C-'Diazinon' in ethanol The reaction vessel was closed with a two-hole stopper, one hole an air inlet, the other carrying the carbon dioxide produced to a trapping tube containing 25 ml of 0·1 N $Ba(OH)_2$ The 'Tygon' tube leading from the reaction vessel to the carbon dioxide trap passed through a peristaltic pump which drew the gases from the reaction vessel into the trapping tube Incubation with labelled ^{14}C-'Diazinon' was maintained for 18 h, after which the precipitated carbonate was rinsed out of the trapping tube with sterile distilled water on to a filter disk. Aliquots of supernates from the reaction vessels and of the cell pellet were also transferred to individual filter disks. The disks were dried, placed on a planchette and the activity was measured in a windowless gas-flow counter No labelled barium carbonate was detected as the result of bacterial respiration On the basis of these results, it was concluded that the *Arthrobacter* sp previously reported to attack the side chain of the molecule was unable to completely metabolize the ring portion of the molecule

Similar results demonstrated that the *Streptomyces*, too, could not by itself convert the pyrimidinyl carbon to carbon dioxide (Table 1). When, however, both the *Arthrobacter* and the *Streptomyces* were incubated together, 15–20 per cent of the carbon-14 appeared as labelled $BaCO_3$ after 18 h, suggesting a synergistic relationship between these two organisms in attacking the pyrimidinyl portion of 'Diazinon'

Confirmation of these results was obtained when cultures of the *Arthrobacter* and of the *Streptomyces* were grown separately and together in a mineral salts medium in which carbon-14 ring-labelled 'Diazinon' served as the sole source of carbon. The contents of the flasks were collected after 7, 14 and 21 days, respectively. The cultures were centrifuged at 9,000 r p m. (g) for 10 min and the supernate was extracted three times with diethyl ether Cell pellets were washed and ground in a glass microhomogenizer and extracted, as were the supernates. Assays for degradation products were made by thin-layer chromatography, using as a solvent chloroform with 0 7 per cent ethyl alcohol and followed by autoradiography with Eastman no-screen X-ray film

Table 1 SYNERGISTIC METABOLISM OF RING-LABELLED ^{14}C-'DIAZINON' BY AN *Arthrobacter* AND *Streptomyces* SPP

Treatment	c p m *Arthrobacter*		*Arthrobacter* + *Streptomyces*	Control
		Streptomyces		
Cells after third wash	26 9	590 4	105 6	
$Ba^{14}CO_3$	0 0	0 0	44 1	0 0
Uninoculated control				202 6

All samples were counted to the level of 5 per cent significance above background Figures are corrected for background

Table 2 INCORPORATION OF SIDE-CHAIN AND RING-LABELLED ^{14}C-'DIAZINON' BY *Arthrobacter*

Incubated with	c p m Cells after three washings	$Ba^{14}CO_3$
Side-chain label	282 4	42 9
Ring label	325 6	0 0

Cells were incubated for 18 h Specific activity of the side-chain labelled 'Diazinon' was 3 21 µCi/mg, of the ring-labelled 'Diazinon' 4 0 µCi/mg Dilutions were made to give comparable initial activities

When the *Arthrobacter* and *Streptomyces* were incubated separately no change was evident in the 'Diazinon' molecule Both the 'Diazinon' in the supernatant as well as the material incorporated in the cell interior remained intact When, however, the *Arthrobacter* and *Streptomyces* were incubated together radical changes were evident After 7 days, only 6 per cent of the parent 'Diazinon' molecule remained, whereas 84 per cent was converted to one unidentified metabolite and 10 per cent to a second unidentified metabolite At 14 days, only 3 per cent of the parent 'Diazinon' remained, whereas metabolite I contained 83 per cent and metabolite II contained 10 5 per cent The parent compound was no longer detected at 21 days, while 86 per cent of the activity was in metabolite I and 14 per cent was present in metabolite II Although the precise identity of these two metabolites remains to be established, thin-layer chromatographic analysis with palladium chloride, a highly specific test for the P=S bond, has shown the presence of this group in both metabolites

Apart from chemical hydrolysis to the pyrimidinyl and phosphorothioate moieties, only carbon dioxide has been recovered as a product of the microbial degradation of 'Diazinon', although the test organism incubated with labelled material showed substantial incorporation of the entire molecule Data from a typical experiment with *Arthrobacter* are shown in Table 2 The absence from pure culture supernates of predicted intermediates ('Diazoxon', tetraethyl dithionopyrophosphate, tetraethyl monothionopyrophosphate, 2-isopropyl-6-mercapto-4-methyl pyrimidine, 6-hydroxy pyrimidine, diethylthiophosphoric acid) would suggest that a significant proportion of the 'Diazinon' molecule may be converted to stable cell constituents and so retained within the organism Cell fractionation studies are currently in progress to test this assumption

Most significant seem to be the indications provided by this study of a synergistic relationship between two organisms in the degradation of a pesticide molecule which neither can achieve by itself Such an interaction, though not to our knowledge previously cited for pesticides, is not uncommon in the soil and elsewhere[4-6]. Mutualistic relationships in the decomposition of cellulose have been frequently noted; a mixed culture is obtained from soil which actively decomposes cellulose whereas the individual cultures will not perform the same task[7]

Whatever the model which will be uncovered for the synergistic activity here reported, these investigations, though preliminary in nature, offer encouragement to the view that molecules hitherto considered recalcitrant to the attack of individual pure cultures of microbes may in fact be biodegradable under the synergistic assault of mixed naturally occurring populations

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[1] Ahmed, M K , and Casida, J E , *J Econ Entomol* , **51**, 59 (1958)

[2] Getzin, L W , and Rosefield, I , *J Econ Entomol* , **59**, 512 (1966)

[3] Gunner, H B , Zuckerman, B M , Walker, R W , Miller, C W , Deubert, K H , and Longley, R E , *Plant and Soil*, **25**, 249 (1966)

[4] Bates, J L , and Liu, P V , *J Bact* , **86**, 585 (1963).

[5] Gale, E F , *Biochem J* , **34**, 853 (1940)

[6] Nurmikko, V , *Experientia*, **12**, 245 (1956)

[7] Alexander, M , *Introduction to Soil Microbiology* (John Wiley and Sons, New York and London, 1961)

Filamentous Phages specific for the *I* Sex Factor

EVERY transmissible bacterial plasmid like a colicin (*col*) factor or a drug resistance (*R*) factor necessarily contains a sex factor responsible for its transmission by conjugation[1-3]. The conjugation bridge is likely to be a sex pilus[4] and examination of some eighty independent plasmids of various kinds isolated from Enterobacteriaceae has shown that only two types of sex pili can positively be identified and therefore that only two types of sex factor are probably present[2,3] These are typified by *F*, the well known sex factor of *Escherichia coli*, and by *I*, the sex factor of *colI* (refs 2 and 3) Phages specific for *F*-like sex pili have frequently been isolated and are of two kinds isometric RNA phages attaching along the length of the sex pilus and filamentous DNA phages attaching to the tip of the pilus[5] The morphological and antigenic characteristics of *F*-like and *I*-like sex pili are distinct[2], so it seemed very probable, because *F* phages do not attach to *I*-like pili, that *I*-specific phages existed These have now been isolated[2], using as hosts bacteria carrying de-repressed (*drd*) mutants of fi^- *R* factors[6], the sex factors of which are known to be *I*-like[2] Strains carrying these mutant *R* factors continually form sex pili and can therefore be used as indicators for the corresponding donor-specific phages

Plating of sewage on *E coli K*12 *drd* R^+ did not yield *I* phages, and so the following method of selection was devised with successful results *F* phages are known to plate on F^+ *Salmonella typhimurium* as well as on F^+ *Escherichia coli* Thus if the phage population of sewage was enriched by overnight incubation with *drd* R^+ *Salm typhimurium* and then plated on *drd* R^+ *Escherichia coli*, or vice versa, only *I* phages would be expected to plate, because the somatic antigens which provide the receptors for many phages differ in the two species In the event, several flagellar phages were unexpectedly isolated because these can adsorb to the flagella formed by the two hosts[7] Enrichment consisted of mixing 1 ml of untreated sewage, 1 ml of overnight culture of the enriching strain (either *Salm typhimurium M*385 or *Escherichia coli K*12 carrying the *drd* fi^- *R* factor, 144 or 64) and 9 ml of Oxoid Nutrient Broth No 2 After incubation overnight, the culture was centrifuged in an angle rotor at 650*g* for 15 min and the supernatant sterilized by filtration through a broth-treated asbestos–cellulose pad. Phage was titrated by depositing 0 02 ml drops from nine ten-fold dilutions on 2 5 ml overlays of 0 35 per cent agar previously inoculated with the indicator and poured on thick plates of Oxoid Blood Agar Base These were incubated overnight at 37° C Four indicators were used *M*385 R^+ and R^-, and *K*12 R^+ and R^-. Inspection of the incubated plates usually showed immediately whether a given plaque type was present on the R^+ but not on the R^- strain, and on *K*12 as well as on *M*385 The drop method is much to be preferred to counts in overlays poured in the usual way, because minute plaques stand out far more distinctly against the surrounding background

Ten independent samples of sewage were examined, of which two, from Billericay, Essex, and from Croydon, Surrey, yielded the *I* phages, *If*1 and *If*2 Both isolates were from samples enriched on *M*385 and plated on *K*12, enrichment on *K*12 followed by plating on *M*385 often

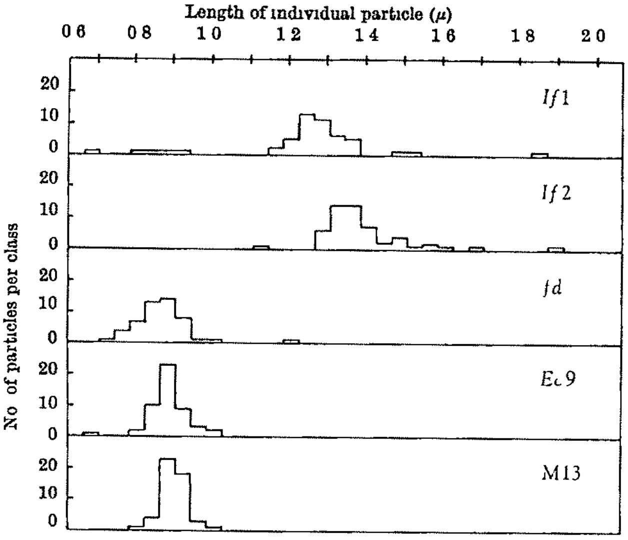

Fig 1 Distributions of lengths of individual particles of the *I* phages, *If*1 and *If*2, and of *F* phages, *fd*, *Ec*9 and *M*13

failed to give plaques of any kind and was abandoned Comparison of the R^+ and R^- plates of the same host showed that about 20 per cent of all sewage phages were restricted by the *R* factor Identification of the phages was greatly simplified by electron microscopy of material taken directly from plaques[8] and often showed immediately the site of attachment of the phage as well as its morphology

Particles of phages *If*1 and *If*2 occurred in large masses like those illustrated for the filamentous *F* phage, *Ec*9 (ref 9) The modal length of individual particles was about 1 3μ (Fig 1), which is markedly longer than the filamentous *F* phages *fd* (ref 10), *Ec*9 (ref 9) and *M*13 (ref. 11) There was no indication in the electron micrographs, such as a central line down the particles or the appearance of split ends, that each particle consisted of a loop twisted on itself as described for phage *fd* (ref 12) Both the *I* phages contained single-stranded DNA, judging from staining with acridine orange[13], with and without previous treatment with DNase or RNase[14] Aqueous suspensions of filamentous *F* phages are exceptionally resistant to inactivation by heat[9,15] Phage *If*1 was also considerably more resistant than a *T* phage[16] although it was slightly less resistant than phage *Ec*9 (Fig 2) Nevertheless, it is evident that heating, for example, to 82° C for 20 min, might provide an additional means for selecting filamentous phages of all kinds

The plaques formed by both *I* phages were usually about 0 5 mm in diameter, even when extremely soft overlays of 0 35 per cent agar were used The most distinct plaques were obtained on Oxoid Blood Agar Base the addition of 0 01 M $MgCl_2$ or 0 005 M $CaCl_2$ was without effect When plaques were exceptionally well defined, infected bacteria produced plaques with a central dot of phage-resistant bacterial growth, whereas free phage formed clear plaques When plaques were less distinct, phage mutants forming clearer plaques could be distinguished One-step growth curves of phage *If*1 resembled those obtained with filamentous *F* phages in that the rise period seemed to be indefinitely prolonged After a latent period of 35 min, the titre of free phage increased at least 1,000-fold at a constant rate during the next 2 h, presumably reflecting the continuing secretion of progeny particles by the infected organisms[17] The host range of the *I* phages has already been described in detail[2,3] and is principally determined by their ability to adsorb tip-to-tip to the sex pili of the test bacteria Thus *I* phages adsorb to the *I*-like pili determined by most *fi*⁻ *R* factors, *colI* and the *I* sex factor associated with *colEla*[18,19], but not to the *F*-like pili determined by *F*, F_0-*lac* and *fi*⁺ *R* factors[2,3]

Table 1 RELATIVE *K* VALUES IN CROSS-NEUTRALIZATION TESTS

	Phage				
Serum	*If*1	*If*2	*M*13	*fd*	*Ec*9
*If*1	1	1	<0 001	<0 001	<0 001
*If*2	1	1	<0 001	<0 001	<0 001
*M*13	<0 001	<0 001	1	0 25	0 25
fd	0 1	0 1	0 2	1	0 6
*Ec*9	0 1	0 1	0 4	1	1

K values estimated from per cent survival after exposure to serial dilutions of antiserum for 60 min at 37° C

Neutralizing antisera with *K* values of about 10^3 were readily produced in rabbits by five intravenous injections of 10^{10} particles during a fortnight These sera, and also antisera to the three filamentous *F* phages, *M*13, *fd* and *Ec*9, were used to determine the degree of antigenic relationship between the two groups of phages (Table 1) Neutralization tests showed that *If*1 and *If*2 behaved identically, as did *fd* and *Ec*9 Anti-*I* sera failed to neutralize any of the *F* phages, although the *I* phages were neutralized by anti-*fd* or anti-*Ec*9 but not by anti-*M*13 There is thus an anomaly in that the *I* phages evidently shared a common antigen with phages *fd* and *Ec*9, yet anti-*I* sera did not neutralize these phages This asymmetrical finding might arise either because the homologous antibody was provoked only by the *F* phages or because the antigen of the *F* phages could unite with the homologous antibody without loss of infectivity Agglutination tests were therefore performed on a microscopic scale by leaving a mixture of 1/40 antiserum and phage on the microscope grid for 1 h at room temperature, when the drops were then stained with uranyl acetate. Although no neutralization occurred, agglutinated phage particles were seen coated with globulin molecules which held the aligned particles some distance apart; whereas particles aggregating spontaneously were closely opposed The results of these tests, which were necessarily only semi-quantitative, showed that anti-*I* sera contained globulin reacting to moderate titres with phages *fd* and *Ec*9 and to a slight extent with *M*13 At this stage it therefore seems that antigenic relationships exist between the *I* and *F* filamentous phages which are not fully revealed by neutralization tests The two *I* phages are closely related

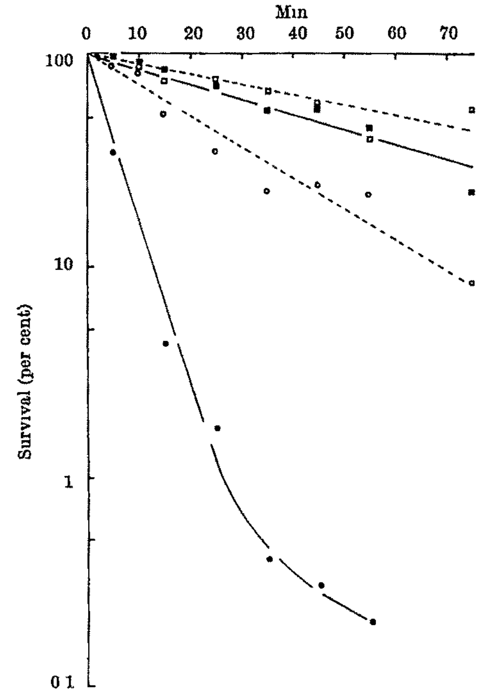

Fig 2 Thermal inactivation of phages *If*1 (●, ○) and *Ec*9 (,■ □) at 88° C (solid points, continuous curve) and at 82° C (open points, dashed curve) The phages were mixed in broth at a concentration of 10^5/ml each, heated, and counted separately on *drd* R^+ and on F^+ indicators

as are *fd* and *Ec9*, whereas *M*13 seems to differ considerably from both groups

In general, the filamentous *I* phages bear a marked resemblance to those attacking *F*-like pili, which suggests a common origin No isometric *I* phages have yet been isolated, although they should have been obtained by the present method of selection because we have used it to isolate isometric *F* phages One *F*-related plasmid, F_0-*lac*, is known to determine *F*-like pili which adsorb filamentous but not isometric *F* phages[2]. It may be that the fi^- *R* factors so far used for the isolation of *I* phages are similarly unable to propagate isometric *I* phages

We thank Dr R Dettori and Dr H Hoffmann-Berling for phages and antisera, and Dr H Williams Smith for providing the material from which phage *If*1 was isolated

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[1] Watanabe, T , *Fed Proc* , 26, 23 (1967)
[2] Lawn, A M., Meynell, E , Meynell, G G , and Datta, N , *Nature*, 216, 343 (1967)
[3] Meynell, E , Meynell, G G , and Datta, N , *Bact Rev* (in the press)
[4] Brinton, C C , *Trans NY Acad Sci* , 27, 1003 (1965)
[5] Hoffmann-Berling, H , Kaerner, H C , and Knippers, R , *Adv Virus Res* , 12, 329 (1966)
[6] Meynell, E , and Datta, N , *Nature*, 214, 885 (1967)
[7] Edwards, S , and Meynell, G G , *J Gen Virol* (in the press)
[8] Bradley, D E , *J R Microsc Soc* , 84, 257 (1965)
[9] Dettori, R , and Neri, M G , *G Microbiol* , 13, 111 (1965)
[10] Marvin, D A , and Hoffmann-Berling, H , *Nature*, 197, 517 (1963)
[11] Hofschneider, P H , *Z Naturforsch* , 18b, 203 (1963)
[12] Marvin, D A , *J Molec Biol* , 15, 8 (1966)
[13] Bradlev, D E , *Nature*, 205, 1230 (1965)
[14] Mayor, H D , and Hill, N O , *Virology*, 14, 264 (1961)
[15] Salivar, W. O , Tzagoloff, H , and Pratt, D , *Virology*, 24, 359 (1964)
[16] Pollard, E , and Reaume, M , *Arch Biochem Biophys* 32, 278 (1951)
[17] Hoffmann-Berling, H , and Mazé, R , *Virology*, 22, 305 (1964)
[18] Meynell, G G , and Lawn, A M , *Genet Res* , *Cambridge*, 10, 323 (1967).
[19] Edwards, S , and Meynell, G G , *Microbial Genet Bull* , No 27, 4 (1967)

GENETICS

Unusual Distribution of Red Cell Acid Phosphatase among Aborigines of Australia

THE Australian Aborigines are an interesting population for the study of human variability due to inherited characteristics The first study of their blood groups was published in 1922 (ref 1) and many extensive surveys have since been undertaken More recently discovered polymorphic characters, including the serum haptoglobins, transferrins, gamma globulins and group specific types, have also been investigated, and the results have been reviewed[2–4]. There is, however, no published information about the distribution of red cell acid phosphatase types among the aborigines and the study reported here was undertaken primarily to provide the information

Blood samples were obtained from the subjects at Darwin and Alice Springs in the Northern Territory and at Cairns in Queensland by staff members of the Commonwealth Health Laboratories Because of the interest which they have provided, many Australian aborigines have been subjected to venepunctures for research purposes. There is now some reluctance to approve the collection of blood for surveys of individual gene markers The problem was overcome in this work by using blood samples which had been collected for clinical pathology investigations Serum for the tests had been removed from all samples and the investigation described here was performed on the residual clots There is no reason to suggest that this selection was in any way related to the genotypes of the subjects Obviously, this material would not be suitable for an extensive survey of both red cell and serum gene markers, but it proved convenient for the test in this survey The samples were kept in a refrigerator after collection, transported to Sydney by air and were tested within 10 days of collection

The red cell acid phosphatase types were determined with minor modifications[5] by the starch gel electrophoresis and staining technique described by Hopkinson, Spencer and Harris[6] The phenotypes were classified using the nomenclature published elsewhere[7] Briefly, the enzyme types formerly called A, B, AB, and so on, have been designated PHs^A, PHs^B and PHs^{AB}, and so on, and the allelic genes PHs^a, PHs^b, and so on, where *P* stands for the gene locus in *Homo sapiens* (*Hs*)

The results are shown in Table 1 Only three phenotypes controlled by two alleles, PHs^a and PHs^b, were found The frequency of the PHs^b is higher than has been observed in any other population. This gene is the commonest in all populations studied so far[8], but the highest frequency previously recorded was 0 805 in a group from the Tristan da Cunha Islands[9]

Table 1 RED CELL ACID PHOSPHATASE IN AUSTRALIAN ABORIGINES

Phenotype	Cairns No observed	Cairns No expected	Darwin No observed	Darwin No expected	Alice Springs No observed	Alice Springs No expected
PHs^A	0	0 2	0	0 1	0	0
PHs^{AB}	8	7 7	5	4 8	0	0
PHs^B	84	84 1	148	148 1	26	26 0
Total	92	92 0	153	153 0	26	26 0
Gene frequency						
PHs^a	0 044		0 016		—	
PHs^b	0 956		0 984		—	

The high frequency of PHs^b among the Australian aborigines could be the result of selection or of the founder principle If selection has operated over the centuries or millennia the gene PHs^b must have been favoured to the extent that the genes, PHs^a and PHs^c, were at least partially eliminated Alternatively, the Australian continent may have been populated by a group which by random chance had a high frequency of PHs^b. The recently restated trihybrid theory[10] involves three successive migratory waves from different parts of Asia with unequal mixing in different geographic areas of Australia. If this theory is correct, selection after arrival seems more likely than the possibility that all migrating groups had the same high gene frequency

I thank the staff members of the Commonwealth Health Laboratories in Darwin, Northern Territory, and in Cairns, Queensland, for the blood samples.

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[1] Tebbutt, A H , and McConnel, S V , *Med J Austral* , 1, 201 (1922)
[2] Simmons, R T , *Proc Seventh Cong Intern Soc Blood Transf* , Rome, 287 (1958)
[3] Kirk, R L , *Occasional Papers in Aboriginal Studies*, No 4, Austral Inst Abor Studies, Canberra, 1 (1965)
[4] Kirk, R L , *Prog Med Genet* , 4, 202 (1965)
[5] Lai, L. Y C , *Nature*, 210, 643 (1966)
[6] Hopkinson, D A , Spencer, N , and Harris, H , *Nature*, 199, 969 (1963).
[7] Lai, L Y C , *Acta Genet* , Basel, 17, 104 (1967)
[8] Lai, L Y C , and Kwa, S B , *Acta Genet* , Basel, 18, 45 (1968).
[9] Hopkinson, D A , Spencer, N , and Harris, H , *Amer J Human Genet* , 16, 141 (1964)
[10] Birdsell, J B , *Arch Phys Anthropol in Oceania*, II, 100 (1967)

the publishers on the very high quality of the plates
BARRY COX

BOOK REVIEWS

TRIBUTE OF FISH

Fossil Vertebrates

Edited by Colin Patterson and P H Greenwood Pp v+260 (London Academic Press, Inc (London), Ltd., New York Academic Press, Inc , 1967) 70s , $8 60

THE presentation of collections of research papers to eminent scientists on their retirement is a happy custom This volume honours Dr Errol White, until recently Keeper of Palaeontology at the British Museum (Natural History) and President of the Linnean Society It contains papers, mostly on aspects of palaeo-ichthyology, by vertebrate palaeontologists from many countries in both Europe and North America, and gives abundant evidence of the respect and affection in which he is held

In 1950 White showed how the changes in their fish faunas could be used to classify the Lower Devonian strata of the Welsh borderland, and later showed that this scheme was valid also for Western Europe Obruchev and Karatajūte-Talimaa now show that it is similarly valid for Eastern Europe, while Dineley demonstrates that it can even be used to correlate the Knoydart faunas of Nova Scotia

Several contributions deal with different aspects of growth and ossification One present controversy is as to whether the carapace of the earliest fish consisted of a small number of large plates (a point of view to which a paper by Tarlo gives further support) or of a large number of small tesserae In an elegantly argued paper, Westoll points out the difficulties involved in accepting either of these extreme positions, and suggests that the different patterns may result from variation in the extent to which the larger, deeper plates may be exposed or concealed by the development of more superficial tesserae Another controversy concerns the methods used to identify and homologize the dermal skull bones of fish and tetrapods Parrington argues strongly that these must be based on the overall relationships of the bones to one another and to underlying structures, accepting that minor variations will occur As he points out, belief in complete uniformitarianism leads to surprising anomalies and the supporting arguments are, in some cases, internally inconsistent A very useful paper by Schaeffer briefly reviews the structure and development of osteichthyan vertebrae He shows that, although the arch components and centra are clearly homologous throughout the Osteichthyes and tetrapods, the latter differ in the resegmentation of the sclerotome and in the fact that the notochordal sheath is not involved in the formation of the centrum

Aspects of fish evolution are discussed by Jarvik and by Gardiner Jarvik demonstrates that the lower jaw of early Dipnoi was already very dissimilar from that of other fish and, like many other aspects of dipnoan anatomy, suggests that the group diverged at a very early date Gardiner describes the way in which modifications in the structure and relationships of the preopercular bone have contributed to the improvements in the mechanism of feeding and gill ventilation in the actinopterygians

These and other contributions together make up a volume which any workers concerned with the anatomy, palaeontology, taxonomy or stratigraphy of fish will find both informative and thought provoking The editors must be congratulated on an almost error-free text, and

ACROSS THE BERING STRAIT

The Bering Land Bridge

Edited by David M Hopkins Pp xiii+495 (Stanford, California Stanford University Press, London Oxford University Press, 1967) 176s net

THIS book consists of twenty-two papers with an introduction and synthesis by the editor The book is related to, but is not a record of, the symposium entitled "Late Cenozoic History and Environments of the Bering Land Bridge" held at the Seventh Congress of the International Association for Quaternary Research (INQUA) Eight of the papers included in the book were not given at the symposium and two of the papers read at the symposium are not included in the book The papers, by American, Canadian, English, German, Icelandic and Russian specialists, provide data and hypotheses on the geology, geomorphology, oceanography, palaeontology, biogeography, archaeology and anthropology of the areas around and under the Bering and Chukchi Seas

Some of the evidence presented in the book has been derived from areas very far from the Bering Strait The arrival of molluscs of Pacific boreal ancestry on the north shores of Iceland more than three million years ago, and the development of Aurignacoid industries in Eurasia, are both significant in the interpretation of the events that took place in Beringia The timing of these events also requires a comparison of Quaternary chronologies developed in different parts of the world The stratigraphy and chronology of the last 70,000 years in North America, Europe and Siberia is shown to be sufficiently well known to enable useful correlation However, the period one million to three million years B P is less well known The record of ten glaciations, the first occurring possibly between 2 4 and 3 0 million years ago, in northern Iceland is stated as probably representing a minimum estimate of the total number of important world-wide Pleistocene glacial cycles, and therefore the Bering Land Bridge may have been renewed as many as ten times by glacio-eustatically lowered sea level during the Pleistocene

As a collection of research papers this book has the disadvantages of a certain amount of repetition of material, duplication of bibliographies and lack of standardization of chronological nomenclature Although most of the authors have published most of their material elsewhere, the editor is to be complimented on bringing together contributions from many disciplines and in several languages Most of the authors have also succeeded in orientating their papers to highlight the central theme, namely, the nature and significance of a land connexion between Eurasia and North America The editor has had a very difficult task in writing his synthesis and he has accomplished it with clarity and confidence It may well be that the major contribution of this book is to highlight outstanding problems and to indicate the great value of the interdisciplinary approach

R J PRICE

POLYCHAETA IN AFRICA

A Monograph on the Polychaeta of Southern Africa

By J H Day Part 1 Errantia Pp viii+1–458 Part 2 Sedentaria Pp 459–878 (London British Museum (Natural History), 1967) 300s the two parts

THIS important and scholarly work has a far wider value than its title would suggest There have been relatively few monographs on the polychaete annelids and none has so far dealt with southern Africa. It is welcome not only in covering this region for the first time in this way, but also for the keys provided, which apply to families and genera everywhere.

The work is divided into two easily handled volumes, one dealing with the Errantia, the other with the Sedentaria. This is convenient for the present purpose although arbitrary. There is a useful and concise introduction and description of methods for collection and examination, which make these books easily usable by the beginner. With the beginner also in mind, there are illustrated keys to the families, and these, together with the initial explanations and instructions for using the monograph with a map of the main localities, are repeated at the beginning of the second volume, so that each can be used without reference to the other. To avoid confusion page numbers run on, the second volume starting at page 459. I like this arrangement, for it simplifies the index. The illustrations are throughout clear and helpful. Some of those of whole worms in the initial key to families (Fig. 0.2, 4–6, 9) are small, and though recognizable, I wonder if they would really be helpful to a beginner. The initial dichotomy of this key is not always as easy as a novice might think. The chronological list of principal references is both useful and interesting to the more general taxonomist, and the arrangement of authority, province collected and depth range relating to each species described is clear and concise.

The order of families is the conventional one. There is a useful definition of every family, each definition being followed by a list of records from southern Africa (south of 20° S.), biological notes, chief diagnostic characters, and a key to genera. These keys and the comments on the diagnostic characters are what workers elsewhere will find useful. Genera not found in southern Africa are marked with an asterisk, and are not thereafter defined. To compile such a work is a prodigious task and it is perhaps impossible without minor errors. These are few and they in no way detract from the accuracy and usefulness of the work. *Tachytrypane* is defined (page 580) although it does not occur in the region, *Pseudoscalibregma* (page 585) should be asterisked in the key, and Apistobranchidae is mis-spelt in the initial key to families. I also found the running title "Cirratulidae and Heterospionidae" (pages 499–517) confusing in that the latter family has not been recorded from this region and hence receives only brief mention. There is a useful glossary at the end of the second volume, and a bibliography. The work includes eighteen new names which are noted in bold type in the index.

Professor Day can only be congratulated for achieving both a definitive monograph on the polychaetes of southern Africa and at the same time providing a work which will be of major value to those collecting in other parts of the world. In spite of the high price, these volumes should find a place on the shelves of libraries of all zoology departments. The trustees of the British Museum must also be complimented on its production, and their printers, Messrs Eyre and Spottiswoode, of Portsmouth, for the very fine job of printing they have done.

R. PHILLIPS DALES

HUMAN HISTOLOGY

Human Histology

Second edition. By Bruce Cruickshank, T. C. Dodds and Dugald L. Gardner. Pp. viii+359. (Edinburgh and London: E. and S. Livingstone, Ltd., 1968.) 84*s*. net.

PRECLINICAL medical students fall into three principal categories and, because their aims in studying medicine are different, the books that they choose, or from which they will derive most benefit, are naturally rather different. The vast majority are anxious in their preclinical days to equip themselves with a sound enough background knowledge of anatomy, physiology and biochemistry to enable them to understand what is going on in the various pathological cases with which they will come into contact and later have to treat. Second, there are a few who not only want to know what is known, but to know how it came to be known, and to be convinced by the evidence that the so-called knowledge is genuine. Many of these are further motivated to enquire more deeply into and to question the "facts" presented to them. Third, there are also a few who aim to acquire only the minimum knowledge of preclinical material necessary to ensure their place at a hospital where they will then learn in "the practical way".

This book is clearly directed towards the vast majority. It is a very clear, dogmatic account of the various tissues and cells of the body as they are at present understood and interpreted. It is very well illustrated with colour photographs, electron micrographs, and with black-and-white figures in various forms. For the non-enquiring mind it gives an excellent picture of human histology. The student with the enquiring mind will not, however, be satisfied with it, though he will certainly find it very useful as a revision book. It does nothing to encourage the reader to evaluate evidence. It does not sufficiently indicate the complexities of tissue interactions or portray cells as living, functioning and adapting entities. The restriction to human tissues, while it may have certain practical advantages, artificially isolates man from other animals and deprives the student of any sense of evolutionary continuity or of species differences. Both of these concepts are of fundamental importance in relation to the evaluation of evidence from experiments on animals, and indeed to the proper understanding of events in the human body.

There are some curious errors, for example, in the chapter on the eye, pathological training has clearly been more influential on the authors than the study of Latin. Bacillum (later, bacillus) was a rod long before it became a bacterium. The macula lutea is yellow because it contains xanthophyll, not because its centre is free of blood vessels. The fovea centralis is within the macula lutea—not the reverse.

Medicine would get on better if histology were more concerned with what cells and tissues do than with what they look like.

E. N. WILLMER

VOLCANIC PAST

The Eruption of Hekla in Historical Times

A Tephrochronological Study. By S. Thorarinsson. Pp. 183+12 plates. (Reykjavik: Visindafelag Islendinga, 1967.) n.p.

TEPHROCHRONOLOGY, or volcanic ash chronology, is a method of study of volcanic areas which depends on the fact that the ash thrown out by a volcanic explosion is dispersed over a wide area, and the resulting ash layer is sensibly of the same age over its whole extent. A succession of such ash layers represents a time sequence relative to which other events, such as the outpouring of lava flows, the retreat of glaciers, or the abandonment of human habitations, can be dated.

A few tens of volcanic eruptions take place in the world each year, but few are studied scientifically and the volume of available data is still quite small. Tephrochronology makes available for study an enormously greater number of eruptions than would occur during one's life-time, and offers the best hope of rapidly acquiring a comprehensive knowledge of volcano behaviour.

This book is one of the most comprehensive accounts yet published on the activity of a volcano over a long period of time, covering as it does a time-span of 1,000 years and a succession of fourteen substantial eruptions Well written (as one has come to expect of the author), it is a remarkable chronicle which, besides scientifically documenting the eruptive products, delves deeply into contemporary Icelandic writings It is illustrated with twenty-eight text-figures, twelve plates, and a 1 120,000 geological map of Hekla and its surroundings (as it was before the 1947–48 eruption) A chapter on the petrology of Hekla's products, written by Jens Tomasson, is included

Isopach maps are compiled for six ash layers, and from them the volume of each ash-fall is computed Taken in conjunction with the volumes of associated lava flows, this gives a figure for the productivity of Hekla, meaningful because it is based on the record of a period of time long in relation to the average repose time between eruptions Chemical studies show that most of the eruptions started with an explosive phase erupting relatively acid pumice, and the composition of the erupted products then became more basic, moreover, the longer the repose period since the preceding eruption the greater was the acidity of the initially erupted material Studies of this kind should have a profound influence on petrogenetic theory which, at present, tends to neglect the time factor

I would have liked to see some data on the grain-size and other textural features of the ash deposits—an opportunity has been lost here to make a notable contribution in this much neglected field—and some more information on the lavas of Hekla The virtual omission of the 1947–48 eruption is excusable, as it is well documented by other publications of which this book is one of a series

Thorarinsson's book is essential reading for all who are interested in volcanology It is a basic work on tephrochronology, written by the pioneer of the method, and is relevant also to the archaeologist and climatologist, or whoever is concerned with the chronology of the last few millennia, nor should it be spurned by the general reader, for it gives an insight into the sociological impact of eruptions from the volcano that was for long believed to be the location of hell

G P L WALKER

AROUND OLLERTON

Geology of the Country around Ollerton

By W N Edwards With contributions by J Phemister and R K Harrison (Explanation of One-Inch Geological Sheet 113, New Series) Second edition (Natural Environment Research Council—Institute of Geological Sciences Memoirs of the Geological Survey of Great Britain—England and Wales) Pp x+297+10 plates (London H M Stationery Office, 1967) 55*s* net

THE principal purpose of this new edition of the Ollerton memoir is to revise the account of the underground geology made possible through the intense search for coal by the National Coal Board and for oil by British Petroleum Much of the new information has been derived from bore-holes, and the main author of this work, Mr W N Edwards, has skilfully brought together these data with observations made by the Geological Survey's officers and a wealth of rock description provided by the petrology section of the Institute of Geological Sciences led by Dr J Phemister and Mr R K Harrison Some of the results obtained during the search for oil have inevitably been published elsewhere, but there is much new information to be obtained from this book

The bulk of the volume is devoted to a description of the Upper Carboniferous rocks In addition to the descriptive account, ribbon diagrams and other text figures graphically display the variations in the sediments throughout the area They show the thinning of the strata in an easterly direction mainly at the expense of the sandstone members The marine "marker" horizons used for correlation are more persistent, traced in part by fossil evidence and in part by geophysical methods, a point perhaps not too clearly stated in the text The easterly extension of the coalfield is well documented and extracts from the principal bore-holes are given Most of the coal extracted is obtained from only four seams by coal mines winding over one million tons annually A little information is given on the quality of the coal and references are made to analyses

Below the Millstone Grit Series occurs that of the Carboniferous Limestone, which includes, towards the base, rocks of Tournasian (C_1) age The fossil evidence in older rocks is meagre, consisting mainly of horny brachiopods, and no decision on the exact age of these rocks is given, they are referred to Devonian and Lower Palaeozoic Systems

Surface rocks of the Permian and Triassic Systems are described in the sixth chapter The description adds materially to that of the first edition, particularly the petrological data which are entirely new Very little change has been made to geological boundaries and the Ollerton map was re-issued with only minor alterations Permian and Triassic rocks can be distinguished despite lateral and vertical facies changes, and the classic terminology of Permian, Bunter, Keuper and Rhaetic is maintained The Muschelkalk is referred to in a footnote (page 120)

Other surface rocks are briefly mentioned including a half square mile of Liassic rocks (seventh chapter and page 152) and the superficial deposits (ninth chapter)

Geological structures are summarized in the eighth chapter These include not only faults and folds but also the effects of unconformities, particularly that of the Permian rocks on the underlying Coal Measures Sedimentary structures are also described in this section

Detailed accounts of the separate divisions of the sequence are arranged in double columns and small type-face as distinct from the single column spread and larger print of the general accounts This improves the lay-out of the text and enables the general reader to avoid detail In places, however, the column is further reduced in width if details of depth are included and only two or three words occur on a line

The publication of this carefully prepared book on an area containing a number of small oilfields is particularly appropriate at this time when the North Sea bore-hole data are becoming available to increase our knowledge of Carboniferous, Permian and Triassic rocks which occur in that region

F M TAYLOR

STUDENT PHYSICAL CHEMISTRY

Physical Chemistry

By Frank T Gucker and Ralph L Seifert Pp xx+824 (London The English Universities Press, Ltd , 1967) 70s net

THE prospective purchaser of this textbook should take heart from the comment on the dust cover that "mathematics is treated as the good servant, but poor master, it has always proved to be to physical science" With this in mind, the authors introduce their work with a short chapter which is only to be regarded as a swift "refresher" survey of selected mathematical areas

The first section on physical chemistry deals with the nuclides, radioactive decay and nuclear reactions This leads to discussion of nuclear structure and, through the introduction of spectroscopic observations, atomic structure Bonding is discussed in terms of the molecular orbital and valence bond approaches leading to hybrid bonds and the resonance concept

A chapter on molecular properties acquaints the reader with nuclear magnetic resonance, Mossbauer spectra, dielectric and dipole phenomena and molecular spectroscopy. Study of the behaviour of gases introduces the kinetic theory, critical phenomena and equations of state A section on thermodynamics and thermochemistry then leads to a discussion of the properties of solids and liquids coupled with structural considerations

At this point the equilibrium concept is brought in, followed by the third law of thermodynamics and statistical mechanics With this background, solution properties of non-electrolytes and electrolytes are reviewed This involves treatment of acid-base equilibria and the electrochemistry of galvanic cells and electrolyte conductance

A chapter on surface chemistry includes a descriptive approach to the colloid state while that on chemical kinetics had both a classical and transition state approach Among reactions dealt with are molecular, ionic, chain, homogeneous and heterogeneous catalysis

The book concludes with a short chapter on heterogeneous equilibria

This textbook is very easy to read and well set out, with a clear use of tabular matter and diagrams It makes refreshing use of short historical introductions to new ideas and makes a good attempt to render the mathematics digestible. Each chapter is provided with a set of problems and a reading list to take the reader further when appropriate

This is a useful book, very moderately priced and, I believe, with an approach which will commend it to undergraduates J C Robb

DIFFERENTIAL EQUATIONS

Nonlinear Partial Differential Equations

A Symposium on Methods of Solution. Edited by W F Ames. Pp xv+316 (New York Academic Press, Inc , London· Academic Press, Inc (London), Ltd , 1967) 112*s*

The history of non-linear differential equations is nearly as old as that of differential equations themselves, for John Bernoulli studied an equation of Riccati's type as long ago as 1694 Some 200 years elapsed before a really intensive theory developed, to be followed by a remarkable and explosive interest during the past thirty years or so For this, two reasons may be suggested in 1939 problems involving non-linear equations cropped up in the military field, and the advent of high-speed computing machines allowed complex and laborious calculations to be performed with ease Apart from this, investigations such as van der Pol's new method for dealing with Rayleigh's equation had the indirect effect of a slight change in emphasis, mathematicians thought less about seeking for a solution of a non-linear equation and more about what the equation can tell us about a function which satisfies it

Because problems in applied mathematics cannot always be adequately treated by assumptions of linearity and of simple superposition, there is an immense field of application awaiting further progress in the study of non-linear equations Useful texts are available, but perhaps the time is not yet ripe for a comprehensive and classical treatise So the present report of a seminar is of considerable value as an interim survey The reader should, of course, have some knowledge of the basic theory of the non-linear ordinary differential equation, such as can be found, for example, in the excellent introductory book by Davies and James He can then use this volume to bring himself into touch with current research work on the non-linear partial differential equation. For instance, Bellman and Kalaba describe briefly some methods which have recently been tried out, particularly those which are suitable for digital computation, a region to which Bellman has made striking and not always conventional contributions Ames stresses the need of the research worker to escape from the traditional background of linear operators and the principle of superposition, while adapting some of the classical exact techniques of the linear partial differential equation to the non-linear Most of the sixteen papers deal with equations from fluid mechanics, elasticity non-linear wave theory, and other physical topics, and the authors are men actively engaged in research

The editor calls the book a small chapter in a largely unwritten history, but believes that it can be both a source of inspiration and a plea for help Older men cannot easily discard the powerful but restrictive machinery of linearity, young men, free from such inhibitions, have a large domain of theory and application awaiting the properly equipped explorer T A A Broadbent

LIPID CHEMISTRY

An Introduction to the Chemistry and Biochemistry of Fatty Acids and their Glycerides

By F D Gunstone Second edition Pp x+209 (London Chapman and Hall, Ltd , 1967) 63*s* net

In the ten years that have passed since this book was first published, the introduction of new techniques such as thin-layer and gas-liquid chromatography has resulted in a considerable accumulation of accurate analytical and structural data of lipids This trend has simplified rather than complicated the author's task, and in the present volume a concise and logical account of lipid chemistry is possible where previously only empirical classification and description could be applied to much of the subject matter It is appropriate, in view of the author's background, that the chemical and physical properties of lipids should be well described The chapter on fatty acids, their derivatives, isolation, properties and structure is likely to be invaluable to both biologists and chemists seeking a better understanding of the physico-chemical basis of lipid biochemistry These chapters are concise, accurate and readable, and do not confuse the reader with excess detail The inclusion of reference lists permits ready access to the original literature

In a sense, most lipid chemistry is biochemical in that lipids are generally naturally occurring Nevertheless, it is to be expected that biochemistry should contain much over and above the chemistry of natural products, and the title of this book might lead one to hope that the author had effected a multidisciplinary approach to the subject However, only a tenth of the content is concerned with the metabolic interconversions of lipids, and this is a fairly standard account The functional and structural involvement of lipids in biological membranes, surely the major problem of lipid biochemistry, receives two lines of passing mention The same ration is allocated to interactions between lipids and proteins Thus the chemist seeking an introduction to the problems in lipid chemistry set by biological systems is unlikely to be challenged, stimulated or even adequately informed by the "biochemical" content of this book, and may therefore take his much-needed talents elsewhere That would be regrettable, and it is to be hoped that a third edition appearing in another decade would be able to relate the physico-chemical structure of complex lipids to their biological function P B Garland

TWO REPRINTS

The Ninth Bridgwater Treatise

A Fragment. New impression of the second edition By Charles Babbage. (Cass Library of Science Classics, No. 6.) Pp 273 (London Frank Cass and Co, Ltd, 1967. First published 1837) 90*s*

Philosophical Experiments and Observations

By Robert Hooke Edited by W Derham (Cass Library of Science Classics, No 8) Pp 391 (London Frank Cass and Co, Ltd, 1967 First published 1726) 90*s*

THERE is now an almost bewildering variety of facsimile reprints of scientific works of historical interest Inevitably, many duplications occur and the price of these books is high The Cass Library of Science has published several of the less hackneyed works and their price is relatively modest Most commendably they have supplied information often neglected Thus they date their reprints and give brief details of earlier editions, and their general editor, Dr Laurens Laudan, has added an index or analytical table of contents to each volume which makes the books more usable than the original editions He has also added an explanatory note to the Hooke volume The books are sturdy and well reproduced.

Unfortunately these two volumes are not of much importance *Philosophical Experiments and Observations* represents minor Hooke at best, what was left in his papers after Waller had published *The Posthumous Works* Here are miscellaneous notes, jottings and letters, random, disconnected and incomplete, about one-third not by Hooke However, many of these notes illuminate both Hooke's activities and those of his contemporaries

Charles Babbage's *Ninth Bridgwater Treatise* is very much a work of its period, the 1830s, when the "argument from design" was thought worthy of eight separate treatises, paid for by a bequest from the Earl of Bridgwater Babbage, perhaps piqued at not being asked to write an official treatise, wrote this brief and truncated discussion illustrated by probability theory and the calculating machine It is a curious but specialized work, more interesting to historians than scientists

MARIE BOAS HALL

OBITUARIES

Dr A T Waterman

ALAN TOWER WATERMAN, who died at the end of last year, did much for science in the United States During the fifteen years when he served, first as Chief Scientist of the Office of Naval Research and later as Director of the National Science Foundation, he worked constantly in the cause of basic research and education in the sciences.

He was born at Cornwall-on-Hudson, New York, in 1892, and did both his undergraduate and graduate work at Princeton, receiving the degrees of AB in 1913, MA in 1914 and PhD in physics in 1916 His research included investigations of the conduction of electricity through solids, thermionic, photoelectric emission and related effects, and electrical properties of solids After receiving his degree, he became an instructor in physics at the University of Cincinnati During the First World War he spent two years with the Science and Research Division of the Army Signal Corps, where he was engaged in meteorological work At the end of the war he joined the faculty of Yale University and stayed at the department of physics until 1942

From 1941 to 1945 Waterman was associated with the Office of Scientific Research and Development (OSRD), and from 1946 to 1951 was Deputy Chief and Chief Scientist of the newly established Office of Naval Research (ONR), Department of the Navy He was one of the principal architects of the policies and programmes of ONR in supporting scientific research He interpreted broadly the types of research which were important to the navy, and provided a means of supporting fundamental research that might otherwise not have been supported at a time when the Federal Government's resources were concentrated on applied science, chiefly concerned with defence and atomic energy Under Waterman the ONR sought advice from scientific organizations, and when necessary established advisory committees outside the navy

The Office of Naval Research became a model for government support of basic research, and pointed out the need for a national foundation to support this kind of research, but on an even broader basis.

On April 6, 1951, President Truman appointed Waterman the first director of the newly formed National Science Foundation, for a six year term In 1957 President Eisenhower reappointed him to this post, and although he reached the compulsory retirement age before the end of his second term Waterman continued in his post until June 1963, at the special request of President Kennedy

While he was in charge of the National Science Foundation, Waterman not only stressed the importance of supporting, through the universities, pure research which was not of immediate significance, but he also encouraged the social sciences, and there was a steady growth in support for these subjects

He always stressed the importance of education and training for scientists, and through a gradually expanding programme of fellowships the Foundation has sought to make graduate training available to all the best students In 1953 the first projects began with summer institutes for teachers of high school and college mathematics and science, and curricula for schools and colleges have since been revised While at the National Science Foundation, Waterman was able to inform the public of the importance of education and research From Congress he obtained an initial grant of 3 5 million dollars which had increased to 40 million dollars by 1957

After retiring, Waterman maintained an active interest in science and education by serving on numerous boards and committees including the Board of Trustees of Atoms for Peace Awards, the Advisory Board of Georgetown University Center for Strategic Studies and the Liaison Committee on Science and Technology of the US Library of Congress He was special consultant to the President of the National Academy of Science and chairman of the Committee on Scholarly Communication with Mainland China, consultant to the Administrator of the National Aeronautics and Space Agency and a member of its Historical Advisory Committee

Waterman was a fellow of the American Association for the Advancement of Science, and served as president in 1963 and chairman of the board in 1964 He also belonged to the American Physical Society and the New York Academy of Sciences He was a member of the American Association of University Professors, the American Institute of Electrical Engineers, the American Rocket Society, the American Polar Society, the Washington Academy of Sciences, the Washington Academy of Medicine, Phi Beta Kappa, Sigma Xi, the Scientific Research Society of America, and the Washington Philosophical Society In October 1967, the American Institute of Physics awarded him the Karl Taylor Compton gold medal for distinguished statesmanship in science

Waterman was an accomplished musician—he mastered the Scottish bagpipes and for many years played the violin, cello and viola in a string quartet, first established during his undergraduate days at Princeton University and later composed of his Washington scientific colleagues He also frequently entertained his associates on the piano and guitar Another great interest was his love of the woods. He was a licensed guide in the State of Maine, and he continued this activity whenever his official duties permitted a rare canoe or camping trip

University News

Edinburgh

Dr D S Falconer, at present deputy director of the ARC Unit of Animal Genetics, has been appointed to a personal chair in genetics Dr Falconer will take up his appointment on April 1 when he will also become director of the ARC unit in succession to Professor C H Waddington

University College of Swansea

Professor E T Nevin, professor of economics at the University College of Wales, Aberystwyth, has been appointed professor of economics and head of the Department of Economics

Announcements

The Paul Ehrlich and Ludwig Darmstaedter prizes have been jointly awarded to Professor W Morgan, director of the Lister Institute of Preventive Medicine, and Professor O Westphal of Freiburg The prizes were awarded for contributions in the fields of physiology and medicine

Erratum In the article "Not Enough Uranium" (*Nature*, 217, 893, 1968) the estimates of available resources of uranium at a price of less than $10 a lb of U_3O_8 should have read 700,000 short tons, with a further 126,000 short tons available as by-products, not 7,000 short tons

Erratum In the report of the symposium of the Experimental Plant Taxonomy Group of the Linnean Society (*Nature*, 217, 902, 1968), *Potentilla anglica* was quoted wrongly as an alltetraploid which forms trivalents at meiosis, it is, in fact, an allo-octaploid which forms only bivalents at meiosis

Erratum In the article "Haemoglobin Torino—α43 (CD1) Phenylalanine→Valine" by A Beretta, V Prato, E Gallo and H Lehmann (*Nature*, 217, 1016, 1968), in lines 22 and 27 of column two, page 1018, the words higher and lower are interchanged The amount of haemoglobin A is higher in heterozygotes for haemoglobin A and α-chain abnormal haemoglobins, and lower with β-chain abnormal variants

CORRESPONDENCE

The Reference Problem

Sir,—One of the most irritating features of the work of editing, typing and publishing a scientific paper must surely be the problem arising in connexion with the bibliography, or citation of references to other literature Yet that problem ought to be readily soluble

The advantages of standardization in literature references will be obvious to author, typist and editor alike in the interests of the author, such standardization would allow the choice of journal to be made with convenience after the manuscript has been edited, for the typist it would mean easy, rapid copying from standard filing cards, while the editor would be spared the boring task of altering punctuation, the ordering of initials, and so on Both the American Standard Association (ASA) and the British Standards Institution (BSI) have prepared style manuals with sections devoted to bibliography It is conceivable that the assessments and recommendations of the ASA and the BSI are occasionally inapplicable for certain reasons Indeed, it is admissible that the forms of documentation needed in different fields are so multifarious that universal standardization is probably unattainable In going from the general to the particular, however, it does seem likely that the difficulties involved would diminish, so that it ought to be feasible to achieve bibliographic standardization among, for example, those journals devoted to biology In 1960, the Committee on Form and Style of the Conference of Biological Editors prepared a *Manual for Biological Journals* dealing with style in writing, and a second edition of this work was published in 1964 Unfortunately, the resolutions passed by this committee have not been acted on generally, this is clearly evident from a perusal of the late issues of just a small sample of biological journals, namely, those containing papers on genetics

To underline the point, here follow some references as they appeared in recent numbers of different journals on genetics

Miles, C P & S D Strong (1962) Nuclear chromocenters of cultured chicken cells *Exp Cell Res* **27** 377–381 (Genetica, **38**, 30, 1967)

Cohen S S & Barner, H D (1954) Studies on unbalanced growth in *Escherichia coli* *Proc natn Acad Sci U S A* **40**, 885–893 (Genet Res , **8**, 99, 1966)

rees h , and thompson, j b 1956 Genotypic control of chromosome behaviour in rye III Chiasma frequency in homozygotes and heterozygotes *Heredity*, **10**, 409–424 (Heredity, **22**, 347, 1967)

Weyers, W H Expression and stability of the marbled allele in maize *Genetics* 46 1061–1067 1961 (J of Heredity, **58**, 156, 1967)

Miles, C P and Storey, S D 1962 Nuclear chromocenters of cultured chicken cells —Exp Cell Res **27** 377–381 (Hereditas, **57**, 216, 1967)

Bender, M A , and P C Gooch, 1962 Persistent chromosome aberrations, in irradiated human subjects Radiat Res **16** 44–53 (Japan J Genetics, **42**, 162, 1967)

Olds, D and Seath, D M (1950) Predictability of breeding efficiency in dairy cattle *J Dairy Sci* , **33**, 721–724 (J of Genetics, **59**, 237, 1966)

Darlington, C D Crossing over and its mechanical relationship in *Chortippus* and *Stauroderus* J Genet **39**, 465–500 (1936) (Chromosoma, **22**, 191, 1967)

Mirsky, A E , and H Ris, 1951 The desoxyribonucleic acid content of animal cells and its evolutionary significance J Gen Physiol **34** 451–462 (Genetica, **57**, 13, 1967)

Lerner, I M , and F K Ho 1961 Genotype and competitive ability in Tribolium species Amer Natur 95 329–343 (Amer Natur , **101**, 274, 1967)

Inasmuch as bibliographic standardization on a broad basis is probably unrealizable, and because in practice geneticists, for example, will only rarely come into professional contact with journals devoted to economics or theology, it may be worthwhile for the geneticists themselves to try to introduce some uniformity into the reporting of work in their own field

The next Congress of Genetics, to be held in Tokyo in the summer of 1968, will certainly convene the vast majority of editors of journals on genetics At that time a meeting could easily be arranged at which all these editors could debate the problem mooted here Other difficulties might be aired and perhaps solved at the same time The matter of illustrations deserves some consideration some journals ask for original drawings with complete lettering, others for drawings without lettering, still others for photographs or photocopies of the drawings, and so on The lay-out of the summary or abstract, of the acknowledgments and of the footnotes could also be discussed

In the event of some agreement being reached, either the officers of the congress, the members of the Permanent Committee for Genetic Congresses, or the members of some other committee yet to be appointed, could easily communicate the suggestions resulting from the editors' deliberation to the International Union of Biological Sciences (IUBS) In its turn, the IUBS could pass on these suggestions to other members of the union and, eventually, to the main body of the scientific community

Yours faithfully,

Frederic A Lints

Laboratoire de Génétique,
F A Janssens,
Parc d'Arenberg,
Heverlee-Louvain,
Belgium

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, March 25

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I Mech E , at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Fluidics and Integrated Circuits" opened by Dr R C Foss and Dr K Foster

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "The Impact of Proposed Vehicle Noise Regulations"

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 6 p m —Dr C H G Oldham "China To-day—Science" (second of three Cantor Lectures)

Tuesday, March 26

Institution of Civil Engineers (at Great George Street, London, SW1), at 5 30 p m —Mr J E Prosser and Mr P A St C Grant "The Tyne Tunnel —Planning of the Scheme", Mr R H Falkiner and Mr S G Tough "The Tyne Tunnel—Construction of the Main Tunnel"

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "High Density Vehicles"

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, SW1), at 6 p m —Dr W I Bengough "Thermal Degradation of PVC in Solution"

Tuesday, March 26—Thursday, March 28

Systematics Association (at the University of Nottingham School of Agriculture, Sutton Bonington)—Symposium on "The Soil Ecosystem (Systematic Aspects of the Environment, Organisms and Communities)"

Wednesday, March 27

Institution of Electrical Engineers (at Savoy Place, London, WC2) at 5 30 p m —Mr G S Buckingham "Elegant Heating—by Electricity" (Sixth Annual Lecture of the Power Division)

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Professor G Kino "Waves in Semiconductors—Possibilities for New Solid State Devices"

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College, London, SW7), at 5 30 p m —Annual General Meeting

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Mr D T N Williamson "The Pattern of Batch Manufacture and Its Influence on Machine Tool Design" (James Clayton Lecture)

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 6 p m —Sir Paul Chambers, KBE "Technological Progress in Industry" (Trueman Wood Lecture)

Society of Chemical Industry, Food Group—Nutrition Panel (at 14 Belgrave Square, London, SW1), at 6 15 p m —Annual General Meeting, followed by Dr T H Grenby ' Some Aspects of Food and Dental Caries"

Wednesday, March 27—Thursday, March 28

Institution of the Rubber Industry, London Section (at the Kensington Close Hotel, London, W8)—Conference on "Modern Polymers—New Developments and Applications"

Royal Society (at 6 Carlton House Terrace, London, SW1), at 10 30 daily—Discussion Meeting on "Results of the Royal Society Expedition to the British Solomon Islands Protectorate, 1965" organized by Professor E J H Corner, FRS

Wednesday, March 27—Friday, March 29

Royal Microscopical Society (at Imperial College, London, SW7)—European Symposium on "Cytochemistry in Electron Microscopy"

Thursday, March 28

University of Liverpool (in the University Lecture Room Building) at 9 30 a m —Symposium on "Mathematical Models in Oceanography and Meteorology"

Gas Chromatography Discussion Group, Liquid Chromatography Sub-Group (at the Institute of Petroleum, 61 New Cavendish Street, London, W1), at 3 p m —Scientific Papers

Institution of Civil Engineers, Hydrological Group (at Great George Street, London, SW1), at 5 30 p m —Informal Discussion on "Investigations Following a Catastrophic Flood" introduced by Mr C Clay

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Professor W H G Armytage "The Engineer in Society"

Institution of Electronic and Radio Engineers, Aerospace, Maritime and Military Systems Group (at 8–9 Bedford Square, London, WC1), at 6 p m —Mr T H Kerr "Cost Effectiveness and Systems Analysis in Defence"

Thursday, March 28—Friday, March 29

Association of Clinical Biochemists (at Newcastle upon Tyne)—Eighth West-European Symposium on Clinical Chemistry—"Immunology in Clinical Chemistry" (Organized in collaboration with the Societies of Clinical Chemistry of Belgium, France, Holland, Switzerland and West Germany)

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, SW1)—Symposium on "Corrosion and Its Prevention in Motor Vehicles"

Mineralogical Society, Clay Minerals Group (in the Geology Department, The University, Southampton)—Meeting on "Clay Mineralogy of Sediments"

Society of Chemical Industry, Fine Chemicals Group and the Birmingham and Midland Section, in association with the University of Birmingham (at the University of Birmingham)—Symposium on "Fluoro-Organic Compounds"

Friday, March 29

Gas Chromatography Discussion Group (at the Royal Institution, Albemarle Street, London, W1)—Informal Symposium on "Gas Chromatography"

Institution of Electrical Engineers (joint meeting with the Automatic Control Group of the I Mech E , at Savoy Place, London, WC2), at 2 p m —Colloquium on "Building Models with Plant Data"

British Mycological Society (at the School of Pharmacy, 29–39, Brunswick Square, London, WC1), at 10 45 a m —Special General Meeting, 11 a m —General Papers

Monday, April 1

Institution of Electrical Engineers (at Savoy Place, London, WC2)—Colloquium on "Injection Luminescence"

Institution of Mechanical Engineers, Medical Engineering Working Party (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Kidney Machines—Technical, Medical and Social Problems"

Royal Society of Arts (at John Adam Street, Adelphi, London, WC2), at 6 p m —"Industry and Commerce" (Last of three Cantor Lectures on "Modern China")

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W1), at 6 p m —Dr R Davies "High Speed Forming of Metals"

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, SW1), at 6 30 p m —Dr N W Pirie, FRS "The Use of Novel Proteins in Human Food"

Monday, April 1—Wednesday, April 3

British Biophysical Society (at the University of Nottingham)—Meeting on "Biological Semiconductors"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer (preferably with special interests in geomorphology and meteorology) in Geography—The Director of Academic Studies, Trinity and All Saints' Colleges, Brownberrie Lane, Horsforth, near Leeds (March 27)

Senior Lecturer or Lecturer, Grade II (qualified to lecture to honours degree standard and with active research interests) in Geography—The Secretary, Sir John Cass College, Jewry Street, London, EC3 (March 28)

Senior Lecturer in Statistics—The Registrar, University College of Wales, Aberystwyth (March 30)

Senior Lecturer (Clinical) in the Department of Pathology, University of Birmingham—The Assistant Registrar, Medical School, Birmingham 15 (March 31)

Lecturer in Botany in the Department of Arts, Science and Education—The Registrar, University of London Goldsmith's College, New Cross, London, SE14 (April 1)

Lecturer or Assistant Lecturer (preferably with an interest in teaching sociology to students of applied science) in Sociology—The Registrar and Secretary, The University, Leeds, quoting Ref No 68/19/3 (April 1)

Temporary Assistant Lecturer or Temporary Lecturer (with a good honours degree in physics and successful school teaching experience) in the Department of Education—The Registrar and Secretary, The University, Leeds, quoting Ref No 68/15/3 (April 1)

Lecturer in Applied Sociology—The Secretary of the University Court, The University, Glasgow (April 3)

Lecturer (preferably with an interest in, and experience of, some branch of experimental botany other than physiology or biochemistry) in Botany—The Registrar (Room 39, O R B), The University, Reading (April 6)

Chair of Electrical Engineering, and Chair of Mechanical Engineering at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Malaya and London, April 8)

Lecturer (experienced graduate teacher of biology and competent to contribute to both the practical and academic work of the University Education Department) in Education—The Secretary, University of Durham, Old Shire Hall, Durham (April 10)

Lecturer (preferably candidate in numerical analysis) in Pure Mathematics—The Secretary, The University, Aberdeen (April 13)

Research Assistant (with a university degree in chemistry or biochemistry or an equivalent qualification) in Biological Chemistry, for work concerned with the biogenesis of membranes, under the direction of Dr Sargent—The Secretary, The University, Aberdeen (April 13)

Lecturer in Physics at the University of Cape Town—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (April 15)

Lecturer (science graduate) in Biochemistry at the University of Otago, Dunedin, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, April 15)

Chair of Electronic Engineering—The Registrar and Secretary, The University, Leeds, quoting Ref No 68/33/1 (April 17)

Research Assistant (with a good honours degree or M Sc in microbiology, bacteriology, botany or other appropriate subject) in Soil Microbiology in the Hartley Botanical Laboratories—The Registrar, The University, Liverpool, quoting Ref RV/397/N (April 22)

Chair of Agronomy at the University of Sydney, Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London, April 30)

Scientific Information Officer (with a degree in agriculture, ability to write good English and a flair for languages)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berkshire (May 15)

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Library Association Scientific Library Services—Report of the Association's Committee on Scientific Library Services Pp v+82 (London Library Association, 1968) [111

Ministry of Transport Road Research Laboratory RRL Report LR79 Cost of Road Accidents in Great Britain By R F F Dawson Pp 68 (Crowthorne Road Research Laboratory, 1967) *Gratis* [111

Mammals (Non-marine) Sixth edition (Instructions for Collectors, No 1) Pp v+55 (London British Museum (Natural History), 1968) 3*s* [121

Bulletin of Special Courses in Higher Technology, Management Studies and Commerce 1968, Part 2 Spring and Summer Terms Pp 153 (London Regional Advisory Council for Technological Education for London and the Home Counties, 1968) 8*s* 6*d* [121

Department of Education and Science The Proposed 300 GeV Accelerator Pp vi+84 (Cmnd 3503) (London H M Stationery Office, 1968) 7*s* 9*d* net [121

Ministry of Transport Road Research Laboratory RRL Report LR78 Hexagonal Motorway Networks By J C Tanner Pp 20+24 figures (Crowthorne Road Research Laboratory, 1967) *Gratis* [121

Department of Education and Science Education Pamphlet No 53 Safety at School Pp v+105 (London H M Stationery Office, 1967) [151

Proceedings of the Royal Irish Academy Vol 65, Section A, No 9 and 10 Note on a Heavy Current Discharge Tube with a High Temperature Capillary By T E Nevin and S O'Connor The Infrared Spectrum of CrH and CrD By S O'Connor Pp 93–112+2 plates 6*s* Vol 65, Section A, No 11 On the Partial Sums of Power Series By J Clunie and P Erdos Pp 113–124 3*s* 6*d* Vol 65, Section B, No 16 Weevils (Coleoptera, Curculionoidea) and other Insects Collected in North West Clare, with special reference to the Burren Region By M G Morris Pp 349–372+plate 13 5*s* Vol 65, Section B, No 17 Some Notes on the "Flying Falcon" Expedition of 1909, Off the South-West Coast of Ireland By C E O'Riordan Pp 373–384 2*s* Vol 65, Section B, No 18 Lower Ordovician Shelly and Groptolitic Faunas from South-Eastern Ireland By P J Brenchley, J C Harper and D Skevington Pp 385–390 1*s* 6*d* Vol 65, Section B, No 19 Pedal Combs of the Larvae of Dragonflies (Odonata) By N MacNeill Pp 391–406+plate 14 3*s* 6*d* Vol 65, Section B, No 20 A Woodland Population of *Apodemus sylvaticus* (L) at Seaforde, Co Down By J S Fairley Pp 407–424 3*s* 6*d* Vol 65, Section B, No 21 The Effect of Environmental Tenperature on Succinic Dehydrogenase Activity in the Ear Skin of the Rabbit By Cecily M Joyce Pp 425–436 2*s* (Dublin Royal Irish Academy, 1967) [151

Bulletin of the British Museum (Natural History) Entomology Vol 21, No 3 A Revision of the Holarctic Genus *Dikraneura* (Homoptera Cicadellidae) By W J Knight Pp 99–201 42*s* Geology Vol 15, No 4 A New *Tempskya* from Kent By Marjorie E J Chandler Pp 169–179+12 plates 30*s* (London British Museum (Natural History), 1967) [151

Theology's Central Problem By Professor John Hick (An Inaugural Lecture delivered in the University of Birmingham on 31st October, 1967) Pp 15 (Birmingham The University, 1968) 2*s* 6*d* [151

Quest for Greatness By Hubert H Humphrey Pp 52 (London United States Information Service, American Embassy, 1968) [151

The Royal Society United Kingdom Report on Research Activities in Physical Oceanography 1963–67 Pp 49 (British National Committee for Geodesy and Geophysics) (London The Royal Society, 1967) [151

Engineering Research and Undergraduate Teaching By Professor P N Rowe (An Inaugural Lecture delivered at University College London, 9 February 1967) Pp 15 (London H K Lewis and Co , Ltd , 1967) 5*s* net [151

Other Countries

United States Department of the Interior Geological Survey Bulletin 1241-F Geology and Petrology of the Greenvillle Quadrangle, Piscataquis and Somerset Counties, Maine By G H Espenshade and E L Boudette Pp v+60+plate 1 Bulletin 1250 Glossary of Uranium- and Thorium-Bearing Minerals By Judith W Frondel, Michael Fleischer and Robert S Jones Fourth edition Pp iii+69 $0 30 Water-Supply Paper 1594-E Artificial Recharge Through a Well Tapping Basalt Aquifers at The Dalles, Oregon By B L Foxworthy and C T Bryant Pp iv+55+plate 1 $0 55 Water-Supply Paper 1662-D Specific Yield—Compilation of Specific Yield for Various Materials By A I Johnson Pp v+74 $0 30 Water-Supply Paper 1832 Hydrology of the Valley-Fill and Carbonate-Rock Reservoirs, Pahrump Valley, Nevada—California By Glenn T Malmberg Pp iv+47+plates 1–5 Professional Paper 524-I Bedrock Geology of the Shin Pond and Stacyville Quadrangles, Penobscot County, Maine By Robert B Neuman Pp iii+37+plates 1–3 (Washington, D C Government Printing Office, 1967) [111

Siebenundzwanzigstr Jahresbericht der Schweizerischen Gesellschaft fur Vererbungsforschung, Société Suisse de Génétique (S S G), 1967, mit Unterstützung der Julius Klaus-Stiftung fur Vererbungsforschung, Sozialanthropologie und Rassenhygiene in Zürich Herausgegeben von Ernst Ochler (Separatabdruck aus Archiv der Julius-Stiftung fur Vererbungsforschung, Sozialanthropologie und Rassenhygiene, Band XLII, 1967, Heft 3/4) Pp 102 (Zurich Art Institut Orell Fussli AG, 1967) [151

United States Department of the Interior Geological Survey Bulletin 1241-G Bedrock Geology of the Chepachet Quadrangle, Providence County, Rhode Island Pp iv+26+plate 1 Bulletin 1241-I Debris Flow and Erosion Control Problems Caused by the Ash Eruptions of Irazu Volcano, Costa Rica By Howard H Waldron Pp iv+37+plate 1 $0 55 (Washington D C Government Printing Office, 1967) [151

Biochemical Genetics Vol 1, No 1, June 1967 Pp 1–71 Published quarterly Annual subscription $18, Outside USA and Canada add $1 80 for postage and handling (New York Plenum Press, 1967) [151

State of California Department of Fish and Game Fish Bulletin 137 Reproduction, Life History, and Ecology of the Round Stingray, *Urolophus halleri* Cooper By John Stanley Babel Pp 104 (Sacramento Department of Fish and Game, 1967) [151

United States Department of the Interior, Fish and Wildlife Service Technical Papers of the Bureau of Sport Fisheries and Wildlife No 5 Ecology of Gila Trout in Main Diamond Creek in New Mexico By Danny M Regan Pp 24 No 10 Marking Fish with Dyes and other Chemicals By Dean E Arnold Pp 44 (Washington; D C Government Printing Office, 1966) [151

United States Department of the Interior Geological Survey Water-Supply Paper 1829 Swatara Creek Basin of Southeastern Pennsylvania—an Evaluation of its Hydrologic System By W T Stuart, William J Schneider and James W Crooks Pp vii+79+plates 1–3 (Washington, D C Government Printing Office, 1967) [161

Commonwealth of Australia Department of National Development Bureau of Mineral Resources, Geology and Geophysics 1 250,000 Geological Series—Explanatory Notes Winton Qld , Sheet SF/54–12 International Index Compiled by D J Casey Pp 11+map Maneroo, Qld , Sheet SF/54–16 International Index Compiled by W Jauncey Pp 11+map (Parkes, ACT Bureau of Mineral Resources, Geology and Geophysics, 1966 and 1967) [161

New South Wales Department of Mines Geological Survey Report No 48 Cullulla Silver-Lead Prospect By D W Suppel and A G Loudon Pp 12 The Mineral Wealth of New South Wales Pp 16 (Sydney Department of Mines, 1967) [161

Bibliographien des Deutschen Wetterdienstes, Nr 20 Agrarmeteorologische Bibliographie 1965 Bearbeitet von Maximilian Schneider Pp xiii+208 (Offenbach a M Selbstverlag des Deutschen Wetterdienstes 1967) [161

Republic of Botswana Ministry of Commerce, Industry and Water Affairs Annual Report of the Geological Survey Department for the year ended 31st December 1966 Pp 44 (Lobatsi Director, Geological Survey, 1967) 50 cents [171

Honeywell Computer Journal, Vol 1, No 1, Fall 1967 Pp 51 (Wellesley Hills, Mass , and London Honeywell, Inc , 1967) [191

Museum of Comparative Zoology, Harvard University Breviora No 271 (17 November, 1967) *Chelonia depressa* Garman Re-investigated By Ernest E Williams, G C Grandison and Archie F Carr, Jr Pp 15 (2 plates) No 272 (17 November, 1967) The Ecological Distribution of the Anoline Lizards Around Kingston, Jamaica By A Stanley Rand Pp 18 No 272 (17 November, 1967) Studies on Neotropical Pompilidae (Hymenoptera) 3 Additional Notes on *Epipompilus* Kohl By Howard E Evans Pp 15 No 274 (17 November, 1967) The Genus *Nops* (Araneae, Capiniidae) in Panama and the West Indies By Arthur M Chickering Pp 19 No 275 (17 November, 1967) A New Scincid Lizard from the Northern Solomon Islands By Allen E Greer and Fred Parker Pp 20 No 276 (24 November 1967) The Generic Relationships of the African Scincid Genus *Eumecia* By Allen E Greer Pp 9 No 277 (24 November, 1967) *Lestidium bigelowi*, a New Species of Paralepidid Fish with Photophores By Michael J F Graae Pp 10 No 278 (24 November, 1967) Notes on the Emberizine Sparrow *Rhynchospiza stolzmanni* By Raymond A Paynter, Jr Pp 6 No 279 (24 November, 1967) A Second Skink with Fragmented Head Scales from Bougainville, Solomon Islands By Allen E Greer and Fred Parker Pp 12 (Cambridge, Mass Museum of Comparative Zoology, Harvard University, 1967) [191

Bulletin of the American Museum of Natural History Vol 137 The Beginning of the Age of Mammals in South America, Part 2 By George Gaylord Simpson Pp 259+46 plates (New York American Museum of Natural History, 1967) $15 [191

United States Department of the Interior Geological Survey Water-Supply Paper 1839–M A Comparison of Methods of Estimating Potential Evapotranspiration from Climatological Data in Arid and Subhumid Environments By R W Cruff and T H Thompson Pp iv+28 $0 15 Professional Paper 515 Paleotectonic Investigations of the Permian System in the United States By E D McKee and S S Oriel Pp xiii+271+plate 1 Professional Paper 575–D Geological Survey Research 1967, Chapter D Pp 297 (Washington, D C Government Printing Office, 1967) [191

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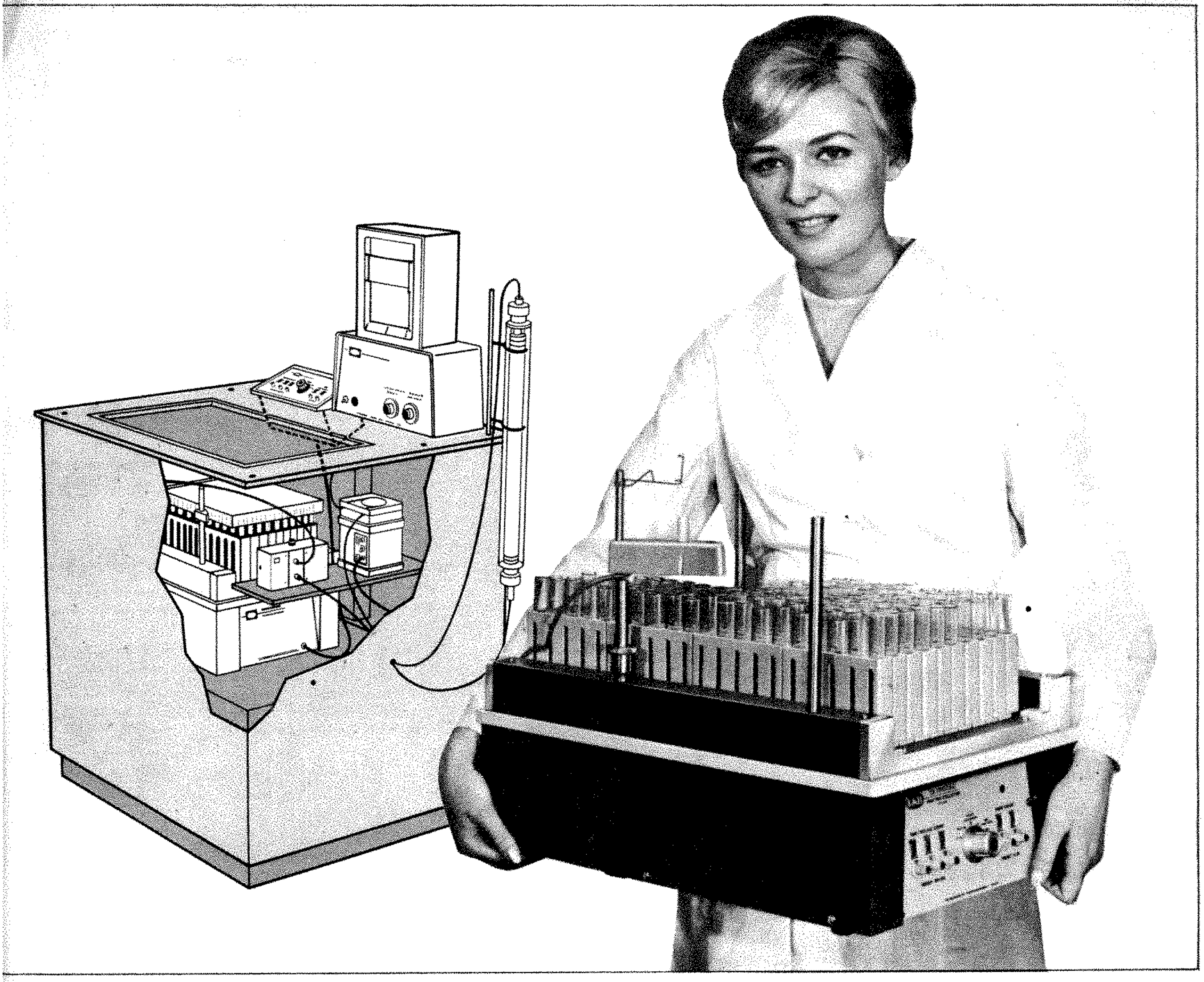

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APPOINTMENTS VACANT

UNIVERSITY OF OXFORD

LECTURER IN ZOOLOGY AND CURATOR OF THE ZOOLOGICAL COLLECTIONS

The University proposes to appoint a Lecturer in Zoology and Curator of the Zoological Collections (which do not include insects). The salary will be according to age on the scale £1,470 to £2,900 (with F.S.S.U.).

Applications (six copies, except in the case of overseas applicants who need only submit one copy), including a statement of age, qualifications and experience, and giving the names of two referees, should be sent to the Secretary of Faculties, University Registry, Broad Street, Oxford, not later than April 27, 1968. The Lecturer and Curator will be expected to take up office as soon as possible after October 1, 1968.

The Lecturer will be responsible to the Linacre Professor for the care of the Zoological Collections in the University Museum and in the Department of Zoology. He will be required to arrange the supply of material and the preparation of displays and other aids for teaching and research, to cooperate in the teaching and administrative work of the Department of Zoology in both term and vacation under the direction of the Linacre Professor, and to engage in advanced study and research. (X1184)

UNIVERSITY OF IFE

NIGERIA

Applications are invited for

(i) Two Senior Lectureships and two Lectureships in CHEMISTRY. For the Senior Lectureships candidates should have special interests in Physical Chemistry or in Organic Chemistry and National Products. For the Lectureships preference will be given to candidates with special interests in Physical and Inorganic Chemistry.

(ii) Professorship and Senior Lectureship in ZOOLOGY. Candidates with interests in any fields (except parasitology) considered; an interest in vertebrate biology particularly welcomed.

Salary: Professor £N3,000 p.a., Senior Lecturer £N2.275 to £N2,575 p.a., Lecturer £N1,200 to £N2,175 p.a. (£N1 = £1 3s. 4d. sterling). Salary supplementation in range £240 to £600 p.a. (sterling) in appropriate cases under British Expatriate Supplementation Scheme. Superannuation; family passages; various allowances; annual U.K. leave where applicable.

Detailed applications (six copies), naming three referees, by April 23, 1968, to Inter-University Council, 33 Bedford Place, London, W.C.1, from whom particulars are available. (1137)

HOUGHTON POULTRY RESEARCH STATION

(Financed jointly by the Agricultural Research Council and the Animal Health Trust)

PHYSIOLOGY AND BIOCHEMISTRY DEPARTMENT

ENGINEER required to assist with the development of controlled environment systems for experimental work with poultry and to supervise the ventilating and heating plant in a new isolation unit. Candidates should have H.N.C. in mechanical or electrical engineering, or an equivalent qualification, and training in technical drawing and workshop practice. Experience of air-conditioning systems in buildings, ships, or aircraft would be an advantage. Appointment in the Experimental Officer grade (£1,365 to £1,734 per annum) according to age, qualifications, and experience.

Further particulars and application forms from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, to whom completed application forms should be returned by March 31, 1968. (1172)

CSIRO
AUSTRALIA

RESEARCH SCIENTIST

ARCHITECTURAL ACOUSTICS

DIVISION OF BUILDING RESEARCH

GENERAL The Division of Building Research of the Commonwealth Scientific and Industrial Research Organization, located at Highett, Victoria, is primarily responsible for long-term research on building and associated engineering. Particular attention is given to studies of building materials and design, thermal environment, tropical building, operational research and architectural acoustics.

DUTIES The appointee will be required to take part in the Division's study of architectural acoustical problems and to initiate such studies especially in noise control and room acoustics both in the field and in the laboratory and to give advice to industry.

QUALIFICATIONS Applicants should have a Ph.D. degree in Physics, Engineering or Mathematics, or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability.

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Senior Research Scientist, $A,6892–$A7,974 p.a., or Principal Research Scientist, $A8,242–$A9,490 p.a. Salary rates for women are $A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

CONDITIONS The appointment, which carries with it Commonwealth Superannuation Fund or Provident Account privileges, will be conditional upon passing a medical examination. An initial probationary period of twelve months may be specified. Fares paid for the appointee and his dependent family. Further particulars supplied on application.

Applications (quoting Appointment No. 390/388) stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, by the **19th April, 1968.** (1162)

Headquarters Allied Forces Central Europe

BRUNSSUM **THE NETHERLANDS**

Vacancy for a

Chief of Operations Research Unit

1. Applications are invited for the post of Chief of Operations Research Unit (NATO-A-7), which will be vacant from 1st July, 1968, at the Headquarters Allied Forces Central Europe, Brunssum, the Netherlands.

2. The duties required will be:

a. to initiate and direct scientific studies of weapons, tactics, equipment and strategy designed to furnish a quantitative basis for command of decisions.

b. to provide the Command with ready and informal access to scientists with specialized training in the techniques applicable to the analysis of modern warfare.

c. to maintain a close liaison with other national and international Operations Research and Analysis organizations.

d. to act as Scientific Adviser to the Commander, AFCE and to keep him and his staff informed of scientific and technical developments related to the mission of the Command.

3. Candidates for this post must have a university degree of good standing in science, preferably in physics, mathematics or a related subject. Candidates must have had some years experience of Operations Research, preferably in the field of air warfare and should have achieved some official standing of reputation in their own countries in this work. Familiarity with the problems of strategic offence, air defence, tactical support and especially with guided weapons and electronics as applied to these fields would be added qualifications. Fluency in one of the NATO official languages is essential; a working knowledge of the other is desirable.

(1171)

AGRICULTURE

General Agriculturists and agricultural specialists of many kinds are frequently required in the effort to match the large demand from the developing countries The vacancies advertised below are a selection from the many openings which exist now While they are being filled, other vacancies are arising

Salaries within the ranges quoted are assessed on qualifications and experience, and the terms of most appointments include free or subsidized accommodation, education grants, family passages, good leave on full pay, etc A contributory pension scheme is available in certain circumstances

Most appointments are limited to nationals of the United Kingdom or the Republic of Ireland who are normally resident in those countries

BOLIVIA

Pasture Research Officer (British Tropical Agricultural Mission). RC 213/22/03

Duties To continue a programme of research on intensive grasses in crop rotations and to advise and work in collaboration with Bolivian technicians on experiments, extensions, seed multiplications, field surveys, etc , with natural and cultivated pastures

Qualifications and Terms An honours degree in botany with post graduate experience of range management in the tropics and of work on intensive grasses A working knowledge of Spanish is desirable Salary £2,500–£3,100 a year, depending on qualifications and experience, subject to British Income Tax, plus a variable non-taxable overseas allowance currently payable at rates from £540–£1,310 a year depending on marital status Contract 2 tours of 18 months

Head of British Mission for Tropical Agriculture RC 213/22/01

Duties To lead team of Agricultural experts in current important programme and to be Adviser to Bolivian Government in Tropical Agriculture

Qualifications and Terms A relevant degree and wide experience of agricultural administration in Tropics at least at Deputy Director level Salary £4,500–£5,000 a year subject to British Income Tax plus a variable non-taxable overseas allowance currently payable at rates from £540–£1,310 a year depending on marital status plus entertainment allowance of £250 a year. Contract 2 tours of 18 months

MALAWI

Senior Agricultural Officer (Irrigation) RC 213/134/015

Duties To work as part of an irrigation team

Qualifications and Terms A degree in agriculture with at least ten years experience, preferably under tropical conditions Salary £2,655 a year plus 15%–25% terminal gratuity A supplement of £100 is also payable direct to an Officer's bank account outside Malawi and Rhodesia Contract 2–3 years

FALKLAND ISLANDS

Grasslands Officer RC 213/60/01

Duties To investigate and experiment on development and improvement of grasslands and possibly to establish a small experimental station

Qualifications and Terms A degree in agriculture with experience in hill grazing improvement Salary £1,500–£2,000 a year 15% terminal gratuity Contract 30–36 months

FIJI

Soil Conservation Officer RC 213/62/07

Duties Supervision and maintenance of technical standards in soil conservation work, by liaison with District Agricultural Officers who administer all soil conservation extension, machinery and field staff Arrange in-service training in soil conservation for field staff Prepare lectures and examinations for the soil conservation course at the Fiji School of Agriculture Assist in preparation of soil conservation publicity Advise on soil conservation matters through the Director of Agriculture or through the Land Conservation Board

Qualifications and Terms A degree in Agriculture or Science with experience in practical soil conservation Experience in tropical agriculture is desirable Salary £ Fijian 1,092–2,181 a year (£ Sterling 1,045–2,087 a year) An inducement allowance at present in the range of £ Sterling 508–829 a year is also payable Terminal gratuity 25% of basic salary and inducement allowance Contract 1 tour of 30–36 months

Soils Scientist RC 213/62/09

Duties To carry out soil surveys on a detailed regional basis to follow on the reconnaissance survey already completed and to be responsible for field soil survey work on proposed development areas

Qualifications and Terms A 1st or 2nd class Honours degree in Geology or Geography and postgraduate experience in soil surveys in tropical areas Salary £ Fijian 1,092–2,181 a year (£ Sterling 1,045–2,087 a year) An inducement allowance at present in the range of £ Sterling 508–829 a year is also payable Terminal gratuity 25% of basic salary and inducement allowance Contract 1 tour of 30–36 months

SABAH

Agricultural Research Officers (Plant Breeders) RC 213/150/03

Duties To deal with the selection and breeding of oil palm and cocoa throughout the State of Sabah

Qualifications and Terms An Honours degree in Natural Science with 2 years post-graduate training A good general knowledge of the subject is essential Field experience desirable Salary $9,000–17,040 a year (£ Sterling 1,226–2,321 a year) plus an inducement allowance at present in the range of £ Sterling 804–1,303 a year Terminal gratuity approximately 20% of basic salary and inducement allowance Contract 2–3 years

Agronomist RC 213/150/04

Duties To manage a cocoa or oil palm research station, to investigate cultivation and fertilizer applications and to implement the departmental research programme

Qualifications and Terms A degree in natural science with two years postgraduate training Salary $9,000–17,040 a year (£ Sterling 1,226–2,321 a year) plus an inducement allowance at present in the range of £ Sterling 804–1,303 a year Terminal gratuity approximately 20% of basic salary and inducement allowance Contract 2–3 years

TANZANIA

Agricultural Economist RC 213/267/011

Duties To lead the Planning and Investigation Unit of the Ministry of Land Settlement and Water Development in the examination and valuation of settlement and irrigation schemes and to train officers engaged on these schemes

Qualifications and Terms A degree in economics with agricultural qualifications or an honours degree in agricultural economics plus some previous experience Basic salary scale E A Shs 33,200–36,000 a year (£ Sterling 1,936–2,100) liable to Tanzanian income tax In addition an inducement allowance, normally tax free, at present in the range of £ Sterling 1,113–1,140 a year will be paid by the British Government direct to an officer's bank account outside East Africa Terminal gratuity 25% of basic salary and inducement allowance Contract 21–27 months

ST. HELENA

Agricultural and Forestry Officer RC 213/151/01

Duties To take charge of the Department which is responsible for forests, grazing and arable land

Qualifications and Terms A degree or diploma in agriculture preferably with overseas experience Salary £1,529–£2,117 a year 12½% terminal gratuity Contract 2 years

For further particulars of these vacancies and an application form please write giving your full name, age and brief details of professional qualifications and experience, and quoting appropriate reference number, to the Appointments Officer, Room 324/N/15, Ministry of Overseas Development, Eland House, Stag Place, London, S W 1.

MINISTRY OF OVERSEAS DEVELOPMENT

(1151)

1966 English China Clays group

SCIENTISTS

The Research Department of English Clays Lovering Pochin & Co. Ltd., the major operating Company of the English China Clays Group and the world's largest producer of China Clay, has a number of vacancies for Scientists and Assistants between 18 and 30 years of age.

CHEMICAL ENGINEERING

Two young graduates are required in the Chemical Engineering Section, in which a wide range of work is undertaken Particular emphasis is laid on research into the processing of fine particles Previous experience is not essential for either of these posts and the discipline of the degree is less important than a genuine enthusiasm for research

PHYSICISTS

Two graduate physicists are required, one for work on particle size measurement of fine powders to study the principles involved and various semi-automatic measuring methods The other would work in the field of mineral studies using electro and X-Ray diffraction and various associated techniques

CHEMISTRY

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SCIENTIFIC ASSISTANTS

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The minimum qualifications required for these positions is likely to be Ordinary National Certificate or equivalent For one of the posts, experience in the Building Industry, with particular reference to concrete aggregates, would be useful

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The well established and expanding Research Department is concerned with all aspects of Research into the nature of China Clay and the Company's production processes China Clay production now exceeds two million tons per annum and over 75% of this is exported The principal industries served are Paper and Ceramics, but China Clay is also used in a wide variety of other manufacturing Industries The Research Department is housed in new Laboratories at St Austell, near the South Cornish Coast

Salaries for the above appointments will be commensurate with age, qualifications and experience

Pension and Life Assurance Schemes operate through the Group, together with a Profit Participation and Long Service Award Scheme Assistance is given towards furniture removal expenses

Please apply in writing, giving brief details of age, qualifications, experience and present salary to

The Company Secretary,

ENGLISH CLAYS LOVERING POCHIN & CO. LTD.,

John Keay House, St Austell, Cornwall

(1196)

NEW ZEALAND

Marine Department

Applications are invited for the undermentioned vacancies

Vacancy B 13/13/17/757

THREE SCIENTIFIC OFFICERS

The Fisheries Research Division of the Marine Department requires three Scientific Officers, two for research on Mollusca and Demersal Fish and one Biometrician for work in population dynamics

Qualifications desired Applicants should have a degree with 1 or 2A Honours in Zoology or Mathematics in the case of the Biometrician

Salary Appointments will be made in the range NZ$2 790 for first-class Honours NZ$2 900/NZ$3 350 for a newly qualified Ph D, and up to NZ$5 670 for suitably experienced scientists

Passages Fares for appointee and his wife and family, if married will be paid

Incidental expenses Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S W 1, with whom applications will close on April 22, 1968

Please quote reference B 13/13/17/757 when inquiring (1136)

NEW ZEALAND

Marine Department

Applications are invited for the undermentioned vacancy

Vacancy B 13/13/19/759

SCIENTIST

The Research Division of the New Zealand Marine Department requires a Scientist for their Wellington Research Laboratory, the appointee to take up duty as soon as possible Work is on freshwater fisheries and allied limnological problems, and the qualifications desired are an Honours degree or Ph D

Appointments will be made in the salary range NZ$2,790 for first-class Honours and up to NZ$3,350 for a newly qualified Ph D

Passages Fares for appointee and his wife and family, if married, will be paid

Incidental expenses Up to £35 for a single man and £100 for a married man can be claimed to cover the cost of taking personal effects to New Zealand

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S W 1, with whom applications will close on April 24, 1968

Please quote reference B 13/13/19/759 when inquiring (1135)

UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of a RESEARCH ASSOCIATE ELECTRON-MICROSCOPIST to join a Cell Research Group engaged in investigating the nature of the adhesive mechanism at the cell surface The research group is at present supported by the Science Research Council but it is due to be taken over by the College The salary is in the post-doctoral range equivalent to an Assistant Lectureship (£1,105 to £1,340) with F S S U provision

Applications, with the names of two referees and enquiries for further information should be sent to Professor B M Jones, Department of Zoology, University College of Wales, Aberystwyth (1193)

NATURE Volume 217
MARCH 30, 1968

Scandal in the Schools

THE compromise which has been reached between the Schools Council and the Standing Conference on University Entrance (see page 1199) is about the only device by means of which an agreement on the sixth-form curriculum in Britain can be reached within the next few years, which is something in its favour With any luck, the working party which is to be set up jointly between the two organizations may even be more successful and decisive than the sheaf of committees which occupy the time of the Schools Council and its servants The result could well be that all concerned will be persuaded that the time has finally come for radical change—and that the schools representatives will somehow be persuaded that the universities are at least half serious when they declare their belief in a broader—not a narrower—pattern of studies in the sixth form The danger, of course, is that the new committee will be used not as a launching pad for a new pattern of teenage education but as a device for muffling honest disagreement and for stifling honest discontent That is why it is important to remember that, even though the formation of the new working group may seem to some to be a remarkable development, it is in the last analysis merely a compromise between reason and obscuranticism It is not in itself a reform but a kind of bran tub from which, with good fortune, reform may yet be snatched.

In the circumstances, a little recrimination is not entirely out of place The conflict which came to a head and a temporary resolution last week has been building up for several years To be fair, the formation of the Schools Council four years ago has helped to strengthen concern among all kinds of educationists for the balance between the schools and the universities In the schools, perceptive teachers were made painfully aware of two quite separate kinds of problems For one thing, some academics among the teachers were conscious of how the increasing difficulty of maintaining what are called academic standards in the face of the increasing pressure of the examination syllabuses and the competition to enter the universities Others, however, were more conscious of the rapidly growing population of the traditional sixth-forms, and of the growing diversity of the interests and capabilities of their older students

To meet these quite separate considerations, the Schools Council has helped the schools to hammer out in quick succession two quite unsuitable and even unacceptable compromises First, two years ago, there was a scheme for a mixed bag of sixth-form courses, with some full-length courses as at present and some half-length courses to permit some degree of broadening in the curriculum To the extent that this device would have allowed the two groups of interested parties in the schools to go their separate ways, some progress seemed to have been made The trouble, of course, was that even if the scheme had been workable—and nobody ever really spelled out whether a half-course was to be regarded as a partial substitute for or a complement to a full course—the educational equivalent of Gresham's law would have ensured that sixth form students would enjoy the worst of both worlds. This is why it is still something of a puzzle that the Schools Council was brash enough to substitute for this scheme, in its proposals published in the autumn last year, a curious suggestion that the pressure of competition for university places would somehow be diminished if most students bent on going on to higher education were to be examined in only two subjects, and not in three as is at present usually the case This is the proposal which the universities seem now gently but firmly to have rejected and it is disingenuous of the Schools Council to suggest that the whole fiasco has served the useful purpose of persuading lions and lambs to lie down together The truth is that the council has seriously damaged its credibility, as the saying goes Nobody can now take very seriously a body which can so seriously misjudge not merely the temper of the universities with which it must negotiate, but even the interests of education with which it is supposed chiefly to be concerned

If therefore the formation of the working party is a hopeful sign, there is also a danger that its work will be hampered by the pettifoggery which has marked the doings of the Schools Council in the past few years In particular, there is a danger that the working party will somehow be so impressed with the solemnity of the task on which it is embarked that it will perpetuate the spirit of compromise in which it has begun, whenever possible, substituting experiment for decision Yet the problems of the transition from secondary to higher education, though formidable, are simple enough, and enough is known about them for the general framework for workable solutions to be established quickly

Quite simply, British schools should be so organized that it is not necessary for potential academics to declare themselves at a tender age In particular, there should be no reason why a student at school should be compelled to decide several years in advance which kinds of courses he will follow at his university Briefly, schools should aim to provide a general education, but this is not the same thing as saying that scholarship should be banished or even that study in depth should be made impossible but merely that premature specialization should be outlawed This is the central point in the report of Dr F S Dainton's committee on the recruitment of young people into science and technology He and his colleagues seem to have convinced themselves that the supply of technical people in Britain would be healthier if they were not invited to don intellectual straitjackets at too early an age More generally, however, the case for a broader curriculum must rest on broad educational principles The narrow curriculum in the British sixth forms is just plain wrong, and must be reformed Whether the outcome should consist of the study of four or five subjects, as Professor J T Allanson and Mr J S Morrison have suggested, or whether instead there should be a more rigidly broad pattern (as in France) or a general course of studies in which a variety of special subjects are embedded (as in the United States) is something to be argued out The plain truth, which no amount of experiment in the schools can conceal, is that there must be a radical change in the pattern

But what will be the consequences of these necessary reforms ? It is, of course, apparent that no radical upheaval in the sixth form would be possible without important changes in the pattern of higher education All kinds of unfamiliar possibilities are on the cards—four year courses, transformed and reformed public examinations, better means for helping students pick the careers that suit them best But most of what seem to be the complications of a broader school curriculum are probably necessary in their own right in any case

Science after the Cultural Revolution

CHINA's target in science, announced in January 1966, was to catch up, on a wide front, with the technologically advanced countries during the next twenty to thirty years The cultural revolution has changed these priorities—the new policy calls for a greater concentration of scientific effort on the problems peculiar to the development of China, and "self-reliance" seems to be the order of the day This was the picture of science and technology in China after the cultural revolution which Dr C H G Oldham, of Sussex University, pieced together, from reports in the Chinese press, in his Cantor lecture delivered last Monday to the Royal Society of Arts

It is beyond dispute that since the establishment of the People's Republic in 1949, the Chinese have been curiously reticent about publicizing their scientific and technological achievements and their expenditure on science and technology Information has been hard to come by and now the cultural revolution has almost completed the blackout No Chinese scientific journals have been received in Britain since October 1966, and the last British scientist to visit China on the Royal Society's exchange programme returned to Britain in November 1966 With announcements in the Chinese press as the only source of information, any assessment of the effect of the cultural revolution on science can at best be only fragmentary and highly subjective But even so it is worthwhile, and Dr Oldham has succeeded conspicuously well

Between 1949 and 1966 China appeared to be making steady if not spectacular progress in scientific development over a broad front The Government in Pekin clearly accepted that the development of economic and military strength depends on science and technology The Chinese Academy of Sciences, the Academy of Agricultural Science and the Academy of Medical Sciences were all expanded, and the explosion of nuclear weapons was a proof of capability in nuclear technology Great emphasis was given to science and technology at all levels of education, and it has been estimated that, by 1967, China had 1 3 million college trained scientists and engineers British scientists returning from China agreed that, on the whole, their Chinese colleagues were well-informed about science elsewhere and that they had a comprehensive supply of literature and an ample supply of domestic and imported equipment And with a few exceptions, such as the synthesis of insulin, the Chinese scientists seemed to be laying the vital, but necessarily unspectacular, foundations for long-term research programmes in basic and applied science No doubt more rapid expansion would have been feasible if senior scientists had not been so scarce As things have turned out, the only real distinction between the Chinese effort and that of other countries at a comparable stage in development has been the tremendous effort which the Chinese have made to teach science to the whole population, not just to an elite The Chinese believed this grass roots policy might lead to discarding old superstitions and to experiment and innovation at all levels of society No doubt there were failures but these were far outweighed by the successes

What has happened since the beginning of the cultural revolution in the summer of 1966 ? According to Dr Oldham, the Chinese Academy of Sciences and some of its institutes have suffered from the excesses of the revolution Fighting broke out in several institutes between supporters and opponents of the revolution The opponents, followers of Mr Liu Shao Chi, had gained the ascendancy in the academy in 1960–61 In Pekin in April 1967 they were accused of promoting research aimed at restoring what was ancient and worshipping what was foreign, of advocating research that was theoretical and divorced from the needs of the country, of supporting the award of academic degrees and scholastic titles such as "professor", and of asking for high salaries for scientists and special awards for those who had made special contributions By implication the pro-Mao group opposed all these policies

As a result of the revolution, a revolutionary committee now runs the academy, and for the first time the military is represented there But the former president and two vice-presidents have kept their positions and work has apparently gone on Several achievements in basic and applied science have been announced in the press since 1966 There has been an expedition of one hundred scientists to the area of Mt Everest, a large radio telescope—begun in 1958 but stopped by the pro-Liu group in 1960—has been rushed to completion ready for the period of intense solar activity in 1968, an all-Chinese digital computer has been developed and a new fossil skull of Pekin man found. It may be that the Chinese have also perfected a gas centrifuge technique for the production of uranium 235. Obviously, in spite of being larded with and attributed to the thoughts of Mao Tse Tung, both applied and basic science have continued through the upheaval. And Dr Oldham made the interesting point that there is nothing wrong with Mao Tse Tung's scientific maxims. When they are stripped of their jargon they boil down to such truisms as "Do not be overawed by what authorities in science say, be willing to challenge their beliefs". No one would quarrel with this. Naturally enough the maxims are aimed at the mass of a poorly educated population and they may not be a great benefit to well educated scientists, but they would not do much harm either.

But although science has not been stopped by the cultural revolution there has been a distinct change of emphasis. Any country in China's position trying quickly to apply science and technology to its economy faces two problems. What technologies are most appropriate for its stage of development and where should the technologies come from ? The country can either try to develop the most advanced technology, even though it is expensive and uses little manpower, or it can use less sophisticated technology and more labour. Equally it can import a technology or try to do its own research and development. As events in most developing countries have shown, striking the right balance between these alternatives is extremely difficult. Before the cultural revolution, China seemed prepared to import science and technology. As Dr Oldham interprets it, the main conflict of the cultural revolution, as far as science and technology is concerned, has been about the extent to which the Chinese should become self-reliant. If economic development were the sole criterion, the policies ascribed to Liu and his followers, who were prepared to rely heavily on imported science and technology, were probably right. But if total development—economic, social and political—is the aim, the self-reliance policy of the revolution makes sense. The new policy is more introspective and isolationist, but it is likely to result in a greater concentration of scientific and technological resources on problems primarily relevant to China's development. That is a loss to world science and to individual Chinese scientists who have lost some freedom of research and will no longer be competing on a world stage. For this reason it is crucial that formal contacts, such as between the Royal Society and the Chinese Academy of Sciences, be maintained and if possible expanded. But Dr Oldham is probably right in saying that, for China as a whole, what has happened may be a gain.

Future Foretold

from our Special Correspondent, Gif-sur-Yvette

THROUGHOUT this week an unusual meeting has been taking place near Paris. At an elegant chateau which forms part of the French National Centre for Scientific Research (CNRS) at Gif-sur-Yvette, experts from all over France—and some from other parts of the world—have been discussing what the world will be like in the year 2020. To keep the discussion within sensible limits, it has been set in a geographical context so that each day has been spent discussing the way in which different technological developments will affect the way land is used. It is clear that this meeting, known as the Symposium on Urban Planning and Advanced Techniques, is intended to be the first of a series which could, no doubt, last long enough for the results of this meeting to be compared with the reality of 2020. But the discussions in the past week have been more limited; they have been restricted to developed countries and have covered five broad topics—power and natural resources, data processing and transmission, communications and telecommunications, agriculture and rural space, and urbanization.

The inspiration for the meeting seems to have derived from Mr Jerome Monod, a French government official responsible for regional development and planning. But the origins are obscure, and it is probable that Mr Jacques Bloch-Morhange, a man with a catholic range of interests including, it is said, the breeding of racehorses, had something to do with it. Mr Bloch-Morhange is acting as secretary-general of the conference. The organizing committee of five, drawn from universities and government service, did most of the preparatory work which included commissioning studies by experts in the subjects concerned. The immediate practical benefit seems to be to help the French Government to formulate the Sixth Plan, but the organizers also see it as a way of helping administrators to avoid making decisions which are immediately overtaken by events.

The conference has been conducted at a philosophical level divorced in the main from immediate considerations such as those of finance. It was possible, for example, for the greater part of a discussion on the benefits of data processing to be completed before anybody asked if the financial investment in France would be great enough to sustain the hopes which had been expressed. But the discussion had not been futuristic—if anything the estimates of power and natural resources had erred on the side of conservatism. To be told that by 2020 half the electricity consumed would be generated by nuclear power, with the other half derived from hydrocarbon sources, astonished nobody. To this extent, the predictions have been unexceptionable, even unadventurous; but that they will help to formulate policies in the next five or ten years is undeniable. Much will depend on those who have to write the final report and draw together the results of what has been rather diffuse discussion. While it has been an interesting exercise, it is not immediately clear how the broad policy implications suggested by the week's discussion can be implemented in the face of the financial restrictions which normally determine events.

Donnish Productivity

BRITISH universities, enlivened no doubt by the scrutiny to which they are now subjected by the Comptroller and Auditor General, have been discussing the efficiency of their operations. To judge from the discussion among university delegates to a conference called jointly by the Committee of Vice-Chancellors and the Association of University Teachers on March 21, the cause of university productivity has made some worthwhile advances since the Committee of Vice-Chancellors first took an official interest in the subject two years ago. It is hard, however, to tell precisely how the universities reacted to some of the proposals now being made, for the universities seem to be as jealous of the privacy of their discussions as of any other academic freedom.

The conference on March 21 was opened by Mr C. F. Carter, vice-chancellor of the University of Lancaster and the British prophet of university productivity. The essence of his case was that teachers, and the way in which their work is organized, are more immediate limitations of the output of qualified students from universities than are buildings and other kinds of equipment, so that "increases of productivity are therefore most likely to be achieved by a better organization of labour or by harder work". Both in teaching and in research, according to Mr Carter, better productivity could be obtained by having fewer small departments, by not using highly trained staff for work that could be done by others, by spending more capital on educational technology, by organizing staff time more efficiently and by working harder. "And why not ? American academics do. It would help if more payment was on piece rates." Mr Carter would also like to see a redefinition of the division between teaching and research, and he considers that the more efficient use of buildings would make it possible for universities to double student numbers "even before using such obvious expedients as lengthening the very short teaching day". In Mr Carter's view, the university year should be organized on a staggered pattern with each student spending two out of three equal terms at the university each year. He wants to see more sharing of expensive equipment, a reduction of committee work and "a drastic reduction in the circulation of paper".

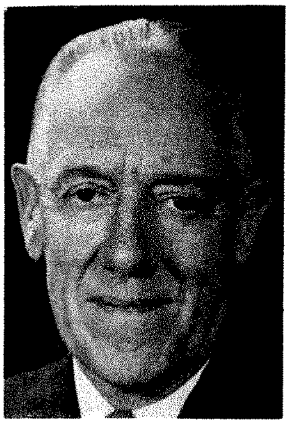
How did all this go down ? Several speakers at the conference were more concerned than Mr Carter with the philosophical definition of what is meant by university productivity. Something of this sort may eventually come from the work of Mr K. S. Davies, seconded to the Committee of Vice-Chancellors as a research officer, who is engaged on a long-term study of the factors which affect university productivity. Dr M. Blaug, of the University of London Institute of Education, took a still longer look at the problem of measurement, and was particularly anxious that the concepts of productivity and efficiency should not be confused. He would like there to be a continuing comparison of scores in student attainment tests at the beginning and at the end of university courses, studies by educational psychologists of the effectiveness of different teaching methods, more information about the socio-economic character of students and about their economic prospects, and some attempt to construct objective yardsticks for the evaluation of research.

It seems that only a minority of those present thought that studies of this sort were undesirable. One count showed that only three out of twenty-four speakers were against, with the rest in favour. This marks a great change of opinion in the past five years. It even seems to have been possible for Mr Carter to get away with the claim that university teachers in the United States work harder than those in Britain. Dr D. G. Christopherson, chairman of the Committee of Vice-Chancellors, said after the meeting that the universities did not feel themselves to be under immediate pressure. They were, he said, looking forward to the next period of fast expansion in the seventies. And the suggestion that the universities fear that if they do not begin to measure their own productivity, somebody less sympathetic will come along and do it for them was indignantly denied. Professor Blaug called it "an uncharitable suggestion".

Rockefeller Without Bronk

New York, March

THE resignation of Dr Detlev W. Bronk as president of the Rockefeller University later this year will amount to something like the end of an era in the affairs of the university, until 1964 the Rockefeller Institute. By common consent, Dr Bronk has been almost entirely responsible for the directions which the institute's growth has followed since his accession to the presidency in 1953 (after several years as president at Johns Hopkins). He has been consistently expansionist, with the result that the faculty has grown in size and lustre. He has also consistently fought for a recognition of the educational responsibilities of the people at the Rockefeller Institute, which is how the institute has acquired the distinction of being an independent university without undergraduates. Although there are few among the faculty who are unmoved by Dr Bronk's vision of the part his institution might play in the academic life of the United States, there have frequently been occasions when the president and the faculty have moved ahead at different speeds. The creation, within the past year, of an academic council at the Rockefeller University should help to ensure that the balance of power between the faculty and Dr Bronk's successor is more even.



There is no doubt that Dr Bronk's career is one of almost unparalleled distinction. For several years he has divided his immense energy between government and universities. His spell as president of the United States National Academy of Sciences, between 1950 and 1962, saw a vigorous growth of the academy's influence in Washington and elsewhere, largely as a result of the drive which Dr Bronk imparted to the job. In the circumstances, it is not surprising that Dr Bronk should plan not to settle back into retirement when his present appointment ends. He says that he will keep on a laboratory at the Rockefeller University, as is the custom for retired professors at the institution. At least until the election campaign is further advanced, he says that he intends to maintain a "flexible" position, which presumably implies that he will be ready to accept a call to another Washington job. In any case, Dr Bronk is personally deeply engaged in trying to bring science and technology to bear on the formidable problems of the city of New York. He says that a deeper involvement in social problems is one of his wishes for the future of the Rockefeller University. He would also like to see the institution become even more successful than at present at interdisciplinary studies.

Of Dr Bronk's successor at the university, there is as yet no news. Several distinguished names have been whispered in this connexion. There seems to be something in the view that Dr Frederick Seitz, now president of the National Academy, has been approached. It would, of course, be something of a feather in the university's cap if there were two examples to support the generalization that presidents of the academy habitually succeed to the presidency of the university.

De-specialization

A JOINT working party is to be set up by the Schools Council and the Standing Conference on University Entrance (SCUE) to examine proposals for broadening the British sixth form curriculum. The position of sixth formers not wishing to go on to university is to be investigated by a second working party which is to be set up by the Schools Council alone. The two parties will work side by side, with a possibility of overlapping membership. While welcoming the move towards broader curricula in schools, the universities see the Schools Council proposals for two A-levels and accompanying "elective courses" (*Working Paper* 16) as an encouragement to further specialization (see *Nature*, **216**, 1260, 1967). A meeting of SCUE last week collected together the comments of the universities and the announcement of the working parties soon followed.

As well as reducing specialization, reform of sixth form curricula will, it is hoped, delay the point at which pupils decide what subjects they will study at university. The universities see experimental curricula as an essential step towards reform, and are prepared to adapt their entrance requirements so as not to prejudice the chances of those pupils who act as guinea pigs. Current curriculum revision by the examining boards and the Nuffield Science Teaching Project is welcomed by the universities, but they would like to see further intensive studies of syllabus-making and examination techniques, with emphasis on the balance between facts, imagination, analysis and presentation. The universities express a doubt that "there yet exists any adequate alternative to externally examined courses as the main basis for sixth form preparation, and selection, of entrants", but at the same time suggest that the "introduction of an element of internal assessment into the examining system merits consideration". A scheme for four or five equivalent two-year courses spanning arts and science subjects is suggested as worthy of experiment, despite teaching difficulties and the resulting lowering of final examination standards. Following on from this the question arises whether an appropriate first degree standard can be reached in three years.

On the basis of this combined view, the universities are joining with the Schools Council to discuss all the proposals so far put forward. The second working party will be concerned with sixth formers not intending to go to university, with regard for qualifications required by industry and commerce. A successful curriculum will obviously have to cater for those both above and below the academic salt, so that pupils are free to decide on their future during their last year at school.

Other aspects of the move away from specialization were discussed at a meeting at Queen Elizabeth College last week of some 140 sixth form science teachers and a sprinkling of university staff. Dr J. E. Spice from Winchester described the workings of the Nuffield physical sciences A-level course which is now on trial in 58 schools throughout the country. In combining chemistry and physics as one A-level subject, the physical science course allows biologists, for example, to take mathematics within the three A-level pattern. The overall comment on the joint course was favourable, from both schools and universities, despite strong words from some quarters—notably from Dr E. R. Roberts of Imperial College, a chemist who regretted the dearth of chemical facts in the course. From the physics side at Imperial College, Dr H. J. Pain retaliated in favour of students who have taken this course, preferring their ability to think to a stream of well-learned facts. There were murmurs from chemistry staff who are beginning to teach the physics side of the course to the effect that they themselves can at last understand the physics they learnt at school.

The new London BSc system, now in its second year, allows students to delay their final choice of subject until the end of the first year. Professor R. E. Burge of Queen Elizabeth College described last week the workings of the system of equivalent course units and the possibilities of inter-college co-operation. By giving each college more control over its courses, the new system is more flexible and adaptable to new knowledge. With a minimum of eight of the equivalent units to be passed for a degree and a maximum of twelve, the new course provides for the wide range of ability now found in universities.

Where To Put It

As everyone had expected, choosing the site for the CERN 300 GeV synchrotron is proving to be an extremely touchy business. Nine countries have offered sites but no decision was reached at the last meeting of the council of CERN which discussed the recently published final report of the Site Evaluation Panel. The panel, consisting of three neutral representatives from countries which had not offered sites, has been working on the report since its appointment in 1966, and a separate geological survey of the sites has been prepared by Dr L. Bjerrum of Norway.

Based on the answers to the three questions of whether this laboratory can be built at the site (A), whether it can be operated there (B) and whether people will go there (C), the panel concludes that Doberdò in Italy is the most suitable location. Le Luc in France is second best and the other sites all less suitable. (See table.)

With the future development of CERN likely to hang on the choice of site for the new accelerator, the panel is understandably cautious in its report. It points out that none of the sites is ideal by all three criteria, that several are perfectly acceptable and that a different set of criteria might change the order The report stresses, however, that the acceptable sites are not of equal value

RATING OF REPLIES TO QUESTIONS A, B AND C

Site	A	B	C	Overall rating
Gopfritz—Austria	γ	γ	γ	γ
Focant—Belgium	γ	β	γ	γ
Drensteinfurt—Germany	β	α	γ	γ
Le Luc—France	α	α	β	β
Aspropyrgos—Greece	δ	δ	γ	γ
Doberdò—Italy	γ	α	α	α
El Escorial—Spain	β	γ	γ	γ
Uppsala—Sweden	α	β	γ	γ
Mundford—Great Britain	γ	β	γ	γ

Needless to say, the report has been accompanied by what amount to special pleadings from the delegations of the disappointed countries The Spanish delegation called for a widening and revision, the Greek delegation contests the view that Aspropyrgos cannot be considered because of the risks of earthquakes, the Austrian delegation disagrees with the geological survey of Gopfritz and the British delegation holds that the cost of construction and operation should be the most important criterion

Back to Chicago

THE capability of the National Science Foundation to hold its own in Washington will be further diminished, in October this year, by the departure of Dr John T. Wilson, at present deputy director at the foundation, for the post of vice-president at the University of Chicago In the past five years, Dr Wilson has become known as a powerful source of new thinking about the policies of the National Science Foundation, and there will now inevitably be speculation that his resignation has been prompted as much by the frustrations of his work in Washington as by the attractions of Chicago

Since the war, Dr Wilson has alternated between the foundation and Chicago He was assistant director for biological and medical sciences at the foundation from the mid-fifties until 1961, when he became a professor of psychology at Chicago and special assistant to the president of the university, Dr George Beadle In his new post as vice-president of the university, he will be responsible for the academic administration If his experience and temperament are any guide, he will expect to play an important part in the development of a strategy for the development of the university under its new president-designate, Dr Edward Levi

Dr Wilson's departure from the foundation may well bring into the open some of the continuing doubts about the management of the foundation which are now widely shared among scientists in the United States There is much to support the view that the direction of the foundation has been less than adequate since its establishment in 1950 For the first decade or so, under the late Dr Alan T Waterman, the foundation was perhaps unduly and unwisely preoccupied with the problems of support for fundamental research, and too little concerned with the relations between research and education on one side and government on the other More recently the foundation s influence seems to have been needlessly muffled On one view, the continuing ill-health of the present director, Dr L J Haworth, is a part of the trouble Elsewhere it is held that a more thorough recasting of the hierarchy at the foundation may now be necessary

Postgraduate Biologists

THE report of the biology sub-committee of the committee of the Royal Society which is surveying postgraduate training in science and technology, the third of six, has just been published Faced with the problem of surveying the multitude of specialized disciplines which came under the umbrella of biology, the sub-committee, under the chairmanship of Professor J L Harley of Sheffield University, decided to collect evidence in four ways It read the propsectuses and proposals of the universities It sent a questionnaire to the heads of departments and research stations (167 replied), and some heads of departments were also interviewed The sub-committee sent another questionnaire to people who had gained their PhD degrees in the past five years from five universities Finally, the committee obtained descriptions of PhD programmes in North American universities from senior biologists who also knew the conditions in Britain Clearly, compared with the physics sub-committee which based its report entirely on the replies of heads of departments to a questionnaire, the biology group has spread its net wide

What are the conclusions ? The report says that "British systems of PhD training in biology are designed essentially for the candidate who is well prepared by his first degree for research and whose success depends principally on his own initiative and that of his supervisor The systems are not so appropriate to those candidates who are less well prepared, nor to some students from overseas" That sounds like a polite way of saying that there are no organized programmes for training PhDs but rather an apprenticeship system in which making a shrewd choice of supervisor at the outset is vitally important This is borne out by the two recurrent themes in the suggestions for improvements sent to the sub-committee They are, first, to improve the system of supervision and, second, to increase the number of lectures and seminars for postgraduates

Altogether, 90 per cent of the recent PhDs recognized that some formal instruction is now necessary, but fewer than 10 per cent of the 167 departments in the survey run lecture courses specifically designed for postgraduates The most favoured proposal, which the sub-committee endorses, is for unexamined lecture courses and surveys spread over the first two years of postgraduate study And, because the standard of the first degree is usually higher in Britain than in the United States, such courses need not be on the scale of those in American PhD programmes In contrast with the views of recent PhDs, the senior staff of 40 per cent of the departments consulted were against formal instruction They no doubt fear the extra teaching load and, as the report points out, the chief reason for the present situation is the small size of most departments, the staff of which is fully committed to undergraduate work This, coupled with the small numbers and diverse interests of postgraduates, often makes special postgraduate courses impracticable and uneconomic The sub-committee is obviously right in saying that closer interdepartmental collaboration is the answer to these problems

The sub-committee has also unearthed figures which show the alarming, in fact disgraceful, paucity of seminars and colloquia in biological departments Only 78 per cent of the departments hold seminars during term—18 per cent weekly, 18 per cent monthly and the rest presumably less often That 82 per cent of British biological departments only average at best a monthly seminar, and that 22 per cent apparently have no regular seminars at all, speaks for itself Small wonder that the report urges that such meetings, which are vital for any thriving department, should be more frequent and inter-departmental where possible

"Good supervision is the keynote of a good PhD system " It is typical for the postgraduate student to see his supervisor several times a week, if not daily

The sub-committee considers that "supervision cannot be improved fundamentally by legislation" but nonetheless it says it would be useful to formulate minimum requirements for meetings between students and supervisors In its subdued way, it also proposes that having committees of three to five supervisors, as is common in the United States, should be considered This system exposes the postgraduate to a wider range of factual knowledge and, more important, to a wider range of experimental skills and approaches without necessarily excluding the personal responsibility of one supervisor for one particular student Indeed, where there is a clash of personalities, the committee system would be an enormous improvement As things are, it is extremely difficult for a student to change his supervisor, no matter how badly they get along with each other

As to the PhD examination itself, although there is concern, especially among recent PhDs, that the standards of the degree and the methods of examination are too variable, the report makes no recommendation for change Arguments for and against the supervisor being an examiner, and whether or not all examiners should be external, are equally balanced, and most departments were not in favour of classifying the PhD degree except for possibly giving a mark of distinction to outstanding theses It seems that 99 per cent of PhDs in biology are completed in three years and only 1 per cent take longer, so the sub-committee, unlike the physics sub-committee, does not recommend provisions for fourth year grants Again in contrast to the physics sub-committee, the biologists believe that, so long as standards are not diminished, changes in the present three year honours degree are not necessary The first degree in biology apparently still provides a satisfactory grounding for PhD training

Old Age

RETIRED people now make up more than 12 per cent of the population of Britain During this century the four-fold increase of people in this age group has far outpaced the increase in lower age groups, although the life expectancy of a 65 year old has only increased by 2 years, while that for a baby has increased by 24 years More than three-quarters of the 300,000 old people who live alone do not have the help at home which they think they need and many thousands are in need of medical attention The Office of Health Economics (OHE) has been studying the facts, figures and problems of old age and makes suggestions as to how the lot of old people can be improved, in its latest paper *Old Age* (OHE, 2*s* 6*d*)

The main priority, the report suggests, is to improve the quality of old people's lives rather than to prolong them At present, care of the old costs more than £1,650 million a year in Britain, £400 million of this being medical care which takes 30 per cent of the National Health Service budget Healthier old people will not only mean happier old people, but a lighter burden on the community If people over 65 become generally healthier, the retiring age could be raised Two particular lines of approach are discussed in the report—the provision of better welfare services for those who are now old and further attempts to prevent illness that will incapacitate people when they get old. Research is required in both directions As well as the obvious investigations into disease and the nature of ageing, the sociological effects of old age must be studied in connexion with housing, health and ignorance of how help can be obtained The OHE recommends that a national council could be set up to co-ordinate the various aspects of research, as a multidisciplinary approach would be most effective

The OHE, sponsored by the pharmaceutical industry, is an independent organization which undertakes research on various economic and social aspects of medical care *Old Age* is its twenty-sixth publication, earlier papers having covered such topics as mental health, polio, drug addiction and medical manpower The director of OHE, Mr G Teeling-Smith, suggests that although the study of old age has less glamour than work with children, it can improve the quality of the retirement years that face us all

Defence Research

from our Parliamentary Correspondent

WITH three public sessions gone, and perhaps twice as many in private, the Select Committee on Science and Technology has learned one thing at least· the civil servants responsible for defence research are unanimous in their approval of the way it is done. Mistakes, it seems, are a thing of the past, banished by the adoption of the Gibbs–Zuckerman procedures, as the techniques of assessment are called This may be true, but the committee would be doing less than its duty if it believed it without independent corroboration Unfortunately, all those who are familiar with the system have an interest in saying how good it is What the committee needs is a company disenchanted with the Ministry of Defence During the most recent session, on March 21, a possible candidate emerged—the hovercraft industry, which seems to have met with little encouragement from the ministry

On March 21, the committee interviewed five representatives from the ministry Most of the questions were on organizational minutiae—the operational requirement committee, eight strong, meets weekly, while the weapons development committee, nineteen strong, gets together every fortnight The witnesses agreed to supply three case studies showing how three projects came to fruition—the CVRT armoured vehicle, the Nimrod aircraft project, and the Type 42 destroyer.

They were confident that good ideas, whatever their origin, got a fair hearing, and that bad ideas could be weeded out before they had gone too far. Some members of the committee suggested that the only pressure brought to bear on the projects was financial pressure. The witnesses agreed that financial pressure alone was enough to put a heavy constraint on defence research, but in addition there was an automatic weeding process.

Mr Eric Lubbock suggested that the British hovercraft industry had had less than fair treatment from the ministry. Although they had made a number of proposals, few had been taken up. Mr Stuart Watson, for the Navy, agreed that the industry had been "pestering us with ideas". The result had been to agree to the building of BH7 hovercraft for the Army and the Navy, although the Army version had now been cancelled because of the withdrawal from the Far East. As for bigger hovercraft, they would have to be shown to fulfil a real military requirement. Mr Lubbock thought this an extraordinary answer. Would the hovercraft not be suitable as a search vessel for submarines, or as a fast armed vessel? The witnesses reiterated that all possible had been done to help the hovercraft industry.

London's Water

The bacteriology, chemistry and biology of London tap water are subjects which most Londoners must prefer not to have to think about when they drink their morning cup of tea. A great deal of work, however, has to be done before the water from the River Thames and the River Lee is even remotely drinkable and stringent standards are operated to make tap water as pure as possible. Some of the routine and research work done by the Water Examination Department of the Metropolitan Water Board during 1965–66 to achieve these standards is described in the forty-second report of the department just published (Metropolitan Water Board, 32*s* 6*d*.).

Continuous sampling and testing is carried out to check the quality of supplies. In 1966 almost 133,000 samples were dealt with—the highest number of samples ever handled by the department. "Ideally all waters intended for drinking should show no coliform bacteria in 100 ml. and it should be the aim of every water undertaking to provide water of this quality", according to Report No. 71 of the Ministry of Housing and Local Government and the Ministry of Health. London water seems to pass the test—out of more than 11,000 samples of treated water leaving the board's filtration works and well stations in both 1965 and 1966, 99.98 per cent were 100 per cent bacteriologically pure as far as *Escherichia coli* was concerned. Results of the coliform test were not quite as good in 1966 as in 1965; 99.68 per cent of samples were free from coliform bacteria compared with a percentage of 99.88 in 1965. This slight decrease in purity can probably be explained, the department says, because a more sensitive test for the isolation of coliform bacteria was introduced at the beginning of 1966.

Detailed descriptions of the work of the various sections of the department are given in the report. The bacteriological section has been continuing its examination of water for *Salmonella* organisms; their presence in the water after storage and primary filtration and on the surface of slow sand filters suggests reinfection of the water by gulls. *Salmonella* organisms have also been isolated from detergent foam. The chemistry section has found that there has been a reduction of the residual amounts of synthetic detergent material in the board's river sources of supply after the complete substitution in packets of washing powder retailed in Britain of anionic detergents which are biologically degradable.

An important part of the department's work is concerned with public health, such as the examination of waterworks employees for the detection of carriers of waterborne diseases. It was reported in the previous annual report that a case of bilharzia had been found among the board's employees, and this has prompted speculation as to whether the disease could possibly spread in Britain. It is thought unlikely but not impossible, and a note of warning is given on the careless introduction of snails—the intermediate hosts for the helminth parasite *Schistosoma*—from countries where the disease is endemic.

No More Noise?

From July this year, British motorists will be subject to yet another roadside spot check. At present drivers have to proceed soberly, in a safe vehicle and at a moderate pace, but by the summer they will have to be quiet as well. Although the regulations announced this week by Mrs Barbara Castle, Minister of Transport, put the maximum allowed decibels at higher levels than those recommended by the 1962 Wilson Committee on Problems of Noise, the limits are experimental and may well be lowered in the future. Noise meters will be used in much the same way as the radar speed checks, with police stopping the vehicles which pass the set limits—90 db for motor cycles over 50 cc, 87 db for passenger cars and 92 db for heavy goods vehicles, agricultural vehicles and buses. During the initial period drivers of lorries with burnt-out silencers and car fiends with "organ-pipe exhausts" will be cautioned only, but later on, offenders will be liable to fines of up to £50 for a first offence.

The noise meters are said by the Ministry of Transport to be very accurate. They will be calibrated by approved laboratories and checks will be made from time to time against gadgets with standard noise output. A three decibel allowance is made in the noise limits for roadside tests to allow for such things as sounds reflected from other vehicles. From 1970, all vehicles will have to be constructed in such a way that they do not exceed the noise limits when tested at full throttle. The two year time lag is being allowed to give manufacturers time to adapt their models.

Golden Touch

The Royal Institution launched last year an appeal for £225,000 and did it so successfully that £270,000 has already been received and the money is still coming in. The donors include some 300 foundations, companies and individuals. The money is to be spent on building a new lecture theatre and museum, renovating the present theatre and laboratories and expanding the institution's educational facilities.

The Royal Institution was founded in 1799 by a native of Massachusetts, Count Rumford, for the promotion of science and the diffusion and extension of useful knowledge Among its resident professors have been Davy and Faraday, and its present director is Professor George Porter The current functions of the Royal Institution, as Professor Porter explained last week to the clinking of champagne glasses, are to serve as a society for the advancement of science, as a national archive for the history of science, as a theatre for scientific lectures and meetings and as a research laboratory The enviable success of the institution's appeal will no doubt help

Computer Control

In the future, British industry will apply computers to production and stock control to the same extent as they are now used in accountancy This was the theme of a symposium organized by the British Computer Society in London on March 20 More than 1,000 delegates heard from British and American experts how computers can be applied to the scheduling of production on the shop floor, to the control of materials needed in the manufacturing processes and to the efficient stocking of the finished product

There still seems to be a variety of pitfalls in the introduction of computers into production control—not the least the problems associated with people One of the speakers pointed out that two of the commonest mistakes made by management are the failure to take into account the limits of comprehension of employees and the neglect of those parts of the control system which are placed outside the computer This means that more attention needs to be paid to gathering data for the computer, and to the way in which the computer output is used by management and personnel on the shop floor And, not unnaturally, there is always the lurking fear that people are being replaced by machines

Several delegates to the symposium seemed doubtful whether computer production and stock control systems had reached the stage of making themselves pay There seems to be no clear-cut answer to this—obviously it is difficult to estimate the financial saving made by benefits such as less capital tied down in stock and work-in-progress, better delivery dates and so on which are claimed by advocates of computer systems Nevertheless, more and more manufacturers are turning to computer systems, especially in the United States, which is clearly showing the way in this field

One of the questions which the symposium tried to answer was whether the smaller firm should go in for production control by computer when big organizations are obviously running into difficulties in this field Mr B G Roberts, computer development manager for Belling and Lee Ltd, defined the smaller firm as one with less than £60,000 per annum to spend on data processing during the first few years of operating a computer He made the point that although the amount of data to be dealt with is less than in a larger company, the number of processes through which the data has to go may be just as great Because the data handling procedures rather than the bulk of data provide many of the problems, and because big organizations have the resources to handle large amounts of data, it seems that small firms are less likely to be successful in introducing a computer than large ones

Nevertheless, the chairman of the symposium, Mr W G E Hulse, who is manager for production planning and material control at Standard Telephones and Cables, said in his summing-up that the emphasis in the future will be on the smaller companies Because there are common ingredients to the production problems of many companies working in the same field, there is a need for co-operation in setting up computer centres This poses problems in the security of business information but, as Mr Hulse pointed out, companies already rely on the confidential nature of bank accounts so why not trust computer centres in the same way ?

New Ideas

The emphasis was once again on the small firm at a conference with the title "Getting New Ideas off the Ground" held in London on March 21 Arranged by the Institute of Directors in association with the National Research Development Corporation, its aim was to tell senior management about the NRDC and the way it can help industry The NRDC was set up in 1948 after it was realized how much had been lost by the failure fully to exploit commercially the discovery of radar and penicillin as well as many other discoveries pioneered in Britain Its aim is to provide support for technological developments of potential commercial importance and to encourage industry to adopt new methods and products invented in government and university laboratories and elsewhere

Sir Donald Stokes, chief executive and deputy chairman of British Leyland Motor Corporation, said in his opening address that no firm, however large or however small, need have any hesitation in putting its problems to the NRDC for consideration He admitted that management was still far too stuffy about new ideas and was reluctant to give revolutionary thoughts a trial

The message of the conference was that small firms should not be reticent in using the NRDC Clearly large companies are aware of the NRDC and several of them are using its facilities, but the organizers felt that the functions of the NRDC needed emphasizing to the smaller firm

Data Centre for Oceanographers

The Natural Environment Research Council is to set up a British Oceanographic Data Service at the National Institute of Oceanography, Godalming The head of the unit will not be appointed for another three months or so, but a steering committee of nine members has been announced and it had its first meeting last week The chairman of the steering committee is Dr G Deacon, director of the National Institute of Oceanography As with many other subjects there is an increasing amount of data which needs handling in a systematic way It is hoped that the centre will use modern methods of data storage—punched tape, a computer, and so on, and that it will have telex links with other oceanographic and research institutions

This will not be the first oceanographic data centre in the world There is one at Copenhagen run by the International Council for the Exploration of the Sea, and others in Hamburg and Ottawa, for example The idea now is to have national data banks co-ordinating with one another instead of world centres There is a small oceanographic data centre in existence in Britain at the moment, but this is for bathythermographic data only and it is run by the Ministry of Defence (Navy), Hydrographic Department The new national centre will cover all oceanographic data from the area of the north-east Atlantic and the North Sea down as far as the bay of Biscay To start with, the centre will collect data on physical oceanography only—temperature, and salinity measurements and so on, but eventually it will store and disseminate information in the whole field of oceanography

Spina Bifida Appeal

AN appeal for funds was launched last week by the Spina Bifida Trust (112 City Road, London EC1) Children born with spina bifida, most of whom have hydrocephalus as well, and those with hydrocephalus only, are now surviving in increasing numbers In Britain there are about 8,000 such children under the age of 16 and their numbers are being added to at the rate of about 900 a year

Spina bifida is a deformity of the spine in which there is a split in the spinal arch, this causes the nervous tissue to lie exposed on the surface of the back at birth Some of the nerve cells have not developed properly and the split spine renders them liable to damage, with the result that there is often partial or complete paralysis below the level of the defect This may affect bladder and bowel control as well as muscles of the limbs

The development of a valve system for treating hydrocephalus, which occurs in most cases of spina bifida, has made treatment of the rest of the disability worth while Because of their paralysis the affected children require special education and training Funds are needed to supply these facilities and to conduct further research into the causes of the condition and its treatment

Operation Habitat Rescue

WILDLIFE in agricultural areas is continually threatened as more hedgerows, copses and banks are removed under the pressure of farm mergers, monoculture and more mechanization To try to reverse the disappearance of wildlife in farming areas in Britain, a Conservation Corps Habitat Rescue is being organized by the Soil Association and the Council for Nature Young volunteers will try to help the survival of wildlife by conserving hedgerows and banks, ponds and ditches, and planting small copses as refuges The scheme has been made possible by the Jack and Mary Pye Trust, which is supporting the Soil Association's participation It is hoped that proper hedge management will not only create and preserve habitats for wildlife but that it will be of direct benefit to farmers in helping conservation of soil fertility and ultimately long-term productivity In many parts of the country removal of hedges has led to quite severe soil erosion by wind, in addition to drastic reduction of some species of animals and plants

Conservation Corps volunteers will work first on the 200 acre experimental farm of the Soil Association in Suffolk Later, it is planned to extend the work to selected areas of wasteland in other parts of Britain

Parliament in Britain

European Technological Collaboration

MR A WEDGWOOD BENN, Minister of Technology, refused to make available a report of the inter-departmental committee meetings on European Technological Collaboration at the request of Mr E Moonman (Billericay) Mr Moonman wanted to know how many meetings of the committee had taken place and urged Mr Benn to make a statement on the future work of the committee. Mr Benn agreed that reports of such committees would be attractive, but suggested that it would make government impossible, and that it would lead to the additional problem of secret meetings of officials to avoid the risk of publication (Oral answer, March 18)

Pharmaceutical Products

MR E J MILNE (Blyth) asked Mr K Robinson, the Minister of Health, to set up Government-owned factories—preferably in development districts—for the manufacture of drugs, pharmaceuticals and other commodities required by the National Health Service Disappointed by the minister's negative reply, Mr Milne stressed the need to look more closely into the matter, adding that pharmaceutical costs "will loom very large in our prices policy in the near future" Also addressing the minister, Mr Maurice Macmillan (Farnham) asked him if he would bear in mind the record of the pharmaceutical industry which has increased its exports about five times since 1948 and which now stands at a net export figure of about £75 million (Oral answer, March 19)

Medical Computing

MR DAVID CROUCH (Canterbury) asked the Minister of Health what plans he had to implement a national plan for medical computing Mr John Hunt (Bromley) was also interested to know what discussions the minister proposed to have with the computer industry regarding the use of computers both in the medical field and for improving the general administration of the National Health Service He tried to find out whether the minister could give any indication of the financial savings from the wider use of computers within the Health Service Mr Robinson replied that discussions were continually being held with computer manufacturers, but he was unable to give any information on Mr Hunt's second point (Oral answer, March 19)

Aldermaston

As usual, Mr Tam Dalyell (West Lothian) had something to say about the type of work done at defence establishments He tried to ascertain what steps had been taken to declassify work at the Atomic Weapons Research Establishment, Aldermaston, and asked if research for commercial purposes was being promoted there Mr Benn said that it had always been the practice at Aldermaston to declassify work as far as possible and to publicize the work extensively He added that, for the past five years, a large part of the site had been an unclassified area Dr Jeremy Bray, Joint Parliamentary Secretary to the Minister of Technology, emphasized that the establishment accepts many orders on commercial terms from government departments and from private customers for research and development outside the atomic weapons field (Oral answer, March 18)

British Schools to go Metric

by

NIGEL HAWKES

THE way in which British schools should adopt the metric units of the Système Internationale was the subject of a one day meeting on March 20 at the Royal Society. As a number of speakers pointed out, it is now no longer a question of whether but of when and how the change is made. The British Government is committed to a complete changeover of units by 1975, and it is becoming increasingly urgent for teachers to be made aware of the change and at the same time given guidance about teaching the new units. As with so many educational changes, the common view seems to be that the new units, as well as bringing Britain into line with most of the world, also offer a splendid educational opportunity if teachers had the courage to seize on it.

Mr Arthur Wynne from the Ministry of Technology and Dr D. H. Sharp from the Confederation of British Industry outlined the progress so far made in industry. It seems that the problems posed by the retail trade have yet to be solved, and Dr Sharp confirmed the report (*Nature*, **216**, 1157; 1967) that the CBI had recommended the formation of a metrication board, to coordinate planning, conduct a massive publicity campaign and arrange for financial assistance. The recommendation has been processed by Mr Wynne's Joint Committee on Metrication, and is now filtering through the machinery of government.

Delegates at the meeting, representing education authorities, schools, learned institutions, teachers, associations, examination boards and the Publishers' Association, agreed that SI units should be the only units permitted in Ordinary level mathematics and science "As soon as possible, and in any case not later than June 1972". The recommendation is to be passed on to the examination boards. The meeting also agreed that "September 1969 should be the date for a change of emphasis in teaching in the primary schools".

A number of speakers at the meeting spoke warmly of the educational benefits of the change. Dr E. D. Tagg, of the unhappily named Accelerated Teaching of Higher Level Mathematics Project at the University of Lancaster (and a former teacher at Oundle School) said that he thought the saving of teachers' and children's time would be worth £100 million a year. Mathematics was unpopular, he said, because at the moment it contained "a maximum of labour and obscurity". Metrication would not be the answer to all Britain's educational ills, but it was a natural and wholly desirable step. Mr F. C. Brown, of the Institute of Education at the University of London, thought that, to be successful, metrication needed a bold touch; it was no good running both systems in the schools at once. "Dualism could be the ruin of the whole process." Other speakers contested this view, but there was a considerable measure of agreement with Mr Brown's view that the examination boards held the key to success.

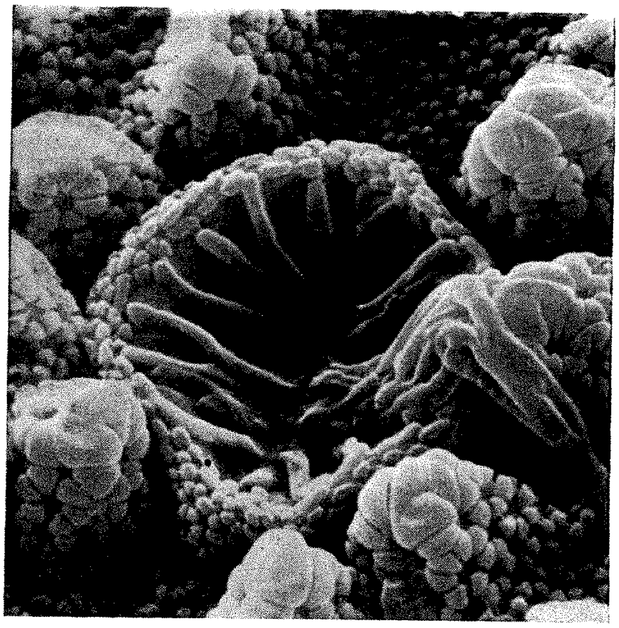
Representatives from the examining boards took a much more cautious line. Their argument was that the examining boards should follow the schools, rather than try to lead. Mr A. E. E. McKenzie, secretary of the Oxford and Cambridge Board, said that the boards were not authoritarian. The Advanced-level examinations would not present a problem, and would all be set in metric units in the next two to three years. Ordinary-level examinations would be harder to deal with, but Mr McKenzie suggested that mathematics and science examinations should be set in the metric system by 1975. Most of the other delegates thought this a depressingly unambitious target, and said so; one pleaded with the boards to be more dictatorial, another suggested that they should take less notice of what the universities said. The upshot was a firm request by the schools that the examination boards should fix a timetable and stick to it.

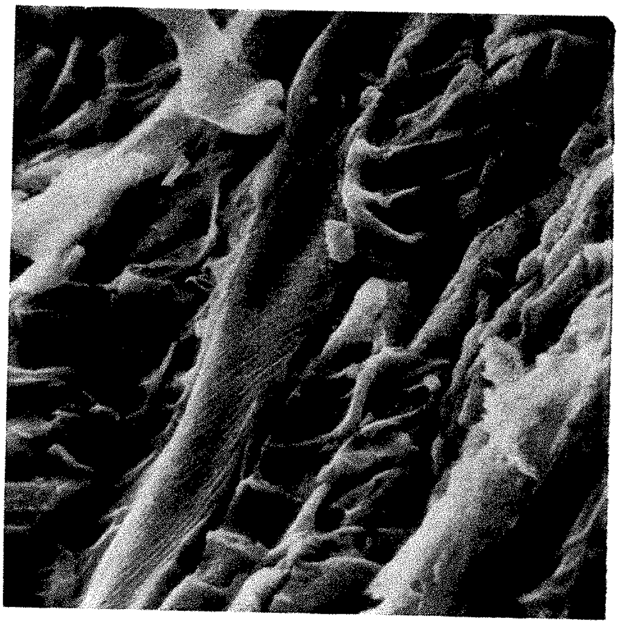
Mrs E. M. Williams, a former principal of Whitelands College of Education, gave a lucid account of how the change would affect the primary schools. She was optimistic, she said, principally because of the changes in teaching which have transformed primary school teaching in the past few years. In particular, the way in which children are now taught to understand numbers by the use of structural apparatus could readily be extended to the teaching of units.

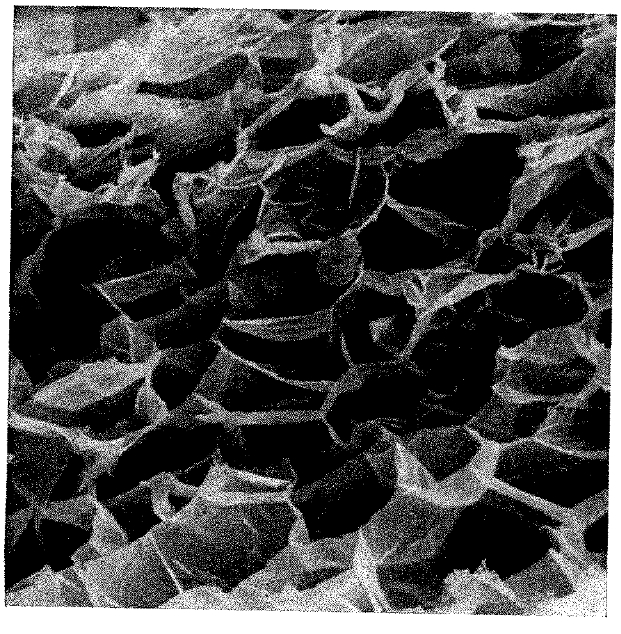
Various speakers from the Publishers' Association expressed alarm about the supply of textbooks. The most urgent need, Mr W. G. Clouter suggested, was for a definite timetable which would enable the publishers to plan ahead. But he was also concerned about the financial aspects of the change of textbooks; would the schools be expected to buy the new books out of their normal capitation allowance ? This was already under strain, he said, and it seemed to him necessary for more money to be made available. Other speakers from the Publishers' Association put the same point more strongly; one pointed out that there were 30,000 textbooks in print. If special grants were not made available, the new SI books would mop up too much of the capitation grant. Most delegates agreed, however, that the revision of textbooks and the writing of new ones would provide an opportunity for a much needed improvement in standards.

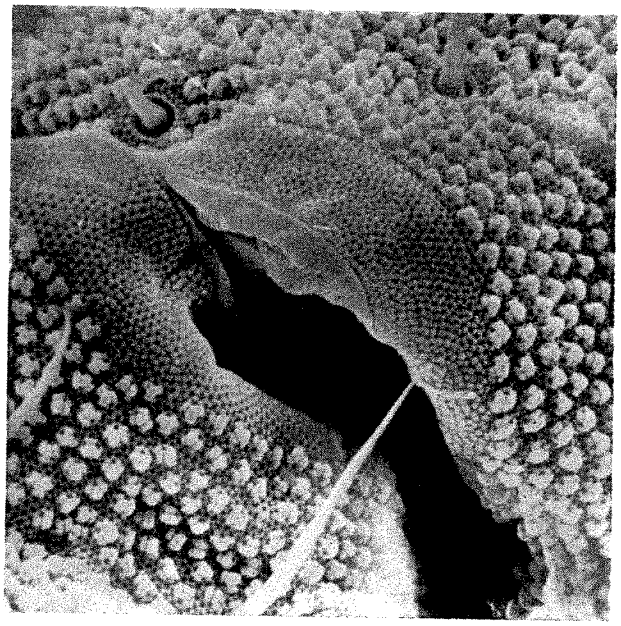
There was some discussion of the ways in which the change could be publicized. Mr R. Sibson from the Schools Council said that the council would soon be publishing a pamphlet for distribution to schools. Professor M. J. Lighthill, who ran the meeting with great skill, suggested that the Royal Society itself might produce a pamphlet, and there was some discussion of a draft pamphlet which the society has already drawn up. There was some feeling that two pamphlets, one for primary and one for secondary schools, would be better, but this, Professor Lighthill said, depended on the resources of the society. After agreeing to submit recommendations to the examination boards, and producing under Professor Lighthill's guidance a suitable form of words, the meeting decided to meet again in September for further discussion.

Scanning Electron Microscope Pictures









Top left : Pseudocellus of *Onychiurus scotarius* of the order Collembola ("springtails"), magnified 6,000 times (Drs W. G. Hale and A. L. Smith).

Bottom left : Expanded polystyrene, coated with a 500 Å layer of gold/palladium, magnified 180 times.

Top right : Fragment of coal with inclusion, possibly metallic, magnified 1,500 times.

Bottom right : Entrance to ventral tube of *Onychiurus scotarius*, magnified 2,300 times (Drs W. G. Hale and A. L. Smith).

Scanning electron microscopes with useful magnification of up to 50,000 and depth of focus 300 times better than that of an optical microscope for similar magnification have been commercially available since 1965. These microscopes, which cost £20,000, have evolved from the research work of Professor C. W. Oatley and his group at Cambridge. The resolution of the present version varies with the operating mode from 150–300 Å to 1000–2000 Å. A beam from a conventional triode electron gun is focused by three pre-aligned electromagnetic condenser lenses to give a fine probe which scans the surface of the specimen.

(Micrographs from the Cambridge Instrument Co.)

NEWS AND VIEWS

British Computer Merger

AFTER eight years of mergers and takeovers, the British computer industry is now under a single management The finalists, International Computers and Tabulators, English Electric and the Plessey Organization, announced last week the formation of a combined company, International Computers Limited, which will be the largest manufacturer of computers outside the United States The spur of American competition has been the principal factor in this process, and no doubt the Ministry of Technology hopes that by doing to computers what has already been done to cars and electrical engineering, it has added another arrow to its Common Market quiver, Indeed, Mr Wedgwood Benn seemed to be pointing his bow at France's failure to protect her computer industry from the American onslaught when he described in January to the Council of Europe the "increasing dominance of European industry by the Americans, this has been so marked in some industries that there must be doubt as to whether these indigenous European enterprises can survive unless there are determined efforts to bring the fragmented entities together to form continental-sized undertakings"

In theory, at least, the unification of the British computer industry is probably wise, and the Ministry of Technology has expressed its confidence in the scheme with £17 million of public money, of which £13½ million is a direct grant for research and development In practice the merger is also a marriage of necessity, not just convenience, and the new company has formidable problems to surmount before the benefits of size become apparent The merging of two groups of people performing more or less identical tasks is likely to be painful and time-consuming The principal ranges of machines produced by the two partners are largely incompatible with each other and much ingenuity will be necessary to devise a new range of saleable computers

The new company will need to mount a considerable research effort, and to be accorded a considerable measure of luck, if it is to achieve this aim The one clear finding that has emerged from the numerous studies of industrial research and invention is that there is no royal road to success In spite of the great size of the new company, there is bound to be some anxiety about its capacity to carry out research on the scale which circumstances probably now require If it really intends to keep up with the IBMs of this world, International Computers Limited will have to change not merely the scale of its research and development but the character as well The strength of the great American companies in computers and communications stems from the way in which they straddle the whole field of innovation Not merely are they thus well placed to take advantage of whatever new developments may fortuitously suggest themselves, but they are also able to impose their own pattern on the development of technology It will be interesting to see how long it takes the new British company to weld together the hotchpotch of research facilities which it has inherited into a single coherent unit, but this may well be the key to its success It is understandable that the partners in the new merger should for several years have been obsessed with the need somehow to sell machinery, but it would be a great misfortune if a quite proper concern for the flow of cash were to divert the interests of the new company from the needs of the decades and not just the years ahead Luckily, the order books of the partners to the merger are full though not bursting, so that there should be a breathing space in which the new company can plan a durable strategy for technological development

On the cheerful side, there is at least one respect in which the British company may have an advantage over its competitors elsewhere The link between the British computer companies and the Plessey Company, which is deeply involved in telecommunications as well as computing machinery, may prove to be particularly apt now that it is plain that the next few years are likely to see a blurring of the separation between communications and computers That, the new company may consider, is a stroke of luck

Self-reversing Oceanic Basalts

from a Geophysics Correspondent

THE fact that it has proved possible to construct, using continental igneous rocks, a world-wide geomagnetic polarity time scale for the past four million years implies predominance of field reversal over self-reversal in explanation of the majority of the world's reversely magnetized rocks Furthermore, the recent discovery that the pattern of linear magnetic anomalies adjacent to mid-oceanic ridges correlates with the pattern of normal and reversed periods from the continental scale, not only gives strong support to the ocean floor spreading hypothesis, but also indirectly strengthens the evidence in favour of field reversal The predominance of field reversal and the use of magnetic anomalies as evidence for ocean floor spreading are thus mutually consistent

Despite this consistency, Ozima and Ozima (*Earth and Planet Sci Letters*, **3**, 213, 1967) have now questioned the assumption that the linear ocean anomalies arise from repeated reversals of the Earth's field

They report that three out of eight submarine basalts obtained from Pacific Ocean deep sea mounts exhibit self-reversal properties under certain conditions Saturation magnetization measurements show that each of the three specimens contains in its virgin state, a single magnetic component with a Curie point of 250° C On heating to 400° C, this component is partially converted to a new magnetic phase possessing a Curie point of 300° C Heating to even higher temperatures produces a single phase having a Curie point of 560° C

What is significant, however, is that if one of these specimens is heated to any temperature between 300° C and 330° C for a few minutes, and then re-cooled in the geomagnetic field, the resulting magnetization is antiparallel to the field The reason is that the unconverted part of the primary magnetic phase, of Curie point 250° C, interacts magnetically with the newly produced component of higher Curie point to produce a net reversed moment Below 300° C there is insufficient new material for this to occur, and above 330° C not enough virgin phase remains, but as long as the temperature of the specimen is not maintained at 300° C to 330° C for too long a time, the balance of old to new material is just correct for the interaction to occur Nor is this merely a low magnetic field phenomenon, for fields of up to 2,600 oersteds are insufficient to suppress the self-reversal

One may argue, of course, that the laboratory processes used by the Ozimas to produce self-reversal over a limited temperature range rarely occur in nature supported by the fact that virgin specimens still contained only the low Curie point magnetic phase), and thus that it is unlikely that self-reversal occurred during the production of the natural magnetization Moreover, the correlations between the linear ocean anomalies and the continental polarity time scale, and between each of these and the reversals patterns in deep sea sediments, are too tight to allow the existence of widespread self-reversal in oceanic basalts For these reasons the work of the Ozimas is likely to be of greater interest to those concerned with the phenomenon of self-reversal itself

Carcinogenic Oil Fractions

IN 1948, it was realized that workers who had been exposed for many years to "cutting oils" applied to the cutting edge of machine tools tended to develop skin cancers In the same year the Medical Research Council set up a committee on the carcinogenic action of mineral oils under the chairmanship of Professor T Ferguson The aim was to carry out an intensive examination of crude oils like those used in industry, in order to isolate and identify the carcinogenic constituents It was also hoped that methods might be developed for the removal of these carcinogens and that specifications for safe oils might be formulated Twenty years later the committee's findings have been published by HMSO in No 306 of the council's special report series, price £2

The research was carried out at the universities of Birmingham, Exeter, Glasgow and Leeds, and the University of Manchester Institute of Science and Technology Biological tests were also carried out at the Chester Beatty Research Institute, and quantitative techniques were evolved at the MRC Statistical Research Unit Using three crude oils from fields in Kuwait, Oklahoma and Lagunillas, the research consisted first of the fractionation of the oils by physical and chemical means, and second of the biological testing of fractions produced in this way Rabbits, which were found to be more sensitive to carcinogens in mineral oil than mice, were used in the biological tests

The principal fractionation techniques used were vacuum distillation, solvent extraction, adsorption chromatography and chemical treatment, and care was taken not to produce permanent chemical changes in the oil constituents The first exploratory studies led to the isolation of some 45 pure compounds, some of which had not previously been recognized as constituents of crude, uncracked mineral oils, and none of which is known to be markedly carcinogenic Some of the more complex hydrocarbons isolated from the higher-boiling point fractions were shown to be weakly carcinogenic, and included 4-methyl-1,2-benzanthracene and 7-methyl-1,2-benzanthracene Other isolated substances approach in complexity the known polycyclic aromatic carcinogens and may contribute to the total carcinogenic activity of the oil In the biological tests, oil fractions were either painted on or injected subcutaneously into experimental animals which were later examined for tumours By diluting the fractions to be tested with medicinal liquid paraffin, it was shown convincingly that the carcinogens reside in the aromatic fractions of the oil which can be extracted with acetone or other solvents

Although the general chemical nature of the active ingredients has been established, the committee has unfortunately failed to develop rapid methods of detecting carcinogens in the oils

Effect of Densely Ionizing Radiation

from a Correspondent

MAMMALIAN cells and higher plant cells are more susceptible to damage from densely ionizing radiations, such as α-particles or fast neutrons, than from hard X-rays In more quantitative terms, the relative biological efficiency (RBE) of these radiations compared with hard X-rays is greater than one The most commonly accepted explanation is that a certain minimum amount of energy deposited in a given length of track is necessary for the production of a biological effect In this interpretation the effectiveness of hard X-rays is due to the densely ionized tails of the tracks of recoil electrons, or to chance interaction between tracks when large doses are used Using data on the RBE of deuterons and helium nuclei of various energies on the killing of human kidney cells *in vitro*, Barendsen (*Int J Rad Biol*, **8**, 453, 1964) estimated that a lethal event was produced when more than about 500 eV (15 "ionizations") was deposited in a target of about 100 Å thick

The sensitizing effect of oxygen decreases with increasing "density of ionization", and this effect has been attributed to the same cause Radicals formed close together along a densely ionized track were thought to interact and produce permanent damage which could not be affected by the presence or absence of oxygen

Recently, however, Neary (*Int J Rad. Biol*, **9**, 477, 1965, **11**, 209, 1966, **12**, 317, 1967) has suggested a different mechanism based on observations of chromosome aberrations in *Tradescantia* microspores irradiated with very soft X-rays. Neary's theory is that production of a chromosome aberration results from interaction between two damaged targets, in each of which the damage is produced by a single energy-loss event. The increased effectiveness of densely ionizing radiation is due to the greater chance of interaction between the two damaged targets when they are produced close together by a single particle. Neary also suggests that cellular killing by radiation is due to the same mechanism. This theory gave results agreeing well with the observations when irradiations were made in aerobic conditions. Under anoxic conditions, however, the RBE was found to rise too quickly with increasing density of ionization. This observation could be explained only by invoking a further hypothesis, namely, that oxygen was actually produced in the tracks of densely ionizing radiation, a hypothesis first made explicitly by Shchepotieva, Ardashnikov, Lurje and Rahkmanova (*Izvestia Akademii Nauk USSR*, biological series No. 4, 642, 1961) and suggested by many previous observations that oxygen is produced when pure water is irradiated by α particles but not by X-rays.

Recently, Alper and her colleagues have been able to test this hypothesis experimentally (*Brit. J. Radiol.*, **40**, 843, 1967, and *Radiation Research*, **32**, 780, 1967). The rests were based on irradiation of bacteria with X-rays and fast neutrons, the latter producing recoil protons with fairly dense ionization. The sensitivity of the bacteria was studied as a function of oxygen concentration using both types of radiation. The results fitted the model of oxygen production by densely ionizing radiation and the concentration of oxygen formed in the secondary tracks from fast neutrons was found to be 0.83 μmole per litre. An independent test was also made by comparing the relative sensitivity in the presence and absence of oxygen (OER) of several strains of bacteria irradiated with X-rays and fast neutrons. A theoretical interpretation of the observed relationship between the values of OER with X-rays and neutrons led to a value for the oxygen produced, in the densely ionized tracks, of 0.76 μmole per litre. The agreement with the first model is remarkable considering the experimental uncertainties. This confirmation of the importance of oxygen production in the tracks of densely ionizing particles is a valuable support for Neary's theory.

Cysteamine as a Radioprotector

from our Medical Biochemistry Correspondent

IT is well known that cysteamine (the amine formed by decarboxylation of the amino-acid cysteine) can protect cells against the effects of ionizing radiation, but how it does this is unknown. It has been suggested that the thiol group (SH) of cysteamine may form a mixed disulphide with the SH groups of tissue proteins, thus protecting the protein SH groups from attack by free radicals which are formed by ionizing radiation. One objection to this theory is the uncertainty about the extent to which proteins form mixed disulphides with small molecular weight thiols under physiological conditions. Modig (*Biochem. Pharmacol.*, **17**, 177, 1968) has now shown that normal preparations of proteins do contain mixed disulphides of proteins and small thiols. He has estimated protein and non-protein sulphydryl groups in crystalline bovine serum albumin and in preparations of protein from Ehrlich ascites tumour cells before and after treatment with sodium borohydride to reduce the disulphide bonds. In both preparations there was an increase in both protein and non-protein sulphydryl groups after treatment with sodium borohydride. Usually, three times as much thiol was bound to protein as was bound to other small molecular weight thiols. The author points out that this accounts for discrepancies in earlier estimations of disulphides in proteins because these have sometimes estimated protein disulphides and small disulphides together. 25 per cent of the thiol compounds liberated from the proteins was glutathione, but the other compounds were not characterized.

The protective effect of cysteamine when irradiation is given at different stages of the cell cycle has been studied in tissue cultures of Chinese hamster cells growing synchronously by Sinclair (*Science*, **159**, 442, 1968). Cysteamine reduced the number of cells killed by X-irradiation at all times. The protective effect was greatest, however, in those stages of the cell cycle when the cells are normally very sensitive to radiation, such as the G_1 period just after mitosis, and least in the stages of the cell cycle when the cells are normally more resistant (the end of the synthetic period S). When relatively high concentrations of cysteamine were added, however, the number of cells killed did not depend on the stage of the cell cycle at which irradiation took place.

Using the same experimental material—synchronously dividing Chinese hamster cells in tissue culture—Klevecz and Puddle (*Science*, **159**, 634, 1968) have shown that the activity of lactate dehydrogenase and glucose 6-phosphate dehydrogenase increases intermittently during the cell cycle. The increases occur during G_1 (three hours after mitosis), in the middle of the synthetic period (seven hours after mitosis) and in the late stages of the synthetic period (ten hours after mitosis). The increases correlated with increases in cell size and total protein, suggesting that enzyme synthesis generally varied in this cyclic manner during the cell cycle. This result suggests that cysteamine radioprotection is not due to an effect on protein synthesis, though there may be a difference in the number of proteins with sulphydryl groups synthesized during the sensitive G_1 phase and the resistant S phase.

Cyclic Antibiotics as Cation Carriers

from a correspondent

THE elucidation by Kilbourn *et al.* (*J. Mol. Biol.*, **30**, 559, 1967) of the molecular structure of a crystalline complex formed between potassium ions and nonactin, one of the cyclic antibiotics, may account for many of the curious properties of this substance and, by inference, for other antibiotics of the same type. These antibiotics (the cyclic polypeptide valinomycin and the gramicidins are more familiar examples) act by allowing potassium ions, protons and other cations but generally not sodium ions, to move across cell membranes and, in particular, across the mitochondrial membrane (Pressman *et al.*, *Proc. US Nat. Acad. Sci.*, **58**, 1949, 1967).

A significant feature of their chemical structure is the preponderance of hydrophobic side chains and the absence of free amino, carboxyl and hydroxyl groupings, which are associated with a high lipid solubility The crystal structure analysis of K^+—nonactin reveals that the K^+ ion is in the centre of the molecule, bonded to 8 oxygen atoms of keto and furane groups This structure is surrounded by the hydrophobic side chains, with the methyl substituents and the methylene group of the furane rings on the outside This structure is quite the reverse of the structure found for globular proteins—which have a hydrophobic centre surrounded by hydrophilic residues—but it is eminently favourable for a molecule which becomes embedded within the lipid side chains of the cell membrane Furthermore the structure of the antibiotics suggests that they act by enabling cations to dissolve in the hydrocarbons and hence cross the membrane In other words, they probably kill bacteria by making them leaky The antibiotic is thus a model for the membrane "carriers", which have been proposed many times over the last thirty years Pressman *et al* specifically put forward this view and called the antibiotics *ionophores* The discrimination between potassium and sodium ions (approaching 10,000 to 1 in certain cases) may depend on the size of the hydrated ion but at least in the case of nonactin the central site can accommodate both K^+ and Na^+

It is encouraging for students of cell membranes to find materials of biological origin, yet of simple structure, which possess such cation selectivity Interestingly as Andreoli *et al* hav shown (*J Gen Physiol*, **50**, 2527, 1967), valinomycin can confer cation selectivity on artificial lipid bilayers which are non-selective in the absence of the antibiotic It can also increase markedly the potassium permeability of the sheep erythrocyte membrane (*ibid*, p 2513) What is even more exciting, Mueller and Rudin (*Nature*, **217**, 713, 1968) have just shown that the antibiotic alamethicin induces action potentials in an artificial bilayer membrane which also contains basic protein How likely is it that transport across cell membranes is normally mediated by similar small molecules ? Pressman *et al* point out that many of these antibiotics contain D-amino acids, which are rare in most organisms, and replacement of the D-residues with the L-isomer severely reduces the antibiotics ability to transport K^- ions Alamethicin, although devoid of D-amino acids, contains 8 residues of the rare amino acid methylalanine Will it be possible to find, or synthesize, a polypeptide carrier from the common amino acids ? And can such a carrier, if it is found, complex with and solubilize sugars, the other major class of substances for which membrane carriers have been proposed ?

Factors for Protein Synthesis

from our Cell Biology Correspondent

The number of factors required for protein synthesis is increasing steadily Two initiation factors are required for the binding of N-formyl-methionine *t*RNA at chain initiation, at least two supernatant factors appear to be involved in the GTP dependent binding of aminoacyl-*t*RNA to ribosomes during chain elongation and now, according to Brown and Doty (*Biochem Biophys Res Comm*, **30**, 284, 1968), there is a specific protein factor—factor 3—which stimulates the binding of *m*RNA to ribosomes

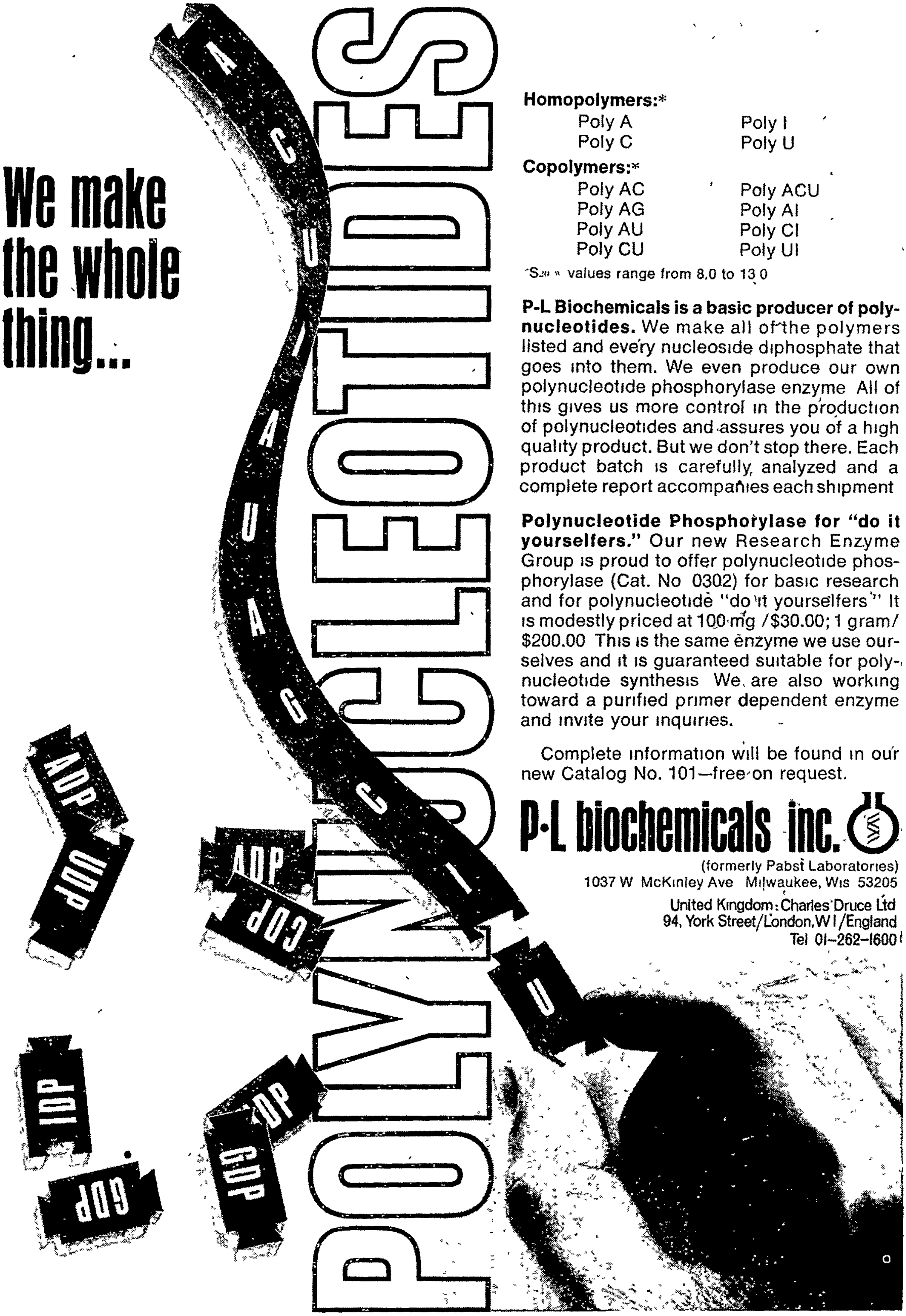
The first clue to the existence of factor 3 was when Brown and Doty found that the binding of synthetic *m*RNAs and *R*17 viral RNA to ribosomes decreased after the ribosomes were washed in 1M NH_4 Cl They inferred from this that a specific messenger binding factor was being washed off the ribosomes and so they set out to purify it Briefly, the purification involved precipitating ribosome washings at 40–60 per cent saturation with ammonium sulphate, followed by column chromatography, on TEAE cellulose, of the redissolved precipitate The fractions from the column were assayed by incubation with ribosomes in the presence of Mg^{++} ions and either the synthetic *m*RNA ApUpG(pA)$_{40}$ or *R*17 RNA As expected, some of the fractions stimulated, 2–4 fold, the binding of *m*RNA to ribosomes Further purification of these fractions yielded the purified factor, which has an ultra-violet spectrum characteristic of protein and not nucleic acid and which migrates as a single component on polyacrylamide gel electrophoresis at *p*H 8 6

Is factor 3 the same as either of the initiation factors—F_1 and F_2—discovered by Stanley and Wahba *et al.* (1966) ? Factors 3 and F_1 behave very differently during column chromatography so they are unlikely to be the same To check that factor 3 differs from F_2, Brown and Doty did an experiment to see if factor 3 could substitute for F_2 during the initiation of translation in a cell-free system programmed with the synthetic messenger ApUpG(pU)$_{30}$ Factor 3 could neither substitute for F_2 in the presence of F_1 nor for F_1 in the presence of F_2 It must therefore be a new and distinct factor, although the experiments do not show whether or not factor 3 is required for translation of *m*RNA as well as binding of *m*RNA to ribosomes

Factor 3 is, however, probably identical to part of an activity which has apparently been discovered by Revel *et al* (in the press, 1968) and is called factor *C* (unfortunately, until all the various factors involved in protein synthesis have been fully characterized, there is bound to be confusion of nomenclature and equivalence) Apparently factor *C*, which was isolated from ribosome washings, is required for the attachment of ribosomes to nascent T_4 *m*RNA In the absence of factor *C*, ribosomes do not stimulate the rate or amount of *m*RNA synthesized by RNA polymease with DNA as template Brown and Doty tested factor 3 for this activity and, in the presence of ribosomes, factor 3 gives about a fivefold stimulation of transcription

F_1 and F_2 and GTP stimulate the binding of F-met-*t*RNA$_F$ to the 30S ribosomal sub-unit in the presence of the AUG code This results in formation of an initiation complex According to Mukundan *et al* (*Nature*, **217**, 1013 1968), F-met-*t*RNA$_F$ binds initially to the amino-acyl binding site and not the peptidyl site on the 30S sub-unit, this is the same site that is used for decoding all the other amino-acids

The binding of all the other amino-acyl *t*RNAs during chain elongation is mediated by GTP and a supernatant factor *T*, which can be separated into two components *Tu* and *Ts* Lucas, Lenard and Haenni (*Proc US Nat Acad Sci*, **59**, 554, 1968) have confirmed that this binding is dependent on factor *T* and GTP and have shown that *T*, GTP and the amino-acyl *t*RNA form a complex This complex will only bind to 70S ribosomes, so both sub-units are necessary and peptide bond formation is a consequence of the binding However, yet another factor, *G*, is required for polymerization to go beyond the depeptide stage Factor *G* presumably promotes the movement of the peptidyl-



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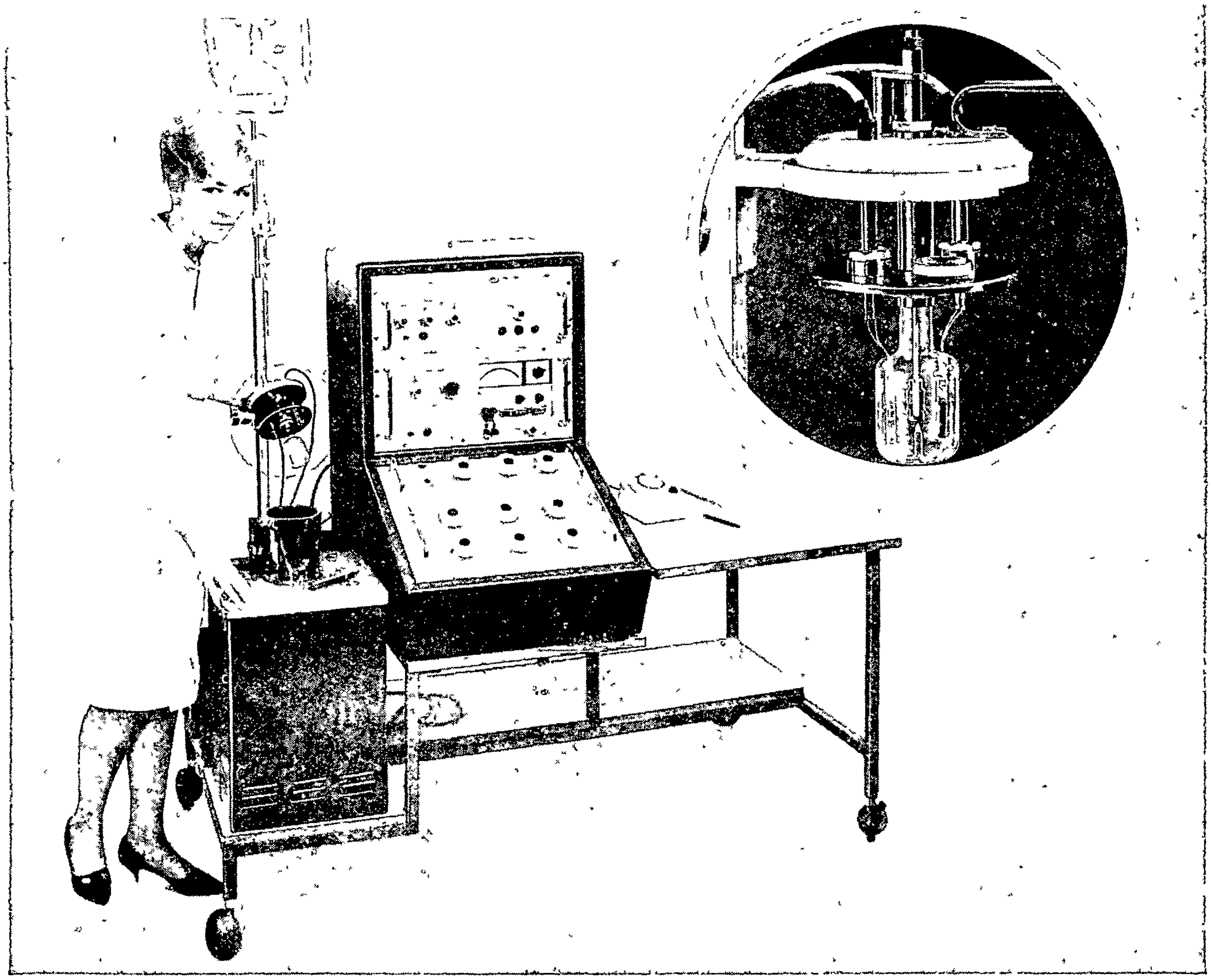

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*t*RNA from the amino-acyl site to the peptidyl site in the 70S ribosome.

Yet More Haemoglobin

from our Molecular Biology Correspondent

THE important concept that the sigmoidal oxygen-dissociation curve of haemoglobin is a consequence of sub-unit exchange, and that the symmetrical dimer $\alpha\beta$ is thus to be regarded as the basic functional entity, is by now well established. An interesting demonstration of this mechanism is provided by some experiments of Enoki and Tomita (*J. Mol. Biol.*, **32**, 121, 1968), who prepared hybrid molecules of human and canine haemoglobins, $\alpha_2^{CAN}\beta_2^{A}$ and $\alpha_2^{A}\beta_2^{CAN}$, in which either the human or the canine chains were in the cyanmet-form, and therefore non-functional. There are four such species, and all were found to show no haem–haem interactions whatever (Hill constant, *n*, of unity). By contrast, a haemoglobin, such as $\alpha_2^{A}\beta_2^{A}$, in which half the haem groups have been oxidized to the ferric state, still shows well-developed haem–haem interactions ($n=1.9$), because functional sub-units of both kinds are present. These persuasive results seem to leave little room for doubt that sigmoidal oxygen binding requires two types of functional sub-unit. In other respects, the interaction between α and β sub-units persists in the hybrid species, in particular the Bohr effect is not destroyed, whereas in species possessing only one type of sub-unit (β_4^{A} for example) the Bohr effect is absent.

Another example of the structural interdependence of sub-units in the absence of haem–haem interactions occurs in the haemoglobin-M species—human variants, in which the iron-bound histidine in one or the other type of sub-unit is replaced by tyrosine. Epstein and Stryer (*ibid.*, 113) have measured the kinetics of binding of azide ions by two methaemoglobin-M variants, one with the mutation in the α-chain, the other in the β-chain: the abnormally linked haems are incapable of binding ligands. The α-chain variant reacts much more rapidly than normal methaemoglobin, and at the same rate as metmyoglobin, whereas the β-chain variant reacts at the same rate as the normal species. Thus the reactivity of a normal β-chain is modified by association with an aberrant α-chain. Also, equivalent modifications in the α- and β-chains have non-equivalent effects on the partnering chains. It is worth pointing out that the α-chain haemoglobin-M variant has no Bohr effect, and these sub-units are thus defective in the way they interact with their partners.

The most important development in haemoglobin chemistry in the recent past has been the discovery by the Benesches of a co-factor, diphosphoglycerate, which profoundly influences the oxygen-binding behaviour and controls the function of haemoglobin *in vivo*. The manner of its interaction is the subject of a further study (*Proc. US Nat. Acad. Sci.*, **59**, 526, 1968). The results are remarkably clear-cut, in the first place, haemoglobin stripped of the co-factor retains its sigmoid oxygen-uptake curve. Addition of the co-factor then leads to a huge displacement of the curve in the direction of lower affinity. The shift is essentially complete when one mole of diphosphoglycerate has been added for each mole of haemoglobin tetramer. Moreover, direct binding experiments show that deoxygenated haemoglobin binds exactly one mole of the co-factor per mole of tetramer, whereas oxyhaemoglobin binds none at all. The change of oxygen affinity with concentration of diphosphoglycerate is then a reflexion of this difference. The stoichiometry of the interaction is altogether at odds with the requirements postulated in the original formulation of the allosteric hypothesis; the hypothesis therefore already stands in need of revision if it is to retain any generality.

Intellectual Style and High School Science

by

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Tests on Australian schoolchildren have revealed a relationship between intellectual style and specialization in science similar to that found in British schools. Convergers, who excel in close logical reasoning and do well in intelligence tests, show a bias for science. Divergers, who have high IQs but show low scores in tests consisting of questions with no one correct answer, tend to avoid science in schools.

HUDSON[1] has shown that British grammar school boys specializing in science are usually convergent in intellectual bias. But, despite the existence of what is now a well established relationship in the case of British schoolboys, Cropley has reported that outstanding Australian undergraduate scientists were mainly divergers[2]. Thus the two sets of findings look to be contradictory. We report here additional Australian data involving, not undergraduates, but schoolchildren, so that a more direct comparison can be made with Hudson's findings. The main point at issue is whether intellectual bias and science specialization are differently related in Australia than in Britain, or whether the Hudson finding prevails when Australian high school children are studied, and is reversed only at university level.

A battery of tests including a divergent thinking test (tin can uses[3]), and a convergent thinking test (the AL–AQ tests of intelligence[4]) was administered to 218 students in the final 2 years of study at two large rural high schools in New South Wales. The 218 students included 106 girls, who had a mean age of 16 years 10 months (range 15 years 8 months to 18 years 2 months), and 112 boys for whom the mean age was 17 years 1 month (range 15 years 8 months to 18 years 7 months). In that the group of students studied included girls as well as boys, the data reported here differ from the studies previously mentioned. The inclusion of girls, however, permits comparisons between the intellectual styles of the two sexes, and so extends earlier work. For this reason, the data gained by scoring the girls' test protocols were included in this report. In the New South Wales educational system, students at fifth and sixth form level may study science at any one of four possible levels, including "first level", "second level (full)", "second level (short)", or "third level". They may also elect not to study sciences at all, joining "non-science" groups. For the purposes of this article, students who had elected either first level or second level (full) were regarded as science specialists, while all students selecting other levels or non-science were regarded as non-specialists. The rationale for treating the first two levels as indicating science specialization rests in the provisions of the so-called "Wyndham scheme" (published by the Australian Council for Educational Research (ACER), and specifically designed for use with above-average groups) of which the organization of science teaching in New South Wales is a result.

The divergent thinking test was scored for originality according to the procedures described by Torrance[3], using the weights suggested by Cropley[5]. The convergent tests were scored according to the published specifications. Subsequently, raw scores on both intellectual tests were transformed into normalized standard scores to eliminate problems associated[4] with differing means and standard deviations. Finally, a bias score was obtained for each student by subtracting the convergent score from the divergent score, according to procedures described before[2]. Each student's rating as either a science specialist or a non-specialist was known, and so it was possible to look at the relationship between science specialization on the one hand, and intellectual bias on the other.

A clear-cut, preliminary finding was that there was a strong relationship between sex and bias. Although the full group of 218 boys and girls contained nearly equal numbers of divergers and convergers (115 divergers, 103 convergers), among the 112 boys, no fewer than seventy-two were divergently biased, forty convergently. The 106 girls, by contrast, included only forty-three divergers, while sixty-three were convergently biased. This relationship was highly significant ($\chi^2 = 10\cdot75$, degrees of freedom $= 1$; $P < 0\cdot01$), and indicated a marked tendency for boys to be divergers, within the context of a group of both sexes. This finding is consistent with data based on Canadian schoolchildren reported elsewhere[6]. In that study a group of students rated as "highly original" consisted mainly of boys, a group rated "unoriginal" mainly of girls.

In view of the large sex differences in bias, the relationship of course preference to bias was examined separately for boys and for girls. The 112 boys included thirty-six science specialists, of whom eighteen were divergers, eighteen convergers, and seventy-six non-specialists, of whom fifty-four were divergers and twenty-two convergers. This distribution of divergence/convergence among science specialists and non-specialists differed significantly from what would be expected by chance ($\chi^2 = 4\cdot71$, degrees of freedom $= 1$, $P < 0\cdot05$). There was a disproportionately large number of convergers among the science specialists. Among the girls, the distribution of divergers and convergers was almost exactly what would have been expected by chance ($\chi^2 = 0\cdot00$). Thus the data show that specialization in science among the boys was related to the convergent bias, a finding supported by a correlation of no less than $0\cdot485$ ($P < 0\cdot01$, degrees of freedom $= 110$) between degree of convergence and level of science achievement. Even among the girls the figure was $0\cdot347$ ($P < 0\cdot01$, degrees of freedom $= 104$).

In demonstrating the existence of a significant relationship between specialization in high school science and convergent intellectual bias, these data suggest that the state of affairs shown to exist among British schoolboy science specialists[1] may well hold for Australian boys too. Taken in conjunction with Cropley's earlier report[2] that, at university level, outstanding undergraduate scientists were not convergers as is the case with schoolboys, but divergers, the data contain an interesting implication. Apparently, although disproportionately large numbers of science specialists entering university are convergers, even as early as at senior undergraduate level, the most outstanding young men come mainly from among the relatively few divergers. This formulation is consistent with Gibson and Light's[7] failure conclusively to separate Cambridge scientists from the general populace, on the basis merely of IQ. It may well be that outstanding scientists are distinguished by the unusual bias of their intellects (unusual among science specialists, that is), rather than by unusual levels of ability.

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[1] Hudson, L., *Nature*, 198, 913 (1963).
[2] Cropley, A. J., *Nature*, 215, 671 (1967).
[3] Torrance, E. P., *Guiding Creative Talent* (Prentice Hall, Englewood Cliffs, 1962).
[4] Board of Senior School Studies, *Science (Syllabus and Notes) Third, Second (Short and Full Courses) and First Levels* (NSW Dept. of Education, Sydney, 1965).
[5] Cropley, A. J., *Creativity* (Longmans, London, 1967).
[6] Anderson, C. C., and Cropley, A. J., *Austral. J. Psychol.*, 18, 218 (1966).
[7] Gibson, J., and Light, P., *Nature*, 213, 441 (1967).

Discontinuities in the History of Sea-floor Spreading

by

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There seems to have been a lapse in the crustal spreading of the sea floor from the Mid-Atlantic Ridge during the late Tertiary period.

GEOLOGICAL and geophysical data obtained from the sea floor in the past decade have led to the hypothesis that the crest of the mid-ocean ridge is a place of injection of new crust into the ocean floor and that the sea floor is as a result spreading away from the crest[1-3]. These ideas have proved fertile for the explanation of the pattern of marine magnetic anomalies suggested by Vine and Matthews[4]. Using this hypothesis workers such as Pitman and Heirtzler[5] have recently given strong support to the idea that the ridge is truly spreading away from its crest at rates of 1 to 5 cm a year.

As new dikes are injected at the ridge axis, they acquire the magnetization of the geomagnetic field at the time they cool through the Curie temperature. The Earth's field has been shown to have reversed its polarity approximately once every few hundred thousand to a million years during late Tertiary and Quaternary time[6]. The oceanic crust has therefore acquired strips of alternately polarized crust which give rise to a remarkable system of linear positive and negative magnetic anomalies which parallel, and are symmetrical about the ridge crest where they were created.

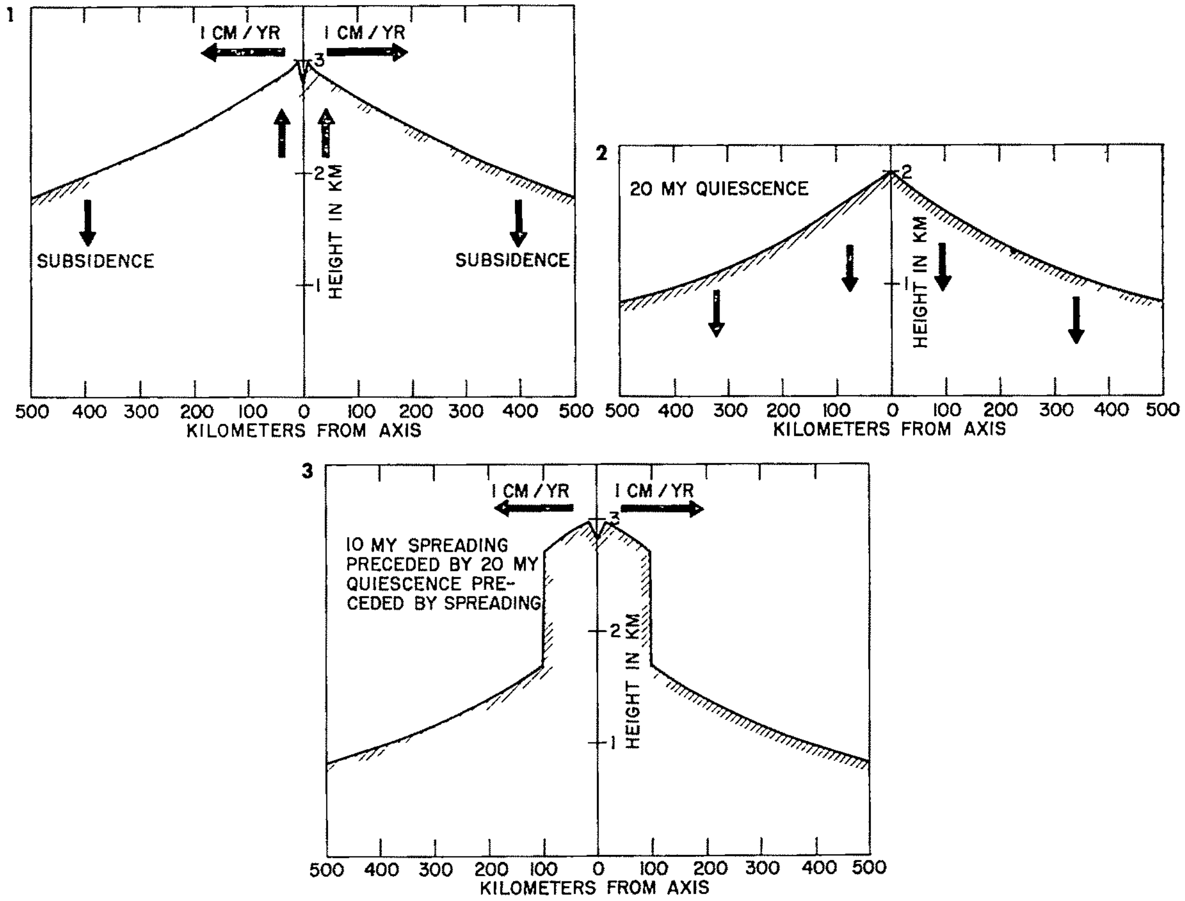

Fig. 1. Hypothetical evolution of a ridge profile during steady-state spreading (1), at the end of a 20 million year lapse (2); and 10 million years after renewal of spreading (3). The profiles were constructed from a steady-state thermal expansion model[11], corrected for isostatic compensation. It was assumed that subsidence depends only on the time after extrusion.

Ewing and Ewing[7] have recently proposed that there was a lapse in crustal spreading which lasted from between 40 or 50 and 10 million years before the present (mybp). As evidence they offer smoothed sediment thickness profiles across mid-ocean ridges. The records of seismic profiles show a relatively abrupt increase in the thickness of unconsolidated sediments at a distance X from the axis of mid-ocean ridges, where X is approximately 100 km on the Mid-Atlantic Ridge and 400 km on the East Pacific Rise. Recent spreading rates seem to have been 1–2 cm/yr and 3–5 cm/yr on the two ridges, respectively[5]. If sedimentation rates have been relatively uniform throughout the Cenozoic, the abrupt thickening of sediment suggests a period of quiescence, or a lapse in crustal spreading.

We shall first examine an idealized steady-state model of ridge spreading and then set out in a generalized fashion the various geological or geophysical criteria by which a lapse in sea-floor spreading might be recognized.

By a lapse in spreading we mean the following. Let X be the distance, measured parallel to the spreading direction, of a parcel of crust from the axis of the mid-ocean ridge. Then a lapse is recorded by two strips of crust, $X_1 < X < X_2$, which were generated at a slower rate when compared with spreading rates S_1 and S_2, before and after the lapse. These strips of crust may be very narrow if spreading stopped and started relatively abruptly. The difference in appearance of the crust $X < X_1$ and that of the crust $X > X_2$ will in general depend on (*a*) the length of the hiatus, (*b*) the difference in spreading rates, S_1–S_2, and (*c*) the time that has elapsed since the end of the hiatus.

On discussion of possible criteria for recognizing a hiatus, several of these criteria, including new magnetic, topographic and seismic profiler data, are then offered as additional support for the hypothesis of Ewing and Ewing[7] that there was probably a middle or late Tertiary lull in crustal spreading, at least on the Mid-Atlantic Ridge. The possibility of other discontinuities in spreading rate is then considered.

The Steady-state Model

Before discussing possible irregularities in the history of crustal spreading, we shall first discuss the possibility and problems involved in an ideal steady-state mid-ocean ridge. This model requires in general that mantle derivatives are injected at a constant rate at any point on the ridge. There may be some injection at a distance X from the axis, causing an increase of spreading rate away from the axis. In a steady-state model, however, any "geophysical operator" G_t which changes the crust at X does not, on the average, change with time The remarkable symmetry of magnetic and topographic profiles across active ridges (which may not be steady-state) suggests that bilateral symmetry is a basic property of ridges[8] Thus $G_t(X) = G_t(-X)$ It seems to be an additional characteristic of ridges that, apart from occasional volcanoes, the upper crust (above the region of possible phase changes) is not appreciably modified after leaving the axial region For example, the work of Sykes[9] has shown that epicentres on the Mid-Atlantic Ridge usually fall between ± 10 km of the axis, suggesting that at present most tectonic activity on the ridge takes place at the crests. Furthermore, the geomagnetic history, when compared with the magnetic anomaly signature, suggests that the spreading rate does not increase appreciably away from the axis[5] Matthews and Bath[10] have shown that the departure of observed magnetic profiles across the Mid-Atlantic Ridge from the classical Vine–Matthews theory is best accounted for if it is assumed that new dikes do not necessarily bisect their predecessors but have a normal distribution about the axis with $\sigma = 5$ km It therefore appears that in the upper crustal layers $G(X) \approx 0$ for $X \gg 5$ km except for occasional vulcanism and the effects of sedimentation

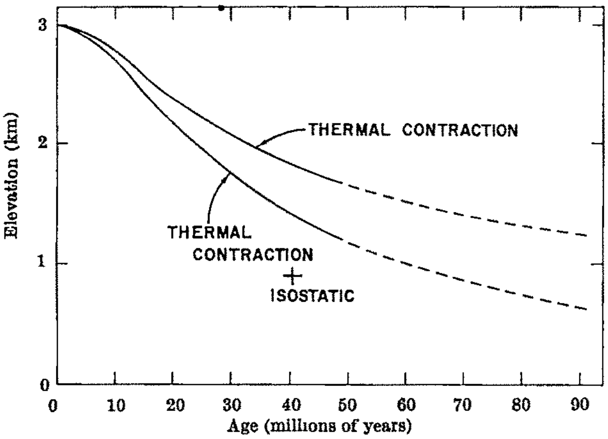

Fig 2 Subsidence against time for a parcel of crust riding down the flanks of a steady-state ridge Solid curve, estimated from ref 12, represents pure thermal contraction Dashed curve includes additional subsidence if increase of water column and thermal contraction of water column is compensated with material of density 3 2

One obvious consequence of a steady-state ridge is that the spreading rate $S(X)$, the subsidence rate $f(X)$ and the regional topographic slope $\theta(X)$ are related by $\tan\,\theta(X) = f(X)/S(X)$. A bathymetric-magnetic profile may yield the slope and spreading rate, allowing the calculation of the subsidence rate Vogt and Ostenso[11] have observed that, if the Pacific–Antarctic Ridge ($S = 4\ 5$ cm/yr) and the Reykjanes Ridge ($S = 1$ cm/yr) are steady-state, f is about 10 cm/1,000 yr for the first $12–15 \times 10^6$ yr after extrusion at the axis. The same quantity $f(X)$ drops to a few cm/1,000 yr on the lower flanks, provided, of course, that the spreading rate has been constant in time The subsidence rates of guyots and the edge of the eastern United States continental shelf have been of the same order of magnitude

A recent attempt at computing a model steady-state mid-ocean ridge[12] based on the assumption that ridge uplift is due entirely to thermal expansion predicts a maximum subsidence rate of 2 or 3 cm/1,000 yr for the first 50 million years. According to this model of Langseth *et al* [12], hot mantle material (1,500° C) is injected at the base of a layer 100 km thick. The material rises to the surface and spreads away from the axis at a constant rate, losing heat by conduction As the hot material spreads away from the axis it contracts In the model computations, the coefficient of thermal expansion, presumably constant, was not specified The conductivity was taken to be constant and equal to 6 c g s throughout the layer. The heat capacity was 0 3 calories/g and the density 3 4 g/cm^3 The thermal equation was solved by iteration for points 10 km apart in the spreading layer The temperature evolution was computed for every point at intervals of 10^5 years, beginning with the equilibrium state in the absence of convection The theoretical mid-ocean ridge models[12] suggest that as long as convection speeds exceed a certain value (0 5 cm/yr), the maximum elevation of the ridges, as well as the rate of subsidence down their flanks is independent of their spreading rate Past variations in the spreading rate may then be reflected by variations in regional slope, because the sinking rate f depends only on the time after extrusion at the axis if $S > 0\ 5$ cm/yr If $S < 0\ 5$ cm/yr, including the limiting case of total quiescence, Langseth's calculations do not yield an exact estimate of f It is, however, clear that, for very slow spreading, the upwelling material loses heat before reaching the surface The crustal elevation of ridges will therefore decline to zero as $S > 0$ Subsidence rates on the flanks of such slow spreading ridges depend on the thermal gradient and therefore also decline when S decreases below 0 5 cm/yr.

There are several significant differences between the Langseth steady-state model and actual ridges These differences, which may be related, are the following

A. Differences between observed and calculated regional topographic slopes—that is, subsidence rates.

B Differences between observed and calculated total uplift

C. Differences between observed and calculated heat-flow anomalies

A. The previous discussion, following from that of Langseth *et al* [12], has assumed that a mid-ocean ridge is the effect of pure thermal expansion, which implies that the mass per unit area below the sea floor remains everywhere constant. But the water column above a parcel of crust increases from 2 or 3 km to more than 5 km as the

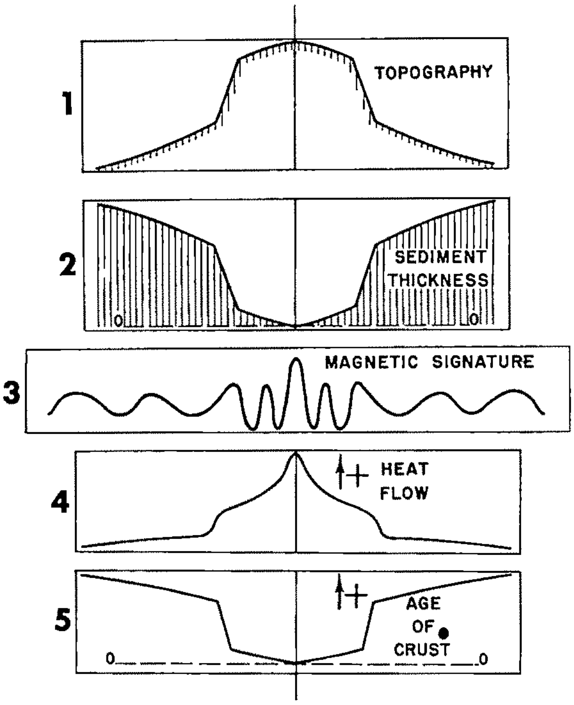

Fig 3 Schematic illustration of several types of evidence for recognizing a lapse in sea floor spreading Relatively abrupt changes in topography, sediment thickness, heat flow and age of crust mark position of lapse Change in magnetic signature could indicate (1) abrupt change in spreading rate, (2) abrupt change in geomagnetic polarity reversal rate, (3) lapse in spreading superimposed on a gradually changing reversal rate, or (4) a combination of (1) and/or (2) with a lapse

parcel moves down the "subsiding conveyor belt" from the crest of the ridge to abyssal depths. This increases the total mass in the crustal column. Talwani *et al* [13] and others have shown, however, that free-air gravity anomalies are nearly zero over the ridge or are, if anything, slightly positive over the crest. The increasing load of the water column above the thermally contracting crust is, therefore, being compensated isostatically. A given thermal subsidence Δh will then be changed because the density and thickness of the crust and the overburden of water is changed. Consider two adjacent crustal columns of identical mass, the one shortened by Δh on account of thermal contraction. Both columns have their tops below sea level and are suspended in a substratum of density ρc. Let the density of water be ρo. Then the difference in water depths to the tops of the two columns is $\{\rho c/(\rho c-\rho o)\}\ \Delta h$, by straightforward application of Archimedes' principle. If $\rho o = 1.03$ and ρc is between 2.8 and 3.4 g/cc, the total subsidence will be between 1.58 Δh and 1.44 Δh. In Fig. 2, an isostatic subsidence curve has been constructed from Langseth's model, taking $\rho c = 3.1$ g/cc. The maximum subsidence rates are about 3.5 cm/1,000 yr after this isostatic correction has been applied to the Langseth model.

The predicted subsidence rates and those "observed" on active ridges (provided the ridges are steady-state) are of the same magnitude. The 10 cm/1,000 yr subsidence rate "observed" for the first 12–15 × 10^6 yr after extrusion is, however, several times that predicted by the Langseth model[12], even after isostatic corrections have been applied. Phase changes under the cooling flanks of this ridge must also contribute to the "observed" subsidence.

B. The Langseth model predicts a maximum elevation of about 2 km for ridges. This seems approximately correct, but may be as much as 50 per cent too small if the equilibrium level of the sea floor is represented by the igneous basement under abyssal plains at large distances from the ridge crest[14]. This discrepancy, if real, might be taken to indicate that the coefficient of expansion is higher, or that phase changes make a significant contribution to the total uplift[3]. If 1 km of the maximum elevation of mid-ocean ridges above the equilibrium level is due to the presence of abnormal mantle with a density 3.25 g/cc, and if this material is being steadily converted to normal mantle of density 3.40 g/cc, the total thickness of anomalous mantle would be 20 km under mid-ocean ridges. The densities are those used by Talwani *et al* [13] in computed crustal models of mid-ocean ridges. The expansion due to phase changes can be no greater than the total, so that the thickness of abnormal mantle converted to normal mantle must be less than 60 km. These figures are very sensitive to the densities of the two phases, which are themselves uncertain. At this stage one can only guess at the phase changes which might be significant in contributing to the subsidence of the flanks of mid-ocean ridges. Three such processes, all of which cause an increase in bulk density, are deserpentization[3], the transformation of basalt to eclogite[15] and the downward freezing of an interstitial liquid basalt fraction in an ultrabasic crystal mush. The last process requires high temperatures and is therefore confined to the mantle except in the region of axial intrusion.

C. The predicted heat flow profile across mid-ocean ridges is broad, high and sharply peaked at the axis, but the active ridges are characterized by relatively narrow belts of rapid heat flow flanked by regions in which heat flow is within 20 per cent of the oceanic average[12]. The high values are found within about 300 km of the crest of the East Pacific Rise and within 100 km of the crest of the Mid-Atlantic Ridge.

Is there any way to adapt the steady-state model to make it fit observational data? The high nearly axial subsidence rate could be due primarily to a phase transformation. Thus, 10 cm/1,000 yr is equivalent to the annual conversion of a layer of density 3.25 g/cc and 2.3 mm thick to the higher density of 3.4 g/cc. This does not account for the discrepancy between observed and predicted heat flow.

The narrow belt of observed high heat flow may suggest that the zone of input is much narrower than assumed in the models[12]. This inference is supported by the existence of a broad magnetic low over the axis of the Mid-Atlantic Ridge. Heirtzler and LePichon[16] ascribed the anomaly to a rise in the Curie isotherm, and their model implies that the Curie isotherm has roughly the shape of an upward pointing wedge with a base 160 km wide at a depth of 30 km, and with its apex about 11 km below sea-level. The melting curve of basalt must form a still narrower wedge, which would then represent the zone of input.

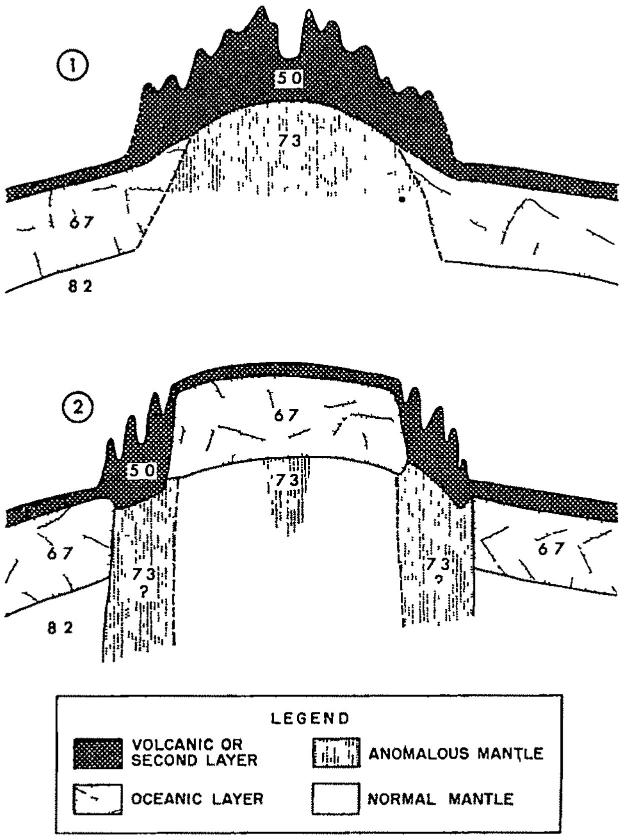

Fig. 4. Crustal structures for two hypothetical spreading histories. In (1) spreading rates were less than about 2 cm/yr before and after the lapse. In (2) the rates exceeded 2 cm/yr except during a period of slower spreading, when crust similar to (1) was generated. Apparently the late Tertiary lapse discussed in the text has been recorded by the Mid-Atlantic Ridge (1) and East Pacific Rise (2) in this manner.

The thermal models of Langseth *et al* [12], on the other hand, predict a broader higher warp in the Curie point surface. The dimensions of this wedge have been interpolated from their Fig. 17, which shows the isotherms due to steady-state spreading at 1 cm/yr. The top of the wedge reaches within about 7 km of the surface and has a base more than 1,000 km wide at a depth of 30 km. Uncertain though the magnetized model calculations may be, it is nevertheless interesting that the width of the zone of input may be only a fraction (1/7 in the rough calculation) of that assumed in the thermal model.

The Curie isotherm also reaches closer to the surface in the thermal models than in the magnetic model. This is consistent with the result[12] that the actual amplitude of the heat flow anomaly is less than that predicted by the thermal model.

The models of Langseth assume constant conductivity

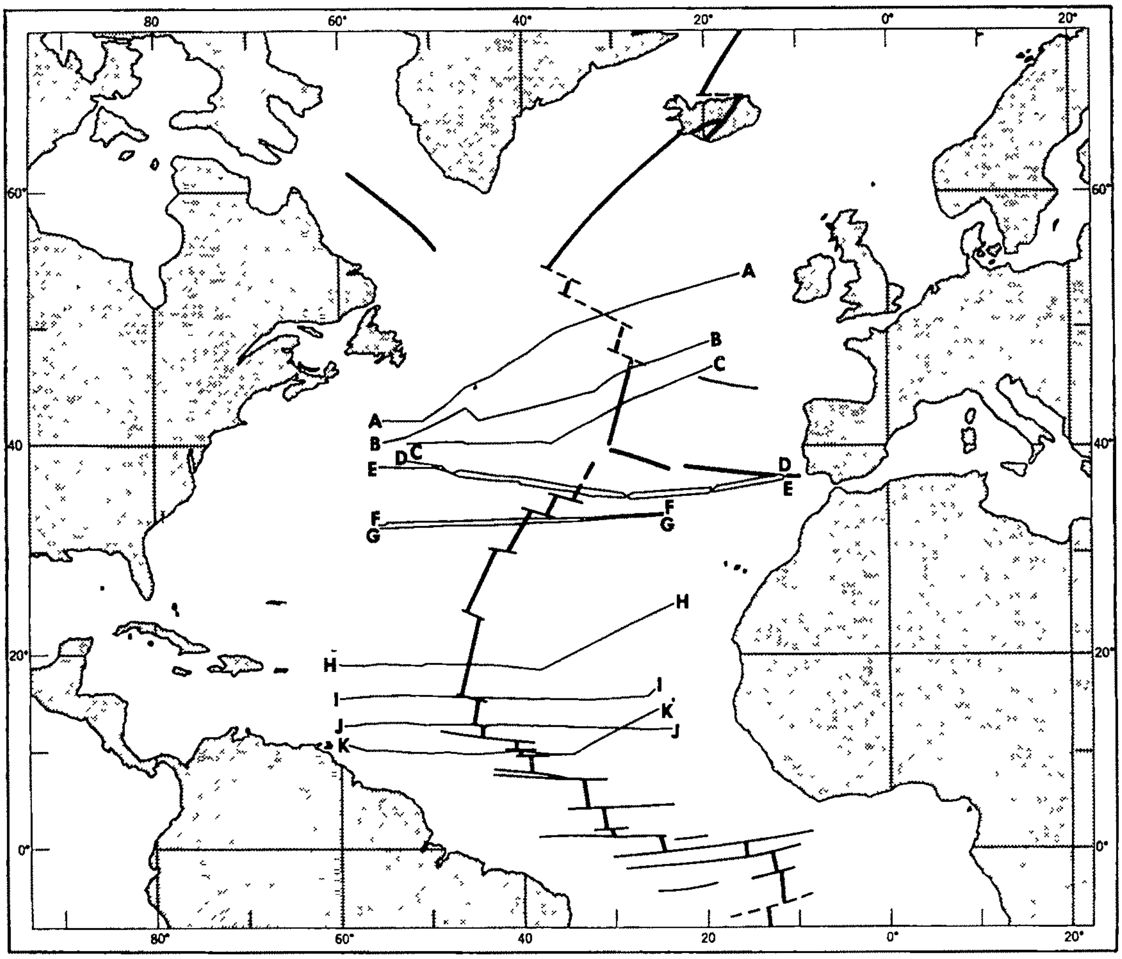

Fig 5 Mid-Atlantic Ridge crest and fracture zones. Superimposed are locations of profiles recording bathymetry, sub-bottom and magnetics Profiles *C*, *D*, *E*, *F*, *G*, *I* and *J* are exhibited in the following figures

It is, however, conceivable that the material under the axis has a much greater effective conductivity at depth, and that the crust has a lower conductivity. The qualitative implication of such a model would be a narrow band of high heat flow in the axial region superimposed on a very flat anomaly extending to the margins of the ocean basins. The latter anomaly must be within 20 per cent of the oceanic average[12]. The high "effective conductivity" in the axial region may simply be a consequence of an effective heat transfer mechanism, as would be provided by the constant injection of new dikes and the emission of lavas

Finally, the possibility must be considered that ridges may be steady-state over periods of 10 to 50 $\times 10^6$ yr but that volcanic cycles of much shorter periods—say 0 1 to 1 $\times 10^6$ yr—may be superimposed on them. Crustal spreading could still be constant while the surface discharge of lavas varied Outbursts of vulcanism may discharge large quantities of heat over short periods of time If the present time is characterized by less than average volcanic and/or plutonic activity, the discrepancy between the model and the observed heat-flow profiles would be understandable Because some active volcanoes occur many hundreds of kilometres from the axis of active ridges, discharges are not confined to the axis Iceland is not typical of the Mid-Atlantic Ridge, but it may be significant that the width of even the very young (0 005 $\times 10^6$ yr) post-glacial volcanic belt on Iceland exceeds the width of the crustal strip generated in 1 $\times 10^6$ yr at 1 cm/yr. The Pleistocene volcanics occur in a complex belt that widens from 100 km in the north to 300 km or more in the south[17]. The actual widening of the crust seems to be 1 cm/yr away from the axis both north and south of Iceland[5,18], implying a widening of only 20 km there during the Quaternary. Svalbard (Spitsbergen) affords similar evidence of exceptional activity during the Quaternary. At distances of 100 to 200 km from the mid-oceanic rift, the continental crust of Spitsbergen has been disturbed by limited Quaternary vulcanism There is no evidence for previous vulcanism since middle Tertiary times[19]

Although it is hazardous to suppose that Pleistocene volcanics occur over such a broad belt on the typical parts of the ridge, the width of the belt of thin sediment (200 km) is about the same as the Pleistocene volcanic zone on Iceland. The possibility cannot be ruled out that the zone of thin sediments on the axis of the Mid-Atlantic Ridge was created when Pleistocene and later volcanic activity obliterated much of the earlier sediments. This would invalidate the interpretation by Ewing and Ewing of the change in sediment thickness as a marked age difference between adjoining parts of the ridge It should be emphasized that such episodic vulcanism is not necessarily inconsistent with a constant spreading rate, although a thickening of the volcanic layer could be expected. Magnetic surveys of Iceland itself will decide whether the island is at all typical with respect to magnetic anomalies and sea floor spreading. There seem to have been several rifts which have been intermittently active on Iceland[17], and this could partly account for the broad Pleistocene volcanic belt.

Although it is conceivable that steady-state models can be found which account for the present observations, particularly if vulcanism is not confined to the central rift or/and is episodic in nature over a short period of time, several difficulties in the steady-state model disappear if crustal spreading does not always proceed at a constant rate.

Non-steady-state Ridges

Assuming the possible non-steady-state ridges, we may distinguish between past changes in spreading rate and discontinuities in time, or lapses during which spreading was at a standstill.

An example of the latter is the hypothesis that no crustal spreading occurred between 40 or 50 mybp and 10 mybp[7] This accounts for the thinness of the axial sediments It also accounts for the narrow band in which heat flow is high. Indeed, it was the disagreement between observation and model that led Langseth *et al* [12] to suggest that the Mid-Atlantic Ridge has become reactivated during the past few million years after a lapse which lasted through most of the Tertiary. The lapse hypothesis accounts for several other characteristics of the ridge

To begin with, we shall examine the likelihood that ridges are not always active There is evidence for rifts in every stage of fossilization The Mid-Atlantic rift bifurcates into a number of major and minor branches north of 50° N. yet, on the basis of seismicity, vulcanism and magnetic anomalies, present activity is almost exclusively confined to a single narrow rift zone There are perhaps three major rifts at 55° N.: (1) the Labrador Sea rift, which is essentially inactive, although occasional small earthquakes occur[20]; (2) the Reykjanes rift, active at present, (3) an older rift between the European shelf and the Rockall–Faeroe ridge[21] South-western Iceland is marked by three rifts active during post-glacial times[17], but only one of these is now seismically active[20] Between Iceland and Jan Mayen Island there is evidence for a fossil rift now represented by a belt of seamounts in the Norwegian Basin east of Jan Mayen Ridge[22] In the Arctic Basin, the active branch is the Nansen Cordillera The Alpha Ridge appears to be a fossil ridge which became inactive during the Tertiary[23]. It is not yet known whether more than one branch was ever active at the same time, or if periods of quiescence preceded cracking in a new area The atypical crestal magnetic pattern and the aseismic nature of the ridge south and east of southern Africa also suggests that it is at present a dormant ridge[21]

In view of the evidence that many rift segments are presently inactive it is not presumptuous to suppose that crustal spreading may be rejuvenated at the axis of a temporarily inactive ridge The ridge is then no longer steady-state, but will reveal somewhere on its flanks a more or less sharp boundary between the old crust and the new. If this lapse in sea floor spreading is recorded by the crust at present between X_1 and X_2 from the axis of the ridge, the following are some of the possible geological and geophysical criteria by which it might be recognized (Figs 3 and 4)

(1) The topographic profile is steepened over a region that may be wider than X_1–X_2 because of heat exchange between old and new crust Fig. 1 shows how the ridge profile might appear after an idealized lapse (quiescent period) which lasted for 20 million years and ended 10 mybp This profile was derived by applying an isostatic correction to the 1 cm/yr model ridge profile[12] and assuming that subsidence of a parcel of crust depends in the first approximation only on the time after extrusion at the axis Fig 1–1 shows the ridge steady-state. Fig 1–2 shows the same ridge considerably subsided, at the end of a 20 million year lapse. The rejuvenated ridge, the crest of which has again attained the maximum of about 3 km elevation above the equilibrium level, is shown in Fig. 1–3 The mid-ocean ridge in the North Atlantic Ocean may be in such a condition

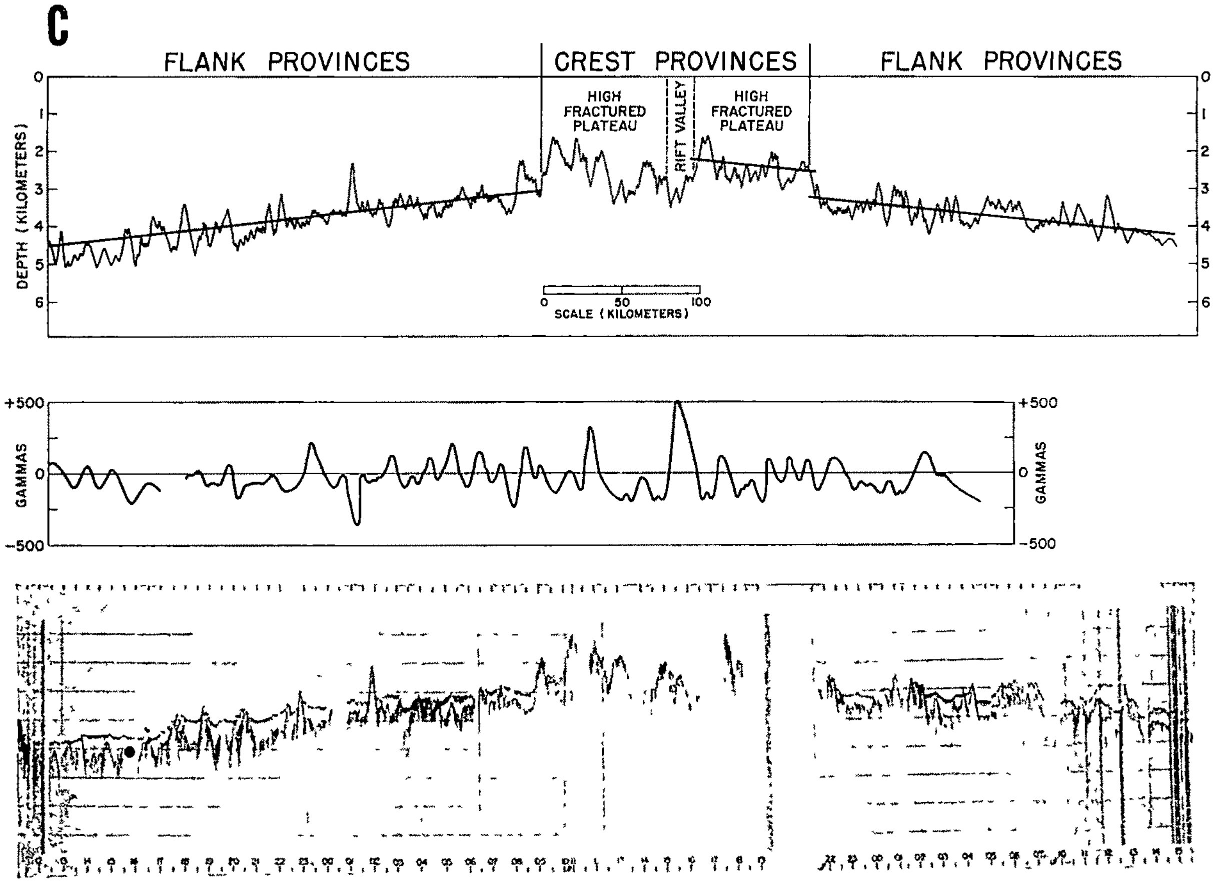

Fig 6 Profile C across Mid-Atlantic Ridge at 46° N Angle between profile and ridge is about 45° Original reflexion record is shown at bottom Straight lines were ruled through basement topography Province designations are based on Heezen *et al* [8]

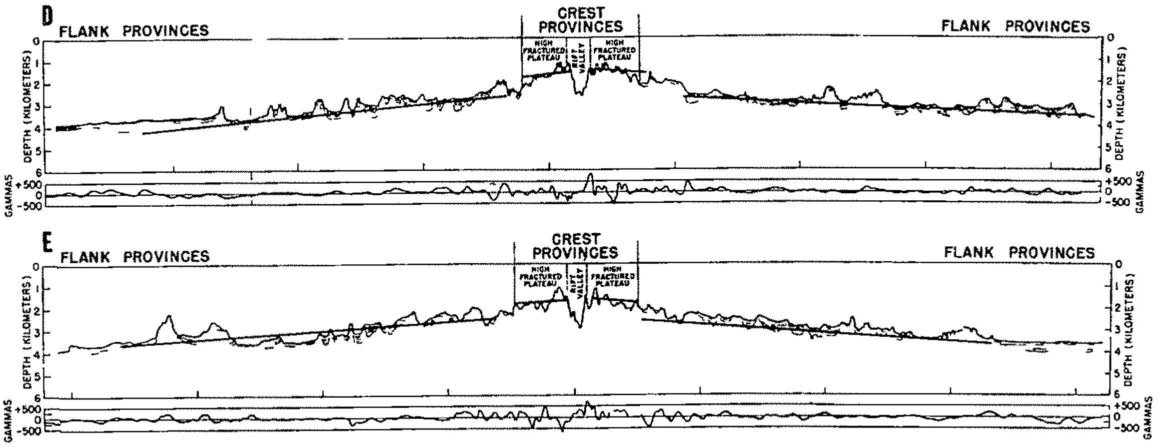

Fig 7 Profiles *D* and *E* across the Mid-Atlantic Ridge at 36° N, illustrating crestal province differing from adjacent flanks in basement roughness, magnetic signature, elevation and sediment thickness Angle between ridge and profiles is about 80° Tick marks at base of profiles are 100 km apart

(2) Sediment thickness increases sharply, if sedimentation rates can be assumed to have been constant

(3) Heat flow values diminish sharply The sharpness will depend on the age and length of the hiatus as well as the conductivity of the crust and upper mantle

(4) After magnetic profiles have been reduced to a constant elevation above the igneous basement, the magnetic pattern may differ on either side of X_1 and X_2 This implies that the hiatus could have marked a change in the frequency of polarity reversals and/or a change in spreading rates of the ridge The most powerful tool in establishing the existence and length of a hiatus would be a direct application of the Vine–Matthews technique[4], which requires a complete history of field reversals, determined from palaeomagnetic measurements and isotope dating, for a period of time at least long enough generously to bracket the hiatus If a certain sequence of reversals is missing in the oceanic crust at $\pm X$, the "magnetic tape recorder" must have been "turned off" for the corresponding period of time

In the absence of such a detailed dated history of polarity reversals—the exact reversal sequence has been extended only to 4 mybp at present—the existence and length of a hiatus could be established by the following relative technique If a complete sequence of reversals, as manifested by anomalies, is recorded on one part of the ridge system, it can be used as a standard of comparison for a part of the ridge where there is a hiatus, reflecting a lapse in spreading The length of the interval for which no crust was generated on the non-steady-state ridge is estimated by the length of the "missing" sequence of anomalies

(5) Isotope and palaeontological ages of the oldest rocks and sediments increase sharply

(6) The character of the subsedimentary crust seems to depend on spreading rate, so that past variations in this rate would have been recorded by the crust Menard[24] has observed that for $S < 2$ cm/yr, the crust that is generated is characterized by a thick "volcanic" layer, rough topography and the absence of an "oceanic" layer Conversely, $S > 2$ cm/yr is characterized by a thin volcanic layer, a smooth basement and an oceanic layer The time rate of generation of the volcanic layer seems to be relatively independent of spreading rate

In Fig 4, two possible combinations are shown schematically Fig 4–1 suggests a present rate less than 2 cm/yr and a past rate greater than 2 cm/yr The interval between is shown dashed It could be merely a transition from one rate to another, or spreading could have been slow or zero during the interval It is not known what the crustal structure might be for spreading rates much less than 1 cm/yr, or indeed if such rates are possible. The second example shows the crustal structure that would be expected if spreading dropped below 2 cm/yr for an interval of time and then rose above 2 cm/yr again

The following additional types of evidence for lapses in sea floor spreading are not restricted to the strips $X_1 < X < X_2$

(7) Volcanic activity might be different in volume and petrology before, during and after the quiescent interval The islands on the ridge would be expected to be most affected, but the possibility that volcanic activity at great distances from the ridge axis is also coupled to spreading rates cannot be discounted, especially if there is differential movement between the magma sources and the overlying crust

(8) The history of volcanic and tectonic activity along island arcs should reveal lapses in sea-floor spreading if the arcs constitute the crustal "sinks" corresponding to the mid-oceanic "sources"

(9) Orogenic and epeirogenic events on adjacent continents may correlate with events on the mid-oceanic ridge

Evidence for a Late Tertiary Lapse

Eleven crossings of the North Atlantic taken between 10° N and 46° N have collected magnetic, bathymetric and sediment thickness data (seismic reflexion) (Fig 5), which have been combined with published geological and geophysical data from the ridge Analysis of these data suggests that many known but unexplained characteristics of the ridge can perhaps be explained in the light of a hiatus in sea floor spreading

Most crossings of the North Atlantic show a distinct break in the topography at a distance of 60 to 130 km from the central rift (Figs 6–10) This break was first noted by Heezen *et al.*[8], who subdivided the ridge into crest and flank provinces with a marked steepening of slope between. The crest province is marked by local topography (800 m relief) which is rough compared with that of the flank provinces (400 m relief)

This steepening of the topographic profile is a distinct vertical topographic offset in the basement and is generally symmetric about the ridge axis The magnitude of the offset ranges from 0 5 to 0 7 km Fracture zones complicate measuring the offset in slope at several crossings of the North Atlantic (Figs 8 and 9), but the raised crestal block of the ridge is a feature that is easily seen in most exaggerated profiles The marked break in slope fits the model, suggested earlier in this paper, of a hiatus in sea floor spreading (criterion 1) During this hiatus the entire ridge subsided by thermal and petrochemical contraction A resurgence of activity was marked by renewed spreading and uplift of the crestal portions of the ridge The rate of uplift is unknown but, for example, the Colorado Plateau, which may constitute a rejuvenated segment of the East

Pacific Rise, has been uplifted at a rate of about 15 cm/1,000 yr since the Pliocene[25]

The variation of sediment thickness with distance from the axis of the Mid-Atlantic Ridge is also consistent with a late Tertiary lapse in sea-floor spreading (criterion 2) Seismic refraction data, cores and bottom photographs have all noted the scarcity of sediment on the crest of the ridge Early seismic reflexion data showed a marked lateral sediment thickness discontinuity on the Mid-Atlantic Ridge[26] Ewing and Ewing[7] have perhaps documented that this discontinuity is of world-wide extent Their best explanation of the phenomenon is a lapse or halt in crustal spreading during the Tertiary, followed by a later resurgence These and earlier workers, noting the thinning of sediment toward the ridge crests, suggested a younger age of the ridge towards the crest[7], but they failed to note that this sedimentary thickness boundary takes place at the well marked physiographic boundary between crest and flank, indicating a marked age difference between these two adjacent provinces

The lateral discontinuity in sediment thickness is evident in the seismic reflexion data despite marked differences in absolute thickness along the strike of the ridge The crossing at 12° N (Fig 10) reveals only 200–400 m of sediment on the ridge flanks, whereas the thickness at 46° N. (Fig 6) ranges from 600 to 1,400 m Yet in both regions the ratio between sediment thickness on the crest and that on the immediately adjacent flanks ranges between 1 2 5 and 1 . 5

Sediment thickness on the ocean floor is controlled by (1) age of the underlying crust, (2) biological productivity and (3) proximity to sediment sources and sediment distribution systems

Air gun and sparker seismic records across the ridge crests show little sediment in this province (less than 100 m) and no noticeable thickening of sediment occurs from the rift to the crest-flank boundary (Figs 6 and 10) Records across the flanks also show no noticeable thickening away from the crest

This observation raises a problem, because uniform sea-floor spreading would appear to demand a monotonic increase of sediment thickness with X, the distance from the crest of the ridge

This dilemma might be resolved by allowance for the steadily changing sedimentation regime "seen" by a parcel of crust as it rides down the "subsiding conveyor belt". For example, the lack of apparent sediment thickening down the flanks may be due to a change from the relatively rapid pelagic rates (1 cm/1,000 yr foram ooze) to the slow rates prevailing in the red clay zone below the level of carbonate compensation (0 1 cm/1,000 yr) In the crest province, the lack of noticeable thickening of sediments away from the younger central rift perhaps stems from techniques of seismic measurement It is also known from bottom photographs that, near the crest, currents are filling in small crevices and valleys with sediment

The heat flow data under "steady-state ridges" are also consistent with the lapse hypothesis High values occur on the high crestal block and are flanked by essentially average values (criterion 3) Magnetic anomaly patterns across the ridge show a change in anomaly pattern from crest to flank with longer wavelength and lower amplitude anomalies prominent on the flanks[13] (criterion 4) This marked change takes place at the fifth major positive anomaly from the crest or at about 10 mybp (ref 21) (Figs 7 and 8) Such change in the character of the anomaly pattern at this boundary either marks (1) a change in the frequency of polarity reversals or (2) a change in the rate of ridge spreading (Fig 3)

Avery *et al* [27] have analysed a detailed aeromagnetic survey of the Norwegian Sea by comparing the anomaly sequence observed there with a "standard sequence" established by several workers at the Lamont Geophysical Observatory (see ref 5 for example) This sequence applies to the mid-oceanic ridge in the North and South Pacific and South Atlantic On the assumption that ridge spreading has been continuous on these ridges, Avery *et al* [27] while admitting the tentative nature of their conclusions, propose the following history for sea floor spreading in the Norwegian Sea The first episode of spreading lasted from 60 to 49 mybp at a rate of 1 4 cm/yr A second period of spreading seems to have lasted for 20 million years (assuming $S = 1$ cm/yr) This interval may or may not have been continuous with the first spreading episode and ended considerably before 10 mybp There was a long period of quiescence, with renewed spreading beginning 10 or 15 mybp on Iceland-Jan Mayen and Mohns ridges The relative magnetic correlation technique (under criterion 4) therefore supports the proposed late Tertiary lapse, at least on the northern extension of the Mid-Atlantic Ridge

As yet, the geomagnetic reversal time scale is not complete enough to establish the exact length of the hiatus The present rates of spreading provided by studies of sea-floor magnetic patterns are approximately consistent with various geological estimates for the time at which rifting began So if we invoke a stoppage in sea floor spreading, it must either be short compared with the overall time of opening the basins or we must invoke higher spreading rates in the past

The distribution of isotope and palaeontological ages away from the ridge crest (criterion 5) is poorly known[11], but the data at least do not preclude the lapse hypothesis

Recent work[28] has revealed a strong correlation between the petrology of oceanic rocks in the Atlantic and Pacific and the distance from the mid-ocean ridge axis Basalt specimens collected within the belt of high heat flow around the crest are strongly oversaturated with silica, whereas undersaturated alkaline rocks appear on the

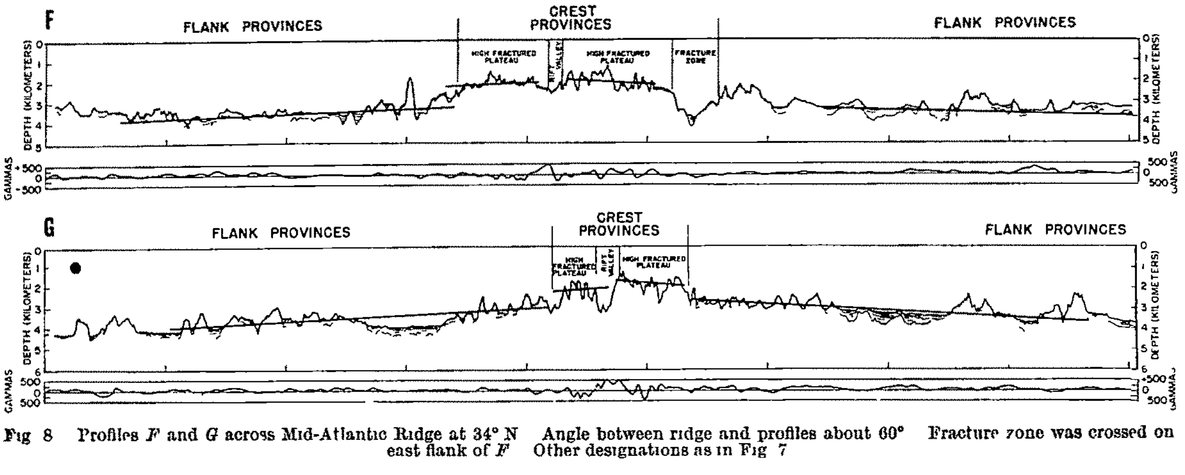

Fig 8 Profiles *F* and *G* across Mid-Atlantic Ridge at 34° N Angle between ridge and profiles about 60° Fracture zone was crossed on east flank of *F* Other designations as in Fig 7

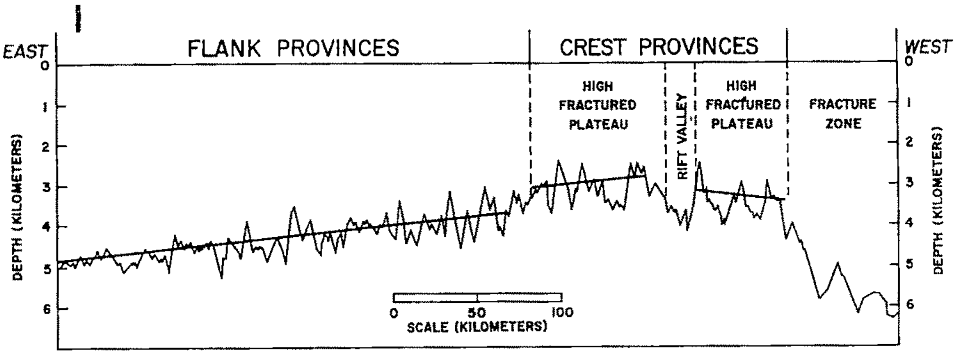

Fig 9 Profile *I*, crossing Mid-Atlantic Ridge perpendicularly at 18° N Profile crosses fracture zone on west flank Other designations as in Fig 6

flanks and in regions far from the crests. Perhaps the petrology of rocks injected since rejuvenation of rifting differs systematically from that of rocks antedating the lapse (criterion 7)

Geological evidence from an active island arc also supports the late Tertiary lapse in crustal spreading (criterion 8) In his studies of the West Indies, Professor T W. Donnelly has concluded that the period between about 40 and 10 mybp was marked by little or no volcanic activity, and the erosion of older lavas Uplift and a renewal of vulcanism began about 10 mybp (personal communication from T W Donnelly) Detailed studies should be made to attempt to correlate orogenic and epeirogenic events on island arcs and continents with the proposed Tertiary lapse in spreading (criterion 9)

Evidence for Other Discontinuities

There is some evidence for several other discontinuities which may, however, only involve changes in rate of spreading Phillips[29] has found an apparent discontinuity in rate which occurred 5 to 6 mybp and involved a decrease of 18 to 27 per cent This change was observed at 22°N and 27° N on the Mid-Atlantic Ridge and resembles a similar discontinuity on the Juan de Fuca Ridge Because the absolute magnetic reversal time scale has not been established, the discontinuity in spreading rate may not be real

Another discontinuity in rate may exist between 300 and 400 km from the axis of the northern Mid-Atlantic Ridge At about 300 km the crustal section is still typical of "slow" ridges, for there is 3 km of second layer overlying low-velocity mantle[13] At 400 km, this abnormal mantle is replaced by 5 km of oceanic layer overlying a normal mantle[13]

The wavelength of this dominant magnetic anomaly changes from 32 km to 36 km at $X=300$ km[16] If no change in the average reversal rate occurred, this implies a 12 per cent decrease in spreading rate The time of this change cannot be established, for the subsequent spreading history is not known precisely. If the average spreading rate has been 1 5 cm/yr, the discontinuity occurred at 20 mybp (if there has been no Tertiary lapse) or at 50 mybp (if there was a lapse of 30 million years as suggested by Ewing and Ewing[7] and by these arguments) An effort should be made to find changes in average topographic slope and in thickness of the volcanic layer on either side of the 300 km discontinuity The change in the magnetic spectrum implies a 12 per cent decrease in both regional slope and thickness of the volcanic layer between 300 and 400 km. The change in slope follows if subsidence is primarily a function of time after extrusion The change in thickness of the volcanic layer follows if the time rate of discharge of this layer is constant in time[24]

At about 700 km, in the North Atlantic, the regional slope decreases from about 3 1000 to 1 5 1000 or less. This break is the outer boundary of the Mid-Atlantic Ridge, as defined by Heezen *et al* [8]. At the same distance, the dominant magnetic wavelength increases from 36 km to 70 km[16], suggesting a 50 per cent decrease in spreading rate if the frequency of polarity reversals did not change at that time The age of this boundary would be 65 mybp if the spreading rate has been 1 5 cm/yr except during the late tertiary lapse This age, which coincides with the Laramide orogeny, may mark the time when the Atlantic rift propagated northward from about 50° N. into the Arctic Ocean[30]. The 50 per cent decrease in spreading rate should have been marked by a 50 per cent increase in the thickness of the volcanic layer, because the time-discharge rate for the volcanic or "second", layer is postulated to be constant for mid-ocean ridges[24] Indeed, seismic refraction profiles in the deep basins of the North Atlantic Ocean do not reveal first arrival returns from the layer[31] Thus if the second layer exists under the deep basins, it must be quite thin

The spreading rate prior to the 700 km discontinuity (about 3 cm/yr if magnetic anomaly wavelengths are indicative) may be extrapolated to the east coast of the United States, suggesting a Jurassic age for the oldest parts of the North Atlantic As a check on this reconstruction of spreading, we note that the isotopic ages of dolerite dikes thought to have been emplaced during the initial stages of North Atlantic rifting fall between 140 and 180 mybp[31]

By contrast, Heirtzler and Hayes[32] have extrapolated a 1 cm/yr rate to the edges of the continents and have concluded that the undisturbed magnetic field near the continental margins should be assigned to the Permo-Triassic, making the North Atlantic up to 250 million years or more old In view of the more recent spreading rate determinations at these latitudes[29] as well as the inferences presented here, the North Atlantic is no older than lower Jurassic Geological evidence suggests that the East African stage of rifting (before there was an ocean) is recorded by the Upper Triassic grabens the sedimentary fill of which is grouped under the Newark series.

We have suggested a major discontinuity in time and possibly spreading rate at about 100 km, and discontinuities in rate at about 300 and 700 km The topographic effect of the late Tertiary lapse is still pronounced. This discontinuity is the boundary between the High Fractures Plateau and the Upper Step in a physiographic classification of the Mid-Atlantic Ridge[8] Similarly, the 700 km discontinuity coincides with the distal boundary of the lower step. It is tempting to suppose that the boundaries Upper/Middle and Middle/Lower also reflect spreading rate discontinuities The upper one may be related to the 300 km discontinuity If marked elevation differences separate the steps, time discontinuities (lapses) may also be involved The absence of marked changes in the thickness of unconsolidated sediment across the features suggests, however, that the lapses, if any, were short.

The late Tertiary quiescent interval recorded by the mid ocean rift in the North Atlantic seems to be correlated with a period of slower spreading (but not stoppage) on the East Pacific Rise Menard[24] has cited evidence that spreading rates were 4 cm/yr before and after this period During the period of slower spreading (1 5 cm/yr ?)

belts of rough topography about 200 to 500 km wide were generated The centres of these belts are now about 500 to 1,000 km from the axis of the East Pacific Rise If the "slow" period corresponds to the North Atlantic hiatus, the width of the belts should equal the spreading rate (1·5 cm/yr) times the length of the hiatus (30 million years), or about 450 km This is in good agreement with the observed width of the belts A further check on this hypothesis is the width of the axial belt of smooth topography on the East Pacific Rise If fast spreading (4 cm/yr) began 10 mybp, the width of the smooth belt should be 2 × 4 cm/yr × 10 my or 800 km, which also agrees with observation The activity on the East Pacific Rise and the Mid-Atlantic Ridge seems to be remarkably synchronized

The East Pacific Rise therefore reveals the type of discontinuity of spreading shown schematically in Fig. 4 Seismic refraction profiles in Menard's "horstgraben provinces" would be of great interest If the second layer is generated at a constant rate in time[24], this layer should be 3 km thick under these parts of the East Pacific Rise By analogy with the Mid-Atlantic Ridge, there should be no oceanic layer but a low-velocity mantle (Fig 4)

Conclusion

Seismic reflexion, topography, heat flow and magnetic data seem to support the suggestion by Ewing and Ewing[7] that there was a late Tertiary lapse in crustal spreading which ended about 10 mybp This lapse has been recorded by the crust and is now about 100 km from the axis of the Mid-Atlantic Ridge The magnetic reversal sequences on the East Pacific, Carlsberg, and South Atlantic branches of the mid-oceanic ridge exhibit one-for-one correlations after linear differences in spreading rate have been removed[27] Because much of the evidence for the late Tertiary lapse applies to these areas as well, the lapse must therefore be assumed to have been a global phenomenon, although the history of crustal spreading seems to have been more complex on the Mid-Atlantic Ridge north of about 50° N

We are not now prepared to suggest a firm length of the period of ridge quiescence or slow-down Sediment thickness ratios between crest and flank are variable and several unknown factors must be considered in calculating the rates of subsidence of a cooling ridge flank. Present values suggest, however, a period of quiescence of 15 to 30 million years Topographic, seismic refraction and magnetic data also suggest that two other discontinuities in the spreading rate (decreases) may be recorded by the crust in the regions 300 to 500 km and, less certainly, at 700 km from the axis of the Mid-Atlantic Ridge Careful statistical work on topography, heat flow, dating, sediment thickness, magnetics and crustal structure in the transitional regions is required to substantiate these events and correlate them with those found on other ridges and with orogenic events in general

The history of spreading is still too incompletely understood to rule out the possibility that rifting may shift between one part of the ridge to another on a global scale in such a way that sea floor is created at a constant rate in time, as measured around a great circle The latter would be expected if the world is presently expanding at a constant rate in time[33,34] If the entire mid-oceanic rift shows synchronized periods of activity and quiescence, the expansion hypothesis must invoke processes that can operate with periods measured in tens of millions of years If sea floor spreading is driven by a global convection cell a simultaneous stopping and starting along the entire Mid-Atlantic Ridge and East Pacific Rise implies that different parts of the cell are very closely coupled in time, with convection speeds varying slowly and apparently regularly along the strike of the ridge Some of this synchronization could be understood if the strength of the healed crust can appreciably exceed the tension stresses on it As stresses build up there will be an area of weakness where rifting begins, and the rifting will propagate, if it is analogous to a crack, over wide regions, passing into weakly stressed and even into unstressed and compressional regions The rift will preferentially follow lines of pre-existing weakness[35] A pre-existing rift will be preferred because it is a "flaw", but the greater the quiescent interval the greater the probability that a new site will be chosen If convection cells are involved, a change in their position would favour a new locus of rifting Interesting possibilities arise, however, if the crust has significant strength The rising limbs of deeper convection cells might be considerably displaced from the ridge axis The ascending cell could be very broad indeed, while mantle derivatives are continuously injected into a narrow zone of constant weakness The regularity of crustal spreading may therefore be partly due to the localization of fracturing along a continuously rejuvenated zone of weakness

If the crust has significant strength, a world-wide cessation of rifting is more difficult to understand, because crustal spreading along pre-existing lines of weakness—along the axis of the mid-oceanic rift—is so much "easier" than a renewal of activity. But granted the tensional forces may decline in time over a large region, possibly the whole world, it is possible to account for a sudden, synchronized stoppage. As tension decreases, spreading

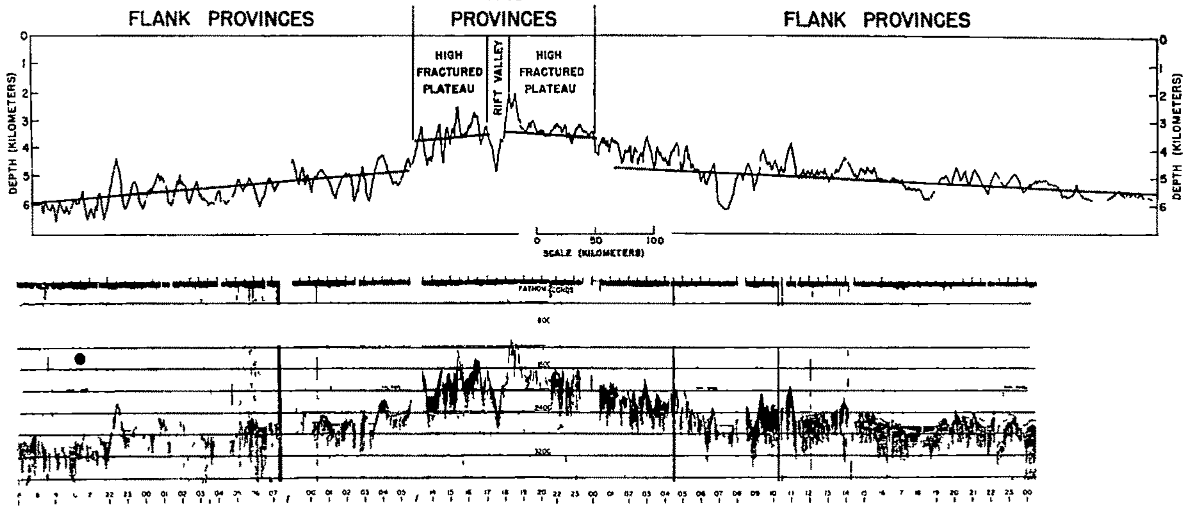

Fig 10 Profile *J*, crossing Mid-Atlantic Ridge perpendicularly at 12° N Other designations as in Fig 6

rates fall. Meanwhile, the thickness of the solid crust increases by downward freezing and by cooling. Tensional fissuring will continue throughout the rift until the weakest or most highly stressed region can no longer sustain crustal spreading by extending the process to neighbouring and stronger regions. There is then a synchronized end to crustal spreading, on a large and possibly global scale.

The evidence for major discontinuities in the history of crustal spreading is still speculative. It is of great interest to know whether the mid-oceanic ridges can be considered steady-state for periods of the order of 10–100 million years. If they can, the variation of crustal structure with X implies a rate. That is, the rates at which physical processes occur in the Earth and the conditions under which they occur could then be deduced by careful study of how a mid-ocean ridge changes away from its crest. Unlike the study of oceanic or atmospheric circulation, where the validity of "steady-state" approximations can be checked by repeated surveying, the upper mantle processes must take millions of years to establish physicochemical equilibrium. The exact history of crustal evolution and spreading thus awaits detailed application of the Vine-Matthews hypothesis[4] to the whole ridge system, and the possibility of discontinuities in the spreading history must be carefully considered and checked out by all possible means.

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[1] Heezen, B. C., *Trans. Amer. Geophys. Union*, 38, 394 (1957).
[2] Dietz, R. S., *Nature*, 190, 859 (1961).
[3] Hess, H. H., *Petrologic Studies. A Volume in Honor of A. F. Buddington*, 599 (Geological Soc. of America, New York, 1962); *Submarine Geology and Geophysics*, 317 (Butterworths, London, 1965).
[4] Vine, F. J., and Matthews, D. H., *Nature*, 199, 947 (1963).
[5] Pitman, W. C., and Heirtzler, T. R., *Science*, 154, 1164 (1966).
[6] Cox, A., Doell, R. R., and Dalrymple, G. B., *Nature*, 198, 1049 (1963).
[7] Ewing, J., and Ewing, M., *Science* 156, 1590 (1967).
[8] Heezen, B. C., Tharp, M., and Ewing, M., *Geol. Soc. Amer. Spec. Paper* 65 (1959).
[9] Sykes, L. R., and Landisman, M., *Bull. Seism. Soc. Amer.*, 54, 1927 (1964).
[10] Matthews, D. H., and Bath, J., *Geophys. J. Roy. Astro. Soc.*, 13, 349 (1967).
[11] Vogt, P. R., and Ostenso, N. A., *Nature*, 215, 310 (1967).
[12] Langseth, N. G., LePichon, X., and Ewing, M., *J. Geophys. Res.*, 71, 5321 (1966).
[13] Talwani, M., LePichon, X., and Ewing, M., *J. Geophys. Res.*, 70, 341 (1965).
[14] Menard, H. W., and Smith, S. M., *J. Geophys. Res.*, 71, 4305 (1966).
[15] Ringwood, A. E., and Green, D. A., *Tectonophysics*, 3, 383 (1966).
[16] Heirtzler, J., and LePichon, X., *J. Geophys. Res.*, 70, 4013 (1965).
[17] Bodvarsson, G., and Walker, G. P. L., *Geophys. J. Roy. Astro. Soc.*, 8, 285 (1963).
[18] Vogt, P., and Ostenso, N., *J. Geophys. Res.* (in the press).
[19] Harland, W. B., *Geology of the Arctic*, 1, 68 (University of Toronto Press, 1961).
[20] Sykes, L. R., *Bull. Seis. Soc. Amer.*, 55, 519 (1965).
[21] Vine, F. J., *Science*, 154, 1405 (1966).
[22] Johnson, G. L., and Heezen, B. C., *Deep Sea Res.* (in the press).
[23] Vogt, P. R., and Ostenso, N. A., *J. Geophys. Res.* (in the press).
[24] Menard, H. W., *Science*, 157, 923 (1967).
[25] Holmes, A., *Principles of Physical Geology*, 1096 (Ronald Press, 1964).
[26] Ewing, M., Ewing, J., and Talwani, M., *Bull. Geol. Soc. Amer.*, 74 (1963).
[27] Avery, O. E., Heirtzler, J. R., and Burton, G. D., *J. Geophys. Res.* (in the press).
[28] McBirne, A. R., and Gass, I. G., *Earth Plan. Lett.*, 2, 265 (1967).
[29] Phillips, J. D., *Science*, 157, 920 (1967).
[30] Grasty, R. L., and Wilson, J. T., *AGU Program Abstracts*, 212 (1967).
[31] Ewing, M., and Ewing, J., *Bull. Geol. Soc. Amer.*, 70, 291 (1959).
[32] Heirtzler, J. R., and Hayes, D. E., *Science*, 157, 185 (1967).
[33] Carey, S. W., *Continental Drift, a Symposium*, 177 (University of Tasmania, Hobart, 1958).
[34] Heezen, B. C., in *Continental Drift, International Geophysics*, 3, 235 (Academic Press, 1962).
[35] Wilson, J. T., *Nature*, 207, 343 (1965).

Rapidly Pulsing Radio Sources

by

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The gravitational lens effect associated with a neutron star binary gives results which agree well with recent observations of a new class of rapidly pulsing radio sources. Further tests of the theory are proposed.

OBSERVATIONS of a new type of rapidly varying radio source have recently been reported[1]. The principal characteristics of the four sources so far discovered may be summarized as follows—sharp pulses of radiation are received, separated by comparatively long intervals which themselves are remarkably constant (with variations of $\lesssim 1$ part in 2×10^7 during the past 3 or 4 months). The pulse duration is no more than ~ 0.016 s, while the period is 1.3372795 ± 0.0000020 s (ref. 1). We understand that the other objects show similar sharp pulses at intervals of the order of 1 s, which, although not identical, strongly suggest that a new type of physical system has been discovered. Hewish *et al.* propose that the objects may be pulsating white dwarfs or neutron stars. Although this may prove to be correct, there are certain difficulties associated with the constancy of the observed period. We suggest an alternative explanation for the observed phenomena.

Gravitational Lens Hypothesis

An obvious method of achieving astronomical regularity is that associated with motion in a binary system. We therefore suggest that the radiation peaks are caused by the nearest member of a binary pair acting as a gravitational lens on the radiation of its more distant companion. The observed interval between emission bursts for objects of equal mass would be one-half the orbital period. It might be thought that such occurrences would necessarily have a negligible probability. Remarkably, this is not the case.

We first embark on an approximate illustrative analysis, to be followed by more detailed computations. Consider the motion of two stars of equal mass $M \sim 0.5\ M_\odot$ and with an orbital period of 3 s. This corresponds to a separation $a \approx 3 \times 10^8$ cm and orbital velocities $\sim 10^{-2}\ c$. Clearly, it follows from the small separation that the stars must be very compact white dwarfs or neutron stars. This requirement is also necessary for the gravitational lens mechanism to be effective.

Liebes[2] has given a theoretical discussion of the gravitational lens and we shall follow his notation throughout. Fig. 1 illustrates the configuration. O, D and R denote the source, deflector and receiver, respectively. $2\varphi_O$ and $2\varphi_D$ denote the undistorted angular diameters of O and D, if they could be seen by the receiver. The semi-apex angle of the "cone of inversion" is given by

$$\theta_0 = \left(\frac{4\,G\,M_D}{(1 + l_D/l_{OD})\,l_D\,c^2}\right)^{1/2}$$

$$\approx\left(\frac{4\,G\,M_D}{c^2\,l_D}\quad\frac{l_{OD}}{l_D}\right)^{1/2} \qquad (1)$$

because in our case $l_D \gg l_{OD}$ M_D denotes the mass of the deflector As the source moves behind the deflector the receiver sees two images I_1 and I_2 on either side of the cone of inversion These images merge to produce an annulus round the cone of inversion in the unlikely case when O, D and R are perfectly aligned Because the brightness remains the same along a light path, the amplification factor A is given by the ratio of the total solid angle subtended by the deflected images to the undistorted solid angle of the source Thus Liebes obtains

$$\begin{aligned} A &\approx 1,\ \alpha>\theta_0 \\ &\approx \theta_0/\alpha,\ \alpha<\theta_0 \end{aligned} \qquad (2)$$

with a maximum value

$$A_{\max} \approx 2\theta_0/\varphi_0 \qquad (3)$$

in the case of perfect alignment These results are subject to the conditions that all deflexion angles are small and that the geometrical cross-section of the deflector is too small to block much radiation This last condition implies that

$$\frac{\theta_0}{\varphi_D} = \left(\frac{4\,G\,M_D}{c^2\,r_D}\right)^{1/2}\left(\frac{l_{OD}}{r_D}\right)^{1/2} \gg 1 \qquad (4)$$

where r_D is the deflector radius Condition (4) can be satisfied only when $l_{OD}/r_D \gg 1$, because the first term in brackets is essentially the Schwarzschild parameter With the estimated value of l_{OD} ($\equiv a$) given here, this implies that the deflector cannot be a white dwarf and must therefore presumably be a neutron star Similarly, to obtain substantial amplification, we require that

$$A_{\max} \approx \frac{2\,\theta_0}{\varphi_0} = 4\left(\frac{G\,M_D}{c^2\,r_O}\right)^{1/2}\left(\frac{l_{OD}}{r_O}\right)^{1/2} \gg 1 \qquad (5)$$

which again demands that the source be a neutron star We are led therefore to a binary system consisting of two neutron stars, each acting as a gravitational lens for the other

In the following discussion we will consider only those cases in which reasonable amplification ($A \gtrsim 2$) is obtained The receiver R will not usually be in the orbital plane of the stars and we define χ to be the angle of closest approach as seen by R The situation is illustrated in Fig 2, in which the path of O is taken to have negligible curvature within the cone of inversion This is a good approximation provided $(4GM_D/l_{OD}c^2)^{1/2} \ll 1$, which is easily satisfied in the present case Clearly the largest amplification $A_{\rm m}$ for a given path is

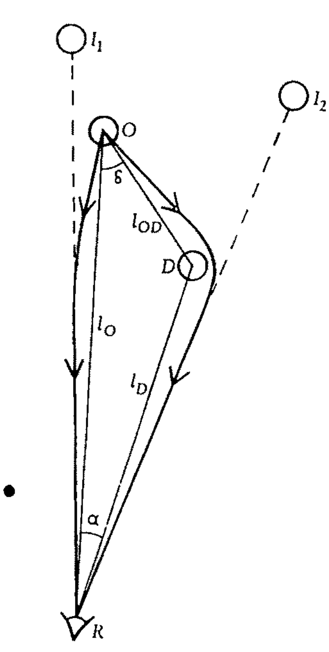

Fig. 1. Geometric configuration and typical light paths for gravitational lens

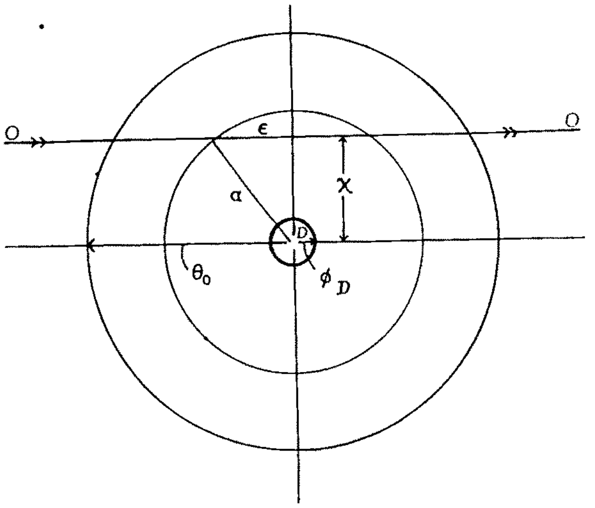

Fig 2 Path of centre of source ($O-O$) compared with cone of inversion

$$A_{\rm m} \approx \theta_0/\chi = \frac{\varphi_0}{2\chi_0}\quad A_{\max} \qquad (6)$$

The angle δ_A ($\angle DOR$) at a given amplification is then given by

$$\begin{aligned} \delta_A &= (l_D/l_{OD})\alpha = l_D\theta_0/A \\ &= (1/A)\ (4GM_D/l_{OD}c^2)^{1/2} \end{aligned} \qquad (7)$$

The time interval between points of given amplification in a single pulse is thus given by

$$T_A = (\delta_A/\pi)\xi P \qquad (8)$$

where

$$\xi = A\varepsilon/\theta_0 = (1-A^2\chi^2/\theta_0^2)^{1/2} \qquad (9)$$

provided, of course, that the required amplification is reached, and that $A \lesssim \theta_0/\varphi_0$ If we demand, for example, an amplification of $A=3$ and use the approximate values of M_D and l_{OD} assumed here, we obtain $\delta_3 \approx 10^{-2}$ and $T_3 \lesssim (\delta_3/\pi)\ P \simeq 10^{-2}$ s This time certainly satisfies the condition $T \lesssim 0\ 016$ s given by Hewish *et al* and suggests that, with entirely reasonable estimates of M, the observed characteristics of the signal can be reproduced

The actual amplification as a function of ε is illustrated in Fig 3 for the cases $\chi=0$ and $\chi=\theta_0/3$ In the former case the maximum is determined by φ_0 Clearly the width at given amplification is directly related to T_A Thus by increasing the amplification factor the steepness of rise can be increased and the width of the pulse reduced The apparent shape of the signal will then depend on the residual signal level (RSL)

If it is assumed that the whole surface of a neutron star acts as the effective source, there is clearly an upper limit to the maximum amplification This is because there is an upper bound M_c to the mass of a stable neutron star configuration, and a corresponding lower bound r_c to its radius Meltzer and Thorne[3] give values of M_c and r_c for two assumed equations of state, one due to Harrison, Wakano and Wheeler (HWW), the other to Skryme, Cameron and Saakyan (SCS) Of these the latter give the most favourable values of r_c and M_c, from which it follows that the maximum possible amplification is

$$A^*_{\max} \approx 17\ P^{1/3} \qquad (10)$$

The HWW values give the coefficient of $P^{1/3}$ as ~ 10 These limits can be relaxed, however, if the stars have significant angular momentum or if a further hard core pressure (the C-field[4], for instance) exists More important, if the radiation comes from smaller regions on the stellar

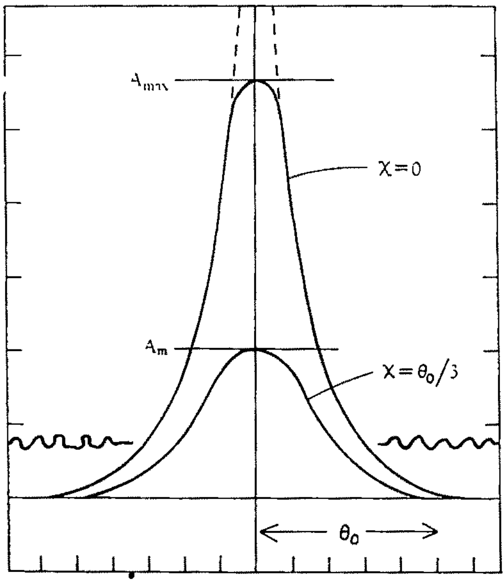

Fig. 3. Typical individual pulse shapes for angular impact parameters $\gamma=0$, and $\chi=\frac{1}{3}\theta_0$.

surface, from flares for example, the value of $A^*_{\max}$ can be exceeded by large factors. This question will be discussed further in the following section in which we attempt a comparison of our proposal with the pulsating star hypothesis.

Comparison of Binary and Oscillation Theories

We now list some of the observational and theoretical problems raised by the new radio sources and examine them in the light of the theoretical explanations so far proposed.

(i) Random Signal Strengths

It appears that the signal amplitudes vary considerably from pulse to pulse, disappearing below RSL for a considerable proportion of the time. On the binary star theory there is clearly no reason to expect correlations between successive pulse intensities because the "clock mechanism" (the gravitational orbit) is completely divorced from the energy source. Random flares occurring on each star or in the region between stars happen to be amplified, and thus observed, only at moments of suitable alignment. We note, however, that if the flare lifetimes were significantly longer than the orbital period one would expect to observe their effects over several pulses. This may be the explanation for the observation[1] that, typically, the pulses are only present for about 1 min, which may occur quite randomly within the 4 min interval permitted by the reception pattern. Indeed any evidence for (even temporary) correlations between alternate pulse strengths would argue strongly for the binary theory, since this would have a natural explanation in terms of unusual activity on one star only. Hewish[1] has reported that several alternate pulses were very weak for one of the objects.

On the other hand, it is difficult to understand how an oscillating star can be tuned so finely that its period changes systematically by less than ~ 1 part in 10^{13} per cycle, yet can trigger pulses of completely random intensity. As a general rule, the shorter and more regular the period of a vibrating star, the more precisely repetitive is the energy output as a function of phase.

(ii) Sharpness of Signal

In the binary theory the apparent sharpness of the signal peak is determined by three main factors—the degree of alignment, the ratio of pulse strength to RSL and the angular diameter of the source (see Fig. 3). As we have noted, there is a maximum possible amplification factor $A^*_{\max}$ (equation (10)) if the emissivity is uniform over the surface of the source. If, as is more likely, flares occur over smaller regions of size $\sim r_f \ll r_O$, the value of $A_{\max}$ is enhanced by a factor r_O/r_f and the energy requirements at the source are reduced by r_f/r_O. The shape or changing position of the flare on the surface of the source could lead to some signal asymmetry, though in general we would expect this to be small. The duration of the flatter portion at the peak relative to the steepness of rise and fall depends on $A_{\max}$ and the residual signal-level and could, provided the source were single, be used in the determination of these parameters.

We should, however, point out that if there were a number of flare zones on a star the signal received would consist of a quick succession of bursts of the type illustrated in Fig. 3. The duration T_c of this combined signal would be determined essentially by the time for the stellar disk to cross the cone of inversion. Thus

$$T_c \approx (1+\varphi_0/\theta_0)\,T_1 \qquad (11)$$

If $\varphi_0 > \theta_0$, T_c could be rather longer than the times computed in the previous section. In this case, the general emission from the star would be unamplified whereas that from each flare zone would be amplified by A as given in equations (2) and (3) with r_O replaced by r_f. In this case, the sum of individual pulse lifetimes must be rather less than the time, T_c, over which the pulses are received, if the basic source is to remain below the level of detection (assumed the same for pulsing and constant sources). (In the previous section we excluded the possibility that the source is a compact white dwarf. If, however, the signals come from a small region on such a star, substantial values of A could still be obtained although the general emission would remain constant. The situation would, however, be far more complicated and is not covered by the small angle analysis.)

The oscillation theory has to explain both sharp rises and falls in the emission at the source. It is possible to imagine a situation in which shocks generated by the pulsations decay in times $\lesssim 0.016$ s, though it is not clear why this should always be the case. Nor is it clear why an oscillating star, tuned to 1 part in 10^{13} per cycle, should produce such shocks for only part of the time. Although such mechanisms may indeed exist, the explanation must certainly be much more complicated than that involving binary stars.

(iii) Probability of Observation

It is obviously necessary in both theories to estimate the probability of observing the required configurations. We take as common ground to both theories that neutron stars are the end point of evolution of stars of mass $\gtrsim 1.5\,M_\odot$. We list below the further assumptions that underlie our calculation.

(i) The luminosity function of the galaxy has remained essentially constant.

(ii) On the upper main sequence $L \propto M^3$, L being the luminosity of a star.

(iii) A star of $\sim 1\,M_\odot$ evolves completely in $\sim 10^{10}$ yr, the age of the galaxy also being of this order.

(iv) The lifetimes of radio bright neutron stars are long.

(v) *a*, A proportion q_1 of upper main sequence (UMS) stars become binary neutron star systems with periods in the region of a few seconds. *b*, A proportion p_1 of UMS stars become single neutron stars with negligible rotation.

(vi) *a*, Orbital planes of binary systems are randomly oriented in space. *b*, A proportion, p_2, of neutron stars have central densities in the correct range to give pulsation periods in the region of a few seconds.

Assumptions (i)–(iii) and (vi)*a* are reasonable and require no comment. We will give the remaining assumptions further attention.

From assumptions (i)–(iii), and using Allen's[5] tables, we find that n, the number density of completely evolved massive stars, is given by

$$n \approx 4 \times 10^{-3}\ \text{pc}^{-3} \tag{12}$$

a value common to both theories.

From assumption (v) *a* the number density of binary neutron star pairs is nq_1. Given (vi) *a*, the probability of the observer lying sufficiently close to the orbital plane to see an amplification A is given by the appropriate δ_A derived from equation (7). Thus the number density n_b of suitably oriented pairs is given by

$$n_b \simeq n q_1\, \delta_A \tag{13}$$

If $A \approx 3$, then $\delta \approx 10^{-2}$, and it follows that the expected number of favourable configurations within ~ 65 pc (the upper limit to distance given by Hewish *et al.*) is $\sim 40\, q_1$. We suggest that q_1 may be of order unity. In support of this, we note that the progenitors in general possess considerable angular momentum, and on contraction are likely to break up into two orbiting masses, according to a well known theory of double star formation[6,7]. Furthermore, a main sequence star is likely to reach the neutron star state via some sort of white dwarf configuration (non-static) which should be in a state of extremely rapid rotation and should break up as soon as any major contraction occurs. Thus an orbital separation of approximately a white dwarf radius ($\approx 10^8$–10^9 cm) and a period of order ≈ 1 s is not implausible. Angular momentum requirements are easily met because typically we require only $\sim 1/100$ of the angular momentum to be retained by $\sim 1/5$ of the mass. Furthermore, we note that because of the rapid rotation of UMS stars it is entirely plausible that multiple fission occurs leading to more than one neutron star binary pair[8]. We emphasize that this is extremely speculative but note however that among UMS stars the number of binaries, presumably formed during the Hayashi contraction phase, is comparable with the number of single stars. Thus a value of $q_1 \approx \frac{1}{2}$ seems not unlikely. It leads to about 20 binary neutron star systems within 65 pc having orbits sufficiently aligned for reasonable amplification. The suggested mechanism cannot therefore be excluded on probability grounds, subject, of course, to the condition that the binary system lifetime is long.

In the oscillation theory the number density of candidates n_p, corresponding to n_b is given by

$$n_p = n p_1 p_2 \tag{14}$$

The angular momentum difficulty suggests $p_1 \ll 1$. The calculations of Meltzer and Thorne[3] indicate that the pulsation period of a neutron star is extremely sensitive to the central density. Thus unless there is some particular mechanism which selects periods of 1 s it follows that $p_2 \ll 1$. (For white dwarfs it is necessary for the oscillation to be in an overtone mode if the observed period of ~ 1 s is to be obtained; this again indicates a low probability.) It therefore appears that n_p cannot significantly exceed n_b and may in fact be rather smaller. Neither theory can, however, be excluded, subject to the assumption of long lifetimes. We now consider this question.

(iv) Constancy of the Period and Energy Requirements

Hewish *et al.* have estimated the energy output of the first source to be $\sim 10^{47}$ ergs yr^{-1} provided it is at a distance of 65 pc. This is an extremely tentative figure, even apart from the uncertainty in the distance, because the spectral distribution is still not known in any detail. We will assume in the following discussion, *faute de mieux*, that this figure is correct. We defer discussion of energy radiated by gravitational waves until later in the paper.

It is important for theoretical purposes to place restrictions on the permissible amount of *systematic* change of period per cycle. From the present data it appears that the period is constant to at least one part in 10^{13} per cycle.

We remark that there is no variable red-shift problem for the binary model. The amplification can occur only when the motion is transverse. We further note that a change in mass would alter the periods in either model. The estimated changes due to accretion are, however, many orders of magnitude too small to have significant effects in either case.

If we assume that the oscillating star contains the maximum possible vibrational energy ($\sim 10^{51}$ ergs), the estimated radiation rate at 65 pc ($\sim 10^{47}$ ergs yr^{-1}) yields a decrease in vibrational energy of ~ 1 part in 10^4 yr^{-1} and presumably a similar change in amplitude. Although in a linear theory the period is independent of the amplitude of pulsations, a residual dependence on terms of higher order would be expected, particularly near maximum amplitude. The observed constancy to 1 part in 10^6 yr^{-1} appears to show a remarkable and, we think, unlikely absence of such non-linear effects. This argument can be countered by reducing the distance to the source by a factor of at least 10, but only at the expense of a further reduction in the expected number of stars to $n_p \simeq 4 p_1 p_2$. This is now disturbingly small.

The figures given here point clearly to a further difficulty. If the estimated emission rate is correct, the lifetime T_p of the pulsations can be at most $\sim 10^4$ yr. (Indeed, T_p is probably less than this because, according to Meltzer and Thorne[3], much of the available energy will be dissipated rapidly (in ~ 10 yr) by various processes including neutrino emission.) On this basis, the probable number of vibrating neutron stars of the type observed should be reduced by a further factor T_p/T_* where T_* is the mean total evolutionary time of neutron star candidates. With $T_* \sim 10^9$ yr, this would again reduce the number of possible candidates within 65 pc to unacceptably low levels. Similar arguments apply in the case of white dwarfs with corrections of order unity for the maximum possible energy and the expected number of stars. One is therefore in some difficulty to explain the observed number of sources of this type on the pulsating star hypothesis.

On the other hand, we run into very similar, though slightly less stringent, difficulties on the binary star hypothesis. Again assuming the source to be at 65 pc, our total energy requirements are increased by the ratio of orbital period to pulse duration and reduced by the amplification factor and, if the emission is coming from n_f smaller flare zones, by the factor $(r_f/n_f r_O)$. (If there were a correlation between flare activity and the position of the binary companion a further reduction would be possible.) Thus our energy requirements are probably of the same order of magnitude as those in the pulsating star theory, with the possibility of some reduction from the $(r_f/n_f r_O)$ factor. The expected number of pairs within 65 pc is thus likewise reduced to unacceptable levels.

The problem of energy supply and reasonable lifetimes thus seems insuperable unless the total emission rate at 65 pc turns out to be a considerable over-estimate. Otherwise only a harder core pressure than that from neutron degeneracy seems to offer any hope of resolving this difficulty. A possibility arises from the work by Hoyle and Narlikar[4] on the resistance to compression offered by the C-field. In this case the masses (to which there is no upper bound) are near their Schwarzschild radius. Clearly this topic requires a more detailed examination which is beyond the scope of this article. We merely note that to maintain the number density the mass cannot exceed $\sim 3\, M_\odot$ and the energy reserve not more than 10^{55} ergs. Thus even at the extreme, the lifetimes are only just comparable with T_* and the problem of expected probable numbers is barely resolved. We therefore feel that if either of these pictures is to prove correct the total emission rate at 65 pc must be reduced by at least $\sim T_*/T_p \sim 10^5$.

Whatever the figure for total emission rate turns out to be, we would, at this point, emphasize one important advantage of the binary star picture over the pulsating star hypothesis. In the latter, any energy taken from internal reserves causes a change in structure and hence in period on the time scale T_p. Thus even if the energy requirement is reduced so that $T_p \sim T_*$ the periods will cover a wide range, ensuring a small value for p_2 and hence of the expected number of sources. On the binary picture, however, these internal reserves and indeed energy stored in the region between the stars (in magnetic fields, for instance) could be utilized without altering the periods, provided the rest mass of the stars remains the same. Even at the estimated emission of $\sim 10^{47}$ ergs yr^{-1} this gives a maximum change in orbital period of 1 part in 10^7 yr^{-1}—still within the observational limits. We have, however, omitted any consideration of gravitational radiation throughout the analysis. We now examine this question in more detail.

Gravitational Radiation

There is still some controversy as to the precise situations in which gravitational waves are emitted. We follow the standard analysis of Landau and Lifshitz[9], who obtain for the characteristic time τ_B for changes in the binary orbit the expression

$$\tau_B \approx \frac{1}{128}\,\frac{c^5 l_{OD}^4}{G^3 M_1 M_2 (M_1+M_2)} = \frac{1}{128}\,\frac{c^5 (M_1+M_2)^{1/3}\,\omega^{-8/3}}{G^{5/3} M_1 M_2} \qquad (15)$$

Using the approximate values $M_1 = M_2 = 0.5\,M_\odot$ and $l_{OD} \sim 3\times 10^8$ cm as before, we obtain $\tau_B \approx 10^8$ s. By reducing one of the masses somewhat τ_B could be increased to at most $\sim 10^9$ s. This clearly implies a relative change of period far in excess of the observational limits and seems to exclude the binary star theory quite conclusively. Before proceeding, however, we would point out that the pulsating neutron star hypothesis also suffers in such an analysis.

For a pulsating star the characteristic decay time due to gravitational radiation is given[9,10] by

$$\tau_p \simeq \frac{5o\, c^5}{G M a^2 e^2\, \omega^4} \qquad (16)$$

where e is the ellipticity. As the loss rate is dominated by the highest harmonics present (16) could be an over-estimate. For a neutron star with a period of ~ 1 s we obtain[3] $M \sim 0.2\ M_\odot$, $a \sim 2\times 10^7$ cm, and thus $\tau_p \simeq 2\times 10^9/e^2$ s. Should neutron stars retain only ~ 1 per cent of the angular momentum of typical UMS stars, they would have marked ellipticity, $e \sim 0.1$, resulting in a decay time τ_p of at most $\sim 10^4$ years. (Similarly a white dwarf with solar angular momentum would have $\tau_p \sim 10^5$ years.) Such time scales are already incompatible with the observational data. There is, however, a suggestion due to Tsuruta and Cameron[11] that a strong magnetic field could remove sufficient angular momentum to reduce e to a negligible value. From their analysis, however, the required magnetic field would itself produce a substantial quadrupole moment. Thus, unless a fine balance exists in which first angular momentum and then magnetic fields are dissipated rapidly, the pulsating neutron star would lose its energy on an extremely short time-scale. The number of stars on the upper main sequence with low (effectively zero) angular momentum could produce the required type of neutron star but only with immensely reduced probability of observation. We feel that if the theory used here is correct the pulsating neutron star hypothesis can be excluded. Pulsating white dwarfs would seem to have the least difficulty concerning gravitational radiation, the associated time-scales always being $\gtrsim \tau_B$. Even in this case, however, one would expect changes in period on a time-scale of $\sim 10^{12}$ s (because most of these stars should be in rapid rotation even if on the main sequence they possessed only the solar angular momentum). This should also have been detected with the present observational data.

Both the binary star and the pulsating star models seem to succumb under the problem of gravitational radiation. On the other hand, our model appears to satisfy a number of other observational requirements in a rather natural way. If observations continue to support the binary star picture, and if general relativity is fundamentally correct, there seem to be two possible ways to resolve the gravitational radiation difficulty.

First, it may be that freely falling particles (binary stars, for example) do not radiate gravitational energy according to general relativity. This possibility has been urged by Bondi[12], who found that time dependent systems without "news" (that is, radiation of energy) can exist. The physical interpretation of his solutions remains controversial but, in view of the recent observations of rapidly pulsing radio sources and their otherwise plausible interpretation as binary stars, it is important that Bondi's suggestion be investigated in detail. We note, however, that this would not resolve the gravitational radiation problem in the case of pulsating neutron stars because the mass in these bodies is not in free fall.

The second possibility is that quantization effects become important in fairly high fields. The gravitational radiation difficulty is similar to that which faced spectroscopy before the Bohr theory. While physicists have long recognized the desirability of quantizing general relativity, no generally acceptable scheme has been proposed. To some extent, this failure may be due to lack of experimental or observational guidance for strong field situations. If our interpretation of these radio sources is correct, the remarkable constancy of the orbital period leads one to suspect some sort of quantization mechanism, that is, radiation free orbits, possibly arising as eigensolutions in the strong field (non-linear) equations. It may be significant that $v/c \approx 1/100 \approx \alpha$ where α is the electromagnetic fine structure constant. Could the observations be hinting that the usual strong field quantization condition $GM/rc^2 \approx 1$ is unrealistic and that $GM/rc^2 \approx \alpha$ is more relevant? We cannot pursue the matter further here, but it seems possible that once again astronomy may be able to provide a laboratory for testing consequences of the general theory of relativity, but this time in strong fields.

Predictions of the Binary Theory

The detailed consequences of our suggestion are now being evaluated. At the present stage, we are able to make some general predictions (applicable to neutron star pairs) which may act as further tests of the proposal.

(*a*) *For a single pulse arising from a homogeneous source region.* (i) The times of onset and disappearance should be the same at each frequency for measurements between given amplification levels. (ii) The duration of a pulse $\lesssim 2\times 10^{-2}\,T$, where T is the time interval between repeated pulses (that is, ~ 1.3 s in the present case). (iii) The *amplification* should be the same at all frequencies. It should be noted that in applying (i)–(iii) due allowance must be made for the residual signal level, which at some frequencies may swamp the amplified signal and cause spurious variations in the predicted temporal coincidences.

(*b*) *For multiple pulses.* In general, the original, unamplified signal may have two different components coming from (i) the whole surface and (ii) flare activity in concentrated regions. Both could be time varying. They should produce an observed signal as follows. (i) A general rise in the entire signal by a modest amount $\lesssim 17$ (cf. section 3) lasting at the lowest amplification levels for $\sim 2\times 10^{-2}\,T$ s. (ii) Sharply peaked signals with maximum amplification factors which may be $\gtrsim 17$ and with widths correspondingly narrower.

(*c*) The integrated unamplified signal received in time T should be comparable to the energy received in each pulse; thus the basic source should be detectable. However, we understand, that this test is quite difficult because of confusion with other, non-pulsing, sources in the area. One would expect to have similar difficulties in detecting the remaining $\sim 4 \times 10^3$ pairs within 65 pc which have alignments unsuitable for amplification

(*d*) If a source can be identified optically, then its light variations should either show sharp bursts similar to those observed at radio frequencies, or no amplification at all It should not show smooth light variations as one might expect from a pulsating star

(*e*) If the radio signals are short compared with the interval between them, as in the present case, an optical spectrum should not show classical white dwarf features Although neutron star spectra are at present unknown one might expect them to exhibit fairly featureless optical continua Should this be found, it might prove worthwhile to reinvestigate the nature of objects with such spectra, hitherto supposed to be a sub-set of the white dwarfs

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Note added in proof Since this article was prepared, further observations have been reported by Ryle and Bailey[13] and Davies, Horton, Lyne, Rickett and Smith[14] Ryle and Bailey's provisional identification of the source with a blue star-like image of $\sim 18^m$ clearly offers the possibility of testing predictions (*d*) and (*e*) above The results of Davies *et al*, in particular the simultaneous onset of emission at all observed frequencies, and the appearance of multiple-pulsing with three or more single pulses per complete pulse, strongly support the binary view put forward in this paper Further observations of individual pulse shapes at several frequencies could distinguish between a pulsating theory in which plasma oscillations (Ryle and Bailey) produce radio bursts and the binary theory Frequency dependent collisional damping with a time constant $\alpha\nu^{-2}$ could produce substantially different pulse widths at different frequencies A ν^{-2} width-dependence would not be so easily explained on the binary hypothesis

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[1] Hewish, A, Bell, S J, Pilkington, J D H, Scott, P F, and Collins, R A, *Nature*, **217**, 709 (1968), and Hewish, A, Cavendish Colloquium, 20 February, 1968

[2] Liebes, jun, S, *Phys Rev*, **113 B**, 835 (1963)

[3] Meltzer, D, and Thorne, K S, *Ap J*, **145**, 514 (1966)

[4] Hoyle, F, and Narlikar, J V, *Proc Roy Soc*, **278**, 465 (1963)

[5] Allen, C W, *Astrophysical Quantities*, second ed, 238 (Athlone Press, London, 1963)

[6] Jeans, J, *Astronomy and Cosmogony*, second ed (Dover Publ, 1961)

[7] Lyttleton, R A, *The Stability of Rotating Liquid Masses* (Cambridge Univ Press, 1953)

[8] Hoyle, F, Narlikar, J V, and Wheeler, J A, *Nature*, **203**, 914 (1964)

[9] Landau, L D, and Lifshitz, E M, *Classical Theory of Fields* (Addison Wesley, 1951)

[10] Zee, A, and Wheeler, J A, as in *Ann Rev Astro Astrophys*, **4**, 393 (1966)

[11] Tsuruta, S, and Cameron, A G W, *Nature*, **211**, 356 (1966)

[12] Bondi, H, *Proc Roy Soc*, A, **269**, 21 (1962)

[13] Ryle, M, and Bailey, J A, *Nature*, **217**, 907 (1968)

[14] Davies, J G, Horton, P W, Lyne, A G, Rickett, B J, and Smith, F G, *Nature*, **217**, 910 (1968)

Possible Model for a Rapidly Pulsating Radio Source

by

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The pulsating radio signals may come from active regions on rapidly rotating white dwarf stars This accounts for some aspects of the periodicity of the signals which are difficult to explain if the sources are pulsating stars.

RECENTLY, the Cambridge radio astronomy group has described observations of a rapidly pulsating radio source[1] Their observations can be summarized as follows (*a*) The signal observed at 81 5 MHz has a period of 1 337 s, the phase being constant to one part in 10^7 The amplitude, however, varies irregularly, the signal appearing and disappearing for periods of a few minutes Each pulse has a length of $\sim 0\ 3$ s (*b*) From the absence of proper motion and a consideration of the dispersive effects of transmission through the interstellar plasma the distance to the source is limited to the range 10^3 AU $< d <$ 65 pc (*c*) Variations of phase can be accounted for by the Earth's orbital motion without the need for additional correction for the motion of the source (*d*) No visible object brighter than twelfth magnitude is apparent in the radio error rectangle (*e*) From the signal width and rate of sweep the source size must be smaller than $4\ 8 \times 10^8$ cm

The authors[1] tentatively suggested that the radio signals come from a pulsating neutron star or white dwarf The calculations of Meltzer and Thorne[2], however, show that stable (as opposed to unstable or metastable) neutron stars have central densities in the range $2\ 7 \times 10^{13} \leqslant \rho_c \leqslant 6\ 0 \times 10^{15}$ and fundamental modes of radial oscillation with periods in the range $5 \times 10^{-2} \geqslant T \geqslant 8 \times 10^{-4}$ Overtones of the fundamental modes have shorter periods still, so pulsating neutron stars seem to be an unlikely origin for the radio signals On the other hand, white dwarf radial oscillations always have periods[2] exceeding 8 s The equilibrium models, however, assume that the nuclear matter of the white dwarfs has been catalysed to the end point of thermonuclear evolution A more realistic equation of state derived by Salpeter[3] produces equilibrium models of higher density[4] and consequently shorter period In any case, observations (*a*) are very difficult to understand if the source is a pulsating star With the large energy that would be stored in a stellar pulsation it is not easy to see how the amplitude could be so variable. If surface pulsations are adopted it is easier to understand how the signal could appear and disappear at irregular intervals but very difficult to understand why the phase should be constant over successive reappearances Because it has been suggested that the radio source may be associated with a planet, it is perhaps worth noting that from (*b*) and (*d*) the associated star must be faint (fainter than eighth magnitude) but is likely to be further from the planet than 1 AU (*c*)

We require an intrinsically faint (*b, d*), small (*e*) star having a natural period of ~ 1 s (*a*) For the reasons mentioned it seems unlikely that the periodic behaviour is associated with a pulsation period Periodic behaviour is also found astronomically in two other cases, orbital motion and rotation, both of which can cause periodic variations in apparent magnitude

We can estimate the shortest possible period for orbiting ordinary stars if we consider a congruent pair of white dwarfs which are assumed to be undistorted by tidal or rotational forces and which are in a grazing circular orbit about one another For stars of mass M and radius R the period P must satisfy the inequality

$$P > 4\pi\left(\frac{R^3}{GM}\right)^{1/2} > 17\,\mathrm{s}$$

The numerical result is obtained by using the mass and radius ($1\ 396\ M_\odot$, $0\ 216 \times 10^{-2}\ R_\odot$) derived[4] for a carbon white dwarf on the verge of implosion due to inverse β

Table 1 MODEL OF A RAPIDLY ROTATING WHITE DWARF

M	$2.26\ M_\odot$
J	3.46×10^{50} g cm^2 s^{-1}
$R_{\frac{1}{2}}$	1.35×10^{48} cm
R_1	4.44×10^{8} cm
ϱ_c	3.90×10^{8} g cm^{-3}
R_e/R_p	2.14
E	-1.21×10^{51} ergs
P_0	1.25 s
$P_{\frac{1}{2}}$	1.61 s
P_1	6.50 s

decay. For a pair of neutron stars the desired orbital period can be obtained, but gravitational radiation caused by the varying quadrupole moment[5] would produce a continuous rate of frequency shift equal to

$$\left(\frac{\Delta\omega}{\omega}\right)=\frac{96}{5}\,\frac{\varepsilon}{(1+\varepsilon)^{1/3}}\,\frac{(GM)^{5/3}}{c^5}\,\omega^{8/3}\Delta t$$

Stars having masses M and εM and circular orbits about their mutual centre of mass have been assumed. For stable neutron stars, $M>0.18\ M_\odot$, $4.0>\varepsilon>0.25$, so that the expected change in frequency would be

$$\frac{\Delta\omega}{\omega}>7\times10^{-5}\ \text{day}^{-1}$$

a possibility which can be ruled out by the observations.

Finally, we consider rotation. The angular velocity at the equator of a rapidly rotating, condensed, star can be estimated by the Keplerian relation

$$\Omega=\left(\frac{GM}{R^3}\right)^{1/2}$$

which, if the period ($P=2\pi/\Omega$) is to be the order of 1 s, gives a "density" ($3M/4\pi R^3$) of the order of 10^8 g/cm^{-3}. This density is characteristic of the matter in the more massive white dwarf stars. Recently[6,7] various sequences of models of rapidly, differentially rotating white dwarfs have been constructed with masses both above and below the mass limit[8] ($1.44\ M_\odot$) for non-rotating white dwarfs. The models with rotation periods as short as 1 s have masses in the range 1–3 $M_\odot$. In Table 1 we summarize the properties of a $2.26\ M_\odot$ model with angular momentum $J=3.46\times10^5$ g cm^2 s^{-1} (characteristic of the main-sequence star having the same mass) and the same angular momentum distribution as in a uniformly rotating uniform sphere.

In Table 1, R is the radius, P the period of rotation, ρ_c the central density and E the binding energy. The subscripts "0", "½" or "1" refer to quantities measured on a cylinder containing that fraction of the mass, subscripts "e" and "p" refer to the equator and the pole. We note, in connexion with observation (e), that the star is smaller than 5×10^8 cm. This very rapidly rotating star ($v\sim5{,}000$ km s^{-1}) might be expected to be in danger of fission caused by an instability to a nonaxisymmetric mode. The lowest ($m=1$, 2) modes have been checked by Tassoul and myself and the star is found to be stable in the absence of viscosity but unstable, with an e-folding time of $\sim10^{10}$ yr, when viscous forces are included, that is, the model is stable.

We can imagine an active region on such a star emitting radiation similar to type II or III solar bursts, each burst lasting the order of a few minutes. During this time radio energy is emitted in the direction of the Earth as a set of pulses, one per period, the period being characteristic of the latitude of the active region. For the time of emission to be as short as inferred from the observations (0.016 s) it is necessary that the radiation be emitted in a cone of width $\sim1°$. A subsequent burst from the same active region would appear on the observer's record as a new set of pulses with amplitude unrelated to the first set but exactly in phase with them. If active regions appear at other points at the same latitude, sudden phase shifts would be observed caused by differential rotation; a shift to active regions at other latitudes would lead to changes in both phase and period.

The details of the model presented here are clearly very tentative. The major point to be made is that rotation provides perhaps the best explanation for the periodicity of the new radio sources, and that rapidly rotating white dwarfs have rotation periods in the experimentally observed range.

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[1] Hewish, A., Bell, S. J., Pilkington, J. D. H., Scott, P. F., and Collins, R. A., *Nature*, 217, 709 (1968).
[2] Meltzer, D. W., and Thorne, K. S., *Astrophys. J.*, 145, 514 (1966).
[3] Salpeter, E. E., *Astrophys. J.*, 134, 669 (1961).
[4] Hamada, T., and Salpeter, E. E., *Astrophys. J.*, 134, 683 (1961).
[5] Landau, L., and Lifshitz, E., *The Classical Theory of Fields* (Addison-Wesley, 1951).
[6] Ostriker, J. P., Bodenheimer, P., and Lynden-Bell, D., *Phys. Rev. Lett.*, 17, 816 (1966).
[7] Ostriker, J. P., and Bodenheimer, P., *Astrophys. J.* (in the press).
[8] Chandrasekhar, S., *Astrophys. J.*, 74, 81 (1931).

Nuclear Uptake of Exogenous DNA by Mammalian Cells in Culture

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The uptake of exogenous DNA by mammalian cells in culture may amount to 5 per cent of the host cell complement. This material is found within the nucleus, but most of it is not incorporated into the chromosomes.

It is well established that mammalian cells, both *in vivo* and *in vitro*, can take up DNA in a macromolecular form, and this phenomenon has been critically reviewed by Ledoux who has emphasized its generality and importance[1]. It has been assumed by many workers, however, without any experimental evidence to support their assumptions, that the exogenous DNA which is taken up in this way becomes incorporated into the chromosomes. The possibility that exogenous DNA can be exchanged with chromosomal DNA in a manner analogous to that which occurs in bacterial transformation is obviously important from a number of viewpoints. For example, it has been claimed that exogenous DNA is effective in the repair of damaged material in the cell[2–5], that it can induce tumours[6] or lead to tumour regression[7], and these effects have sometimes been ascribed to such an exchange. A hypothesis of carcinogenesis involving freely circulating DNA has already been proposed[8]. Furthermore, the specificity of ^{3}H-thymidine as a label for cells in DNA synthesis would need to be re-examined for experiments in which measure-

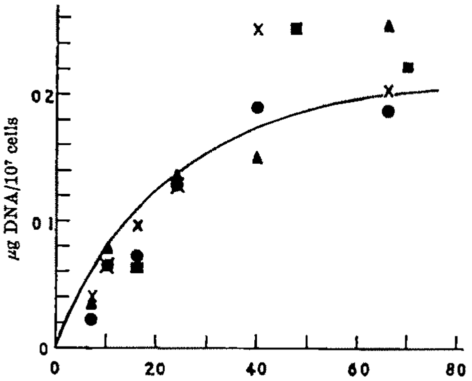

Fig. 1. Incorporation of ^{14}C-DNA by cells in exponential growth as a function of time expressed as ■, whole DNA, ●, adenine, ▲, guanine, ×, thymine Solid curve expresses the function incorporation = 0 21 $(1-2^{-t/15})$

ments of the cell labelling index were to be made at intervals greater than a few hours after the administration of the tracer

In this article, a distinction will be made between the uptake of DNA by a cell, and its true, chromosomal, incorporation The experiments were designed to examine the possibility of a true incorporation, using cultures of lymphoma cells, and exogenous DNA labelled with carbon-14, isolated from the same strain of cells The amount of DNA which had entered the nucleus was measured and then an estimate was made of the maximum amount of this material which could be regarded as having been incorporated It has been found that this amount is very small, and may be insignificant

Murine lymphoma cells (*L5178Y*) were labelled during exponential growth with 0 25 μCi of ^{14}C-formate/ml of medium at 37° C for 65 h, the final cell population being 5×10^5 cells/ml The medium used throughout these experiments was the full medium developed by Fischer[9] The cells were washed free of active medium with isotonic saline, and shaken in 2 M NaCl for 10 min One-tenth the volume of 10 per cent dodecyl sulphate was added, and the whole shaken overnight After centrifugation at 3,000 r p m the DNA was precipitated from the clear solution by gently stirring in two volumes of ethanol It was redissolved immediately in a minimal amount of 10^{-3} M NaCl and subjected to ribonuclease digestion (20 μg of ribonuclease/ml for 4 h), after which the solution was made 1 M in NaCl and the DNA again precipitated with ethanol The resultant DNA contained less than 5 per cent protein, and had a viscosity-average molecular weight of 2×10^7 An aliquot of the nucleic acid was hydrolysed with 90 per cent formic acid for 45 min at 165° C, and electrophoresed on Whatman *3MM* paper for 9 h at 7 V/cm in pyridine-acetate buffer, *p*H 3 5 Bases were located under ultraviolet light, eluted with 0 1 N HCl, and measured spectrophotometrically The carbon-14 activity was determined in a liquid scintillation counter ('Tracerlab *LSC*-30'), with an efficiency of 90 per cent In later experiments in which larger volumes of aqueous phase had to be employed because of their low radioactivity, an emulsion scintillator was used This consisted of 70 per cent Nuclear Enterprises *NE* 214 and 30 per cent 'Triton *X*-100', the efficiency in this case was 75–80 per cent In the conditions which have been described, the carbon-14 activity of the isolated DNA was between 2 1 and 2 3 μCi/μg of DNA

To study the incorporation of homologous DNA by cells, two experimental procedures were followed (*a*) prolonged incorporation at a low DNA concentration during exponential growth of the cells, and (*b*) a 30 min "pulse" incorporation at much higher DNA concentrations[10] With procedure (*a*), a concentration of approximately 1 μg ^{14}C-DNA, prepared as outlined, was present in each ml of culture for varying lengths of time up to 70 h Under procedure (*b*), concentrations of DNA were varied from 1 to 400 μg/ml of suspension, the cell concentration in this case being standardized at 2×10^7 cells/ml at 37° C Following both types of incorporation, collected cells were washed four times with 0 08 M iodoacetate, 0 14 M NaCl, *p*H 7, to remove much of the adhering DNA[10] Nuclei were isolated at 4° C by suspending in Creasey–Stocken medium[11] and performing 70–80 hand reciprocations in a Teflon–glass homogenizer size A. After a centrifugation at 1,000 r p m, the nuclei were pelleted by centrifugation at 40,000*g* in 2 2 M sucrose The DNA was extracted and analysed in an identical manner to that described for the original "donor" DNA

The uptake of ^{14}C-DNA in condition (*a*) is shown in Fig 1 as a function of the time allowed for uptake Although the results are expressed in μg of DNA/10^7 cells, these have been calculated from the separate values for the three bases guanine, adenine and thymine The agreement between these values suggests that macromolecular uptake is more likely than a breakdown to, and re-incorporation of, precursors Further support for this was found in the fact that the uptake of a nucleotide mixture (produced by enzymatic hydrolysis of ^{14}C-DNA) was 3 μg of DNA per 10^7 cells for adenylate and guanylate, and 20 μg of DNA for thymidylate after 65 h—that is, a factor of 15 to 100 times greater than the values in Fig 1 The curve drawn in Fig 1 was calculated by assuming that cells which are synthesizing DNA before division derive a constant fraction of this material from ^{14}C-DNA in the medium The incorporation per cell is given by $I = k\,(1-2^{-t/d})$, where the doubling time, *d*, is 15 h and *t* is the time of exposure of cells to DNA within the medium A value of $t = 70$ h was used to approximate to the condition $t = \infty$ and to calculate *k* The data of Fig 1 fit this relation, although this does not, of course, prove the original premise on which the curve was based

The uptake of ^{14}C-DNA under condition (*b*) is shown in Fig 2 as a function of the DNA concentration Again the uptake is expressed as μg of DNA/10^7 cells, the points representing values for the individual bases The specific activity ratios for (guanine adenine) and (thymine · adenine) for the donor DNA were $0 99 \pm 0 06$ (*S D*) and $0 55 \pm 0 10$, respectively, for fourteen preparations The corresponding values for the same ratios in seventeen preparations of host DNA (after uptake) were $1 07 \pm 0 21$, and $0 56 \pm 0 10$, respectively Such constancy of the ratios before and after incorporation suggests that the DNA has retained its macromolecular form[10] The incorporation of a nucleotide mixture such as that used under condition (*a*) was also investigated under "pulse" conditions, and here the uptake was between five and ten-fold lower for the nucleotide mixture than for intact DNA under the same conditions This suggests that the incorporation of intact DNA follows a different pattern from that of the con-

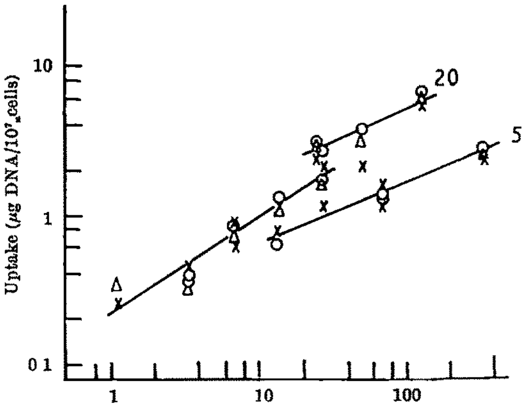

Fig 2 Uptake of ^{14}C-DNA by cells in 30 min at 37° C, as a function of DNA concentration, expressed as ○, adenine, △, guanine, ×, thymine

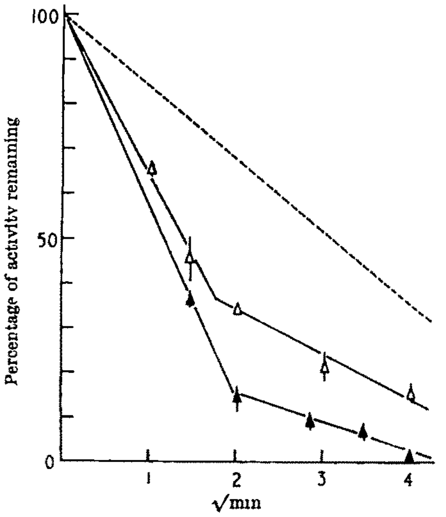

Fig. 3. Percentage original total carbon-14 activity within nuclei which remained after t min of treatment with DNase, as a function of $t^{1/2}$. Concentration DNase 35 μg ml⁻¹. Uppermost line is the regression line for four experiments on nuclei labelled with ^{14}C-formate. △▲, Two experiments with nuclei labelled with ^{14}C-DNA during 30 min "pulse".

stituent nucleotides, a result which has also been reported by Ledoux[12].

The three regression lines in Fig. 2 express results from experiments in which the cell population contained different proportions of "dead" cells, that is, cells which under the phase-contrast microscope were opaque. The enhanced uptake which seems to occur when non-viable cells are present would suggest that some, at least, of the uptake does not depend on the metabolism of the cell. When similar experiments to those of Fig. 2 were carried out in the presence of inhibitors, only a slight effect upon uptake was seen. For example, 100 μg of barbiturate/ml. of cell suspension containing 30 μg of DNA/ml reduced the uptake from 5.0 ± 0.3 to 4.1 ± 0.1 μg of DNA/10^7 cells. The corresponding effect in the presence of 100 μg of oligomycin/ml was an increase to 5.8 ± 0.3 μg of DNA/10^7 cells. In the absence of inhibitors but at 2° C instead of 37° C the uptake was decreased to 3.0 ± 0.5 μg DNA/10^7 cells. Evidently the uptake of DNA is not greatly affected by the inhibition of oxidative phosphorylation and active transport processes in the first two cases.

There are reports of DNA incorporation similar to that described here in other biological systems, and it has been demonstrated that the DNA probably retains its macromolecular form[12-14]. In all such instances, the chief difficulty is to show whether or not the material is truly incorporated into the chromosome[15]. In order to resolve this uncertainty, two lines of approach can be followed. Either the exogenous DNA can be separated from the host cell DNA after the incorporation, or traces of "donor" DNA can be made recognizable within the host cell complement. It is experimentally very difficult to realize the first objective, but one indirect method was attempted. After the extraction of nuclei from cells which had taken up ^{14}C-DNA, these were subjected to the action of deoxyribonuclease I in a manner identical to that described elsewhere[16]. The result of such treatment is shown in Fig. 3, and it is apparent that there is a sharp initial fall in the activity relative to the control value, after which a more gradual loss in activity follows. This slower rate of activity loss is roughly parallel to that which occurs when control nuclei, labelled chromosomally with ^{14}C-formate during exponential growth, are similarly treated with enzymes. Possibly there are two different modes of retention of the DNA, namely a temporary and a more permanent fixation[13].

The alternative approach, namely that of recognition of donor DNA, was followed by the use of the density marker 5-bromouracil. Cells were grown in the presence of ^{14}C-formate to yield ^{14}C-DNA, but during the last 24 h of exponential growth, 5-bromodeoxyuridine was added to a concentration of 6×10^{-6} M. After extraction the DNA was hydrolysed in the usual manner, and the bases separated by two-dimensional thin-layer chromatography on cellulose[17]. The specific activity of the bases was found to be identical to that observed previously, and the replacement of thymidine by 5-bromouracil was between 45 and 50 per cent. Incorporation experiments in "pulse" condition (*b*) showed that the BU-DNA was taken up by cells to the same extent as unsubstituted DNA. Samples of donor BU-DNA, and of host cell DNA after incorporation were subjected to density-gradient centrifugation in caesium chloride, and in three experiments out of a total of five a physical mixture of BU-DNA and unlabelled, unsubstituted DNA was also employed for comparison.

In each experiment between 100 and 150 μg of DNA in 0.3 ml of water were added to 3 ml of caesium chloride solution (60 per cent w/w British Drug Houses, Ltd.) in a 5 ml polypropylene tube and covered with 1 ml. liquid paraffin. Centrifugation was carried out for 65 h at 29,000 r.p.m. and ambient temperature in the swinging bucket rotor of an MSE 'Superspeed 40' centrifuge. Tubes were pierced subsequently and the gradient fractionated into 75–80, five-drop samples which were diluted by the addition of 0.7 ml of water. Optical densities were measured in thick-walled 1 cm cells in a spectrophotometer (Cary 15)

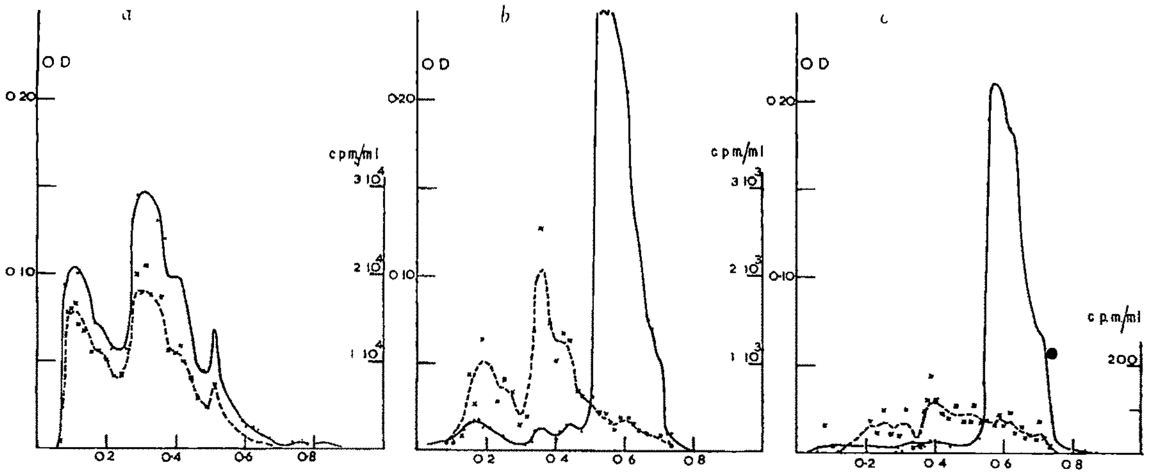

Fig. 4. Optical density, and activity profiles of carbon-14 after caesium chloride centrifugation of (*a*) donor BU-^{14}C-DNA, (*b*) donor DNA + unsubstituted, unlabelled DNA, (*c*) host cell DNA after incorporation (Table 1, run 5). (●) optical density, (×) carbon-14 activity.

Table 1 SPECIFIC ACTIVITIES AND AMOUNT OF BU-DNA TAKEN UP BY LYMPHOMA CELLS

Run	Specific activity under "light" peak (c p m /μg DNA) Calculated	 Observed	 Difference	Uptake BU-DNA / Host cell DNA %	Bound BU-DNA / Host cell DNA %
1	14 7±1 0	21 2±2 5	6 5±1 4	5 2	0 31±0 07
2	26 6±3 6	32 8±3 5	6 2±2 5	5 2	0 29±0 12
3	Approx 6 5	10 0±1 7	3 5	4 3	0 11
4	9 3±0 7	7 4±1 7	−1 9±0 9	4 3	−0 10±0 04
5	3 1	5 0±4 5	1 9	1 1	0 06

and aliquots counted for carbon-14 activity The results of one run in which "donor", "incorporated" and "mixture" DNA samples were centrifuged are shown in Fig 4 The BU-DNA peaks are well separated from each other and from the "light" DNA, although in different experiments the proportion of the DNA in the peaks was found to vary For example, in two experiments, 60 to 65 per cent of the donor DNA was found to be labelled in both strands whereas, in the other samples, 60 per cent was found to be labelled in one strand This was probably a reflexion of the age of the culture medium and other factors which affect the growth rate of the cells

In Fig 4, a third small peak can be seen in the donor BU-substituted DNA which corresponds to a proportion of the DNA which had escaped BU substitution In the experiment of Fig 4, the proportion of this peak was 7 5 per cent but the proportion varied for different preparations This unsubstituted material leads to contamination of the host cell DNA with carbon-14 activity which, although originating in the donor material, will run with the host material, and allowance must be made for this In practice, the average specific activities of the host cell DNA in the neighbourhood of the optical density peak were compared with an "estimated specific activity" calculated from that of the donor DNA, the amount of incorporation and the proportion of non-BU-substituted DNA (Table 1) In four cases, the experimental values are greater, and in one case less, than the calculated values The difference between observed and calculated values may be taken as the specific activity within the host cell which has originated from the donor DNA and which is sufficiently tightly bound, or incorporated, to move with it on centrifugation In the last column of Table 1, this difference is expressed in terms of the total donor DNA which it represents, as a percentage of the host cell material. The values are in all cases less than 0 3 per cent and therefore it is very doubtful if any chromosomal incorporation has occurred in these conditions

To summarize, it has been demonstrated that although the uptake of exogenous DNA by cells may amount to as much as 5 per cent of the host cell complement, and that this material is found within the nucleus, most of it is not chromosomally incorporated. This fact must also exclude the possibility of breakdown of the DNA to precursors and re-utilization of these. Two unsettled questions which remain are whether the large amount of DNA taken up into the nucleus performs any function, and whether even the extremely small amount of true incorporation—if real—could have genetic consequences Some preliminary experiments have been performed to investigate this latter possibility, but so far without any positive proof

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[1] Ledoux, L , *Prog Nucleic Acid Res* , **4**, 231 (1965)

[2] Wilczok, T , and Mendeki, J , *Intern J Radiat Biol* , **9**, 201 (1965)

[3] Petrovic, D , Miletic, B , Ferle-Vidovic, A , and Han, A , *Radiat Res* , **27**, 41 (1966)

[4] Savkovic, N , Kacaki, J , and Hajdnukovic, S , *Nature*, **211**, 1179 (1966)

[5] Horikawa, M , Sagahara, T , and Doida, Y , *Exp Cell Res* , **34**, 198 (1964)

[6] Cantarow, A , Williams, T L , and Goddard, J W , *Cancer Res* , **26**, 652 (1966)

[7] Glick, J L , and Goldberg, A R , *Trans NY Acad Sci* , **28**, 741 (1966)

[8] Bendich, A , Wilczok, T , and Borenfreund, E , *Science*, **148**, 374 (1965)

[9] Fischer, G A , *Methods in Medical Research*, **10**, 247 (1964)

[10] Meizel, S , and Kay, E R M , *Biochem Biophys Acta*, **103**, 431 (1965)

[11] Creasey, W A , and Stocken, L A , *Biochem. J* , **72**, 519 (1959)

[12] Ledoux, L , Gerber, G B , Charles P , Remy, J , and Remy-Defraigne, J , *Experientia*, **23**, 16 (1967)

[13] Ledoux, L , and Huart, R , *Biochim Biophys Acta*, **134**, 209 (1967)

[14] Ledoux, L , Charles, P , and Srógl, M , *Nature*, **214**, 1241 (1967)

[15] Ledoux, L , and Charles, P , *Arch Intern Physiol. et Biochim* , **75**, 363 (1967)

[16] Robins, A B , *Nature*, **215**, 1291 (1967)

[17] Bond, P S , *J Chromatog* (in the press)

Effect of Dehydration on Bacterial Recombination

by

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When water is removed from DNA by semi-dehydration there is a pronounced effect on its *in vivo* activity, and the effect may be more severe in some regions of the chromosome than others. The effects of dehydration on the ability of cells to take part in recombination have been studied in three non-lysogenic strains of *Escherichia coli K12*

Two principal factors seem to be involved in the mechanisms by which bacterial cells lose their viability when they are held in aerosols at various levels of humidity (RH): first, the removal and reorientation of water molecules which are bound to cell macromolecules, resulting in the loss of the structural integrity of the latter[1]; and second, the oxidation by oxygen of cell components leading to the possible destruction of flavin-linked enzymes[2,3] The former type of damage occurs when bacterial cells are held at RH levels ranging from 80–40 per cent and the latter type when cells are held at RH values below 40 per cent[2,3].

Experiments designed to determine the type of macromolecules which are affected or destroyed by the complete or partial loss of their bound water have shown that the loss of water leads to (*a*) the inability of cells to manufacture new protein[1,4,5], (*b*) the destruction of free and intracellular DNA bacteriophages[1,6,7]; (*c*) the production of mutant cells[8,9], and (*d*) the induction of prophages within lysogenic bacterial cells[10,11] The inability of some cells to manufacture new protein after periods of dehydration has led some investigators to suggest that the activity of cellular RNA is impaired as a result of structural alterations in RNA molecules when these lose their bound water Evidence of this has been presented by DeJong[12], who has shown that certain RNA viruses lose their infectivity as a result of partial dehydration Other researchers consider that a failure in *s*RNA synthesis leads to lack of manufacture of protein and cell death[3]. The mutagenic action of partial dehydration and the destruction of free and intracellular DNA phages, however, indicate that the biological activity of cell DNA is affected by the loss of its bound water Because the structure of isolated DNA is known to rely on the presence of correctly placed water molecules[13,14], the failure of *s*RNA synthesis is therefore reasoned to be the result of DNA damage

Because all attempts to demonstrate damage to enzymes existing in cells before they are dehydrated have failed, the induction of lambda prophages by desiccation seems to be the result of a direct effect of dehydration on DNA The removal of bound water has therefore been suggested to separate prophage DNA from host DNA in accordance with a mechanism of prophage induction suggested by some workers The destruction of a protein normally able to repress the maturation of the prophage is, however, currently the popular concept of induction, and if this repressor protein is susceptible to damage by very small doses of radiation, then it is conceivable that it is also susceptible to damage by dehydration

In many respects recombination following the transfer of DNA from one cell to another during conjugation resembles the incorporation of viral DNA into the genome of a susceptible host cell Hayes[15] has likened the transfer of the fertility factor *F* to lysogenation and moreover has shown that the irradiation of donor cells with small doses of radiation vastly increases the number of recombinants obtained Because at present there seems to be no function for repressor protein in recombination phenomena, it seemed pertinent to undertake an investigation into the effects of partial dehydration on the ability of cells to take part in recombination

The three non-lysogenic strains of *Escherichia coli K*12 used in the studies were (1) NCIB 9481 $F^+(Met^-)$, (2) NCIB 9482 $F^-(L^-T^-B_1^-Lac^-)$ and *HfrH* (Met^-), kindly supplied by Dr Ashwood-Smith and modified to non-lysogeny The cells were grown at 37° C for 24 h in brain–heart infusion broth, collected by centrifugation, resuspended to form a cell concentration of approximately 5×10^8 cells/ml in fresh media and reincubated for 4 h After this incubation the cells were washed in distilled water, and resuspended to give a cell concentration of approximately 1×10^{10} cells/ml in distilled water or 5 per cent w/v myo-inositol The cells in these suspensions were atomized into a rotating stainless steel aerosol drum in which the relative humidity had been pre-set to a desired level Immediately after and at subsequent intervals of time, samples of the airborne cells were collected in liquid impingers operating at 12 l /min and containing 8 ml of 1 per cent glucose in 0 85 per cent NaCl (saline) Hayes[15] has shown that in order to obtain an increase in the number of recombinant cells when the donor strain is irradiated with small doses of ultraviolet light, the cells should be incubated in nutrient broth for 60 min before they are mixed with the recipient strain To each of the samples collected from the aerosol 3 ml of double strength nutrient broth (NB) was therefore added and the cells were incubated at 37° C for 60 min Following this, two 1 ml aliquots of the cells were removed, incubated for 45 min at 37° C, washed twice in distilled water and one was plated on minimal salt medium to check for reverse mutation The other was serially diluted in NB and

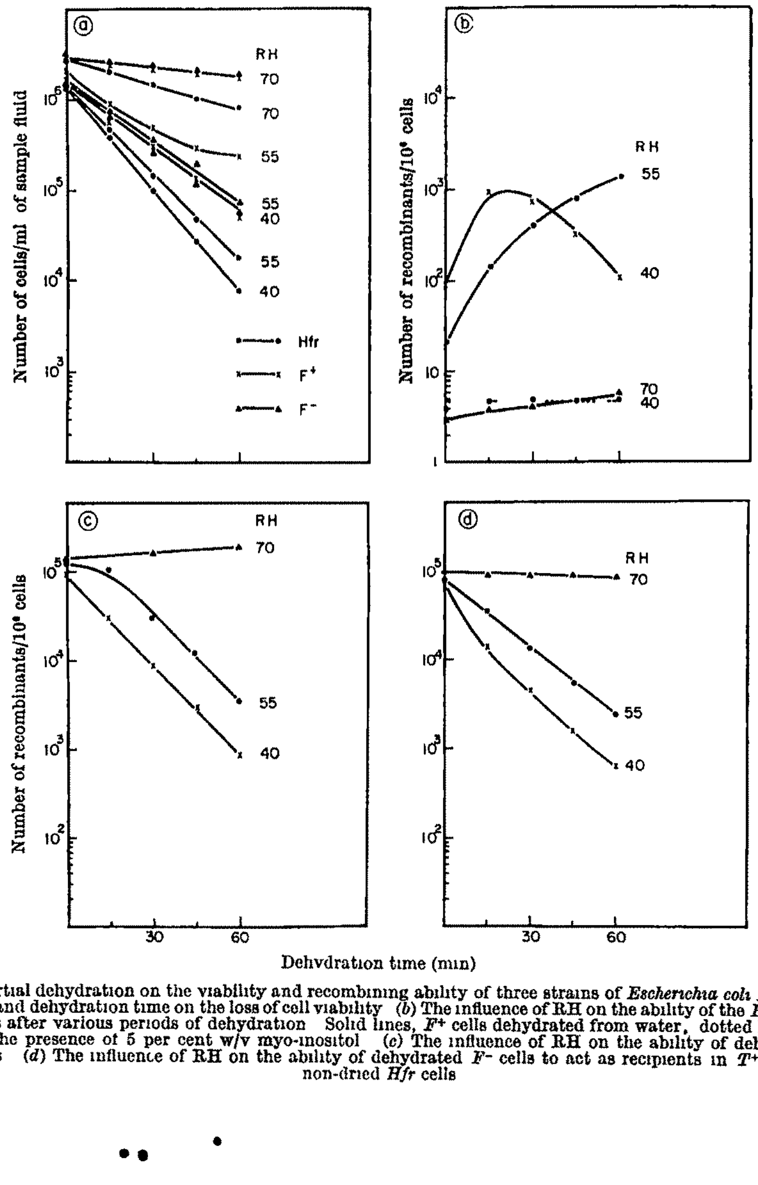

Fig 1 The effect of partial dehydration on the viability and recombining ability of three strains of *Escherichia coli K*12 (*a*) The influence of relative humidity (RH) and dehydration time on the loss of cell viability (*b*) The influence of RH on the ability of the F^+ strain to act as a donor in T^+L^+ recombinations after various periods of dehydration Solid lines, F^+ cells dehydrated from water, dotted line, F^+ cells dehydrated at 40 per cent RH in the presence of 5 per cent w/v myo-inositol (*c*) The influence of RH on the ability of dehydrated *Hfr* cells to act in T^+L^+ recombinations (*d*) The influence of RH on the ability of dehydrated F^- cells to act as recipients in T^+L^+ recombinations with non-dried *Hfr* cells

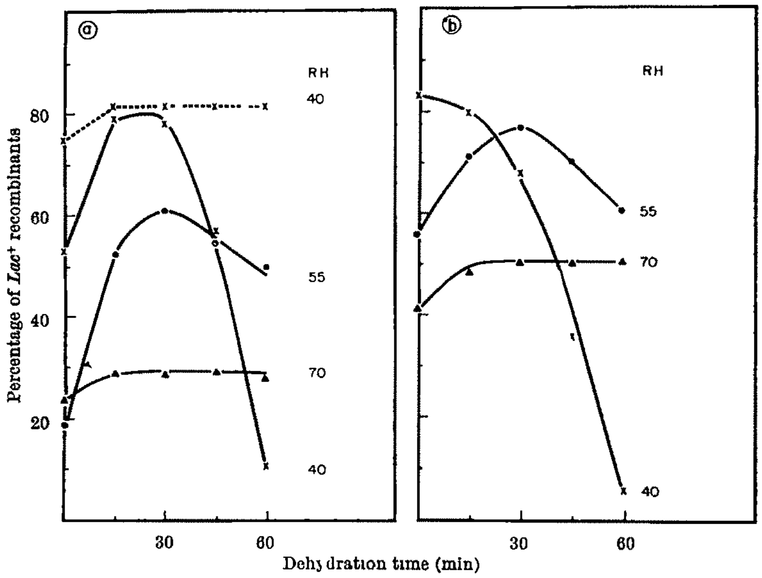

Fig. 2. The effect of partial dehydration on the percentage of T^+L^+ recombinants possessing the Lac^+ character. (*a*) The influence of relative humidity (RH) on the percentage of T^+L^+ recombinants possessing the Lac^+ character after crosses between semi-dried F^+ cells and non-dried F^- recipients. Solid lines, F^+ cells not exposed to lactose prior to drying; dotted line, F^+ cells exposed to lactose before drying. (*b*) The influence of RH on the percentage of T^+L^+ recombinants possessing the Lac^+ character after crosses between semi-dried *Hfr* cells and non-dried F^- recipient cells.

0.2 ml of each dilution was plated onto nutrient agar for viable counts. The cells in the remaining portion of the sample were concentrated by centrifugation and recipient or donor cells from a 6 h culture in NB were added so that the proportion of donor : recipient cells was approximately 1–2 × 10^7/ml, respectively. The mixed cells were incubated at 37° C for 45 min, washed twice in distilled water, resuspended in 5 ml of water, serially diluted and 0.5 ml of each dilution plated onto a minimal medium (MM) consisting of the following/l: NH_4Cl, 5 g, Na_2SO_4, 1 g, K_2HPO_4, 3 g, KH_2PO_4, 1 g, $MgSO_4$, 100 mg, glucose, 5 g; asparagine, 1.5 g, thiamine, 2 mg. The nutrient agar plates used for viability counts were incubated at 37° C for 24 h, after which time the colonies were scored and the MM plates were incubated for 48 h at 37° C before recombinant colonies were counted. To test for T^+L^+ Lac^+ recombinants the T^+L^+ recombinant colonies were picked off and plated on MM containing lactose instead of glucose and EMB agar.

In initial experiments with crosses of non-aerosolized cells, the $F^+ \times F^-$ cross produced from three–six T^+L^+ recombinants/10^6 donor cells, of which approximately 20 per cent received the Lac^+ marker. In the *Hfr* × F^- cross, 16–17 per cent of the donor cells produced recombinants, of which from 40–43 per cent possessed the three markers studied. Back mutation to prototrophy of the recipient cell was not observed and approximately one prototroph/10^7 donor cells occurred in both *Hfr* and F^+ strains. With *Hfr* and F^+ cells held in aerosols of 40 or 55 per cent relative humidity, the number of prototrophic colonies produced by reverse mutation increased to values between 20 and 30/10^7 cells recovered. In view of the large number of recombinants obtained with aerosolized *Hfr* and F^+ cells, however, this reverse mutation was ignored in the interpretation of the experimental results described here.

The recovery of cells after various periods of time of storage in air at the three relative humidities selected is shown in Fig. 1*a* for the three strains of organisms used. The *Hfr* strain was more susceptible to partial dehydration at the three relative humidity levels than either of the other two strains. No significant changes in the number of T^+L^+ recombinants were observed when the F^+ donor cells were held in aerosols at 70 per cent relative humidity. When they were held at 55 or 40 per cent relative humidity, however, an increase in the number of T^+L^+ prototrophs occurred even with cells collected immediately after their aerosolization. It takes 5 min to generate the aerosol, so this increase in recombinants seems to be caused by processes occurring in the cells during the first 5 min of partial dehydration at 55 or 40 per cent relative humidity. When the cells were held at 55 per cent relative humidity the number of T^+L^+ recombinants obtained steadily increased as the time of storage in the aerosol increased, until their numbers reached approximately 0.1 per cent at the end of the 60 min period studied. If the cells were stored at 40 per cent relative humidity, the number of recombinants obtained increased by more than a hundred-fold and reached a maximum number after 15 min of storage. Holding the cells for longer periods resulted in a drop in the number of recombinants obtained (Fig. 1*b*). The fact that the number of viable cells decreased posed a problem as to whether the results should be presented in terms of the number of recombinants/10^6 viable cells present in the initial samples collected from the aerosols, or the number of viable cells obtained in the samples collected at the various intervals of time.

For the sake of clarity it was decided to present them in both ways, and in Fig. 1 and Table 1 the former and latter methods have been used, respectively. If the two sets of data are compared, it can be seen that when the F^+ cell was held at 55 per cent RH, the number of recombinants obtained represented approximately 0.12 per cent of the viable cells present in the initial samples collected, or approximately 0.44 per cent of the viable cell number present in the 60 min sample. When the cells were held at 40 per cent relative humidity the percentage of viable cells donating DNA present in the sample at the time of collection increased during the first 45 min of storage, reaching a maximum value of 0.35 per cent, and decreased thereafter. These results suggest therefore that either a larger proportion of the surviving cells donate DNA to the recipient cells as the time of storage increases, or else some cells unable to form colonies are able to donate active DNA.

Based on the number of viable cells present in the initial sample, no increase in the number of recombinants was observed when the *Hfr* strain was subjected to periods of partial dehydration at 70 per cent RH, but when these cells were held at 40 and 55 per cent relative humidity the number of recombinants obtained decreased

Table 1 EFFECT OF SEMI-DEHYDRATION ON BACTERIAL RECOMBINATIONS

Cross	RH	Time of drying in air 0	15	30	45	60
F^+(D) × F^-	40	0.08*	0.13	0.27	0.35	0.31
	55	0.002	0.031	0.085	0.23	0.44
	70	0.0003	—	—	—	0.0005
Hfr(D) × F^-	40	7.2	7.8	9.1	10.3	8.4
	55	9.6	21.3	18.4	23.6	16.5
	70	11.3	18.6	17.4	21.2	24.3
Hfr × F^-(D)	40	8.3	2.1	1.5	1.2	0.96
	55	9.1	5.3	3.7	3.6	3.2
	70	11.2	9.6	12.3	12.1	8.4

Cells of *Escherichia coli K*12 were held at various levels of relative humidity for set periods of time and then mixed with suitable donor or recipient cells.

D, Semi-dried cell. RH, Relative humidity.

* Results expressed as percentage of viable semi-dried cells taking part in recombination.

rapidly with time (Fig. 1*c*). When based on the number of viable cells present at the time of sampling and recombinants obtained, however, the percentage of cells transferring DNA seemed to remain fairly constant. These results indicated that the ability of the *Hfr* cell to donate active DNA to the F^- cell decreased at approximately the same rate as its viability or ability to form colonies. Exposing the F^- cell to periods of partial dehydration resulted in a rapid decrease in the number of recombinants obtained when these cells were mated with non-dried *Hfr* cells. The rate at which the percentage of recombinants decreased seemed to be more rapid than the loss of F^- cell viability, particularly when the recipient cell was held at 40 per cent relative humidity (Fig. 1*d*, Table 1).

If the *Hfr*, F^+ or F^- cells were aerosolized from 5 per cent myo-inositol, none of the effects of partial dehydration were observed. These results are represented by the dotted line in Fig. 1. It seemed therefore that the presence of myo-inositol prevented both the increased ability of F^+ cell to donate its DNA and the decreased effectiveness of the F^- to receive it because of their partial dehydration in aerosols.

The percentage of T^+L^+ recombinants possessing the Lac^+ character is shown in Fig. 2. When *Hfr* or F^+ cells were held at 70 per cent relative humidity, the percentage of Lac^+ recombinants increased very slightly during the first 15–30 min and then remained constant. With the former strain of cell, between 42 and 48 per cent, and with the latter strain, between 24 and 27 per cent, of the recombinants were Lac^+, regardless of the holding time in the aerosol. The holding of the cells in aerosols of 55 per cent relative humidity, however, resulted in a substantial increase in the percentage of recombinant cells possessing the Lac^+ character. With the *Hfr* and F^+ cells, respectively, the percentage increased to 79 per cent and 53 per cent after a 30 min period of storage and then gradually decreased. When these cells were held at 40 per cent relative humidity, a difference in the behaviour of the two types of cell became evident. With the *Hfr* strain, 84 per cent of the recombinants obtained using the cells in the initial aerosol samples proved to be Lac^+ ones, but continued holding resulted in a rapid decrease in their numbers until, after 60 min, they represented less than 6 per cent of the T^+L^+ recombinants obtained. As a result of holding the F^+ cell at 40 per cent relative humidity, the percentage of Lac^+ recombinants increased from 55 per cent to 80 per cent during the first 15 min, remaining relatively constant during the next 15 min, and then fell during the remaining 30 min. These findings seemed to explain the permanent or temporary failure of many cells surviving semi-dehydration at 55–35 per cent RH to manufacture enzymes concerned in the metabolism of lactose.

From the results presented in Fig. 2, the Lac^+ locus seemed to be more susceptible to damage than the T^+L^+ loci. In the knowledge that cells exposed to lactose before they are dehydrated do not readily lose their ability to manufacture enzymes that metabolize lactose, the validity of the foregoing hypothesis was tested by examining the behaviour of F^+ cells grown in nutrient broth supplemented with 2 per cent lactose. When these cells were subjected to periods of semi-dryness in the aerosol, the percentage of Lac^+ recombinants subsequently obtained increased to about 80 per cent and then remained constant. The decrease observed with cells not exposed to lactose did not occur. This is shown by the dotted line in Fig. 2*a*. Thus it would seem that contact between the cell and lactose before the cell was dehydrated stabilizes the Lac^+ portion of the DNA against the destruction by dehydration.

The genetic implications of the results presented are not understood and will require much more experimentation for a reasonable explanation to be offered. From the standpoint of dehydration damage, however, the increase in the number of recombinants obtained when F^+ cells are dehydrated at 40 or 55 per cent RH and the prevention of this phenomenon by myo-inositol are remarkably similar to the effects of dehydration and inositol on the induction of lambda prophages. If prophage induction by dehydration results from the separation of phage DNA from host DNA, then the increase in recombinants seems to occur as a result of a more efficient transfer of F^+ donor DNA to the recipient cells as a result of some kind of DNA fragmentation. The involvement of permeability changes in this phenomenon seems to be ruled out because (*a*) dehydration and inositol both increase cell permeability, yet inositol will prevent the increase in recombinants; and (*b*) the efficiency of the recipient cell to receive DNA is decreased, not increased, by dehydration. The latter phenomenon may reflect an effect of dehydration or DNA synthesis which prevents recombination. The occurrence of more T^+L^+ recombinants possessing the Lac^+ character than would normally be expected in both $F^+ \times F^-$ and $Hfr \times F^-$ crosses after a short period of donor cell dehydration and the decrease in their number after prolonged dehydration suggest that either the T^+L^+ Lac^+ unit is transferred more easily after cell dehydration and the Lac^+ DNA is more easily inactivated than the T^+L^+ DNA, or that the characters are transferred independently as a result of separation of the T^+L^+ and Lac^+ DNA by chromosome breakage. Even in the latter case, however, the Lac^+ DNA would seem to be inactivated more quickly than the T^+L^+ DNA. Clearly, the removal of water from DNA by semi-dehydration has a pronounced effect on its *in vivo* activity and the effect may be more severe in some regions of the chromosome than others.

The use of recombination phenomena in studies of dehydration damage to microbial cells should therefore prove useful in determining the reasons for the various known effects and may assist in understanding the mechanisms of recombination. Detailed studies using selected and unselected markers and blender separations of conjugants after given times of incubation are under way and will be reported in the near future.

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[1] Webb, S. J., *Bond Water in Biological Integrity* (Thomas, Illinois, 1965).

[2] Benbough, J. E., *J. Gen. Microbiol.*, 47, 325 (1967).

[3] Webb, S. J., *Canad. J. Microbiol.*, 13, 733 (1967).

[4] Webb, S. J., *Canad. J. Microbiol.*, 6, 71 (1960).

[5] Anderson, J. D., *J. Gen. Microbiol.*, 45, 303 (1966).

[6] Webb, S. J., Dumasia, M. D., and Bhorjee, J. S., *Canad. J. Microbiol.*, 11, 141 (1965).

[7] Cox, C. S., and Baldwin, F., *J. Gen. Microbiol.*, 44, 15 (1965).

[8] Webb, S. J., *Nature*, 213, 1137 (1967).

[9] Webb, S. J., and Walker, J. L., *Canad. J. Microbiol.* (in the press).

[10] Webb, S. J., and Dumasia, M. D., *Canad. J. Microbiol.*, 13, 33 (1966).

[11] Webb, S. J., and Dumasia, M. D., *Canad. J. Microbiol.*, 13, 303 (1966).

[12] DeJong, J. C., *Phenomena of Growth and Decay with the Viruses of Poliomyelitis and Measles* (Drukkerij Uitgeverij H. Gianotten N.V. Tilburg, 1967).

[13] Sutherland, G. B. B. M., and Tsuboi, M., *Proc. Roy. Soc.*, A, 239, 446 (1967).

[14] Falk, M., Hartman, J. A., and Lord, R. C., *J. Amer. Chem. Soc.*, 85, 387 (1963).

[15] Hayes, W., *Cold Spring Harbor Symp. Quant. Biol.* 18, 75 (1953).

LETTERS TO THE EDITOR

ASTRONOMY

Interpretation of Cosmic Microwave Background

THE discovery of microwave background radiation by Penzias and Wilson[1] is one of the most interesting events in observational astronomy in recent years. On the basis of the first observations at a single wavelength, the radiation was interpreted[2] as being of the black-body type, and it was concluded that this was the remnant of the primordial radiation of a big-bang universe. Later results by different observers have strengthened this interpretation. Indeed it is now claimed[3,4] that the black-body curve applies for a wavelength range of 1 90, and corresponds to a temperature $T=2.68°$ K. The observers[5] go as far as to state that the exact black-body intensity curve

$$\mathcal{J}(\nu) \propto \frac{\nu^3}{e^{h\nu/kT}-1} \tag{1}$$

fits the observations better than the "grey-body" approximation at long wavelengths—namely

$$\mathcal{J}(\nu) \propto \nu^2 \tag{2}$$

The purpose of this communication is to examine this interpretation in the light of available information about the universe. We begin by noting that if the observations are taken at their face value, one of the two possible conclusions can be drawn.

(I) The entire observed cosmic background is in fact the remnant of the primordial black-body radiation.

(II) If there are other processes operative in the universe capable of producing a microwave background of the observed magnitude, the present observations disprove (I).

It must be emphasized that for (II) to hold, it is not necessary that the microwave radiation produced by the other processes should follow the law (1) or (2). It is only necessary that the contribution be comparable with that observed in the relevant frequency range.

A few months ago one such process was indeed suggested[6,7]. The process consists of the absorption of optical and ultraviolet radiation from stars and its re-radiation at longer wavelengths by impurity oscillators in the interstellar dust grains of galaxies. This process could operate in all galaxies provided interstellar dust is present. It was also shown that in the steady state universe, because of the red-shift effect, radiation along a line with frequency $\nu=\nu_0$ is smeared out in the form of a spectrum

$$\mathcal{J}(\nu) \propto \begin{cases} \nu^3 & \nu \leqslant \nu_0 \\ 0 & \nu > \nu_0 \end{cases} \tag{3}$$

The actual spectrum observed would be a superposition of several such spectra, arising from different ν_0.

Although this calculation refers to the steady state universe, the process in question could equally well operate in a big-bang universe, but there are two points of difference. The red-shifts permitted in the steady state universe can be infinite, in the big-bang universe, there is an upper limit set by the epoch of galaxy formation. At present we have no definite theory of galaxy formation available to fix this epoch, but red-shifts up to $z \simeq 100$ may still be permitted. Such a factor is sufficient to bring the far infrared frequencies into the microwave range[7]. The second point of difference arises in that the evolutionary cosmologies permit the use of arbitrary epoch-dependent functions—a freedom denied to the steady state theory. Such functions have been extensively used[8,9] to make the predictions of big-bang universes agree with observations.

On the assumption that there is no epoch-dependence, however, the function $\mathcal{J}(\nu)$ corresponding to a single line $\nu=\nu_0$ may be computed in a relativistic cosmological model. The calculation is particularly simple if we assume that only the matter density makes a significant contribution to T^{ik} in the Einstein equation—an assumption which seems to be justified from our present knowledge of the universe. The result can be expressed in terms of q, the deceleration parameter[10]. We have

$$\mathcal{J}(\nu) \propto \begin{cases} \dfrac{\nu^{3/2}}{\sqrt{2q\nu_0-(2q-1)\nu}} & \text{for } \nu_1 \leqslant \nu \leqslant \nu_0 \\ 0 & \text{otherwise} \end{cases} \tag{4}$$

Here ν_1 is the lower limit set by the epoch of galaxy formation. (This form of $\mathcal{J}(\nu)$ can be altered almost at will by the use of arbitrary epoch-dependent functions, so that we attach no special significance to it.) For the Einstein–de Sitter universe, $q=\frac{1}{2}$ and $\mathcal{J}(\nu) \propto \nu^{3/2}$. For the empty hyperbolic universe $q=1$ and $\mathcal{J}(\nu) \propto \nu$.

It remains to discuss the order of magnitude of this radiation. Shakeshaft and Webster[4] have argued that the black-body radiation density is about 400 times greater than the energy density of star-light from all galaxies, and quote this result from a review by Davidson and Narlikar[10]. We wish to point out that the factor arose on the hypothesis that the radiation was of the black-body type. The radiation density corresponding to a black-body radiation temperature is

$$\sim 3.8 \times 10^{-13} \text{ erg cm}^{-3} \tag{5}$$

This energy density corresponds, however, to radiation over all frequencies. We are here concerned only with a limited wavelength range over which radiation has actually been observed, namely, from 36.6 GHz to 408 MHz, the factor of 1 90 quoted by Shakeshaft and Webster[4]. (In fact the process considered by us does not operate over all frequencies, as is clear from (3) and (4).) The radiation density over the observed range is less than

$$\sim 6 \times 10^{-15} \text{ erg cm}^{-3} \tag{6}$$

a figure comparable with the optical radiation density (Davidson and Narlikar, *op cit* page 553). Furthermore, we had allowed ourselves a factor 10–100 over and above the optical radiation density in young galaxies (not the local galaxy as quoted by Shakeshaft and Webster). We conclude therefore that there is no energy difficulty associated with this process.

Shakeshaft and Webster[4] have raised objections to the mechanism of themalizing starlight. We fail to see the relevance of their arguments. While it is true that there are essential and vital differences between the electrical properties of graphite and those of alkali halides, these differences are clearly not significant for the process we consider. A weakly bound impurity atom or ion in any crystal lattice would be expected to have a localized low frequency oscillation mode, the frequency of which is determined almost entirely by the ratio of its mass to that of the host crystal atom and by the force constants of its attachment to the lattice. We cannot also follow their arguments against the possibility that all the impurities may be lodged in the outer dielectric mantles of the grains. On the basis of grain models which agree with extinction and polarization data we require the outer dielectric mantle to be about 100 times more massive than the graphite core. It would indeed seem likely that most of the impurities are lodged in the dielectric mantles, and about 10^4 such impurity atoms[6] suffice to re-radiate the absorbed starlight in the far infrared.

We therefore feel that such a process could very well operate in galaxies with interstellar dust and make a substantial contribution to the microwave background We do not suggest that this is the only source of microwave background It is of interest in this connexion that Partridge and Wilkinson[11] have recently reported a slight increase in the background radiation at the 3 2 cm wavelength from a region of the sky which is close to the galactic plane Kurilchik and Kokin[12] have also detected a microwave flux at 8 cm wavelength from two individual quasi-stellar objects BSO 1 and Ton 730 Very recent infrared surveys (Low[13]) have actually revealed the remarkable fact that several galaxies emit ~100 times more radiation in the far infrared than in the visible spectral region It is entirely conceivable that later and more systematic surveys of discrete sources at long wavelengths would produce further evidence of a contribution to the microwave background from such sources

We now turn to the cosmological aspects of the problem If the aforementioned process takes place in a big-bang universe, we fail to see how the combined curve of this process plus the primordial black-body radiation could lead to the purely black-body curve (1) Our own view is that the "black body" explanation has been overstated and that the actual form of $\mathcal{J}(\nu)$ may not be so simple For this reason, it is also premature to conclude from the present observations that the steady state theory of the universe is untenable

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[1] Penzias, A A , and Wilson, R W , *Astrophys J* 142, 319 (1965)
[2] Dicke, R H , Peebles, P J E , Roll, P G , and Wilkinson, D T *Astrophys J* , 142, 414 (1965)
[3] Howell, T F , and Shakeshaft, J R , *Nature*, 216, 753 (1967)
[4] Shakeshaft, J R , and Webster, A S , *Nature*, 217, 339 (1968)
[5] Stokes, R A , Partridge, R B , and Wilkinson, D T , *Phys Rev Lett* , 19, 1199 (1967)
[6] Hoyle, F , and Wickramasinghe, N C , *Nature*, 214, 969 (1967)
[7] Narlikar, J V , and Wickramasinghe, N C , *Nature*, 216, 43 (1967)
[8] Longair, M S , *Nature*, 211, 949 (1966)
[9] Davidson W , and Davies, M *Mon Not Roy Astro Soc* , 127, 241 (1964)
[10] Davidson, W , and Narlikar, J V , *Rep Prog Phys* , 29, 539 (1966)
[11] Partridge, R B , and Wilkinson, D T , *Nature*, 215, 719 (1967)
[12] Kurilchik, V N , and Kokin, Yu F , *Soviet Astron* , 11, 374 (1967)
[13] Low, F J , *Tucson Conference on Seyfert Galaxies*, February, 1968

Interstellar Extinction by Quartz Grains

KNACKE[1] has pointed out that quartz particles possess strong absorption bands at $\lambda \sim 10\mu$ Although these features occur in the spectral region 3–10μ, where Johnson[2] has found humps in interstellar extinction curves, by itself this cannot be taken as indicating a silica component in the interstellar grains It has already been pointed out[3] that impurities in any solid with a Debye frequency close to that of graphite would give optically active lattice bands at $\lambda \sim 10\mu$* Further evidence would be necessary before quartz could be considered as a serious alternative to interstellar graphite grains

From an approximate calculation, Knacke[1] has claimed that silica grains of radii ~ 0 2μ would fit the observed interstellar extinction curve in the wavelength range 0 3–1 5μ to within about 15 per cent, but the extension of the observed extinction curve into the far ultraviolet region[4,5] has greatly enhanced the sensitivity of this test[6] It is therefore of interest to compute accurate extinction cross-sections for quartz particles and particles with a quartz core and ice mantle for the entire wavelength range of the observations We have performed such calculations for single-sized quartz particles, quartz particles with an Oort–van de Hulst[7] distribution of radii and for quartz particles covered with ice mantles

Calculations of the cross-sections for homogeneous quartz particles were done using the Mie theory and those for the composite particles using the Guttler theory (see, for example, ref 8) The results of our calculations are shown in Figs. 1–3 We have adopted a normalized extinction F_λ defined by

$$F_\lambda = \frac{0\ 5\,[Q(\lambda) - Q(0\ 5556\mu)]}{Q(0\ 4464\mu) - Q(0\ 5556\mu)}$$

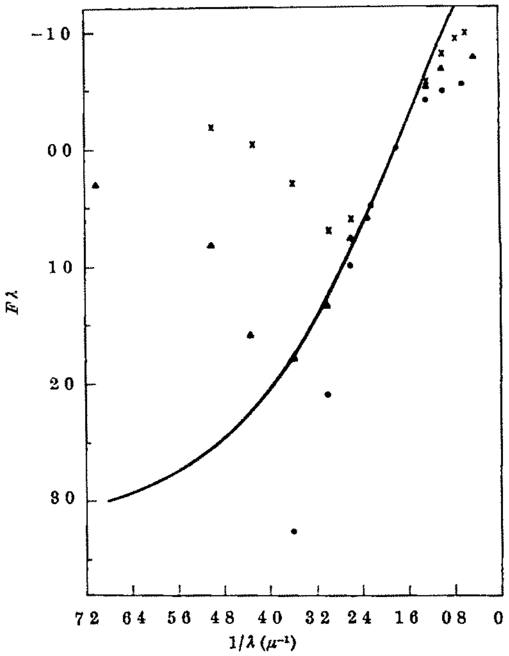

Fig 1 Comparison of the theoretical extinction curve for single homogeneous quartz grains with observations The solid line denotes the smoothed curve drawn through the observations ●, $r = 0\ 10\mu$, ▲, $r = 0\ 15\mu$, ×, $r = 0\ 20\mu$

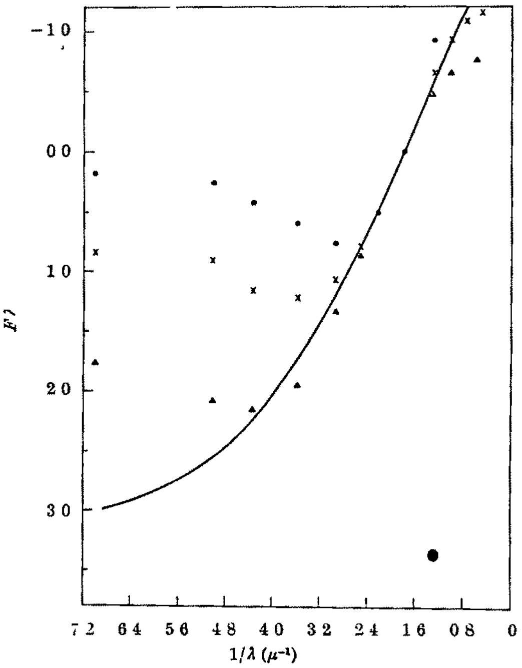

Fig 2 Comparison of the theoretical extinction curve (for quartz particles integrated over Oort–van de Hulst size distribution) with observations The solid line denotes the smoothed curve drawn through the observations ●, $\gamma_1 = 0\ 10\mu$, ×, $\gamma_1 = 0\ 08\mu$, ▲, $\gamma_1 = 0\ 06\mu$

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* The phonon spectrum of reactor grade graphite shows the strongest pile up of phonon states at ~ 7μ (ref 9)

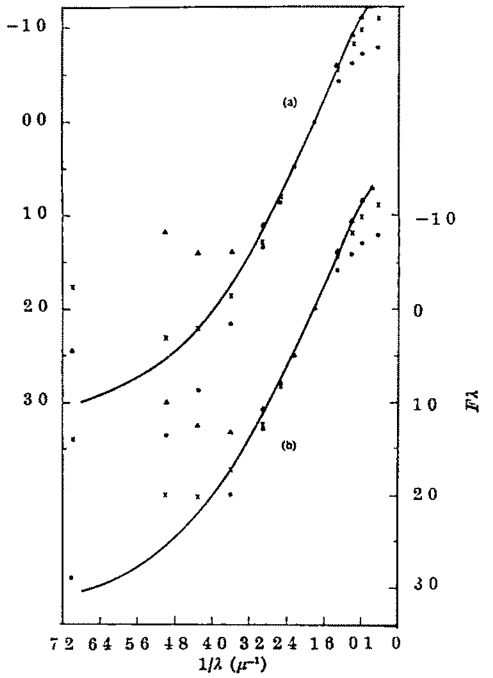

Fig 3 Comparison of the theoretical extinction curve (for quartz ice particles) with observations The solid line denotes the smoothed curve drawn through the observations (*a*) Core radii = 0 04μ ●, outer radius = 0 14μ, ×, outer radius = 0 19μ, ▲, outer radius = 0 24μ (*b*) Core radii = 0 05μ ●, outer radius = 0 15μ, ×, outer radius = 0 20μ, ▲, outer radius = 0 25μ

where $Q(\lambda)$ is the integrated extinction cross-section The solid curves represent the observed extinction[5] normalized in the same way

In all the cases we have considered, no agreement is possible over the entire wavelength range of the observed interstellar extinction curve

Although pure quartz is a pure dielectric, if quartz particles exist in the interstellar medium they may be expected to contain an appreciable quantity of impurities which contribute an imaginary component to their refractive index Accordingly we repeated our calculations taking the complex refractive index to be $m(\lambda) = n(\lambda) - 0\ 1\,i$, where $n(\lambda)$ is the refractive index appropriate to pure quartz The results for the impure quartz are not significantly different from those plotted in Figs 1-3 for pure quartz

Thus it seems most unlikely that quartz particles can form any appreciable component of the interstellar grains We have also recently examined in some detail the conditions required for the formation of silica grains in M stars (our unpublished results) We performed chemical equilibrium calculations including about 60 molecular species and concluded that the condensation of silica requires temperatures ~1,000° K—less than the boundary temperature of these stars

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[1] Knacke, R F *Nature*, **217**, 44 (1968)
[2] Johnson, H L, *Astrophys J*, **141**, 923 (1965)
[3] Wickramasinghe, N C, *Nature*, **216**, 249 (1967)
[4] Boggess III, A, and Borgman, J, *Astrophys J*, **140**, 1636 (1964)
[5] Stecher, T P, *Astrophys J*, **142**, 1683 (1965)
[6] Krishna Swamy, K S and O'Dell, C R, *Astrophys J*, **147**, 937 (1967)
[7] Oort, J H, and Van de Hulst, H C, *Bull Astro Inst Netherlands*, **10**, 187 (1946)
[8] Wickramasinghe N C, *Interstellar Grains* (Chapman and Hall, 1967)
[9] Young J A, and Koppel, J U, *J Chem Phys*, **42**, 357 (1965)

Is Interstellar Hydrogen capable of Maser Action at 21 Centimetres?

THREE sets of observations suggest that the density of interstellar neutral hydrogen may be less than the value usually inferred from 21 cm emission line measurements This possible discrepancy is found in observations of (*a*) the incoming high velocity neutral hydrogen clouds which, according to Oort[1], deposit material on to the galactic disk at an alarmingly large rate, (*b*) the ultraviolet spectra of stars in Orion which show an unexpectedly weak interstellar Lyman-α absorption line[2,3], and (*c*) the X-ray background at ~50 Å which suggests that the opacity of the Galaxy is less than would be estimated from the observed column density of neutral hydrogen[4]

Shklovskii[5], following Varshalovich[6], has attempted to resolve (*a*) by invoking maser action in the 21 cm line of interstellar hydrogen, made possible by a population inversion of the hyperfine levels of the ground state arising from Lyman-α pumping This mechanism would operate selectively on the incoming clouds and would lead to an overestimate of their mass Fischel and Stecher[7] have made a similar suggestion to explain (*b*) One might also consider this process in connexion with (*c*) (ref 8) We wish to point out that a detailed consideration of the Lyman-α pumping mechanism shows that it is unlikely to be of importance in resolving these problems

In his theoretical analysis, Varshalovich has shown that population inversion of the hyperfine levels of the ground state of neutral hydrogen can occur in a region of high Lyman-α density, where the excitation conditions are determined by Lyman-α absorption and not by spin-exchange collisions The criterion for inversion in such a region is

$$\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu} > 2\times 10^{-32}\ \text{photons Hz}^{-2}\ \text{cm}^{-3} \qquad (1)$$

where $n(\nu)\,\mathrm{d}\nu$ is the number density of photons of frequency between ν and $\nu+\mathrm{d}\nu$ in the Lyman-α radiation field When inversion occurs, the spin temperature in degrees absolute is given by

$$T_s = -6\ 81\times 10^{-2}\ \frac{n(\nu)}{\nu_0\,\dfrac{\mathrm{d}n(\nu)}{\mathrm{d}\nu}} \qquad (2)$$

where $\nu_0 = 1\ 42\times 10^9$ Hz, and the gain at the centre of the 21 cm line for a region of length L cm is given by the expression

$$\tau_0 = \frac{3}{32\pi\,\Delta\nu_0}\frac{c^2}{\nu_0^2}\,A_{12}\,N_{\mathrm{H}}fL \qquad (3)$$

where $A_{12} = 2\ 85\times 10^{-15}\ \text{s}^{-1}$, Δv_0 is the Doppler width of the 21 cm line, typically $\sim 5\times 10^4$ Hz, N_{H} is the density of neutral hydrogen in atoms cm^{-3} and the degree of inversion f is given by

$$f = \frac{g_0 N_1}{g_1 N_0} - 1 = \frac{-6\ 81\times 10^{-2}}{T_s} \qquad (4)$$

The subscript 1 refers to the $F=1$ triplet state of the $1S_{\frac{1}{2}}$ level of neutral hydrogen and the subscript 0 to the $F=0$ singlet state

At the edge of a typical HII region, the Lyman-α radiation density $\sim 4\times 10^{-3}$ Lyman-α photons cm^{-3} (ref 8) If the Lyman-α profile in the region of half maximum in the red wing of the line has a Doppler shape of width $\Delta\nu_D \sim 8\times 10^{10}$ Hz, corresponding to a temperature of 10^4 °K in the HII region, then in this part of the line profile

$$\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu} > 10^{-26}\ \text{photons Hz}^{-2}\ \text{cm}^{-3}$$

and

$$\frac{n(\nu)}{\nu_0\,\dfrac{\mathrm{d}n(\nu)}{\mathrm{d}\nu}} \sim \frac{(\Delta\nu_D)^2}{2\nu_0\,\Delta\nu} \qquad (5)$$

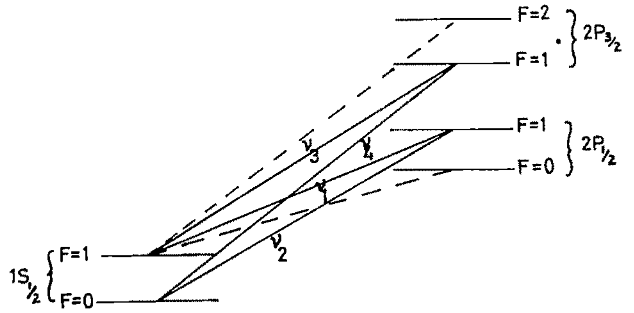

Fig. 1. The $1S$ and $2P$ energy levels of neutral hydrogen, showing the allowed transitions excited by Lyman-α – – –, Complementary transitions.

where $\Delta\nu$ is the displacement from the central maximum. For $\Delta\nu = 2\Delta\nu_D$

$$\frac{n(\nu)}{\nu_0 \dfrac{dn(\nu)}{d\nu}} \sim 14$$

Thus if blue-shifted Lyman-α radiation from an HII region is incident on neutral hydrogen, population inversion in the $1S_{\frac{1}{2}}$ state will occur, with $T_s \sim -1°$ K from (2) and $f \sim 0.07$ from (4).

Fischel and Stecher consider a model of the Orion Nebula in which the neutral hydrogen is moving with a velocity $\sim +10$ km/s with respect to the central HII region. They assume that, in these conditions, a maser region ~ 5 pc thick will be formed, with a gain $\tau_0 \sim 0.8$ (from equation (3) with $f \sim 0.07$ and $N_H \sim 1$ cm^{-3}). This, however, neglects the very rapid thermalization of the Lyman-α spectrum as the radiation enters the neutral hydrogen region. With an absorption coefficient in the line centre, $k_0 = 5.4 \times 10^{-13}$ cm^2, 5 pc of neutral hydrogen of density 1 cm^{-3} has an opacity for Lyman-α of $\tau_a = 8 \times 10^6$. The transfer problem for Lyman-α in neutral hydrogen has not been accurately solved, but the results of Unno[10] and Field[11] show that complete thermalization of the profile would occur well within a region of optical depth $\tau_a = 10^2$. Thus appreciable inversion can only occur in a zone of thickness $\sim 6 \times 10^{-5}$ pc at the edge of the neutral hydrogen region.

To ensure that, within this zone, the total rate of Lyman-α absorption is greater than the spin exchange collision rate for a gas of density ~ 1 cm^{-3} (which is required for inversion to occur at all) it is necessary for $\Delta\nu$ to be less than 4 $\Delta\nu_D$. If this inequality holds then

$$\frac{n(\nu)}{\nu_0 \dfrac{dn(\nu)}{d\nu}} > 7$$

(from (5)), giving a maximum possible degree of inversion $f \sim 0.14$ with $T_s \sim -0.5°$ K (for negative spin temperature a smaller value of $|T_s|$ gives a larger degree of inversion).

Thus the very large opacity of neutral hydrogen for Lyman-α reduces the gain of the maser zone in the Fischel and Stecher model of Orion from $\tau_0 \sim 0.8$ to a maximum of $\tau_0 \sim 2 \times 10^{-5}$. Accordingly no significant amplification of the background radiation will occur. The same conclusion has also been reached independently by Van Bueren and Oort[12].

Shklovskii has considered the possibility of Lyman-α pumping in neutral hydrogen clouds of density $N_H \sim 10^{-3}$ cm^{-3} and diameter ~ 100 pc in the galactic corona. He attempts to overcome the problem of the rapid thermalization of the Lyman-α profile by assuming that, if the incident Lyman-α spectrum is growing exponentially, most of the absorption will occur in the far violet wing of the absorption profile of the neutral hydrogen. In the far wing the absorption cross-section $k \sim 10^{-18}$ cm^2 and the clouds will be optically thin. However, if most of the absorption does occur in the violet wing, the theoretical analysis of Varshalovich cannot be used to calculate the degree of inversion produced by the Lyman-α radiation field.

Consider the energy level diagram of the $1S$ and $2P$ states of neutral hydrogen (see Fig. 1). Of the six allowed hyperfine transitions $1S \rightarrow 2P$ excited by Lyman-α, four produce mixing of the ground state populations. (The two complementary transitions $1S_{\frac{1}{2}} \rightarrow 2P_{\frac{3}{2}}$, $1S_{\frac{1}{2}} \rightarrow 2P_{\frac{1}{2}}$ cannot mix the populations and will not be considered in the following discussion.) Each of these four transitions has an absorption profile centred on its transition frequency, as shown schematically in Fig. 2. The width of each line $\gamma \sim 10^8$ Hz (the natural width of the $2P$ state) and the separation of the lines $\nu_2 - \nu_1 \sim 10^9$ Hz. At frequencies close to the absorption peaks there is very little overlapping of the separate absorption profiles (since $\gamma \lll \nu_2 - \nu_1$). Appreciable overlapping does occur, however, in the wings (see Fig. 2), where the absorption coefficient for the ν_n hyperfine transition has the form

$$K_n(\nu) = \frac{K_{n0}}{1 + \left(\dfrac{2(\nu - \nu_n)}{\gamma}\right)^2}$$

For $\nu - \nu_n \sim 10^{11}$ Hz, $\gamma \sim 10^8$ Hz the absorption coefficients for the separate hyperfine transitions differ by at most 3 per cent. This difference decreases as $\nu - \nu_n$ increases.

In his analysis, Varshalovich assumes that the Lyman-α absorption in the wings is negligible. If absorption occurs near the line centre, where there is little overlap of the individual absorption coefficients, the relative populations of the ground state hyperfine levels will be determined by the gradient of the Lyman-α spectrum near the line centre and equations (1) and (2) can be used to calculate the resultant population inversion. If, however, most of the absorption takes place in the wings where the separate absorption lines overlap, any preferential absorption into a particular hyperfine state will be caused not by the rapidly growing Lyman-α spectrum but by the small difference between the overlapping absorption coefficients. Because such differences are so small and decrease as $(\nu - \nu_n)$ increases, the resultant degree of inversion will be less than that obtained by Shklovskii using the results of Varshalovich. An approximate calculation shows that it is not possible to get a degree of inversion $f > 5 \times 10^{-4}$ by means of this slight preferential absorption in the violet wing. No significant overestimation of the mass of the coronal clouds would result from this small degree of masing and no significant selection of incoming clouds would occur.

We conclude that the very large opacity of neutral hydrogen for Lyman-α makes it exceedingly difficult to form an extended region of appreciable population inversion of the hydrogen ground state by means of Lyman-α pumping. The very small masing zone formed at the surface of an HI region moving relative to an HII region can-

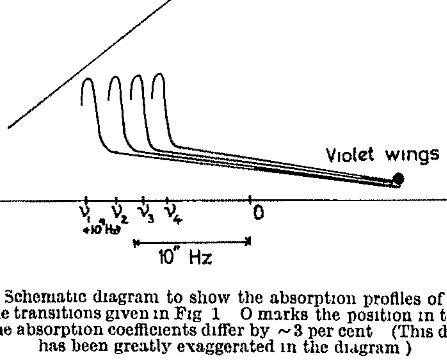

Fig. 2. Schematic diagram to show the absorption profiles of the four hyperfine transitions given in Fig. 1. O marks the position in the wings where the absorption coefficients differ by ~ 3 per cent. (This difference has been greatly exaggerated in the diagram.)

not produce significant amplification of the background radiation Accordingly, the problems raised by the observations (*a*), (*b*) and (*c*) described at the beginning of this communication probably have to be resolved without invoking a maser mechanism in interstellar hydrogen

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[1] Oort, J H *Bull Astro Netherlands*, **18**, 421 (1966)
[2] Morton, D C , *Ap J* , **147**, 1017 (1967)
[3] Jenkins, E B , and Morton, D C , *Nature*, **215**, 1257 (1967)
[4] Bowyer, C S , Field, G B , and Mack, J E , *Nature*, **217**, 32 (1968)
[5] Shklovskii, I S , *Sov Astro AJ*, **11**, 240 (1967)
[6] Varshalovich, D A , *Sov Phys JETP*, **25**, 157 (1967)
[7] Fischel, D , and Stecher, T P , *Ap J Lett* , **150**, 251 (1967)
[8] Rees, M J , Sciama, D W , and Setti, G , *Nature*, **217**, 326 (1968)
[9] Field, G B , *Proc Inst Rad Eng* , **46**, 240 (1958)
[10] Unno, W , *Pub Astro Soc Japan*, **3**, 158 (1952)
[11] Field, G B , *Ap J* , **129**, 551 (1957)
[12] Van Bueren, H G , and Oort, J H , *Bull Astro Netherlands* (in the press)

Cosmogonic Fragmentation

Cosmogony probes the evolution of a cosmic system by entertaining a variety of hypotheses about its origin It is valuable to distinguish these, as far as possible, from any specific mechanisms they may assume Thus many hypotheses call for some, but not necessarily a particular, mechanism for fragmenting cosmic masses[1-5] This communication concerns one possible fragmentation mechanism—gravitational instability in anisotropic expansion—which can operate effectively under diverse hypotheses

In this communication, I argue that this is a feasible mechanism for cosmogonic fragmentation in that (1) linear instability can be strong, and (2) nonlinearities need not bar permanent fragmentation, but (3) they can control excessive subfragmentation I then indicate some of the hypotheses yielding anisotropic expansion, with special reference to star formation In that case, the value of distinguishing mechanisms from hypotheses will be particularly in evidence, in that (4) under a generally accepted hypothesis, suitably elaborated, the proposed mechanism can yield acceptable protostar masses

Gravitational stability theory shows that anisotropic expansions can permit exponential growth of density fluctuations ($\delta\rho/\rho$), in contrast to the weak algebraic instabilities of a spherical expansion The essential physical reason for this is that the density, and therefore the fluctuations of self-gravity ($\propto \rho\ \delta\rho$), can decrease with time sufficiently slowly in anisotropic expansion To see this mathematically, I shall confine attention to the limit of long waves in uniform media for which (sum convention)

$$\left(\frac{\mathrm{d}^2}{\mathrm{d}t^2} - 4\pi G\rho\right)\frac{\delta\rho}{\rho} = 2\frac{\partial u_i}{\partial x_j}\frac{\partial v_j}{\partial x_i} \text{ and } \frac{\mathrm{d}}{\mathrm{d}t}\frac{\delta\rho}{\rho} = -\frac{\partial v_i}{\partial x_i} \qquad (1)$$

where u_i are cartesian components of unperturbed flow and v_i their linear perturbations This extends Jeans' static case, $u_i = 0$, $\rho =$ constant, with instability $\delta\rho/\rho \propto \exp\sqrt{4\pi G\rho}\ t$, and may be generalized further (Layzer, private communication) Clearly, expansion tends to be less unstable because ρ decreases with t In fact, for a uniform spherical expansion $u_i = -\frac{1}{3}(\rho/\rho)\ x_i$, equation (1) yields the known[6] maximum instability

$$\frac{\delta\rho}{\rho} \sim t^n, \quad n = -\frac{1}{6} + \sqrt{1/36 + 4\pi G\rho t^2} = \frac{2}{3} \qquad (2)$$

because $\rho \sim 1/6\pi G t^2$ at best (zero energy) For an expansion such that $\rho \sim t^{2\varepsilon-2}$ with $\varepsilon > 0$, however, equation (1) shows that the dominant behaviour as $t \to \infty$ remains exponential

$$\frac{\delta\rho}{\rho} \propto \exp\int\sqrt{4\pi G\rho}\ \mathrm{d}t = \exp k\ t^{\varepsilon} \qquad (3)$$

($k =$ constant) provided the velocity term in (1) is asymptotically weak compared with $4\pi G\delta\rho$ for at least some mode This is possible for some anisotropic expansions

As an extreme example, for a one-dimensional explosion (or ' high energy jet", see ref 7) for which $u_i = -(\dot\rho/\rho)\ \delta_{i1} x_1$ and $v_i = \delta_{i1} v_1$, equation (1) gives

$$\frac{\delta\rho}{\rho} \sim \rho\exp\int\sqrt{4\pi G\rho}\ \mathrm{d}t \sim \frac{1}{t}\exp k\ t^{\frac{1}{2}} \qquad (4)$$

because $\rho \sim k^2/16\pi G t$ with $k^2 = 16\pi G\rho_0\tau_0$ where ρ_0, τ_0 are density and explosion time at $t = 0$ For a more general spheroidal expansion, analysis—to be given in detail elsewhere—gives, for axial modes and zero energy,

$$\frac{\delta\rho}{\rho} \sim \rho^{\frac{1}{4}}\exp\int\sqrt{4\pi G\rho}\ \mathrm{d}t \sim \frac{1}{t^{1/3}}\exp k\left(\frac{t}{\log t}\right)^{1/3} \qquad (5)$$

(in equation (1), $v_i = \delta_{i3} v_3$, $\partial u_i/\partial x_3 = -\frac{1}{2}(\rho/\rho)\ \delta_{i3}$, and $\rho \sim k^2/36\pi G t^{4/3}(\log t)^{2/3}$) Other expansions present similar possibilities, provided $\rho > 1/t^2$, although detailed examination is required to see if effective instability is realized at finite wavelengths, for non-uniform ρ, and so on Moreover, linear analysis breaks down as $\delta\rho \to \rho$ Second order effects may, however, be expected to assist any instability because the total density $\rho + \delta\rho$ of a condensation, and its self-gravity then decrease still less rapidly than ρ

The virtues of an expansion become apparent in the nonlinear regime First, incipient fragmentation is not menaced by nonlinear interactions, as it would be in a contraction[8] Thus Layzer has argued cogently that in a contracting system the nonlinear interaction between such fragments as might form would make them ephemeral, incipient fragmentation would degenerate into turbulence In an expansion, however, the interfragment forces are diminishing and might be expected to attenuate to impotency[9] Second, once fragments begin to condense out of the expanding milieu, these same nonlinearities within a fragment can curb subfragmentation This neatly avoids another difficulty of fragmentation during a contraction—the problem of stopping the mechanism once started if excessive fragmentation is to be avoided (see ref 1, p 360)

Fragmentation need not, of course, occur in all expansions, any more than in all contractions (and for a cosmological expansion, Layzer[1] proposed the antithesis "clustering"), but the possibility does arise in anisotropic expansions

Hypotheses (and observations) which involve expansion are not uncommon Pre-eminent among these is the universal expansion, which, however, at present seems closely isotropic In any case, these considerations do not apply to cosmological problems proper except to the extent that appropriately anisotropic Newtonian cosmology would be an adequate approximation On a smaller scale, it has recently been suggested that asymmetric gravitational collapse may rebound to an expansion which is approximately Newtonian[10] On a galactic level, many observations suggest explosive events[11] Ejections from galactic nuclei were an early hypothesis, and the associated concept that quasars are relics in delayed development from a superdense past also involves expansion[12] Furthermore, in star formation theory, whereas the familiar hypothesis (*A*) "interstellar cloud→star cluster" usually assumes a mechanism of fragmentation during contraction[13], Ambartsumian[2] long ago proposed an alternative hypothesis (*B*) "superdense body→stars (+ gas)" which calls for fragmentation during expansion In fact it is interesting that the hypothesis (A) which is perhaps more plausible, leads to a similar result if the assumption of fragmentation during contraction is not made, it is not an indispensable assumption and there is reason not to adopt it Thus to explain stellar association phenomena it was suggested[3] that contraction *en masse* may lead to (nuclear) explosion In other words, (A) is elaborated to the form (A') "interstellar cloud→massive condensation→stellar association", and detailed calculations have lent this support[4] Explosions, perhaps of a related nature, are suggested by many other phenomena[3,4,5,7,14,15]

Anisotropy of any of the hypothesized expansions—except the cosmological—is probable It can arise from appropriate initial conditions or from constraining forces For example, the massive condensation in (A') evolves to a dense, hot, rotating disk which, by an unconventional pulsation instability, is liable to explode[4] This would produce initial motion predominantly along the axis, so that the spheroid would expand from oblate to prolate form Resistance from a surrounding medium could constrain the motion essentially to one dimension, at least for a time Magnetic constraint is another possibility

For the star formation theory based on (A'), detailed analysis of a self-gravitating, rotating, spheroidal expansion (see equation (5)) shows that modes with axial wavelength greater than transverse wavelength can grow exponentially at such a rate as to produce fragmentation when the disk has expanded to a cigar shape of length some tens times its radius, provided the (total) energy is not as large as self-gravity at that time From a generalized Jeans criterion of instability, it can be deduced that the fragments would have minimum mass typically $\sim \frac{1}{100}$ to $\frac{1}{10}$ $M_{\odot}$, and so may evolve to acceptable stars Without going into detail, we may note that in other respects, however, the fragments can differ remarkably from the conventional picture of "protostars" This could, for example, explain the apparent anomaly of T Tauri stars below the main sequence (see page 64 in ref 12), although such phenomena may also be understood, for not too small masses, as a natural outcome of the evolution of rotating protostars of the conventional sort[16]

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[1] Layzer, D , *Ann Rev Astron Astrophys* 2, 341 (1964)

[2] Ambartsumian, V A , *Rev Mod Phys* , **30**, 944 (1958)

[3] Gold, T , in *La Structure et l'Evolution de L'Univers, Eleventh Solvay Congress Proc* , 276 (R Stoops, Brussels, 1958) McCrea, W H , ibid

[4] Bird, J F , *Rev Mod Phys* , **36**, 717 (1964)

[5] Burbidge, G , *Nature*, **216**, 1287 (1967)

[6] Bonnor, W B , *Mon Not Roy Astr Soc* , **117**, 104 (1957)

[7] Arp, H C , *Astrophys Lett* ,**1**, 1 (1967)

[8] Layzer D , *Mém Soc Roy Sci Liège Ser* 5, **15**, 325 (1967)

[9] Layzer, D , *Astrophys J* , **137**, 351 (1963)

[10] Israel, W , *Nature*, **216**, 148 (1967)

[11] Burbidge, G R , Burbidge, E M , and Sandage, A R , *Rev Mod Phys* , **35**, 947 (1963)

[12] Ambartsumian, V A , in *The Structure and Evolution of Galaxies, Thirteenth Solvay Congress Proc* , 1 and 172 (Interscience, New York, 1965)

[13] Spitzer, L , in *The Structure and Evolution of Galaxies, Thirteenth Solvay Congress Proc* , 54 (Interscience, New York, 1965) Reddish, V C , *Vistas*, **7**, 173 (1965)

[14] Westerlund, B E , and Mathewson, D S , *Mon Not Roy Astr Soc* , **131**, 371 (1966) Bok, B J , *Ann Rev Astron Astrophys* , **4**, 95 (1966)

[15] McCrea, W H , *Nature*, **213**, 239 (1967) Sugimoto, D , *Prog Theor Phys* , **33**, 339 (1965)

[16] Bird, J F , *Prog Theor Phys* , **35**, 615 (1966)

Discussion of the "Forbidden" Intercombination Transition of C_2 in Comets

THE only comet emissions known to arise from the C_2 radical are the Swan bands of the triplet electronic system ($A\,^3\Pi_g \rightarrow X'\,^3\Pi_u$) (ref 1) The absence of definitive evidence for singlet system emissions has led to speculation that C_2 must be formed directly in the triplet state either by a primary photochemical process or by direct sublimation from the icy nucleus[2,3] These speculations have been supported by the widely accepted view that the singlet and triplet states of C_2 cannot be exchanged by either a radiative or a collisional process Because the radiative intercombination transition for C_2, $X'\,^3\Pi_u \leftrightarrow x\,^1\Sigma_g^+$, is "forbidden", it has been assumed not to occur More specifically, Ballik and Ramsay[4], state that a lifetime of several days would not be unlikely for this transition Because the gas density in the coma is very low, collisional processes have also been assumed not to occur The exclusive observation of triplet C_2 is particularly disturbing because the $x\,^1\Sigma_g^+$ state has been shown to be the ground state and the observed triplet system originates from the $X'\,^3\Pi_u$ state which is 610 cm^{-1} above the ground state[4] This emission is in contrast to all other emissions which are observed in fluorescence from the ground state

The purpose of this note is to demonstrate that the "forbidden" radiative process is likely to occur during the travel time of the radical in the coma, and to point out some of the consequences of this process Escape velocities of radicals in a comet coma are of the order of thermal velocities (1 km s^{-1}) and the radius of the coma may exceed 3×10^5 km (ref 2) This results in a radical travel time of approximately 3 or 4 days Although collisions probably do occur in the inner coma[5], any effect they might have on the singlet–triplet interchange will be neglected and only the radiative process will be discussed here

The relationships determining the radiative lifetime of an excited state can be written simply as follows To a first approximation, the spontaneous transition probability is

$$A_{v'v''} = K\nu^3 R_e^2\,(\bar{r}_{v'v''})\,q_{v'v''} \tag{1}$$

where $A_{v'v''}$ is the Einstein coefficient, K is a constant involving units and geometry, ν is the frequency of the transition, R_e is the electronic transition moment, $(\bar{r}_{v'v''})$ is the r centroid of the transition and $q_{v'v''}$ is the Franck–Condon factor The Franck–Condon factor, $q_{v'v''}$, associated with the (v,v'') band is defined by the square of the vibrational overlap integral

$$q_{v'v''} = [\int \psi_{v'} \psi_{v''} \mathrm{d}r]^2 \tag{2}$$

The lifetime of the v' level for transition to v'' is simply the reciprocal of the Einstein coefficient

$$\tau_{v'v''} = \frac{1}{A_{v'v''}} \tag{3}$$

This is the time required for decay of all but exp(-1) of the molecules in state v' The mean lifetime of all the levels of v' for transition to all possible lower levels, v'', is

$$\tau = \Big[\sum_{v'v''} Q_{v'} A_{v'v''}\Big]^{-1} \tag{4}$$

where $Q_{v'}$ is the fractional number of molecules in the v' level Using these relationships and with the assumption that the ratio of the spontaneous transition probabilities for the transitions are proportional to the ratio of the factors $\nu^3 q_{v'v''}$ for the respective transitions, the lifetime of the intercombination transitions for C_2 can be estimated by comparison with a known lifetime for a system of similar forbiddenness The lifetime derived using this scheme depends on the validity of drawing an analogy between the electronic transition moments for different electronic transitions, and therefore is only approximate It can be used, however, to generate estimated lifetimes in lieu of experimental knowledge of the transition probabilities Potential curves for the lower electronic states of interest are readily available and will not be included here[6,7]

A transition similar to the intercombination transition of C_2 is the $C\,^3\Pi_u \leftrightarrow X\,^1\Sigma_g^+$ transition of N_2 Both transitions violate only the weak selection rule $\Delta S = 0$ and in both cases the excited triplet states have singlet states of the same symmetry nearby (C_2, $b\,^1\Pi_u$ and N_2, $b'\,^1\Sigma_u^+$), which can mix with the corresponding triplet states giving the latter some singlet character and making the transitions more probable The lifetime of the $v' = 0$ level of the $C^3\Pi_u$ state of N_2 with respect to transition to the $v'' = 0$ level of the $X\,^1\Sigma_g^+$ state is approximately $2\ 5 \times 10^{-3}$ s This lifetime has been derived from the data of Wilkinson and Mulliken[8] using the recently determined Franck–Condon factor for the transition[9] The mean lifetime of the C_2 transition will be estimated below using this value

The absence of vibrational–rotational transitions of C_2 in the coma results in a vibrational population corresponding to an approximate Boltzmann temperature of 6,000° K (ref 10) In order to determine the mean lifetime of the upper electronic state it is therefore important to consider decay from the upper vibrational levels because some of them will be appreciably populated at this temperature The levels up to and including $v = 4$ have been considered here because they comprise approximately 90 per cent of the total population of the C_2, $X'\,^3\Pi_u$ state at 6,000° K Table 1 lists the normalized Franck–Condon factors and the band origins in cm^{-1}, $\omega_{v'v''}$, for the transitions between the vibrational levels of the electronic states, calculated from the constants tabulated by Ballik and Ramsay[6] The Franck–Condon factors have been evaluated graphically using the eigenfunction for the harmonic oscillator Although the use of this wave function can lead to serious errors, the fact that the values reported here satisfy the sum rule, $\sum_{v'} q_{v'v''} = 1$, indicates that they are adequate for our purpose The C_2 radiative lifetimes for these levels $\tau_{v',v''}$, calculated by comparison with the (0,0) band of the $C^3\Pi_u - X\,^1\Sigma_g^+$ transition of N_2, are also listed in this table Using these values, the mean lifetime of the $X'\,^3\Pi_u$ state calculated from equation (4) is 3 min Because of the similar energies of the two electronic states, radiative transitions can also occur from the "lower" $x\,^1\Sigma_g^+$ state to the "upper" $X'\,^3\Pi_u$ state The mean lifetime for the $x\,^1\Sigma_g^+$ state, assuming a 6,000° K vibrational temperature, is 4 min The calculated lifetimes for the $X'\,^3\Pi_u$ and the $x\,^1\Sigma_g^+$ states of C_2 are more than three orders of magnitude smaller than the radical travel time This result therefore strongly suggests that the intercombination transition does occur in the coma

Table 1 WAVENUMBER OF THE BAND ORIGINS ($\omega_{v',v''}$, CM^{-1}), FRANCK–CONDON FACTORS ($q_{v',v''}$) AND LIFETIMES (τ, S) FOR SOME OF THE MOST PROBABLE TRANSITIONS FOR THE LOWER LEVELS OF C_2, $X'\,^3\Pi_u \leftrightarrow x\,^1\Sigma_g^+$

v''	v' 0	1	2	3	4	
0	610 0	2,228	3,823	5,394	6,942	—ω
	0 47	0 38	0 10	0 00	0 01	—q
	9,364	245	178	—	—	—τ
1	1,218	400 0	1,995	3,566	5,114	
	0 33	0 04	0 28	0 22	0 21	
	1,717	—	468	105	38	
2	3 019	1,401	193 3	1,765	3,313	
	0 11	0 20	0 01	0 07	0 25	
	355	1,907	—	2,784	112	
3	4,794	3,176	1,581	10 1	1,538	
	0 05	0 20	0 08	0 09	0 05	
	182	158	3,029	—	5,722	
4	6,541	4,924	3,329	1,758	209 9	
	0 01	0 10	0 20	0 02	0 15	
	—	83	137	936	—	

In all cases except (0,0), the life-times are for transition from the higher numbered to the lower numbered vibrational level

The consequences of this radiative transition in the coma must be considered in relation to a possible mechanism suggested by Ballik and Ramsay for the production of a triplet from the singlet C_2 radical[4] The mechanism involves optically pumping the vibrational–rotational levels of C_2, $x\,^1\Sigma_g^+$ by absorption and emission in the Phillips system, $b\,^1\Pi_u \rightarrow x\,^1\Sigma_g^+$, followed by a perturbational crossing from the $x\,^1\Sigma_g^+$ state to the $A'\,^3\Sigma_g^-$ with subsequent radiation to the $X'\,^3\Pi_u$ state The production of the $x\,^1\Sigma_g^+$ from the $X'\,^3\Pi_u$ by the reverse radiationless process is less likely because of the negligibly small concentration of $A'\,^3\Sigma_g^-$ with respect to $x\,^1\Sigma_g^+$ The possibility of this mechanism for radiationless production of the triplet state from the singlet state concurrent with the possibility of the radiational interchange of the singlet and triplet states suggested here would result in a steady state concentration of the $x\,^1\Sigma_g^+$ and the $X'\,^3\Pi_u$ states in the coma which would be independent of the state of production of C_2 The probability of observing Phillips emission near the comet nucleus depends on the state of production of C_2 but observation further out in the coma depends on the steady state concentration of the singlet and triplet which would be controlled by the rates of the competing processes If the steady state favoured the triplet concentration, the Phillips emission would be weak even if C_2 was formed entirely in the singlet state Failure to observe singlet system emission therefore cannot be used as evidence for the production of C_2 in the triplet state

Relatively few experimental observations of cometary spectra in the Phillips region have been reported Also, the measurements in this wavelength region are usually less sensitive than those in the visible because of the lower sensitivity of photoelectric and photographic techniques and because of the lower solar intensity Some reported photoelectric data suggest that the (3,0) and (4,1) bands of the Phillips system have been observed in comet Candy (1960*n*) (ref 11) The limited number of observations as well as the lower sensitivity of the measurements might account for the lack of more definitive evidence Higher resolution studies and observations at different distances from the comet nucleus would be helpful in resolving the C_2 question

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[1] Wurm, K , in *The Moon, Meteorites and Comets* (edit by Middlehurst, B M , and Kuiper, G P) (University of Chicago Press, Chicago, 1963)

[2] Swings, P , *Quart J Roy Astro Soc* , **6**, 28 (1965)

[3] Stief, L J , and DeCarlo, V J , *Nature*, **205**, 1197 (1965)

[4] Ballik, E A , and Ramsay, D A , *Astrophys J* , **137**, 61 (1963)

[5] Dolginov, A Z , and Gnedin, Y N , *Icarus*, **5**, 64 (1966) Jackson, W M , and Donn, B , *Collisional Processes in the Inner Coma* (Goddard Space Flight Center, Greenbelt, Md , 1965)

[6] Ballik, E A , and Ramsay, D A *Astrophys J* , **137**, 84 (1963)

[7] Read, S M , and Vanderslice, J T , *J Chem Phys* , **36**, 2366 (1962)

[8] Wilkinson, P G , and Mulliken, R S , *J Chem Phys* , **31**, 674 (1959)

[9] Benesch, W , Vanderslice, J T , Tilford, S G , and Wilkinson, P G , *Astrophys J* , **143**, 236 (1966)

[10] Arpigny, C , *Ann Rev Astron and Astrophys* , **3**, 351 (1965)

[11] Wyller, A A , *Observatory*, **82**, 73 (1962)

Observations of Atmospheric Absorption in the Wavelength Range 2 mm to 300 μm

WE present here the results of experiments started in 1957, to which have been added some observations made in December 1967 using a new telescope at Hochalpine Forschungssation Jungfraujoch (altitude 3,580 m)

In 1957 we used a coelostat with 12 in diameter mirrors to illuminate a lamellar grating interferometer to obtain submillimetre solar spectra but in 1967 we had the benefit of a 30 in telescope recently installed by Grubb Parsons This had an NPL modular Michelson interferometer fitted at the coudé focus, and the combination gave us approximately ten times the light grasp of the earlier equipment For the recent observations, however, the total water vapour content was not as low as in 1957 because the length of the slant path was greater by at least a factor of two

A limited account of the 1957 results for wavelengths shorter than 1 mm was published shortly after the observations were made[1] and since then we have accounted for the chief features which were not explained in 1957 by assigning them to lines in the magnetic dipole pure rotation spectrum of molecular oxygen[2] The new observations completely support the 1957 results in this wavelength region with the oxygen features showing even stronger (Fig 1), in accordance with the increased air mass

The principal interest of the new observations, however, is at wavelengths longer than 1 mm (Fig 2) Between 6 cm^{-1} and 12 cm^{-1} there are two strong ground state rotational lines of H_2O^{16} at 6 12 cm^{-1} and 10 85 cm^{-1} but in the spectra recorded in 1957 there were in addition well marked features in the region of 7–9 cm^{-1} These show up even more clearly in the 1967 spectra

The strength of this feature seems to be correlated with the total amount of water vapour in the absorbing path and so we tentatively assign it to absorption by a complex molecule containing H_2O^{16}, of which the most likely is the dimer $(H_2O^{16})_2$ We have now started laboratory observations of the absorption of water vapour in the region below 10 cm^{-1} using long absorption paths First results give some support to the solar observations, and we intend to investigate the temperature and pressure dependence of these features

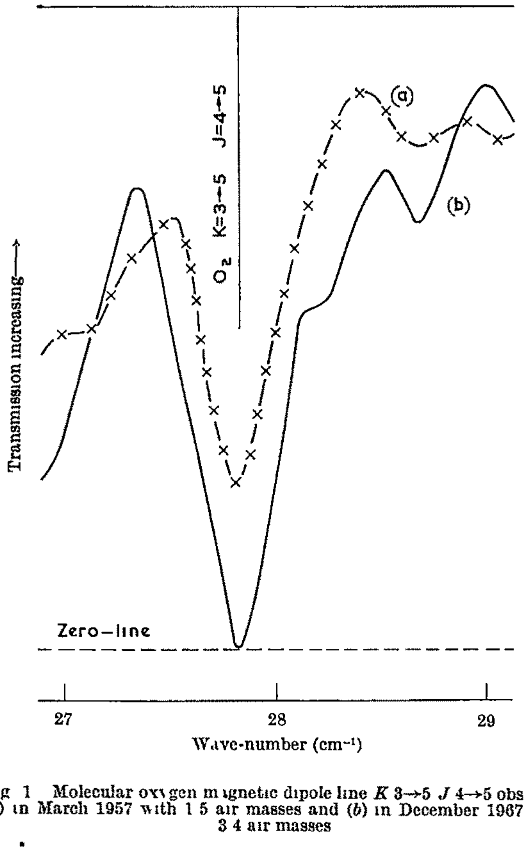

Fig 1 Molecular oxygen magnetic dipole line K 3→5 J 4→5 observed (*a*) in March 1957 with 1 5 air masses and (*b*) in December 1967 with 3 4 air masses

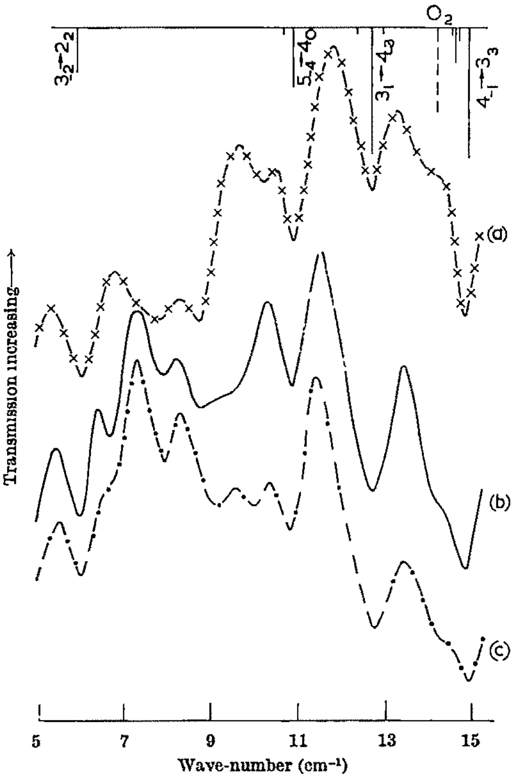

Fig 2 Observed spectrum at 0 5 cm^{-1} resolution of the Sun through the atmosphere (*a*) in March 1957 with 1 5 air masses and 2 mm precipitable water, air temperature +2° C, (*b*) in December 1967 with 3 4 air masses and 3 mm precipitable water, air temperature −26° C, (*c*) with 3 6 air masses and 5 mm precipitable water, air temperature −24° C

The existence of water dimers in atmospheric conditions was predicted by Viktorova and Zhevakin[3], who invoked their presence to explain why higher absorption is observed between the monomer water lines than the strength and shape of the latter would imply These authors also made a numerical prediction, based on a spectroscopic model of the dimer, that there would be an isolated feature at 7 07 cm^{-1} This might at first sight seem gratifying but the numerical values for their molecular constants were derived from the assignment to dimeric water of absorption, at 49 5 cm^{-1}—a feature which was reported by Yaroslavsky and Stanevich[4] We cannot accept that the existence of this line is established, for our own laboratory observations, as well as some recent aircraft observations, show no sign of it at anything like the strength reported in ref 4 We believe that the 49 5 cm^{-1} feature may be an artefact arising from deficiencies of far infrared grating spectrometers The determination of values of molecular constants for dimeric water must therefore await further observation, and assignment

We thank Professor Von Muralt for making available to us the new telescope facility at Jungfraujoch, and Professor M Migeotte, Dr G Roland and Dr L Delbouille for their help

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[1] Gebbie H A , *Phys Rev* 97 1174 (1957)
[2] Gebbie, H A , Burroughs, W J , Robb, J A , and Bird, G R *Nature* 212 66 (1966)
[3] Viktorova, A A , and Zhevakin, S A , *Doklady*, 11, 1065 (1967)
[4] Yaroslavsky, N G , and Stanevich A E , *Opt and Spec* , 7, 380 (1959)

PLANETARY SCIENCE

Measurement of d*T*/dΔ

This communication describes a method of estimating directly the derivative of the travel time curve of seismic body waves, d*T*/dΔ, as a function of epicentral distance Δ, by combining data from several seismometer arrays To test the method the d*T*/dΔ curve for P waves for the range 67°–82° has been estimated The results provide further evidence for rather rapid changes in the velocity gradient in the Earth's deep mantle

The equation of condition for determining the azimuth α, and phase velocity V, of a plane wave crossing an array of n seismometers is

$$C - \frac{R_i \cos(\theta_i - \alpha)}{V} = t_i \qquad (1)$$

where (R_i, θ_i) are the polar co-ordinates of seismometer i (relative to some arbitrary origin), t_i is the arrival time at seismometer i relative to some arbitrary baseline and C is a constant The arrival time t_i will usually be in error, V, α and C can be estimated in the presence of this error by least squares provided n is greater than three The derivative of the travel time curve is then D/V, where D is the number of km per degree of Δ By choosing events at different distances from the array a d*T*/dΔ curve can be constructed Chinnery and Toksoz[1] and Toksoz *et al* [2] have presented d*T*/dΔ curves derived in this way using the Large Aperture Seismic Array, Montana, USA

This method of measuring d*T*/dΔ has the disadvantage that each event must be treated separately so that at each distance there is a scatter of points through which some mean curve must be drawn The method also takes no account of possible effects of structure beneath the array although Chinnery and Toksoz[1] have attempted to minimize this by confining their study to events that lie within a restricted range of azimuth from the array If, however, d*T*/dΔ is determined for a number of events simultaneously the effects of the array structure can be estimated and removed—at least in part—and if the constraint that d*T*/dΔ is constant for all events that fall in the same interval of epicentral distance is applied, the best (least squares) estimate of d*T*/dΔ in each distance interval can be directly obtained The confidence limits on the estimates are also readily obtained

Equation (1) is now rewritten for event j that falls in the kth distance range as follows

$$S_i + C_j - \frac{R_i \cos(\theta_i - \alpha_j)}{V_k} = t_{ij} \qquad (2)$$

where S_i is a correction to the arrival time at seismometer i, and results from the structure at the array site In equation (2), S_i and C_j are linearly dependent so the further assumption that $\Sigma_i S_i = 0$ has to be made By choosing events from a wide variety of azimuths it should be possible to estimate all S_i, C_j, V_k and α_j Because α_j will, however, usually be known fairly accurately from the position of the event epicentre relative to the array site, α_j can be restrained to this known value

Unfortunately, this method is difficult to apply because events are usually poorly distributed in azimuth and distance around any one array To overcome this, data from all four UKAEA designed arrays has been combined The equation of condition now becomes for array h

$$S_{hi} + C_{hj} - \frac{R_{hi} \cos(\theta_{hi} - \alpha_{hj})}{V_k} = t_{hij} \qquad (3)$$

and $\Sigma_i S_{hi} = 0$ for all h

Methods of estimating d*T*/dΔ based on equations (2) and (3) have a further advantage By including the equation of condition

$$\frac{D_1}{V_1} + \frac{D_2}{V_2} \cdot\cdot + \frac{D_k}{V_k} \qquad \frac{D_m}{V_m} = T_m$$

where D_k is the interval in km over which the phase velocity is V_k, the area under the d*T*/dΔ curve can be constrained, if necessary, to what it should be, that is, to T_m, the travel time across the range D_1 to D_m For the results described later, however, application of this constraint has not been necessary

Table 1 d*T*/d*Δ* RESULTS AND 95 PER CENT CONFIDENCE LIMITS FOR THE RANGE 67°–82°

Distance (degrees)	d*T*/d*Δ* (with 95 per cent confidence limits)
67	6 57±0 10
68	6 29±0 12
69	6 07±0 11
70	6 06±0 17
71	6 05±0 11
72	5 97±0 17
73	5 85±0 09
74	5 86±0 27
75	5 68±0 09
76	5 67±0 22
77	5 61±0 10
78	5 38±0 12
79	5 27±0 13
80	5 17±0 10
81	5 00±0 14
82	

Using data from the arrays at Eskdalemuir (Scotland), Yellowknife (Canada), Gauribidanur (India) and Warramunga (Australia), the d*T*/dΔ curve for P waves for the ranges 67°–82° has been estimated by least squares using equation (3) Eventually this curve will be extended to cover the range 30°–105° but work has been concentrated in the range 67°–82° because of the increasing evidence of rapid changes in the velocity gradient in the deep mantle as revealed by observations in the range 70°–80° Thus Carpenter *et al* [3] using explosion amplitude data show a sharp perturbation in the P wave amplitude–distance curve at around 75° suggesting a possible low velocity layer, and Chinnery and Toksoz[1] and Toksoz *et al* [2] from d*T*/dΔ measurements in the vicinity of 75° conclude that at depths of around 2,000 km the P wave velocity increases much more slowly with depth than normal 67°–82° is therefore an interesting range in which to test the method of measuring d*T*/dΔ described here

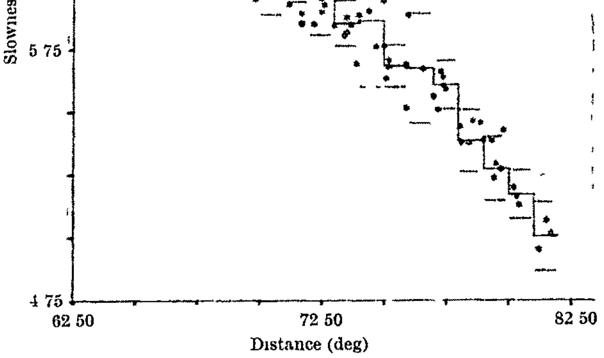

Fig 1 Estimated d*T*/d*Δ* curve for the range 67°–82°
, 95 per cent confidence limits
• d*T*/d*Δ* estimates for individual events using corrected arrival times

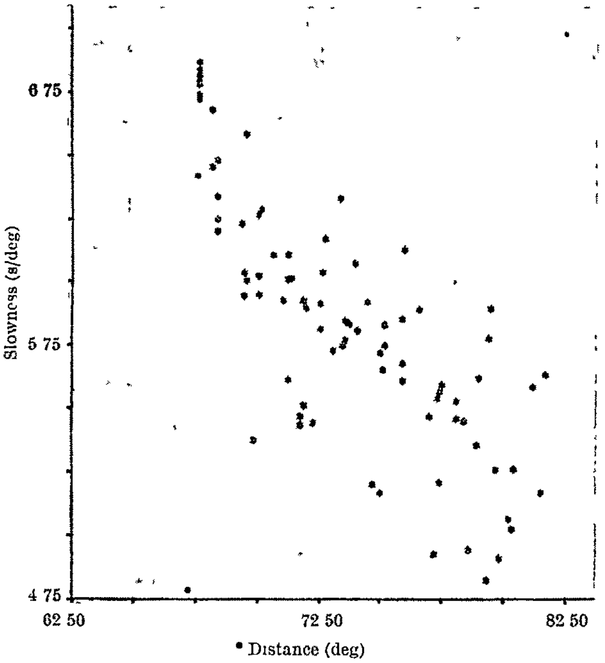

Fig 2 $dT/d\Delta$ estimates for each individual event using uncorrected arrival times

Table 1 and Fig 1 show the $dT/d\Delta$ results and 95 per cent confidence limits for the range 67°–82°, using a total of eighty-four shallow events The $dT/d\Delta$ values estimated for each event individually using the corrected arrival time $t_{hij} - S_{hi} - C_{hj}$ are also shown The arrival times, t_{hij}, were measured by a method proposed by Cleary in a private communication one of the seismometer outputs is taken as standard, the first few seconds of the P phase are traced and the traced curve is matched by eye to each of the other outputs in turn The displacement of the traced curve relative to the standard output is then the arrival time

Fig 2 also shows the $dT/d\Delta$ estimates for each event individually but without applying any corrections for array structure, that is, using equation (1) From a comparison of Figs 1 and 2 it is clear that combining data so that the effects of array structure can be estimated and removed greatly reduces the scatter of the data

The $dT/d\Delta$ curve shown in Fig 1 shows the same inflexion as suggested by Toksoz and Chinnery and is further evidence for anomalous velocity gradients in the deep mantle Detailed comparison of the $dT/d\Delta$ curve and the amplitude–distance curve of Carpenter *et al* [3] has not yet been made but the perturbation in the amplitude–distance curve is consistent qualitatively at least with an inflexion of the $dT/d\Delta$ curve Comparison of travel times and the area under the $dT/d\Delta$ curve shows good agreement The Jeffreys–Bullen time, for example, is 86 40 s and the integral of the $dT/d\Delta$ curve is 86 51 s, a difference of less than 0 2 s This is without any travel time constraints being placed on the $dT/d\Delta$ data These results of the test of the method are thus very encouraging

Inversion of the $dT/d\Delta$ curve to produce a velocity depth structure for the Earth is the ultimate aim of this work The chief purpose of this communication is to describe a method of measuring $dT/d\Delta$ which combines data from a number of arrays and to demonstrate some of the advantages of this method over those that treat each event individually

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[1] Chinnery, M A , and Toksoz, M N , *Bull Seis Soc Amer* , **54**, 2271 (1966)
[2] Toksoz, M N , Chinnery, M A , and Anderson, D L , *Geophys J R Astr Soc* , **10**, 127 (1967)
[3] Carpenter, E W , Marshall, P D , and Douglas, A , *Geophys J Roy Astro Soc* , **13**, 61 (1967)

Two-component Electromagnetic Ship's Log

NAVIGATION on the deep ocean, away from accurate shore-based radio aids, is an important problem for the oceanographer The use of the US satellite navigation system allows accurate position fixes to be obtained at intervals depending on how many satellites are operational, but there remains a need for a system to interpolate between these, particularly when complex manoeuvres are being carried out To fill this need this institute is developing an improved dead-reckoning system, in which the ship's track is calculated from its velocity through the water based on measurement of fore-and-aft speed, sideways speed, or "leeway", and ship's heading An electromagnetic log is available commercially which measures the fore-and-aft speed to an accuracy of about 0 1 knot, but to obtain leeway as well we have developed a new type of electromagnetic log The prototype has been tried successfully at sea, and though full calibration has not yet been possible, there is every indication that an accuracy of 0 1 knot will be obtainable The chief problem in calibration is that account has to be taken of the varying pattern of flow around the hull of the ship for different combinations of fore-and-aft speed and leeway

Apart from its application to finding the ship's position, the new log has been found useful when handling equipment over the side of the ship at low speeds

The principle of operation of the log is shown in Fig 1 A flat coil with a vertical axis and in a streamlined housing is fixed to a spar projecting through the hull of the ship

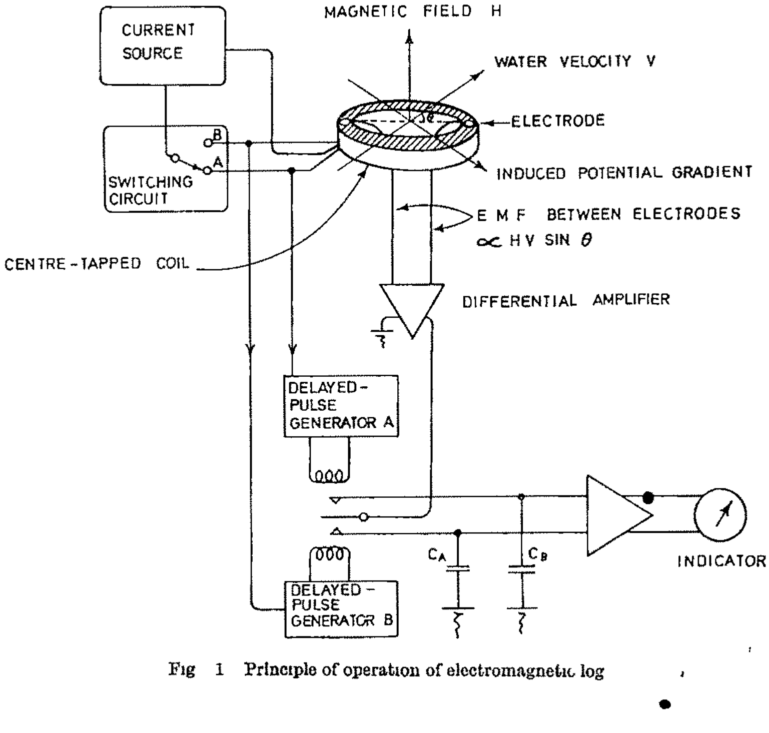

Fig 1 Principle of operation of electromagnetic log

This coil produces a vertical magnetic field, and the water flowing through this field generates a potential gradient perpendicular to both the field and to the direction of flow. Two pairs of electrodes (for simplicity, only one pair is shown in Fig. 1) pick up e.m.fs proportional to the fore-and-aft and athwartships components of the potential gradient, and these e.m.fs are therefore proportional to the athwartships and fore-and-aft components, respectively, of the water velocity past the measuring head.

With a direct current through the coil, the e.m.f. between the electrodes consists of a component caused by the water flowing through the magnetic field, together with components from other sources such as electrochemical effects between the electrodes. If the magnetic field is reversed, the former component is also reversed but the others are unaffected. The electronic circuit therefore measures the change of e.m.f. between the electrodes when the magnetic field is reversed. To reverse the magnetic field it is convenient to use a centre-tapped field coil and to switch the current from one half of the coil to the other.

The reversal of the magnetic field induces transient voltages in the electrode circuit, and the sampling of the e.m.f. between the electrodes is therefore delayed until these have died away. Referring to Fig. 1, and considering the sequence starting with the current switched to coil A, an interval is allowed for the transients to die away and the delayed-pulse generator A then connects the amplifier output to capacitor C_A, which charges to a voltage proportional to the e.m.f. between the electrodes. The amplifier output is then disconnected and the coil current switched to coil B. After the correct interval, the delayed-pulse generator B connects the amplifier output to capacitor C_B. Both capacitors remain at constant potential when disconnected from the amplifier, so that the difference in their potentials is proportional to the component in the electrode voltage caused by the water flow. This difference in potential is amplified and can, for example, be displayed on a meter calibrated in terms of water velocity. The switching is carried out at a rate of about 8 Hz.

Previous electromagnetic flowmeters have used sinusoidal a.c. for energizing the coil, but then it is difficult to prevent spurious e.m.fs from being induced electromagnetically and electrostatically into the electrode circuit, causing a zero offset which has to be balanced out and which can be a source of zero drift. In our device the shape of the measuring head has to be such that the wanted e.m.fs produced (approximately 75 μV knot^{-1}) compared with the voltage (10 V) and current (1 A) in the energizing coil are considerably smaller than in single component logs, so that the directly induced e.m.fs would have presented a serious problem if, say, 50 Hz a.c. had been used. Our measuring system overcomes this effect, and if the zeros of the amplifiers are set with the coil de-energized, the shift in zero when the power is switched on to the coil is too small to detect.

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PHYSICS

Surface Energy and Evaporation Rate of Spherical Particles of Radii less than 500 Å

THE vapour pressure of both liquid and solid particles is expected, on thermodynamic grounds, to differ from that of the bulk material. Investigations of the increased vapour pressure for spherical particles have been mainly limited to liquid droplets of a radii greater than 500 Å (see, for example, refs. 1 and 2). The only investigation of solids involving very small particles seems to be that by Pruz[3] (particle diameters 500 Å), who used a radioactive weighing technique to determine the mass loss at 840° K. He obtained a value for the mean surface energy of silver of 6,000 ergs cm^{-2} which is about six times the value found by Udin[4] from the creep of silver at high temperatures.

Simple kinetic theory shows that the vapour pressure of a spherical particle is related to the rate of change of drop radius r with time t by the following equation

$$\left(\frac{\mathrm{d}r}{\mathrm{d}t}\right)_r = \mathrm{const}\, p_r/T^{\frac{1}{2}} \tag{1}$$

where T is the temperature of the drop and p_r the vapour pressure of a drop of radius r. This relation is substituted into the Kelvin equation

$$p_r = p_\infty \exp\left[\frac{2\sigma M}{\rho R T r}\right] \tag{2}$$

where ρ is the density, M the molecular weight, R the gas constant per mole, and T the absolute temperature. For liquids, σ will be the surface tension; for solids with a spherical surface σ will be the surface energy per unit area. It then follows from (1) and (2) that

$$\left(\frac{\mathrm{d}r}{\mathrm{d}t}\right)_r = \left(\frac{\mathrm{d}r}{\mathrm{d}t}\right)_\infty \exp\left[\frac{2\sigma M}{\rho R T r}\right] \tag{3}$$

This equation can be integrated to give the following relation between the radius and the time

$$\Delta t = \frac{2\sigma M}{\rho R T \left(\frac{\mathrm{d}r}{\mathrm{d}t}\right)_\infty}\left[\Delta E_0\left(\frac{2\sigma M}{\rho R T r}\right) - \Delta E_1\left(\frac{2\sigma M}{\rho R T r}\right)\right] \tag{4}$$

where

$$E_0(x) = \left[\frac{\exp(-\mu)}{\mu}\right]_x^\infty$$

and

$$E_1(x) = \int_x^\infty \frac{\exp(-\mu)\,\mathrm{d}\mu}{\mu}$$

Experiments have shown that the variation of drop radius with time is consistent with equation (2), and this variation can therefore be used to determine surface energies. The substances examined were liquid lead, liquid bismuth and solid silver.

The specimens were produced *in situ* in an electron microscope by depositing material on to a carbon substrate maintained at a temperature which gave a convenient evaporation rate. Selected particles were then photographed at regular intervals until they vanished. In order to use the theory it was necessary to ensure that the particles were spherical. Preliminary experiments showed that in suitable experimental conditions there was no difficulty in obtaining spherical liquid drops. In the case of silver spherical particles were produced at a temperature in the region above 1,040° K in agreement with the work of Sundquist[5,6]. Electron diffraction patterns taken *in situ* confirmed that these spherical particles consisted of crystalline silver.

Results for typical experiments are shown in Figs. 1*a* and *b*. In both cases the continuous curve is that obtained from equation (4). The value of σ for lead is that for massive liquid at this temperature. For bismuth, very similar curves were obtained at $T = 677°$ K with $\sigma = 374$ dynes cm^{-1} (which is the value for the massive liquid).

For silver, good agreement is obtained with theory with $\sigma = 1{,}130$ ergs cm^{-2} at 1,045° K. This value is the

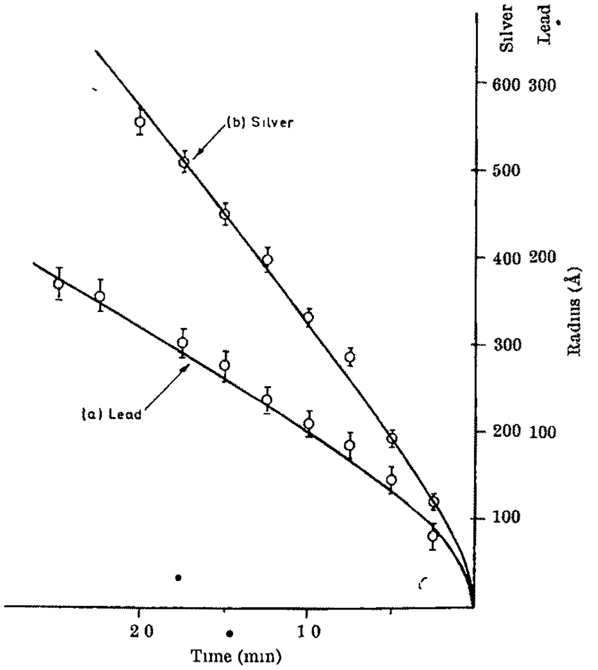

Fig 1 *a*, The evaporation of lead droplets as a function of time, $\sigma = 434$ dynes cm^{-1}, $T = 726°$ K *b*, The evaporation of spherical silver particles as a function of time, $\sigma = 1,130$ ergs cm^{-2}, $T = 1,045°$ K The origin of the time axis is chosen so as to coincide with the time of disappearance of the particles

same as that found by Udin[4] at roughly the same partial pressure of oxygen, but at a higher temperature, 1,200° K While the variation in σ caused by this temperature difference is not expected to be large, it should be noted that grain boundary effects are significant in the experiments of Udin, whereas the work presented here refers to single crystals possessing a spherical surface

An examination of the errors associated with the experiment show that the values quoted above are subject to an error of about ± 10 per cent Further experiments are being carried out with the aim of reducing the error and also to study particles of smaller diameter

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[1] Gudris, N , and Kulikova, L , *Z Physik*, **24**, 121 (1924)
[2] LaMer, V K , and Gruen, R , *Trans Faraday Soc* , **48**, 410 (1952)
[3] Piuz, F , *Helv Phys Acta*, **37**, 620 (1964)
[4] Udin, H , *Metal Interfaces*, 114 (Amer Soc for Metals 1952)
[5] Sundquist, B E , *Acta Met* ,**12**, 67 (1964)
[6] Sundquist, B E , *Acta Met* **12**, 585 (1964)
[7] Tolman, R C , *J Chem Phys* ,**17**, 333 (1949)
[8] Buff, F P , *J Chem Phys* ,**23**, 419 (1955)

Nucleation of Freezing in Supercooled Water by Cavitation

Nucleation of freezing in supercooled liquids by cavitation has been ascribed to a momentary large increase in the degree of supercooling, induced by the high transient pressures generated by a collapsing cavity This is a plausible mechanism for substances which contract on freezing, for substances such as water, which expand on freezing, it seems less satisfactory[1] Hickling[2], however, has proposed that the pressures associated with cavitation in water are sufficient to stabilize one of the high-pressure modifications of ice Nuclei of these high-pressure forms presumably transform to ordinary ice as the pressure decays, and nucleate freezing of the bulk liquid occurs

Hickling assumed that compression during cavitation is a reversible adiabatic process Hunt and Jackson[3] have questioned this assumption on the grounds that the process should be irreversible, more nearly resembling a shock wave compression They have calculated an incremental temperature increase caused by this irreversibility, and conclude that the liquid phase is stable in all states accessible by a shock compression of water from an initial state at 0° C and 1 atm , thereby implying the impossibility of Hickling's proposed mechanism for nucleation

Hickling[4] has defended his assumption of reversibility The purpose of this communication is to show, however, that this assumption is not critical, and that Hunt and Jackson's objection is probably not valid Their calculation of the temperature rise in water during shock wave compression does not agree with the results of other studies of this problem, and there is indeed experimental evidence for a phase transition in water as a result of shock wave compression

There is still some uncertainty concerning the relationships between pressure, volume, and temperature for water at high pressures Several calculations of the temperature rise along the Hugoniot curve (the locus of all states accessible by shock wave compression from a given initial state) give somewhat different results For example, Kirkwood and Montroll[5,6] in an early theoretical study predicted that the Hugoniot curve for water centred at an initial state at 20° C and 1 atm would cross the equilibrium curve between liquid water and "Ice VII" at about 25 kbar The solid phase was predicted to be stable at all higher pressures Their calculation was based on data from static high-pressure investigations of the water–ice system A more recent study by Rice and Walsh[7] agrees with Kirkwood and Montroll's work at lower pressures, but predicts somewhat higher temperatures at higher pressures The primary data in Rice and Walsh's calculations were

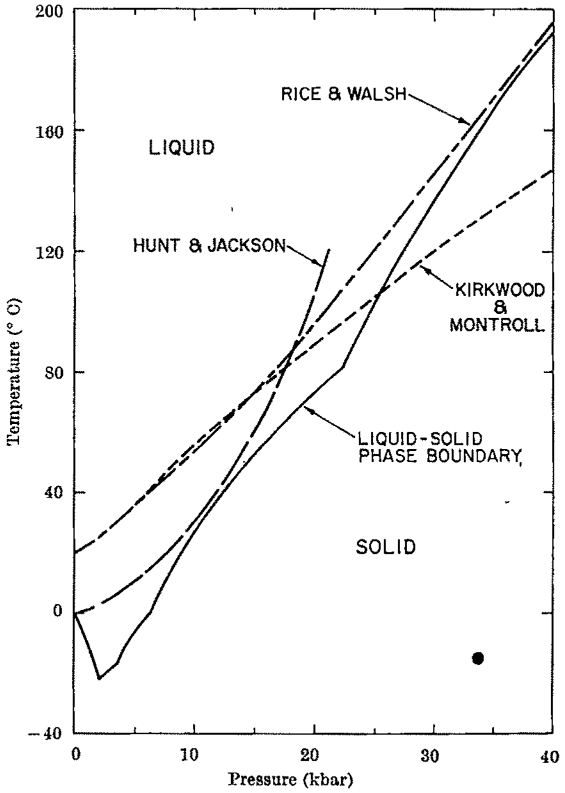

Fig 1 Hugoniot curves of Rice and Walsh, Kirkwood and Montroll, and Hunt and Jackson, superimposed on a plot of the solid–liquid phase equilibrium curve for water

P-V data from shock wave compression experiments, temperatures were derived by making simplifying assumptions concerning the specific heat of water at high pressures In view of these differences, the Hugoniot curves of Rice and Walsh and of Kirkwood and Montroll are superimposed on a P-T plot of the liquid–solid equilibrium line of water in Fig 1, along with Hunt and Jackson's results It is obvious that a displacement of either Kirkwood and Montroll's or Rice and Walsh's Hugoniot curves to an initial temperature of 0° C or below would predict stability of the solid phase from 20–25 kbar to much higher pressures Hunt and Jackson's curve deviates strongly from the others as pressure increases In the absence of more detailed information concerning their calculation, it is difficult to ascertain the reasons for this lack of agreement

Experimentally, Walsh and Rice[8] found no evidence for a phase transition during shock compression of water to pressures up to 100 kbar Al'tshuler, Bakanova, and Trunin[9], however, have reported both optical and hydrodynamic evidence for a phase transition at 115 kbar Unfortunately, they did not specify their initial temperature. Because the positions of both the liquid–solid equilibrium curve and the Hugoniot curve are not known at these pressures, one cannot estimate the degree of transient supercooling in their experiments Nevertheless, it is clear that the Hugoniot curve does cross the phase equilibrium line, and that transient high pressures can induce nucleation

Hickling and Plesset[10], using a numerical model, have estimated the peak pressures to be expected from cavity collapse in water Their values range from several tens to several hundred kbar, depending on the amount and thermal behaviour of the gas in the cavity The inclusion of real gas effects at high temperatures usually gave rise to the higher peak pressures It therefore seems that Hickling's proposed mechanism for nucleation of freezing in supercooled water by cavitation cannot be dismissed on the grounds cited by Hunt and Jackson

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[1] Chalmers, B , *Principles of Solidification* 86, (John Wiley and Sons, New York, 1964)

[2] Hickling, R , *Nature*, **206**, 915 (1965)

[3] Hunt, J D , and Jackson, K A , *Nature*, **211**, 1080 (1966), *J Appl Phys* , **37**, 254 (1966)

[4] Hickling, R , *Nature*, **214**, 379 (1967)

[5] Kirkwood, J G , and Montroll, E , *OSRD Report* 676 (1942)

[6] Cole, R , *Underwater Explosions*, 38 (Princeton Univ Press, New Jersey, 1948)

[7] Rice, M H and Walsh, J M , *J Chem Phys* , **26**, 824 (1957)

[8] Walsh, J M , and Rice, M H , *J Chem Phys* , **26**, 815 (1957)

[9] Al'tshuler, L V , Bakanova, A A , and Trunin, R F , *Dokl Akad Nauk SSSR*, **121**, 67 (1958)

[10] Hickling, R , and Plesset, M S , *Physics of Fluids*, **7**, 7 (1964)

New Mounting for Concave Grating Spectrometers

A METHOD has been devised for designing an X-ray spectrometer which requires no movement of the grating or the electronic counter Spectrometers widely used in the past have contained a fixed light source and a movable counter The development of sensitive electronic counters with delicate and complex cooling systems makes movement of the counter undesirable Our mounting makes it possible to use a fixed counter, and spectra can be obtained using a multiple-channel-scalar or displayed on a Braun tube directly

In Fig 1, X-rays radiating from a point source $F(\rho,\varphi)$ are diffracted by a grating G of radius R and focused at a point $S(r,\theta)$ We have $d(\cos\varphi-\cos\theta)=n\lambda$, where d, λ and n are the grating constant, the wavelength and the spectral order, respectively

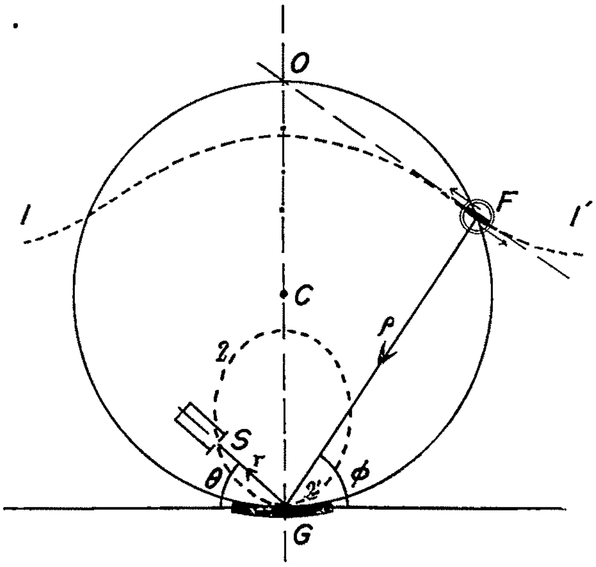

Fig 1 Principle of the Sawada mounting for a concave grating spectrometer *C*, Centre of Sawada circle, *G*, grating, *F*, X-ray tube provided with a cathode-ray deflector, *S*, slit of an electronic counter

We can determine the direction of the diffracted beams by the grating equation, and the magnitudes of the vectors[1–3] of the incident and diffracted beams can be determined by the following two conjugate equations

$$\frac{\sin^2\varphi}{\rho}-\frac{\sin\varphi}{R}=-\frac{1}{D}$$

$$\frac{\sin^2\theta}{r}-\frac{\sin\theta}{R}=\frac{1}{D}$$

where D is a parameter When D is infinite, the two equations reduce to a single self-conjugate circle—the Rowland circle If D has any other value, the (r,θ) curve becomes an oval, and its conjugate (ρ,φ) curve becomes W-like or omega-like, having two asymptotes and two inflexion points The shortest distance from the mid-point of the grating G to a point on the (ρ,φ) curve lies in the direction given by $\sin\varphi=2R/D$, where $D>2R$ It also lies on the circumference of a circle $\rho=2R\sin\varphi$, the centre of which is at C, and which has a radius that of the grating itself We will tentatively refer to this circle as the Sawada circle If we put an X-ray source $F(\rho_0,\varphi_0)$ on the Sawada circle and scan this source back and forth along a straight line which is a tangent at F to the curve (1–1′), described by

$$\rho=R\sin^2\varphi/[\sin\varphi-(\tfrac{1}{2})\sin\varphi_0]$$

so that the scanning direction lies on the straight line perpendicular to the vector GF, then the diffracted spectra are always at the slit $S(r_0,\theta_0)$ of the counter, the co-ordinates of which are

$$r_0=R\sin^2\theta_0/[\sin\theta_0+(\tfrac{1}{2})\sin\varphi_0]$$

This point lies on the curve (2–2′) in Fig 1

A comparison of the astigmatic and spherical aberrations[4,5] between the Sawada and the Rowland cases gives

$$^{S}E_{ast}-{}^{R}E_{ast}=\frac{l^2}{4R}\;\frac{\sin^2\varphi-\sin^2\theta}{\sin^2\theta\sin\varphi}$$

and

$$^{S}E_{spher}-{}^{R}E_{spher}=\frac{(w^2+l^2)^2}{8R^2}\;\frac{\sin^2\varphi-\sin^2\theta}{\sin^2\theta\sin\varphi}$$

where w and l are the width and length, respectively, of the rulings measured from the centre of the grating These expressions show that in the positive spectral orders the astigmatic and spherical abberations are small for the Sawada mounting compared with the Rowland mounting Another merit of the Sawada mounting is that the total optical path lengths are practically constant compared with the Rowland mounting, so that cumbersome intensity corrections can be avoided

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[1] Baily, W , *Phil Mag* ,15, 183 (1883)

[2] Kayser, H , *Handbuch der Spektroscopie*, 1, 456, 468 (S Hirzel, 1900)

[3] Baly, C C , *Spectroscopy*, 1, 161 (Longmans, Green and Co , New York, 1926)

[4] Beutler, H G , *J Opt Soc Amer* , 35, 311 (1949)

[5] Namioka, T , *J Opt Soc Amer* , 49, 446 (1959)

Measurement of Normal Stress Differences in Solutions of Macromolecules

THE measurement of normal stress differences generated in the steady laminar shearing motion of solutions of macromolecules has attracted a great deal of attention[1] The experimental work has been performed almost exclusively at low shear rates using three well known flow geometries the concentric cylinder, parallel plate and the cone and plate geometry The use of these three curvilinear geometries has been described in detail elsewhere and the current position is that determination of the three normal stress differences (usually labelled $p11-p22$, $p22-p33$, $p33-p11$) involves the use of more than one shearing geometry[1]

Recently I introduced the ring and plate geometry[2], which readily enables the medium range of shear rates to be investigated An outline of the arrangement, modified to include the central capillary tube to allow normal stress measurements to be obtained, is shown in Fig 1

In the original work the ring width (r_2-r_1) was adjusted so that with a mean ring radius of about 1 in the variation of shear rate over the ring surface was 10 per cent With a gap between ring and plate of 0 001 in mean shear rates of 40,000 s^{-1} were attained without much difficulty using the geometry in the Weissenberg rheogoniometer This shear rate is well within the range of industrial significance

The introduction of a capillary tube in the upper platen as shown in Fig 1 allows the pressure of the fluid within the region $0 \leqslant r \leqslant r_1$ to be sensed The corresponding normal force between the ring and plate over this same interval is

$$F_1 = \rho g \pi r_1^2 \Delta h \qquad (1)$$

where ρ is the density of the fluid in the capillary tube and Δh is the increment in the meniscus height from the undisturbed state at zero shear rate There will also be a contribution caused by centrifugal effects but we assume for the moment that these are small enough to be neglected, although the effect can easily be incorporated

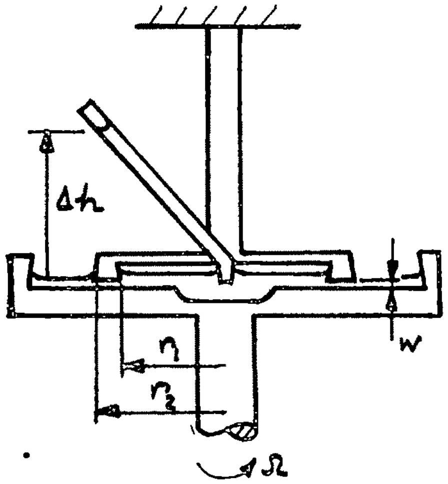

Fig 1 The ring and plate geometry.

If F is the total normal force between the ring and plate, then the force on the ring alone is

$$F_2 = F - F_1 \qquad (2)$$

and this is experimentally determinable (For example, by using the Weissenberg rheogoniometer)

At $r=r_1$ the radial stress in the material in the sheared annulus is

$$-(\widehat{rr})_1 = \rho g \Delta h \qquad (3)$$

Neglecting centrifugal effects again, the radial equation of equilibrium within the annulus is

$$\frac{\partial \widehat{rr}}{\partial r} + \frac{\widehat{rr} - \widehat{\theta\theta}}{r} = 0 \qquad (4)$$

where $\widehat{\theta\theta}$ is the normal stress in the circumferential direction at the radius considered

The shear rate within the annulus is given by

$$\gamma = \frac{\Omega r}{w} \qquad (5)$$

where Ω is the (steady) relative rate of rotation of the plates

Equation (4) can be integrated

$$-(\widehat{rr})_1 = \int_{r_1}^{r_2} \left(\frac{\widehat{\theta\theta} - \widehat{rr}}{r} \right) \mathrm{d}r \qquad (6)$$

The radial stress at $r=r_2$ is (nearly) the ambient pressure, here taken as datum

Differentiating equation (6) with respect to the lower limit yields

$$\frac{\partial (\widehat{rr})_1}{\partial \ln(r_1)} = (\widehat{\theta\theta} - \widehat{rr})_1 \qquad (7)$$

where the suffix 1 denotes quantities evaluated at $r=r_1$ Equation (7) represents a normal stress difference—it may be written as

$$(\widehat{\theta\theta} - \widehat{rr})_1 = -\rho g \frac{\partial(\Delta h)}{\partial \ln(r_1)} \qquad (8)$$

The force on the ring alone is

$$-F_2 = \pi \int_{r_1}^{r_2} \widehat{zz}\, \mathrm{d}\,(r^2) \qquad (9)$$

Differentiating equation (9) with respect to the lower limit

$$(\widehat{zz})_1 = \frac{1}{\pi} \frac{\partial F_2}{\partial\, r_1^2} \qquad (10)$$

Hence, combining equations (1), (2), (3) and (10)

$$(\widehat{rr})_1 - (\widehat{zz})_1 = -\rho g \Delta h - \frac{1}{\pi}\left(\frac{\partial F}{\partial {r_1}^2} - \frac{1}{2}\rho g \pi r_1 \frac{\partial(\Delta h)}{\partial r_1}\right) \quad (11)$$

Experiments are required with rings of constant outer radius r_2 and various inner radii r_1.

Equations (8) and (11) are the equations required. A third normal stress difference can, of course, be derived from these two. It seems that this curvilinear flow geometry is unique at the moment because it is capable of yielding all three normal stress differences. Furthermore, by using sufficiently narrow gaps between ring and plate it should be possible to extend greatly the shear rate range beyond those currently attainable with curvilinear flows. The subject of self-alignment of the platens will be dealt with elsewhere.

In experimental arrangements with narrow menisci at the platen perimeters there is the possibility of anisotropic surface tensions developing. It is already known that polymer surface films can exhibit elasticity and it is reasonable to suppose, in accordance with bulk behaviour, that normal surface tensions may develop in simple surface shearing. Accordingly, results which take no account of this possibility should be viewed with some reserve at the moment. In the experimental arrangement in Fig. 1 surface velocity gradients will undoubtedly be very small in comparison with the gradients between the ring and plate and hence surface effects will be minimized.

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[1] Lodge, A. S., *Elastic Liquids* (Academic Press, 1964).

[2] Bogie, K., and Harris, J., *Rheol. Acta* (in the press).

Dimensional Problem of the Power Law in Rheology

I HAVE discussed points raised in my recent communication[1] with Scott Blair and we think that it may not have been made quite clear that the treatment applied only to certain conservative systems with flow properties which vary with time. It has been applied to two such systems, starch solution[2] and rennetted milk[3]. It is not, of course, possible to isolate three independent parameters from the monotonic flow curve obtained with many systems.

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[1] Prentice, J. H., *Nature*, **217**, 157 (1968).

[2] Nedonchelle, Y., and Schutz, R. A., *C.R. Acad. Sci.*, **265**, 16 (1967).

[3] Scott Blair, G. W., and Tuszynski, W., *Nature*, **216**, 367 (1967).

THE SOLID STATE

Grain Boundary Diffusion Effects in Films of Gold on Chromium

SOME interesting and important effects have been observed when gold films are annealed. The films were prepared on borosilicate glass substrates. They were about 4000 Å thick and had underlayers of chromium 100 Å thick. Examination of the film surfaces has shown that certain diffusion effects occur predominantly at grain boundaries. Two distinct phenomena have been observed—the gradual emergence of ridges of materials

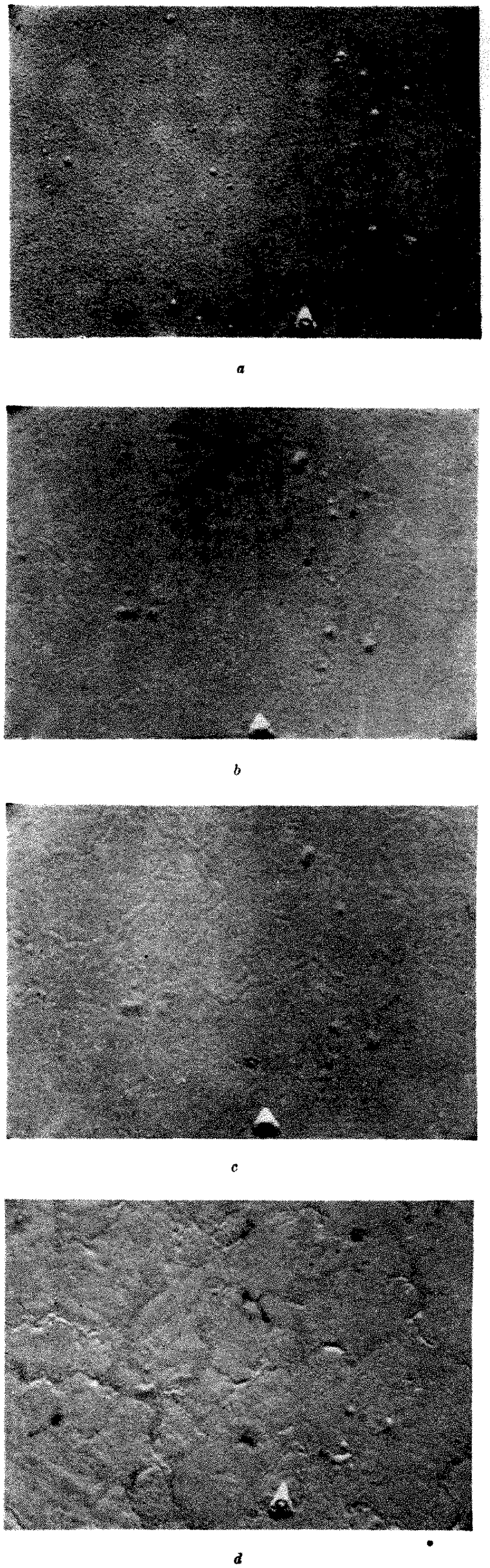

Fig. 1. Effect of annealing at 300° C: (*a*) as deposited; (*b*) after 3 h; (*c*) after 5 h; (*d*) after 11 h. (× 16,500).

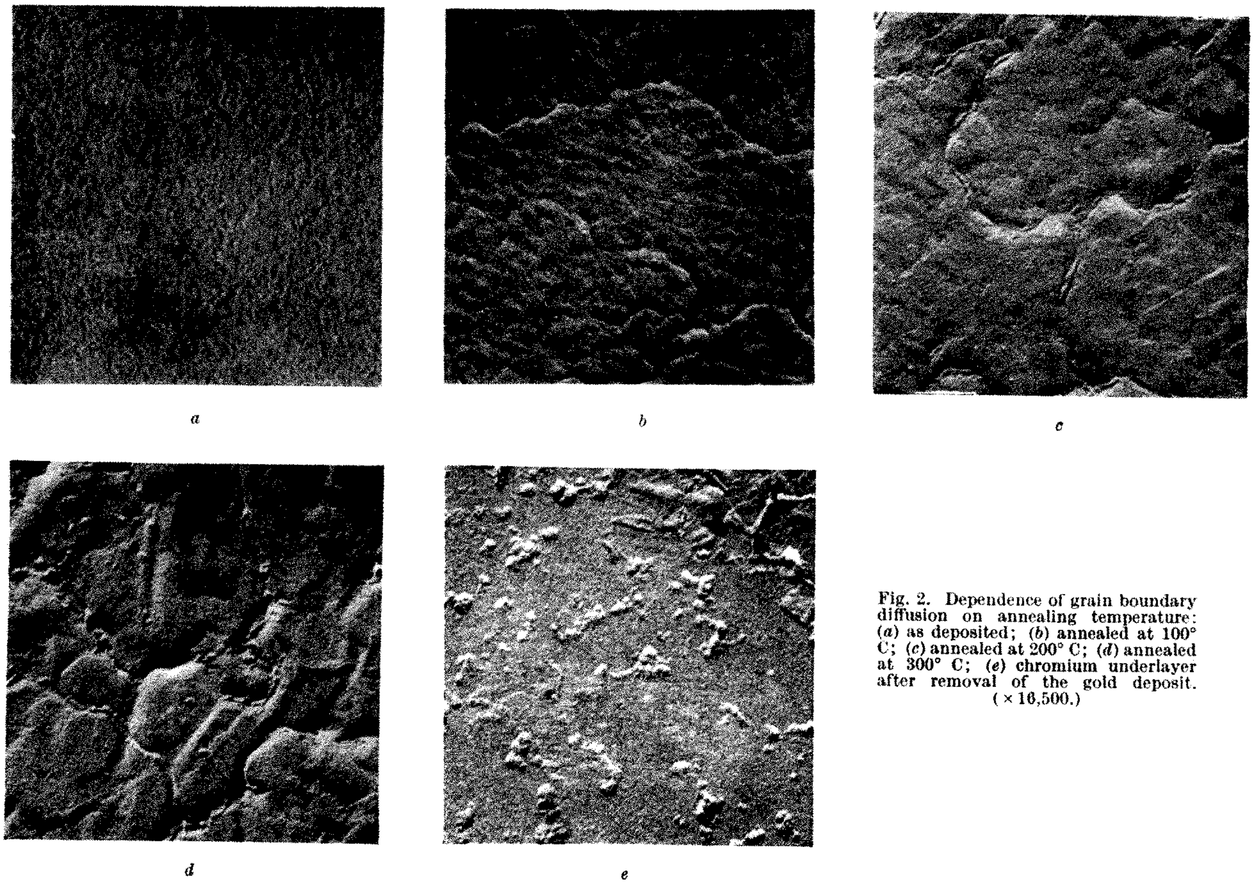

Fig. 2. Dependence of grain boundary diffusion on annealing temperature: (*a*) as deposited; (*b*) annealed at 100° C; (*c*) annealed at 200° C; (*d*) annealed at 300° C; (*e*) chromium underlayer after removal of the gold deposit. (× 16,500.)

decorating the grain boundaries and a more catastrophic change of surface in which the crystallites stand out as islands.

A controlled technique was applied to produce a smooth surface, gold-on-chromium film. The film was then annealed and examined by stages. It was annealed at 300° C, first for 3 h then for a further 2 h and then for a further 6 h. At each stage a collodion carbon replica of a selected area of the surface was prepared and examined in an electron microscope. Fig. 1 shows the micrographs obtained. Fig. 1*a* shows the smooth surface of the original film. After the first annealing, ridges appeared in the surface indicating the positions of grain boundaries (Fig. 1*b*). After successive periods of annealing these became more strongly emphasized.

This film was typical of other gold-on-chromium films produced. Some films were annealed for short periods of time and others for up to 20 h at various temperatures. The crystallites had an average diameter of a few microns which remained constant throughout the annealing treatment. It is thought that the crystallites had grown to this size on deposition: in one case the positions of grain boundaries could be seen in the film as deposited. A film of gold with no underlayer exhibited crystal grains of a similar size when heated to 300° C for 2 h. The grain boundaries showed up as lines on the surface, but were not emphasized by ridges because there was no underlying material present to decorate the boundaries. The series of micrographs in Fig. 2 clearly shows how grain boundary decoration develops as annealing proceeds by 100° C increments. Fig. 2*e* shows the form of the chromium underlayer after the gold film had ultimately been etched away. The material remaining has migrated into lumps and ridges distinctly outlining flat areas of a similar size

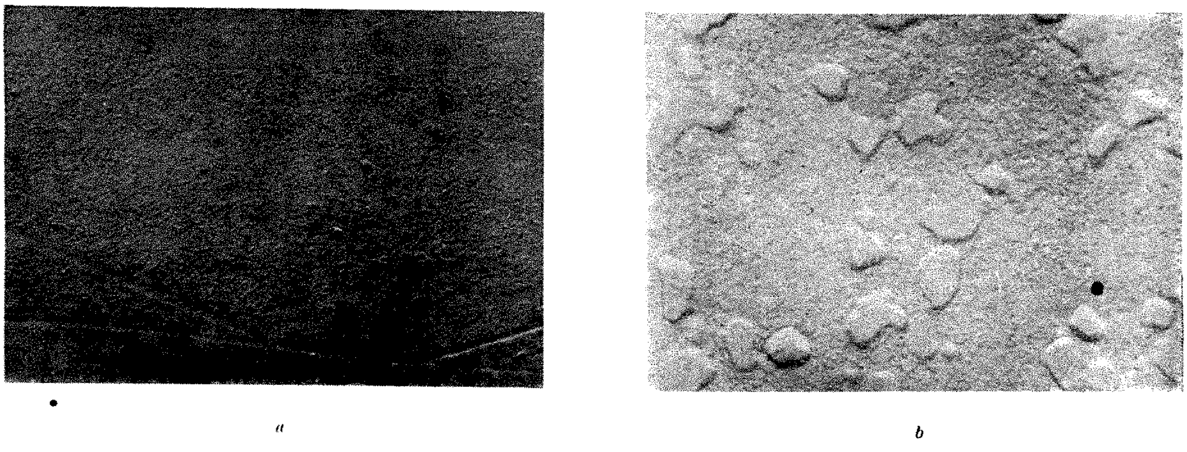

Fig. 3. Violent change of structure: (*a*) as deposited; (*b*) after annealing. (× 16,500.)

to the gold crystallites. This is further evidence that the underlayer material has diffused along the chromium/gold interface and through the gold film between crystal grains.

A more violent effect which occurred with some of the films is illustrated in Fig. 3. The structure of this film including its many scratches had not changed on heating to 100° C for 2 h. But when heated to 200° C, all previous surface features disappeared and were replaced by large crystalline islands. The crystallites were completely free of background texture and stood up about 500 Å from the irregular, original surface level of the film. This effect has occurred in a number of films, but always those which have not exhibited obvious grain boundary diffusion. It is thought that the change may have been initiated by thermal shock because some of the films were seen to turn milky in removal from a hot oven. It has been observed that films deposited fast (30 Å/s) exhibited grain boundary diffusion whereas those deposited more slowly (3 Å/s) underwent a catastrophic change in structure or none at all. This violent change usually followed a succession of severe annealing and replication processes. Another variation in procedure which was found to affect the behaviour of these films on annealing was pre-heating of the substrate. Some substrates were heated under vacuum to 100° C for ½ h and then cooled by means of a water cooled copper block with which they were in contact. Films deposited onto these substrates were very similar in texture to others produced on normal substrates, but on heating the grain boundary structure did not appear. Surprisingly, neither vapour phase mixing of the chromium and gold deposits, nor partial oxidation of the chromium underlayer had any significant effect on the behaviour of the films on annealing.

Certain other underlayer materials have been considered. Iron exhibited an effect very similar to chromium. Reactively sputtered bismuth oxide did not diffuse in the same way although annealing caused a gross surface structure to emerge. Nickel–chromium alloy did not diffuse through overlying gold films but a lumpy surface resulted from the annealing treatment.

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Production of Epitaxial Films of Antimony on Disordered Substrates

THERE has been widespread interest in thin films of metals and metalloids during the past decade because of their varied technological and fundamental scientific significance[1-3]. The mechanism of their formation is intimately associated with nucleation theory[4] and epitaxy[1] (the growth of a substance into a single crystal or well oriented polycrystalline form on a different substance as substrate); and their properties are relevant to the study of electrical, magneto-optical and catalytic phenomena[1,5,6]. On the physical side, much effort has been expended in assessing the influence of such factors as the symmetry, lattice spacing and temperature of the substrate in the growth of epitaxial films. On the chemical side, it has recently been recognized that, although evaporated films have greatly stimulated the study of heterogeneous catalysis, the crystallographic heterogeneity of misoriented polycrystalline or amorphous films complicates interpretation of adsorption phenomena, and makes comparison invidious with field ion and other studies which utilize single crystals.

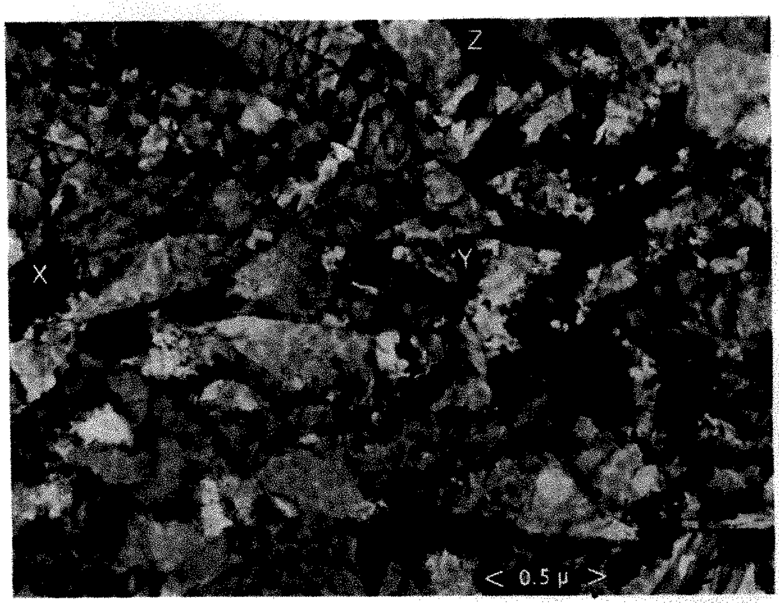

Fig. 1. Transmission electron micrograph of evaporated antimony film (average thickness 1000 Å) showing extinction contours and, in particular, extended dislocation networks in the region marked *XYZ*.

This communication describes the preparation of well oriented evaporated films of antimony in unusual, but readily realizable, conditions, in which the substrate possesses little or no crystallographic symmetry and long-range order, and the substrate temperature is surprisingly low[7]. Some recent speculations[8-10] as to the importance of source material in determining the microstructure of antimony films are tested, together with general statements[11,12] regarding the ease of producing ordered structures on disordered substrates. The results suggest a convenient method of generating single-crystal films of relatively large area suitable for adsorptive studies using low energy electron diffraction[13], work function and related[6] measurements. Together with the work of Yu[12] on the microstructure of films of ionic solids, the results may contribute to the elucidation of certain biochemical processes, especially some aspects of the phenomena recently discussed by Lonsdale[14].

High purity (99·999 per cent) antimony was evaporated from a tungsten spiral in a vacuum of 3×10^{-4} to 5×10^{-5} torr (varied systematically) at an average rate of 30 Å s^{-1} up to a thickness of about 1000 Å. The films were floated off the substrates and examined in transmission using an electron microscope. The temperature of the source was in the range 500°–700° C, and that of the substrate surface, which was 5–6 cm away, never exceeded 30° C during deposition. Three kinds of substrate were used: (1) outgassed 'Pyrex' glass (microscope) slides; (2) 'Pyrex' slides onto which a thin layer of detergent was added, by "rubbing in" a concentrated solution of 'Teepol' and allowing the water to evaporate *in vacuo*, which leaves a waxy film of soap on the glass; (3) collodion mounted on a copper grid for electron microscopy.

Substrates (1) and (2) gave identical results. The films are continuous though of variable thickness, as revealed by the extinction contours (Fig. 1); and the occurrence of an extended dislocation network demonstrates that a remarkable degree of crystalline perfection exists over areas of about 2 μm². Significantly, selected-area diffractions taken from most of the regions shown in Fig. 1 yielded a spot pattern indicative of a single crystal, rather than a well oriented crystalline film, the orientation being such that the {111} planes are parallel to the substrate surface. In a few regions both {100} and {111} orientation occur about equally. Small-angle boundaries, with regions of misorientation up to about 20°, are also present.

With substrate (3) there is again pronounced epitaxy, but there are no signs of dislocation networks, the film readily fragments on floating, and from the diffraction pattern (Fig. 2) it is concluded that a well oriented polycrystalline, rather than a single crystal, film is formed.

When a gold–antimony alloy (atomic ratio Au : Sb = 40 : 60) is used, from which, on heating, monatomic and diatomic as well as the usual tetra-atomic species are vaporized[10], essentially the same results are obtained with substrates (1) and (2) but not with (3), which yields an unoriented polycrystalline film (Fig. 3). Thus the chief differences reported between antimony films produced from the element and from an antimony alloy are not fully repeated here. Aluminium, which has a melting point (660° C) close to that of antimony (631° C) yields a discontinuous polycrystalline film when evaporated to the same thickness on all three kinds of substrate, showing that the ease of oriented growth is not a simple function of melting point.

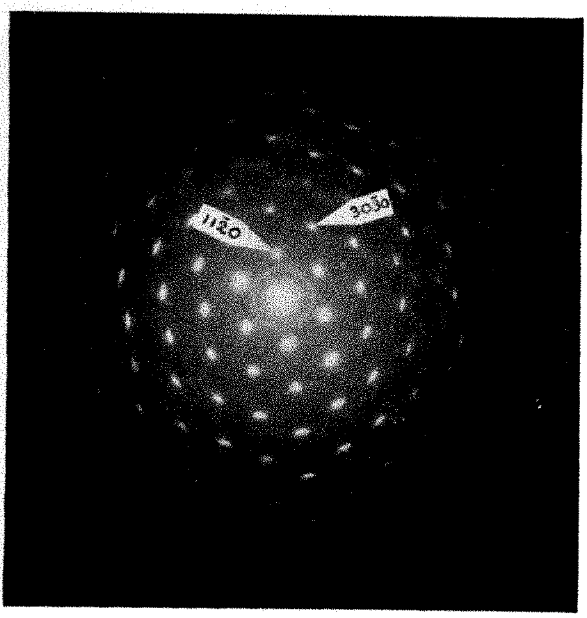

Fig. 2. Selected area diffraction pattern from an area of 15 μm² of a film deposited on collodion.

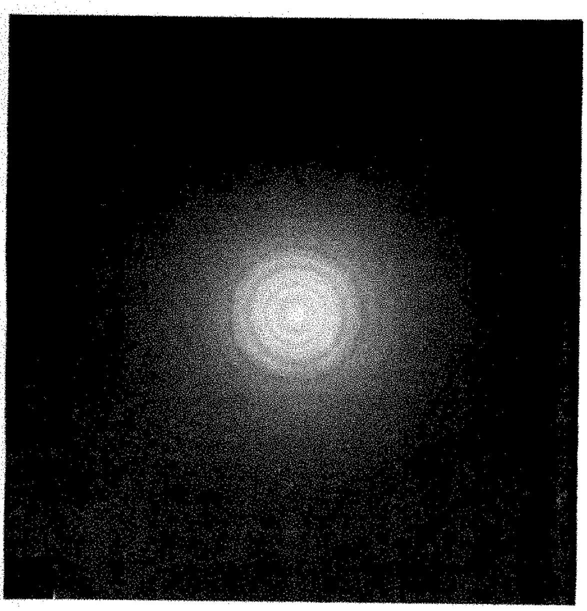

Fig. 3. Diffraction pattern of film deposited from Au–Sb source on a collodion substrate.

It is impossible to decide, as yet, which of the three principal factors—nucleation orientation, growth orientation or recrystallization—known to facilitate the production of epitaxial films dominates here. It is clear, however, that condensed aggregates of antimony formed from gaseous species of varied atomicity are capable of migrating over large distances on non-crystalline surfaces. The highly specific nature of surface migration of metal crystallites on non-metallic, but crystalline, substrates has previously been noted and partly explained[15,16] in terms of Brownian motion.

Preliminary parallel experiments with bismuth indicate that films of this metalloid also show some tendency to be oriented when formed on disordered substrates at very low temperatures.

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[1] Pashley, D. W., *Adv. Phys.*, **14**, 327 (1965).

[2] Francombe, M. H., and Sato, H., *Single Crystal Films* (Pergamon Press, 1964).

[3] Anderson, J. C., in *The Use of Thin Films in Physical Investigations* (edit. by Anderson, J. C.) (Academic Press, 1966).

[4] Rhodin, T. N., in *The Use of Thin Films in Physical Investigations* (edit. by Anderson, J. C.), 187 (Academic Press, 1966).

[5] Heavens, O. S., and Miller, R. E., *Proc. Roy. Soc.*, A, **266**, 547 (1962).

[6] Thomas, J. M., and Thomas, W. J., *Introduction to the Principles of Heterogeneous Catalysis*, chap. 3 (Academic Press, 1967).

[7] Francombe, M. H., in *The Use of Thin Films in Physical Investigations* (edit. by Anderson, J. C.), 37 (Academic Press, 1966).

[8] Sommer, A., *J. App. Phys.*, **37**, 2789 (1966).

[9] Rosenblatt, G. M., *J. App. Phys.*, **38**, 888 (1967).

[10] Baumann, F., Kessler, J., and Roessler, W., *J. App. Phys.*, **38**, 3398 (1967).

[11] Mader, S., in *The Use of Thin Films in Physical Investigations* (edit. by Anderson, J. C.), 439 (Academic Press, 1966).

[12] Yu, R. M., *Phil. Mag.*, **13**, 1167 (1967).

[13] Jona, F., *Surface Science*, **8**, 57 (1967).

[14] Lonsdale, K., *Nature* **217**, 56 (1968).

[15] Sears, G. W., and Hudson, J. B., *J. Chem. Phys.*, **39**, 2380 (1963).

[16] Thomas, J. M., and Walker, P. L., *J. Chem. Phys.*, **41**, 587 (1964).

CHEMISTRY

Heterogeneous Oxidation of Methane over Palladium Catalysts

A brief investigation has been made of the effect of different forms of palladium catalysts on the heterogeneous oxidation of methane. The experiments are based on the idea that, if crystals of metal are formed by thermal decomposition of a metal salt which is deposited within the pore structure of a zeolite, the maximum size to which the metal crystallite can grow is equal to the size of the pore or cavity of the zeolite within which decomposition occurs. Because the dimensions of these cavities and pores are in the range of 0·4–4 nm for most zeolites, it is possible to study catalytic reactions on crystallites of these dimensions without interference from the much larger crystallites present in most supported metal catalysts. These catalysts could be prepared by thermal decomposition of a complex metal salt in which the catalytic metal is in an anionic form so that ion exchange with the zeolite metal ions does not occur. In this study we have compared three catalysts: massive palladium metal (catalyst 1), palladium crystallites within a zeolite structure (catalyst 2) and a zeolite catalyst containing separated metal atoms (catalyst 3) produced in the normal way by ion exchange[1].

Catalyst 1 was prepared by thermal decomposition of an aqueous ammonium chloropalladite solution. The resulting mixture of palladium and palladium oxide was reduced in a 12 per cent methane–air mixture[2] at 900° C. Catalyst 2 was prepared by immersing Linde 13 × molecular sieve in an aqueous solution of ammonium chloropalladite. The sieve was drained, washed with water and dried at 400° C in air. Catalyst 3 was prepared by immersing Linde 13 × molecular sieve in an aqueous solution of tetramminopalladium chloride, prepared by treating ammonium chloropalladite with excess ammonia, draining, washing with water and drying in air at 400° C. Catalysts 2 and 3 contained 3·55 and 0·55 per cent by weight of palladium respectively. Reduction of these two catalysts with hydrogen at 300° C produced a slight increase in activity in catalyst 2 but had no effect on the kinetic results. Poisoning experiments with hexamethyl disiloxane[3] confirmed that in both catalysts 2 and 3 in the prepared and reduced states no metallic palladium was present on the external surface of the zeolite. X-ray diffraction measurements on these catalysts confirmed that catalyst 1 was metallic palladium but failed to detect the presence of palladium in catalysts 2 and 3, indicating that the crystallite size of any metallic palladium in these catalysts was less than about 5 nm.

Methane oxidation over each catalyst was studied by the microcalorimetric method[2]. A large excess of oxygen for complete oxidation to carbon dioxide and water was used in each experiment. The methane pressure range was 0–2 kNm^{-2} and the oxygen pressure range 8–80 kNm^{-2}. Nitrogen was used as a diluent gas to bring the pressure up to 10·1 kNm^{-2}. The zeolite containing no palladium did not oxidize methane in the temperature range studied here. In each case only carbon dioxide and water were detected as reaction products. The relevant kinetic data are given in Table 1.

Table 1. REACTION PARAMETERS FOR METHANE OXIDATION

Catalyst	Form of catalyst	Reaction order in methane	Reaction order in oxygen	Apparent activation energy kJ $mole^{-1}$	Temperature range °C
1	Palladium metal	1	0	50	300–450
2	Palladium in 13 × zeolite. Prepared from ammonium chloropalladite	1	0·3–0·4	50	300–400
3	Palladium in 13 × zeolite. Prepared from palladium ammine	1	0·05	88	300–400

The apparent activation energies were the same on catalysts 1 and 2 but different on catalyst 3, indicating that palladium is present as a metallic phase in catalyst 2 and showing that the presence of the zeolite support in this catalyst does not markedly effect the reaction. The reaction orders indicate that on each catalyst methane is weakly adsorbed and oxyten is strongly absorbed. No negative effect over the ten-fold range in oxygen concentration was observed on any catalyst, which is strong evidence that methane and oxygen are not competing for the same sites on the catalyst. Oxygen, however, is not strongly chemisorbed by zeolites themselves and, because in catalyst 3 the only catalytic sites are the separated palladium ions, such ions are capable of simultaneous adsorption of both oxygen and methane. Moreover, because the complete oxidation of methane requires two oxygen molecules for each methane molecule, the separate palladium ions are able to absorb partially oxygenated methane intermediates as well as oxygen atoms.

The apparent activation energies for the reaction on catalysts 1 and 2 indicate that a similar reaction sequence is occurring on each catalyst and that the bond strengths of the adsorbed intermediates are similar. The decrease in crystallite size in catalyst 2 increases the reaction order in oxygen, however. Such an effect will be produced if the ratio of adsorbed methane to adsorbed oxygen is greater on catalyst 2 than on catalyst 1. This would result in an increase in the rate of creation of vacant adsorption sites by the increased rate of reaction of adsorbed oxygen with adsorbed methane. It is unlikely that this could be caused by cracking of methane by the zeolite itself before adsorption on to the palladium; cracking of methane would result in some oxidation before adsorption, which is contrary to the low order in oxygen on catalyst 3 and the lack of activity for methane oxidation displayed by the zeolites themselves. Palladium metal has a face-centred cubic structure and as the crystallite size decreases the ratio of atoms in the edges of the crystal to those in the faces increases. If methane is adsorbed on the edges of the crystal faces and reacts with oxygen which is strongly adsorbed on the crystal faces and edges, then below a certain crystallite size an increase in the order in oxygen will result. It therefore seems likely that on palladium metal methane adsorption in oxidation reactions occurs at surface imperfections such as steps. Such mechanisms have been proposed for other heterogeneous catalytic reactions[4].

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[1] Rabo, J. A., Shomaker, V., and Pickert, P. E., *Proc. Third Intern. Congr. Catalysis*, 1264 (1965).

[2] Firth, J. G., *Trans. Faraday Soc.*, **62**, 2566 (1966).

[3] Firth, J. G., and Holland, H. B., *Nature*, **212**, 1036 (1966).

[4] Uhara, I., Yanagimoto, S., Tani, K., Adachi, G. Y., and Teratami, S., *J. Phys. Chem.*, **66**, 2961 (1962).

Stereochemistry of the Allyl Ligand in some Allyl–Palladium Complexes

SEVERAL X-ray analyses of the structures of allyl complexes of transition metal ions have been completed[1]. Apart from the symmetrical bonding found in π-allyl palladium chloride[2,3] and π-allyl palladium acetate[4], the results also indicate considerable changes of the stereochemistry of the allyl group on co-ordination. For example, in both *bis*-π-methallylnickel[5] and triphenylphosphine-2-methallylpalladium chloride[6], the methyl group is bent out of the plane containing the three carbon atoms of the allyl ligand while in π-$C_3H_4.CH_3PPh_3PdCl$[6] and *bis*-π-allyl rhodium chloride[7] the metal–carbon allyl bond lengths are non-equivalent. The very close relation between the bond lengths in these last two molecules rules out the suggestion[8] that the asymmetric allyl to metal bonds are due to "adventitious packing distortions". The non-planarity of the methyl groups in $(\pi\text{-}C_3H_4.CH_3)_2Ni$ and π-$C_3H_4.CH_3.PPh_3.PdCl$ may indicate σ–π electron mixing in the ligand[9] and other methyl-substituted allyl complexes have now been studied to provide further information on this point.

π-2-Methallyl palladium chloride crystallizes in the triclinic system, being the dimeric molecule required to be centrosymmetric in the space group P$\bar{1}$; its geometry is shown in Fig. 1. The methyl groups are displaced 0·3 Å towards the metal from the plane of the allyl–carbon atoms, so that the result noted earlier[6] is not a consequence of asymmetry in the metal–allyl bond. The dihedral angle which the two crystallographically equivalent allyl groups make with the plane containing the palladium and chlorine atoms is 112°. The average estimated standard deviations in the bond lengths are Pd–Cl 0·005 Å, Pd–C 0·018 Å and C–C 0·026 Å ($R = 0{\cdot}078$ for 1,123 independent reflexions).

π-1,1,3,3-Tetramethallylpalladium chloride is also dimeric in the space group P$\bar{1}$. The molecular geometry (Fig. 1) results from a refinement of 1,040 independent reflexions ($R = 0{\cdot}087$). As in the π-2-methallyl complex, the Pd_2Cl_2 bridge system is required to be planar and the dihedral angle of the allyl carbon plane with respect to this plane is 121°. Estimated standard deviations of the bond lengths average 0·007 Å (Pd–Cl), 0·026 Å (Pd–C) and 0·039 Å (C–C).

In both of these molecules the palladium–carbon and carbon–carbon bond lengths in the allyl ligand are identical within experimental error. This result, together with the observed planarity of the Pd_2Cl_2 rings in both structures, makes the reported geometry of the π-1,3-dimethallylpalladium chloride dimer[10] extremely difficult to understand. In the π-1,1,3,3-tetramethallylpalladium chloride dimer, each pair of methyl groups is distorted from the plane of the allyl carbon atoms to produce a conformationally eclipsed arrangement; atoms C4 and C7 are displaced 0·4 Å towards the metal, atoms C5 and C6 being pushed 0·8 Å away from the metal. A simple way of picturing the allyl stereochemistry is that the average deviation of C(1,3) from the plane containing C2, C(5,6) and C(4,7) is 0·2 Å; if C1 and C3 were trigonal, this deviation would be zero, whereas if they were tetrahedral it would be approximately 0·5 Å.

π-1,1,3,3-Tetramethallyltriphenylphosphine palladium chloride has also been investigated to determine whether the deviation of C1 and C3 from trigonal stereochemistry depends on the symmetry or otherwise of the metal–ligand bond. The refinement is not yet complete (the present discrepancy index is 0·097) but it can be stated that the methyl groups are again displaced from the allyl plane to give a conformationally eclipsed structure, the average deviations of the two pairs of methyl carbon atoms being −1·2 Å and +0·4 Å.

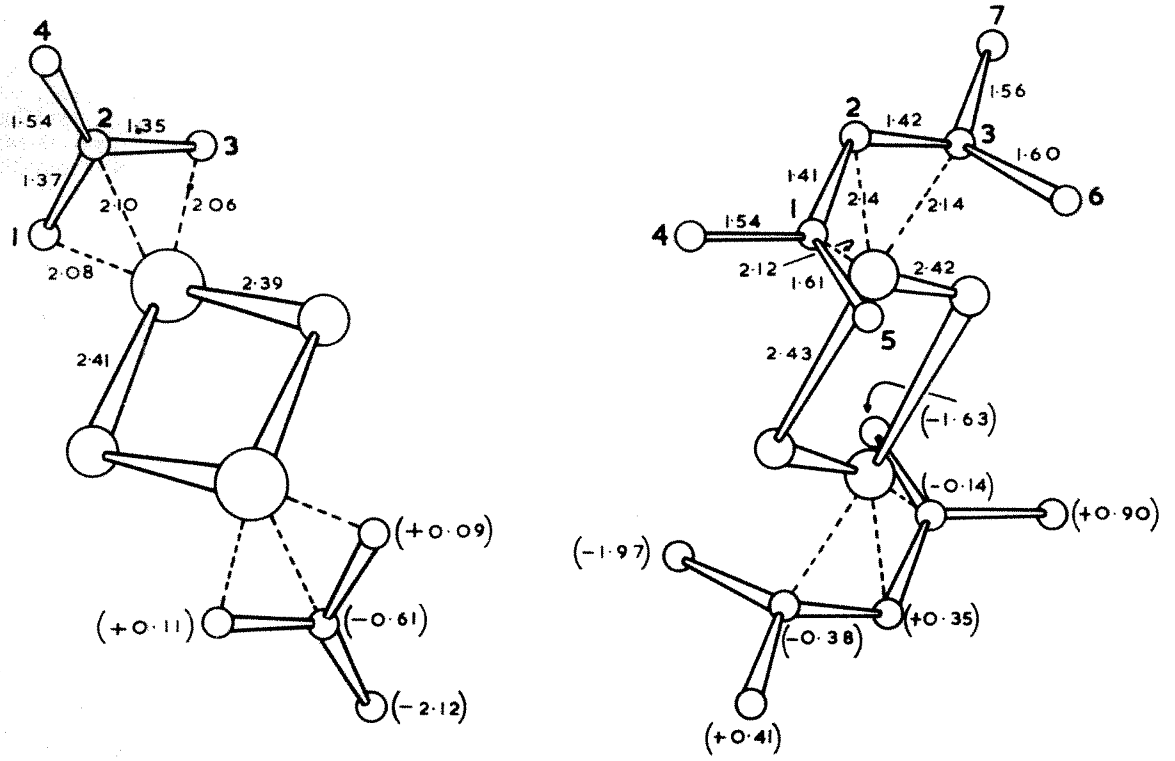

Fig. 1. The molecular geometries of the π-2-methallyl palladium chloride and the π-1,1,3,3-tetramethallyl palladium chloride dimers. Numbers in parentheses refer to deviations (Å) of carbon atoms from the Pd_2Cl_2 planes.

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[1] Churchill, M. R., and Mason, R., *Advances in Organometallic Chemistry* (edit. by Stone, F. G. A., and West, R.), **5**, 93 (Academic Press, 1967).
[2] Smith, A. E., *Acta Cryst.*, **18**, 331 (1965).
[3] Oberhanski, W. E., and Dahl, L. F., *J. Organometallic Chem.*, **3**, 43 (1965).
[4] Churchill, M. R., and Mason, R., *Nature*, **204**, 777 (1964).
[5] Uttech, R., and Diettrich, H., *Zeit. Krist.*, **122**, 60 (1965).
[6] Mason, R., and Russell, D. R., *Chem. Commun.*, 26 (1966).
[7] McPartlin, M., and Mason, R., *Chem. Commun.*, 16 (1967).
[8] Cotton, F. A., Faller, J. W., and Musco, A., *Inorg. Chem.*, **6**, 182 (1967).
[9] Kettle, S. F. A., *Inorganic Chim. Acta*, **1**, 303 (1967).
[10] Davies, G. R., Mais, R. H. B., O'Brien, S., and Owston, P. G., *Chem. Commun.*, 1151 (1967).

Influence of Carbon Dioxide in the Calcite–Sodium Oleate Flotation System

FATTY acids are usually employed in the flotation of carbonate minerals as collectors; among them oleic acid in the form of sodium oleate is important. Mortenson and Ljøkjell[1] reported that flotation of calcite using sodium oleate as the collector is improved by conditioning the pulp with carbon dioxide. Biswas[2] pointed out that the improvement due to the use of carbon dioxide is not related solely to the lowering of *p*H but is largely attributable to the surface chemical reaction in the system. We report further studies on this system.

To study the calcite–carbon dioxide–water system transparent calcite crystals were wet ground and sieved. About 20 g of moist samples of $-120+140$ ASTM mesh size range were treated with 150 ml. of distilled and deionized water in a 500 ml. reagent bottle and then carbon dioxide was bubbled through the system for various times at a constant rate of 3 l./min. The bottle was shaken for 30 min and kept in a thermostat (operating at 30 ± 0·1° C) for 1 h. For each set, the slurry was filtered and the Ca^{++}, $CO_3^=$ and HCO_3^- concentrations in the filtrate estimated.

Calcium estimation was done by the ammonium purpurate method described by Williams and Moser[3]. The *p*H was maintained at 11·3 and percentage absorbance was measured at 505 mμ. It was established that neither carbon dioxide nor sodium oleate interferes with the estimation of calcium by this method. Carbonate and bicarbonate were estimated by titration[4]; the results are presented in Table 1.

Table 1. CALCITE–CARBON DIOXIDE–WATER SYSTEM

Time of bubbling of carbon dioxide at the specified rate (min)	Carbonate (moles/l.)	Bicarbonate (moles/l.)	Calcium (moles/l.)	$[Ca^{++}][CO_3^=]$	*p*H at the end of the experiment
0	$2{\cdot}87\times10^{-3}$	—	$0{\cdot}19\times10^{-3}$	$54{\cdot}5\times10^{-8}$	7·26
5	$0{\cdot}10\times10^{-3}$	$1{\cdot}26\times10^{-3}$	$1{\cdot}56\times10^{-3}$	$15{\cdot}6\times10^{-8}$	—
10	$0{\cdot}18\times10^{-3}$	$1{\cdot}17\times10^{-3}$	$2{\cdot}30\times10^{-3}$	$41{\cdot}4\times10^{-8}$	—
20	$0{\cdot}20\times10^{-3}$	$0{\cdot}16\times10^{-3}$	$3{\cdot}35\times10^{-3}$	$67{\cdot}0\times10^{-8}$	5·56
30	$0{\cdot}13\times10^{-3}$	$0{\cdot}12\times10^{-3}$	$3{\cdot}48\times10^{-3}$	$45{\cdot}2\times10^{-8}$	5·80

To study the calcite–carbon dioxide–sodium oleate–water system a similar set of experiments was performed with moist calcite immersed in 100 ml. of sodium oleate solution of known concentration and the procedure already described was followed.

In this set of experiments, estimations of sodium oleate and calcium ion were made at different stages. A method of colorimetric estimation of sodium oleate using Nile blue as the complexing reagent was developed on the basis of earlier findings by Goiffin and Conchand[5]. It was necessary to buffer the solution at *p*H 11·2. Colorimetric estimation at a wavelength of 620 mμ was found to be unaffected by the presence of carbon dioxide. Presence of carbon dioxide was found not to interfere with the estimation of sodium oleate. The data obtained are given in Table 2. These results suggest the following.

(*a*) As more carbon dioxide is bubbled through the calcite suspension, the concentrations of Ca^{++} and HCO_3^- in the solution increase, presumably as a result of a surface chemical reaction. The equilibrium constant values for the various dissociative reactions in this system were worked out by Garrels and Christ[6]. According to them, if a calcite suspension is equilibrated with pure water without any gas such as carbon dioxide, the ionic concentration values should be $0{\cdot}126\times10^{-3}$, $0{\cdot}398\times10^{-4}$ and $0{\cdot}891\times10^{-4}$ moles/l. for Ca^{++}, $CO_3^=$ and HCO_3^-, respectively. It should be pointed out that, according to Mellor[7], the solubility of $CaCO_3$ is 0·0014 g/100 ml. of water at 25° C and thus the concentration of Ca^{++} at equilibrium should be $0{\cdot}14\times10^{-3}$ moles/l. Our data reported in Table 1 ($0{\cdot}19\times10^{-3}$ moles/l.) is slightly higher. Garrels and Christ[6] further postulated on the basis of equilibrium constants that equilibration with atmospheric air containing carbon dioxide (partial pressure $10^{-3.5}$) would raise the concentrations of Ca^{++} and HCO_3^- to $0{\cdot}398\times10^{-3}$ and 10^{-3} moles/l., respectively, and lower the carbonate concentration to $0{\cdot}126\times10^{-4}$ moles/l. These trends are reflected and established in data presented in Table 1. The value for the ionic concentration product $[Ca^{++}]$ $[CO_3^=]$, however, was not constant in the experimental conditions, and differs widely from the theoretical value: $0{\cdot}2\times10^{-8}$. This inconstancy and discrepancy reflect non-attainment of equilibrium conditions.

Table 2. CALCITE–CARBON DIOXIDE–SODIUM OLEATE–WATER SYSTEM

Initial concentration of sodium oleate solution: $9{\cdot}85\times10^{-4}$ moles/l.

Time of bubbling of carbon dioxide at the specified rate (min)	0	5	10	20	30
Concentration of calcium (moles/l.)	$0{\cdot}32\times10^{-3}$	$0{\cdot}66\times10^{-3}$	$1{\cdot}02\times10^{-3}$	$1{\cdot}71\times10^{-3}$	$2{\cdot}49\times10^{-3}$

Time of bubbling of carbon dioxide (min)	Final concentration of oleate (moles/l.)	Amount of oleate adsorbed (moles/g)
Initial concentration of sodium oleate solution: $2{\cdot}35\times10^{-4}$ moles/l.		
0	$1{\cdot}61\times10^{-4}$	$0{\cdot}41\times10^{-6}$
5	$0{\cdot}54\times10^{-4}$	$1{\cdot}04\times10^{-6}$
10	$1{\cdot}74\times10^{-4}$	$0{\cdot}39\times10^{-6}$
Initial concentration of sodium oleate solution: $4{\cdot}70\times10^{-4}$ moles/l.		
0	$1{\cdot}13\times10^{-4}$	$2{\cdot}20\times10^{-6}$
5	$0{\cdot}52\times10^{-4}$	$2{\cdot}72\times10^{-6}$
10	$0{\cdot}89\times10^{-4}$	$2{\cdot}30\times10^{-6}$
Initial concentration of sodium oleate solution: $9{\cdot}40\times10^{-4}$ moles/l.		
0	$1{\cdot}02\times10^{-4}$	$5{\cdot}35\times10^{-6}$
5	$3{\cdot}03\times10^{-4}$	$3{\cdot}94\times10^{-6}$

(*b*) Even in the presence of sodium oleate, this surface chemical reaction mentioned is evident. The concentration of calcium in the solution increases as more carbon dioxide passes (Table 2). The slower increase in calcium concentration could be caused by an adsorbed layer of oleate hindering the diffusion of carbon dioxide or by the parallel precipitation reaction which results in the formation of calcium oleate.

(*c*) Adsorption of sodium oleate on calcite increases with a greater initial concentration of sodium oleate and with the use of a limited amount of carbon dioxide. With more carbon dioxide, however, a process of desorption is initiated.

The difference in the adsorption of oleate on calcite in the carbonated and uncarbonated pulp is pronounced for low concentrations of sodium oleate, for example, $2{\cdot}35\times10^{-4}$ moles/l. For a high concentration such as $9{\cdot}4\times10^{-4}$ moles/l., adsorption actually decreases with the use of carbon dioxide.

(*d*) Thus it seems plausible that bubbling a limited amount of carbon dioxide increases the concentration of the calcium in the solution through surface chemical reaction, which in turn induces heightened adsorption of collector (oleate) and increases flotability. The beneficial effect of carbon dioxide (in terms of collector adsorption and flotation) is discernible only at low collector concentration[2].

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[1] Mortenson, M., and Ljøkjell, D., *K. Norske Vidensk Selsk Forhand*, **33**, 12, 155 (1960).

[2] Biswas, A. K., *Indian J. Technol.*, **5**, 6, 187 (1967).

[3] Williams, M. B., and Moser, J. H., *Anal. Chem.*, **25**, 9, 1414 (1953).

[4] Caven, R. M., *Quantitative Chemical Analysis and Inorganic Preparations*, 196 (Blackie and Son, Ltd., London, 1962).

[5] Goiffin, R., and Conchand, *Anal. Biol. Clin.*, Paris, **11**, 327 (1953).

[6] Garrels, R. M., and Christ, C. L., *Solutions, Minerals and Equilibria*, 75 (Harper and Row, New York, 1965).

[7] Mellor, J. W., *Inorganic and Theoretical Chemistry* (New Impression), **3**, 825 (Longmans Green and Co., London, 1957).

BIOCHEMISTRY

Recognition of DNA Base Sequence Differences among Vertebrates

UNLIKE the wide variation of DNA base composition found in bacteria, higher organisms tend to have a restricted average base composition (vertebrates ~42 moles per cent guanine + cytosine)[1]. Thus physical methods such as thermal denaturation or equilibrium density gradient sedimentation, which are useful in distinguishing bacterial DNAs[2], do not discriminate to any extent among vertebrate DNAs, excluding the question of satellite bands. Phylogenetic relationships among bacteria, based on other criteria, are largely borne out by DNA–DNA hybridization[3]. While the latter technique can discriminate among distantly related higher organisms[4] it can be applied with only limited confidence to more closely related vertebrates.

Another approach is to fractionate single strands of DNA according to base sequence and to compare the resulting fractions. One method presently available for fractionation of DNA strands is countercurrent distribution[5] in solvent systems different from those used for sequence-dependent fractionation and purification of transfer RNA species[6]. Countercurrent distribution of single-stranded DNA shows a base composition gradient, with guanine-rich fractions more soluble in the aqueous (lower) phase and adenine-rich fractions more soluble in the organic (upper) phase[5]. The relation between secondary structure and the behaviour of double-stranded DNA in the same solvent systems[7,8] indicates that partition of DNA in these systems is dependent on the availability of free amino groups. This observation, the varying ratios of guanine : cytosine and adenine : thymine in single stranded DNA fractions and the fact that DNA from a given source gives a reproducible fractionation profile, indicate that, as with molecular species of transfer RNA, countercurrent distribution of single stranded DNA molecules in this solvent system is at least partly dependent on base sequence as well as net base composition. We have further examined the specificity of fractionation profile by comparing DNAs from several vertebrates. This study was initially designed to obtain information about relatedness among Australian marsupials but clearly it is of more general application.

DNA was isolated by one of three phenol methods[9-11] from liver, spleen or kidney. Contamination by protein[12] was greatly dependent on the source and on the extraction method (Table 1) (compare ref. 13). For countercurrent distribution, DNA was isolated from a given source by the procedure giving consistently low protein contamination. Nucleotide base compositions of DNA preparations were calculated from the melting temperature (*Tm*) in 0·15 M sodium chloride–0·015 M sodium citrate[2] (Table 2) and were found to conform to the generally restricted range observed for other vertebrates[2].

Table 1. ISOLATION METHOD AND PROTEIN CONTENT OF DNA PREPARATIONS

Tissue	Method PAS*	 PAS–TIPNS*	 SDC–PPP*	Protein (per cent
Rat liver	×			< 1
Rat liver		×		< 1
Rat kidney			×	< 1
Rat spleen			×	< 1
Mouse liver		×		1·7
Ringtail possum liver	×			20
Ringtail possum liver		×		< 1
Ringtail possum kidney		×		1
Koala liver		×		No DNA
Koala liver			×	No DNA
Koala kidney		×		3·5
Wombat liver	×			42
Wombat liver		×		< 1
Wombat liver			×	1·8
Wombat kidney			×	1·2
Echidna liver		×		< 1
Echidna kidney		×		17
Echidna kidney			×	3
Fowl liver		×		3
Bluetongue lizard liver		×		2
Shingleback lizard liver		×		1
Toad liver		×		80
Toad liver			×	< 1

* Abbreviations and method references: PAS, sodium 4-aminosalicylate, 6 per cent (ref. 9); PAS–TIPNS, PAS, 6 per cent–sodium tri-iso-propylnaphthalenesulphonate, 1 per cent (ref. 10); SDC–PPP, sodium dithiocarbamate, 0·3 M–sodium phenolphthalein diphosphate, 0·015 M (ref. 11). The phenol mixture used was phenol–*m*-cresol–water–8-hydroxyquinoline (500 : 70 : 55 : 0·5, ref. 14).

Table 2. NUCLEOTIDE BASE COMPOSITIONS CALCULATED FROM *Tm*[2]

DNA	*Tm*	Percentage G+C
Rat liver	86·7	42·4
Mouse liver	86·3	41·2
Wombat liver	86·0	40·7
Koala kidney	86·0	40·7
Ringtail possum liver	86·5	41·9
Echidna liver	86·6	42·2
Fowl liver	86·5	41·9
Lizard liver	87·5	44·5
Toad liver	87·3	43·9

Before fractionation, DNA preparations were dialysed against 0·0013 M trilithium citrate, denatured by heating 100° C for 10 min and cooled rapidly on ice (in contrast to earlier studies[5], the strands were not further fragmented). Countercurrent distribution was then carried out in an all-glass automatic apparatus, 3 ml. each phase, in an alkoxyethanol–trilithium citrate system[5], using 4 mg of denatured DNA. The fractionation profile was reproducible with respect to major peaks for different DNA preparations from a given species (Fig. 1). Similar reproducibility was obtained for DNA preparations from different tissues of the same species. This profile is critically dependent on ionic strength[5] and can thus be altered by varying the lithium ion concentration in the solvent system but reproducibility is good in a standard set of conditions.

Comparison of a number of vertebrate species (Fig. 2) demonstrates remarkable differences in countercurrent distribution of single stranded DNA. With the possible exception of fowl and lizard there was little identity of pattern between any two species examined, even in the case of the rodents (Wistar rat, Swiss mouse). Differences among the marsupials (ringtail possum, koala, wombat) were particularly striking.

Studies with bacteria and bacteriophage have produced evidence interpreted variously as favouring the occurrence of specific[15] or non-specific[16] strand breaks during isolation and subsequent handling of DNA. There is as yet little knowledge of the nature or nucleotide base sequence specificity of such DNA breaks in higher organisms. The reproducibility of the fractionation profiles and their apparent species specificity suggest that these breaks are not entirely random, so that countercurrent distribu-

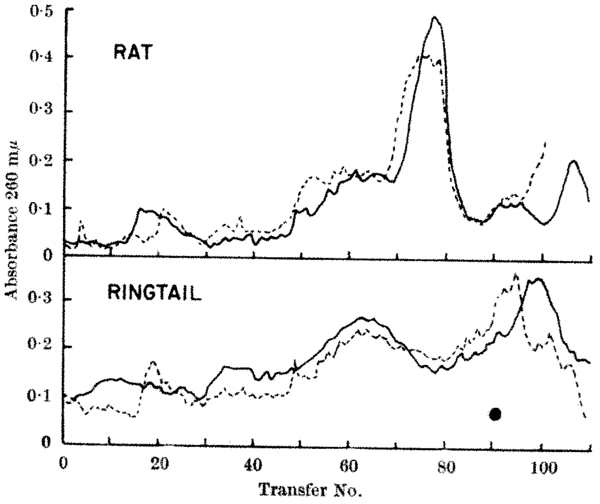

Fig. 1. Reproducibility of countercurrent fractionation profile for single stranded DNA from different individuals of a single species. Countercurrent distribution carried out as in ref. 5; ionic strength, I = 0·028.

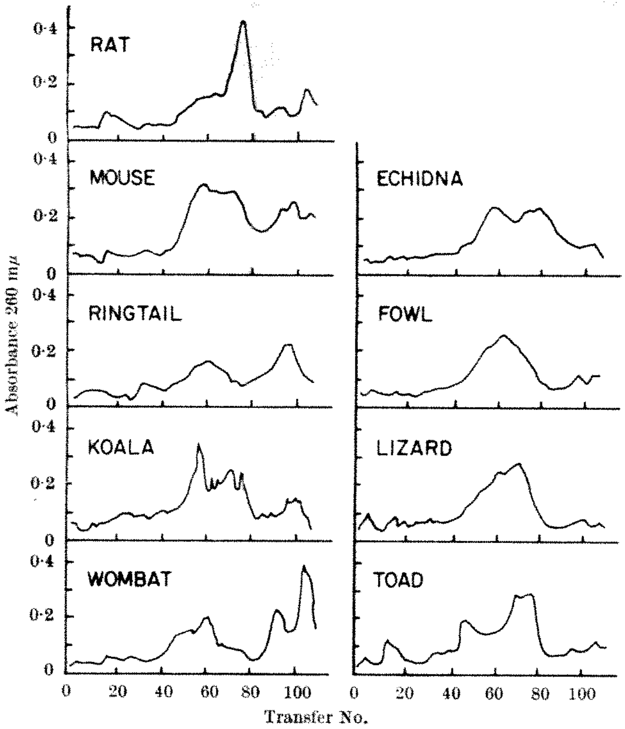

Fig. 2. Species specificity of countercurrent fractionation profile of single stranded DNA. Experimental conditions as for Fig. 1.

tion of single stranded DNA could perhaps serve a useful purpose as a crude fingerprint for a given species. Clearly, by this method alone, it is not possible to ascertain the extent of sequence homologies, but the data do suggest the existence of marked variations in base sequences in DNA of related vertebrate species.

Countercurrent distribution permits the comparison of non-radioactive DNAs, an important practical feature because phylogenetic studies of higher organisms cannot always utilize tissue culture. Countercurrent distribution of radioactively labelled DNA strands may also find useful application in combination with hybridization[4] in the more detailed examination of base sequence homologies.

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[1] Sueoka, N., in *Evolution of Macromolecules* (edit. by Bryson, V., and Vogel, H. J.), 479 (Academic Press, 1965).
[2] Sueoka, N., *J. Mol. Biol.*, **3**, 31 (1961).
[3] Marmur, J., Falkow, S., and Mandel, M., *Ann. Rev. Microbiol.*, **17**, 329 (1963).
[4] Hoyer, B. H., McCarthy, B. J., and Bolton, E. T., *Science*, **144**, 959 (1964).
[5] Kidson, C., and Kirby, K. S., *Biochim. Biophys. Acta*, **76**, 624 (1963).
[6] Goldstein, J., Bennett, J. P., and Craig, L. C., *Proc. US Nat. Acad. Sci.*, **51**, 119 (1964).
[7] Kidson, C., and Kirby, K. S., *Biochim. Biophys. Acta*, **91**, 627 (1964).
[8] Kidson, C., *J. Mol. Biol.*, **17**, 1 (1966).
[9] Kirby, K. S., *Biochim. Biophys. Acta*, **36**, 117 (1959).
[10] Parish, J. H., and Kirby, K. S., *Biochim. Biophys. Acta*, **129**, 554 (1966).
[11] Kirby, K. S., *Biochim. Biophys. Acta*, **55**, 382 (1962).
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
[13] Kirby, K. S., in *Prog. in Nucleic Acid Res.*, **3**, 1 (1964).
[14] Kirby, K. S., *Biochem. J.*, **96**, 266 (1965).
[15] Roger, M., *Proc. US Nat. Acad. Sci.*, **51**, 189 (1964).
[16] Hershey, A. D., Burgi, E., and Ingraham, L., *Biophys. J.*, **92**, 206 (1962).

Morphogenetic Properties of Follicle-stimulating Hormone: Evidence from Effects of Anti-gonadotropin Sera on Developing Chick Embryos

FOLLICLE-STIMULATING hormone (FSH) is able to induce the formation of neural tissue and, in some instances, support differentiation of notochord-like and somitic tissues in chick embryonic systems. Recent experiments have suggested that the primary mesoderm is the target tissue for FSH[1-6]. The latter observation may be of some interest in view of the part played by the mesoderm in the establishment of the embryonic axis. The antigen–antibody reaction is a sensitive and specific reaction. We have attempted, using specific antisera, to obtain some information about the possible importance of FSH or FSH-like glycoproteins in embryonic development.

Antiserum from rabbits against human FSH (anti-FSH-R) and antisera from turkeys against human FSH, HCG and LH (anti-FSH-T, anti-HCG-T and anti-LH-T, respectively) were used. Chick embryos at the definitive primitive-streak stage (stage 4) were explanted *in vitro* according to New's technique[7], and 0·005 ml. of antiserum in 0·2 ml. of Pannett–Compton (PC) saline was added on the embryos which were cultured for 18 h. Controls were maintained using normal rabbit serum or turkey serum obtained before immunization. The results are summarized in Table 1.

Embryos which were cultivated in the presence of normal rabbit serum developed normally (86·4 per cent). About 9 per cent showed general malformation. When treated with anti-FSH-R about 32 per cent of the embryos developed normally while 46 per cent showed malformation of brain and somites often accompanied by degeneration of these tissues (Fig. 1). Development appeared to be arrested at stage 4–5 in 16·3 per cent of the embryos.

Nearly all of the forty-three embryos treated with anti-FSH-T stopped development at stage 4–5. Only about 2 per cent showed malformation. Arrest of development produced by anti-HCG-T was, however, considerably less. But a greater proportion of the embryos treated by the latter produced malformation. Control turkey serum produced very low developmental arrest or mal-

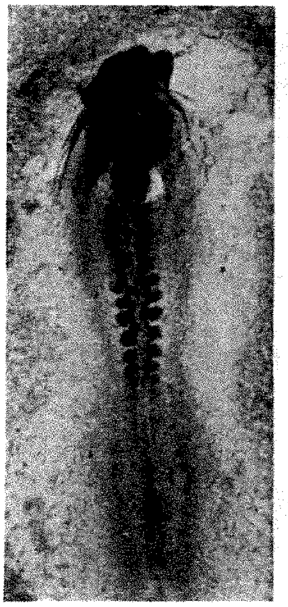

Fig. 1

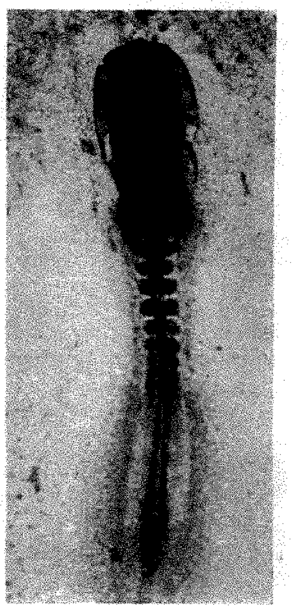

Fig. 2

Fig. 1. Chick embryo treated at stage 4 with anti-FSH-R. Malformation of the brain and degeneration of neural tube and somites to be noted.

Fig. 2. A control chick embryo.

formation. Chicken gamma globulin produced about 7 per cent developmental arrest but a much higher incidence of malformation. It can be reasonably concluded therefore that the developmental arrest was produced by the antibodies themselves and not by indifferent proteins present in the serum.

At a concentration of 0·4 mg (equivalent to 0·005 ml. of antiserum) anti-LH-T produced developmental arrest in 22·4 per cent of treated embryos. When the concentration was doubled arrest occurred in 35·3 per cent and another 53 per cent showed general abnormality. This slight enhancing effect may be due to azide which worked out to a concentration of 0·0008 M. But the lack of any significant rise in developmental arrest with the doubling of anti-LH-T concentration would seem to indicate that arrest produced even at the lower anti-LH concentration may only be a consequence of the presence of small amounts of anti-FSH as an impurity.

The embryos which had undergone developmental arrest were examined histologically. In embryos arrested by anti-FSH-T and anti-HCG-T there was an extensive degeneration of the mesoderm (Fig. 3). In these embryos the epiblast and the hypoblast were apparently not affected by the antisera. Degeneration of the mesoderm in embryos arrested by anti-LH was neither as extensive nor encountered as frequently as in those embryos arrested by anti-FSH-T or anti-HCG.

Differences between the effects produced by the various antigonadotropin sera are obvious. Anti-FSH-R caused predominantly abnormal development but developmental arrest in only a few embryos. Anti-FSH-T and anti-HCG-T, on the other hand, produced chiefly arrested development. Anti-HCG-T also caused malformation to some extent. These differences could be attributed to the fact that the antisera were derived from widely divergent species like rabbits and turkeys. It is to be expected that antisera from turkeys (homologous antibodies) will be more effective than those from rabbits. Anti-HCG-T is able to cross react with FSH (ref. 8). This may explain why the effects of anti-HCG-T are somewhat similar to those of anti-FSH-T. Anti-HCG-T is not as effective as anti-FSH-T in arresting embryonic development.

Gastrulation is a significant event in embryonic development and is accompanied by a sudden rise in the transcription of the genetic information[9]. It is very sensitive to the action of structural analogues of purines and pyrimidines, histones, poly-*l*-lysine and actinomycin[10]. Complete arrest of development at this critical stage by anti-FSH-T and anti-HCG-T sera may indicate the importance of molecules similar to FSH for normal development.

The selective degeneration of mesodermal layer and also cytolysis of the neural tissue and the somites might be caused by immune lysis which is a characteristic feature of the antibody–antigen–complement reaction[11]. The selective effect on the mesodermal layer observed in the arrested embryos seems to confirm our earlier observation that mesoderm is probably the target tissue of FSH. It also suggests a number of other possibilities. It seems plausible that during or subsequent to the invagination of the epiblast through the primitive-streak the cells may be synthesizing or elaborating molecules similar to FSH, or changes might take place in the conformation of glycoprotein molecules present on the cell surface as a result of the processes of invagination. Invagination through the primitive-groove involves a breakdown of the epithelial structure preparatory to differentiation of mesoderm. This process itself might render the mesoderm vulnerable to immune lysis, because organized tissues have not been shown to be as much susceptible to immune lysis as are unorganized or isolated cells[12].

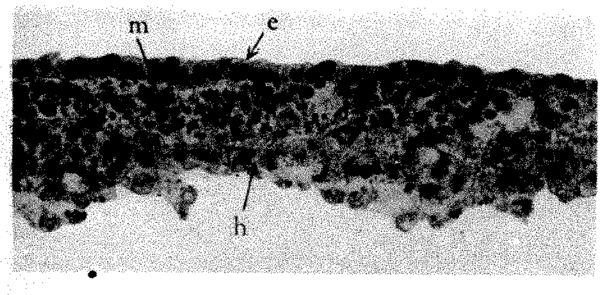

Fig. 3. Section through an embryo arrested by anti-FSH-T showing extensive degeneration of the mesoderm (m). e, Epiblast; h, hypoblast.

Table 1. EFFECTS OF ANTIGONADOTROPIN SERA ON THE DEVELOPMENT OF CHICK EMBRYOS *in vitro*

Treatment	No. of embryos treated	Normal (per cent)	Delayed development (per cent)	Arrest at stage 4–5 (per cent)	Abnormal (per cent)
PC saline	35	79·9	14·3	2·9	2·9
Azide 0·0006 M*	17	76·8	5·8	5·8	11·6
Rabbit serum plus azide 0·00035 M	19	79·2	10·4	5·2	5·2
Rabbit serum in PC	22	86·4	4·6	—	9·0
Anti-FSH-R	53	32·1	5·6	16·3	46·0
Control turkey serum	51	51·0	41·2	3·9	3·9
Chick gamma-globulin	14	64·3	—	7·1	28·6
Anti-FSH-T	43	—	—	97·7 †	2·3
Anti-HCG-T	37	2·7	32·4	48·7 ‡	16·2
Anti-LH-T	58	57·0	3·4	7·1	17·2

* Anti-FSH-R contained 0·00035 M sodium azide as preservative. Some embryos were therefore treated with 0·00035 M or 0·0006 M azide and their development compared with untreated ones.

† Mesodermal degeneration in 90 per cent of these developmental arrests.

‡ Mesodermal degeneration in 77 per cent of these arrested embryos.

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[1] Sherbet, G. V., *Naturwissenschaften.*, **49**. 471 (1962).
[2] Sherbet, G. V., *J. Embryol. Exp. Morphol.*, **10**, 227 (1963).
[3] Sherbet, G. V., and Lakshmi, M. S., *Nature*, **215**, 1089 (1967).
[4] Sherbet, G. V., and Lakshmi, M. S., *Experientia*, **23**, 969 (1967).
[5] Sherbet, G. V., and Mulherkar, L., *Roux. Arch. Entwickl. Mech.*, **154**, 506 (1963).
[6] Sherbet, G. V., and Mulherkar, L., *Roux. Arch. Entwickl. Mech.*, **155**, 701 (1965).
[7] New, D. A. T., *J. Embryol. Exp. Morphol.*, **3**, 326 (1955).
[8] Butt, W. R., Crooke, A. C., and Cunningham, F. J., in *Immunoassay of Hormones*, Ciba Foundation Colloquia on Endocrinology (edit. by Wolstenholme, G. E. W., and Cameron, M. C.), 310 (J. and A. Churchill, London, 1962).
[9] Denis, H., *J. Mol. Biol.*, **22**, 285 (1966).
[10] Sherbet, G. V., in *Histones, Their Role in the Transfer of Genetic Information*, Ciba Foundation Study Group 24 (edit. by De Reuck, A. V. S., and Knight, J.), 81 (J. and A. Churchill, London, 1966).
[11] Wissler, R. W., *Ann. Rev. Microbiol.*, **16**, 265 (1962).
[12] Benaceraff, B., and McCluskey, R. T., *Ann. Rev. Microbiol.*, **17**, 263 (1963).

Effects of Tannic Acid on Erythrocyte Enzymes

MANY properties of the red cell have been studied with the aid of tannic acid since the original observations by Roberts[1]. Treatment with this acid causes reduction in anion permeability[2], agglutination[3], alterations in osmotic resistance[4] and renders erythrocytes capable of adsorbing proteins from solution[5]. The latter characteristic serves as a basis for the widely used procedures of passive haemagglutination. We have shown that the exposure of human erythrocytes to tannic acid, in conditions commonly employed in passive haemagglutination techniques, results in irreversible inactivation of acetylcholinesterase (AChE)[6]. The effect on this membrane enzyme[7] was dependent on concentration, *p*H, time and temperature. Although the mechanism by which tannic acid exerts its action on the red cell has not been elucidated, it has been suggested that in low concentrations this agent traverses the erythrocyte membrane[4]. To test this hypothesis we have investigated the effect of tannic acid on two intracellularly located enzymes, glucose-6-phosphate dehydrogenase (G6PD)[8] and acid phosphatase[7].

Blood obtained from normal adult individuals was centrifuged and the plasma and buffy coat were removed by suction. The erythrocytes were washed thrice with twenty volumes of ice-cold 0·15 M NaCl. A 50 per cent cell suspension was prepared after the last centrifugation and used immediately. A 0·1 per cent stock solution of tannic acid, Fisher Certified Reagent, was prepared in 0·1 M sodium–potassium phosphate buffer adjusted to the required *p*H. Red cells were treated with tannic acid as indicated in Table 1. The activity of AChE was measured at 412 mμ in 0·1 M phosphate buffer, *p*H 8·0, with acetylthiocholine as substrate and 5 : 5′-dithiobis-(2-nitrobenzoic acid) as colour reagent[9]. The activity of G6PD was determined spectrophotometrically at 340 mμ and acid phosphatase was assayed colorimetrically at *p*H 5·0 using 0·1 M acetate buffer and *p*-nitrophenyl phosphate as substrate. Enzyme activity was related to the haemoglobin concentration, which was determined at 540 mμ as cyanmethaemoglobin.

Table 1. EFFECT OF TANNIC ACID ON ERYTHROCYTE ENZYMES

*p*H	G6PD*		Acid phosphatase†		AChE‡	
	Tannic acid	Control	Tannic acid	Control	Tannic acid	Control
6·0	5·9	4·1	0·15	0·15	11	141
6·5	7·5	5·4	0·15	0·14	17	134
7·0	8·0	6·2	0·14	0·15	25	142

Solutions of tannic acid containing 0·1 mg/ml. were prepared in 0·1 M phosphate buffer adjusted to the *p*H indicated. Samples of these solutions (4 ml.) were added to replicate 0·5 ml. samples of a 50 per cent suspension of three times washed red cells. Buffer was added to controls. After 30 min of incubation at 37° C, erythrocytes were washed three times with twenty volumes of chilled 0·15 M NaCl. A 50 per cent suspension was prepared after the last centrifugation. Enzyme activity was determined as indicated in the text.

* Specific activity expressed as μmoles of TPN reduced/min/g of haemoglobin.

† Specific activity expressed as μmoles *p*-nitrophenol liberated in 30 min, at 37° C/mg of haemoglobin.

‡ Specific activity expressed as *ΔO.D.*/min/mg of haemoglobin.

Table 1 shows the results obtained when G6PD and acid phosphatase activity was measured after intact erythrocytes had been incubated for 30 min with 0·1 mg/ml. of tannic acid at 37° C and at three different hydrogen ion concentrations. For comparison the activity of AChE is also given. From these results it is evident that tannic acid had no effect on the activity of the two cytoplasmic enzymes and that only the membrane-located AChE was inactivated by this agent. Similarly, incubation of red cells with tannic acid in a range of concentrations of 0·02 to 0·2 mg/ml., while causing AChE inactivation, did not produce a reduction in the activity of G6PD or acid phosphatase. Exposure to higher concentrations of tannic acid resulted in marked haemolysis. The enzymes of erythrocytes from Negro individuals with genetically determined G6PD deficiency behaved like those from normal subjects.

To make certain that the absence of an inactivating effect on G6PD and acid phosphatase was not a result of a general indifference of these red cells enzymes to tannic acid, the action of this agent on cell-free assay systems was investigated. For this purpose, increasing amounts of the acid were mixed with haemolysates before the addition of substrates and coenzyme. Tannic acid was omitted from the controls. As can be seen in Table 2, both enzymes were inhibited by tannic acid; but whereas 0·02 mg/ml. caused a 72 per cent reduction in G6PD activity, inhibition of acid phosphatase was apparent only at a concentration of 1 mg/ml. The observations are reminiscent of the effects of proteases on erythrocyte enzymes[7]; that is, intracellularly located enzymes are affected in cell-free haemolysates[8], but only AChE activity is destroyed when whole red cells are exposed to small amounts of trypsin, chymotrypsin, papain or bromelin[7]. The finding that cytoplasmic enzymes were not affected when intact erythrocytes were exposed to tannic acid, in conditions which caused irreversible inactivation of AChE[6], does not support the contention that tannic acid is able to traverse the red cell membrane[4], but indicates that this agent only interacts with specific segments of the outer surface of the human erythrocyte membrane[2].

Table 2. EFFECT OF TANNIC ACID ON REACTIONS OF CYTOPLASMIC ENZYMES

Tannic acid (mg/ml.)	G6PD		Acid phosphatase	
	ΔO.D./340 mμ	Percentage activity	*ΔO.D.*/410 mμ	Percentage activity
1·0			0·136	47
0·2			0·260	96
0·1	0·000	0	0·270	98
0·02	0·051	28	0·284	99
0·01	0·113	72	0·298	104
0·002	0·145	92	0·294	102
0·001	0·153	97	0·280	98
None	0·158	100	0·287	100

A stroma-free 1 : 20 aqueous lysate[10] was prepared from a 50 per cent suspension of three times washed erythrocytes. For G6PD, 1·2 ml. of tannic acid was added to replicate cuvettes containing 1·0 ml. of 0·19 M *tris* buffer, *p*H 8·0; 0·1 ml. of 0·3 M $MgCl_2$; 0·1 ml. of 0·02 M glucose-6-phosphate and 0·4 ml. of lysate. Reaction was started immediately by adding 0·2 ml. of 0·002 M TPN and changes in absorbance at 25° C were recorded at 1 min intervals. *ΔO.D.* indicated corresponds to 5 min. For acid phosphatase, 1·0 ml. of tannic acid in 0·1 M acetate buffer, *p*H 5·0, was added to replicate 0·5 ml. of lysate, followed by 1·0 ml. of 0·016 M *p*-nitrophenyl phosphate. After incubation at 37° C for 30 min, the reaction was stopped with 10 per cent trichloroacetic acid and absorbance was determined at 410 mμ in supernatants alkalinized with 0·25 M NaOH.

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[1] Roberts, W., *Quart. J. Microsc. Sci.*, **3**, 170 (1863).
[2] Edelberg, R., *J. Cell. Comp. Physiol.*, **40**, 529 (1952).
[3] Hunter, F. R., *J. Cell. Comp. Physiol.*, **55**, 175 (1960).
[4] Bohlmann, A., *Arch. Exp. Pathol. Pharm.*, **203**, 317 (1944).
[5] Boyden, S. V., *J. Exp. Med.*, **93**, 107 (1951).
[6] Herz, F., *Proc. Soc. Exp. Biol. and Med.* (in the press).
[7] Herz, F., Kaplan, E., and Stevenson, jun., J. H., *Nature*, **200**, 901 (1963).
[8] Firkin, B. G., Beal, R. W., and Mitchell, G., *Austral. Ann. Med.*, **12**, 26 (1963).
[9] Kaplan, E., Herz, F., and Hsu, K. S., *Pediatrics*, **33**, 205 (1964).
[10] Herz, F., *Proc. Soc. Exp. Biol. and Med.*, **125**, 68 (1967).

Chemical Transfer of Learned Fear

RESULTS reported earlier[1–5] have suggested that acquired information can be transmitted to naïve animals by giving them material extracted from the brain of trained subjects. In most of these experiments, as in others[6–11], transfer of information was suggested by varying degrees of statistical probability and it was challenged by negative results obtained in other laboratories[12].

The experiments reported here are easily and rapidly reproducible and yield unequivocal results which clearly demonstrate the possibility of a purely chemical transfer of some types of acquired information. The design of these experiments is based on the well known preference shown by rats and mice for dark rather than lighted enclosures. The preference is reversed if the animals, when they take refuge in the dark, are submitted to a painful stimulus, such as electric shock. This learned fear or passive avoidance, often used for learning studies, was first investigated for the possibility of transfer by Gay and Raphelson[13], who found that naïve recipients injected with extracts of brain taken from trained donors avoided the dark.

In our experiments, donor animals (male Sprague–Dawley rats weighing 200–250 g) were trained in two boxes (the starting box, *S*, made of translucent plastic with a transparent lid, 30 × 20 × 15 cm, and the dark box, *D*, of black plastic on five sides and an electrified grid floor, 25 × 10 × 15 cm) connected by a passage 5 cm long with a cross-section of 8 × 8 cm with a gate. The rat was placed in *S* and, usually, after a few seconds ran into *D*. The gate was then closed and the animal received electric shocks (2 mA) for 5 s through the grid floor. When the gate was lifted, the animals ran back into the starting box or were returned to it. The operation was repeated five times: each shock period of 5 s was separated by an interval of 10 to 15 s. One such session was given to the donors daily for 6 or 8 days, unless otherwise stated.

Between 1 and 2 h after the last training session the rats were decapitated, their brains were rapidly removed, frozen in dry ice and stored at − 20° C until use. Brains from groups of animals similarly trained were pooled, weighed and homogenized with water in a glass tube with a motor driven 'Teflon' pestle. In the first experiment, the homogenate was stirred for 5–6 h in an ice bath, kept in the refrigerator for 14–16 h and centrifuged at 80,000*g* for 1 h. The volume of the supernatant was adjusted so that 1 ml. was equivalent to 2 g of brain. In the most recent experiments, the homogenate was poured into 'Cellophane' bags and dialysed against 20 volumes of distilled water for 20–24 h. The dialysate was lyophilized and the residue was redissolved in distilled water. Other extraction procedures will be described later together with the chemical properties of the transfer factor. The extracts were kept frozen at − 20° C until use.

The recipients were male Swiss mice weighing 20–25 g. The same boxes were used as for the training of donors, but a third compartment was attached to the starting box. This white box (*W*), made of the same material as *S*, was connected to it by a passage identical with the one linking *S* to *D*.

Before injection, mice were screened so as to eliminate those that did not show a definite dark preference; animals that did not spend in the dark at least 90 out of a total of 180 s were eliminated. In 348 mice, the mean time in the dark box was 138 ± 19 (*S.D.*) s.

After screening, the mice were injected intraperitoneally with the brain extracts and tested at varying intervals. Testing, like screening, consisted of placing the animal in *S* with both gates open. By means of stop watches or, in more recent experiments, with a photoelectric system, the time spent in all three compartments was recorded for 180 s. The observers had no knowledge of the treatment received by the animals. The results are shown in Table 1 in terms of the mean number of seconds spent in *D* (TDB) 1, 2 and 3 days after injection.

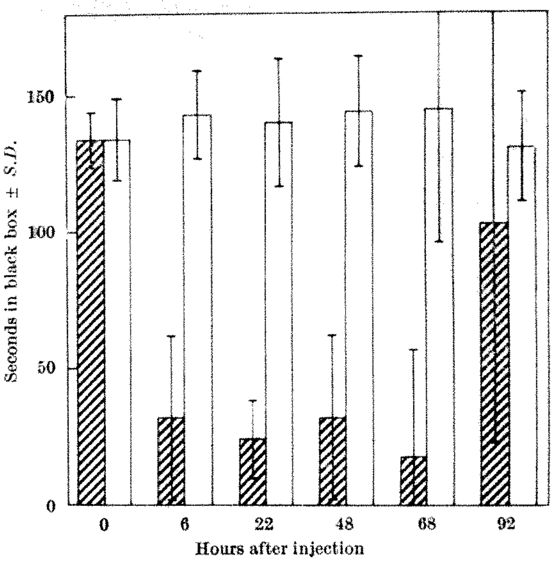

Fig. 1. Action of brain extracts on dark-light preference in naïve mice. Abscissa, interval between injection of extracts and test; ordinate, time spent in dark box out of a total of 180 s. White columns, mice injected with untrained brain (12); hatched columns, mice given extract of brain from rats which had acquired fear of dark (18). Vertical lines indicate standard deviations.

In the first experiment, thirty mice were divided into two groups: eighteen of group 1 mice received the supernatant of the homogenate of brain taken from donors trained for 8 days—amounts equivalent to 1 g of rat brain were injected into each mouse; in group 2, twelve mice were injected with the same dose of similarly prepared extract of untrained rat brain. The results, shown in Fig. 1, indicate that the extract from untrained animals had no significant effect on the behaviour of the recipients, but the trained extract considerably shortened TDB. The significance between the means, computed by the *t* test, indicates $P < 0{\cdot}001$ until the fourth day when most of the animals reverted to the preinjection pattern of behaviour.

Table 1. INFLUENCE OF DONOR TRAINING AND DOSE OF BRAIN INJECTED ON AVOIDANCE IN RECIPIENTS

Training of donors (days)	Amount of brain injected (g)	TDB (s) ± *S.D.* recipients		Units/g ± *S.D.*	
2	1·0	74	23	1·66	0·50
	1·5	26	14		
4	0·3	98	27	2·73	0·67
	0·6	67	25		
	1·0	24	28		
6	0·4	72	29	3·38	0·56
	0·6	63	22		
	0·8	27·5	9		
8	0·3	86	15	3·46	0·30
	0·6	56	17		
	0·9	16	6		
11	1·0	54	22	2·01	0·62
15	1·0	82	25	1·22	0·71
8 (+5)*	1·0	33	17	2·60	0·45
8 (+10)*	1·0	76	22	1·37	0·64

All donors were subjected to five periods of 5 s shock (total 25 s) daily. Groups of six mice were used.

* Numbers between parentheses indicate number of days between last training day and removal of the brain.

In the next series of experiments, the effect of varying amounts of brain was studied. As shown in Table 1, the effect increased with the dose of brain material injected. Another significant factor was the length of training given to the donors and Table 1 shows that the transfer effect increased with the length of training up to 8 days. For longer periods of training, the effect declined sharply. The decline was less marked with donors trained for 8 days and given 5 or 10 days rest before removal of the brain.

Given the quantitative relationship between dose and effect, it was possible to establish a unit of activity defined as the amount of material reducing TDB to 90 s. The content of the brain in transfer factor could be expressed in these units (last column of Table 1).

In all these experiments, the donors were given five periods of 5 s of shock every day. In other experiments, varying amounts of shock were given daily. It is seen in Table 2 that one 5 s period per day for 6 days (total of 30 s) did not give satisfactory results. When shock was given for 50 s daily, the results resembled those of 11 days training with 25 s/day. It therefore seemed likely that the total exposure to shock may bear some relation

Table 2. EFFECT OF TOTAL DURATION OF SHOCK GIVEN TO THE DONORS AND THE INTERVAL BETWEEN TRAINING AND BRAIN REMOVAL ON AVOIDANCE IN RECIPIENTS

Days in training	Total duration* of shock (s)	Interval between last session and brain removal (h)	TDB (s) ± *S.D.*		Units/g ± *S.D.*	
6	30	2	91	31	0·97	0·89
6	300	2	51·5	29	2·08	0·53
1	150	0·1	135	19	0	
1	150	2	107	38	0·52	0·34
1	150	6	66	31	1·68	0·88
1	150	12	38	25	2·46	0·59
1	150	18	51	38	2·10	1·07
1	150	24	43·5	34	2·31	0·96
1	150	36	70	41	1·56	1·15
1	150	48	85	43	1·14	1·21

Groups of six mice were used.

* Given in periods of 5 s separated by intervals of 10 to 15 s.

Table 3. CHEMICAL PROPERTIES OF THE TRANSFER FACTOR

Extract	TDB	± *S.D.*
Supernatant	27	15
Dialysate	30·5	28
Retentate	136·5	12
Phenol phase	40	20
Aqueous phase	143	13
Trypsin treated	134	23
Chymotrypsin treated	32·5	25
Ribonuclease treated	26	21
Incubated control*	23	16

In all experiments groups of six mice were used. The injected amount was 1 g/mouse.

* Incubated without enzyme at *p*H 8 and room temperature for 1 h to serve as control to the trypsin treated sample.

to the amount of transfer factor present in the brain. Experiments were set up to see whether massing thirty periods of 5 s in 1 day would give results similar to five periods of training for 6 days. In these experiments, however (Table 2), the result depended also on the interval between the last trial and the removal of the brain. It seems that although the training and the electric shocks trigger the formation of the transfer factor, it requires several hours for the synthesis of sufficient amounts of this factor. In these experiments, the best results were obtained after 12 to 24 h. The decreasing effect of extracts obtained after longer intervals suggests a rapid destruction of the transfer factor.

The chemical properties of the transfer factor have been investigated. Table 3 shows that the substance responsible for the factor is dialysable. When the dialysate was submitted to phenol partition the activity was found in the phenol phase. When the dialysate was incubated for 1 h with trypsin, chymotrypsin or pancreatic ribonuclease at the optimum *p*H of these enzymes, trypsin destroyed the activity while the other enzymes had no effect. The transfer factor has been purified by precipitation with acetone, passage on 'Sephadex *G*-25', 'Dowex 50' and 'Dowex 1'. At the present state of purity, one unit of activity corresponds to approximately 5 μg of partially purified extract.

The experiments just described have shown that fear of the dark, acquired by training, can be transferred to naïve animals by material extracted from the brain of trained donors. The degree of transfer depends on at least three factors: the amount of brain administered, the length of training of the donors and the interval between training and removal of the brain. The transfer factor is probably a basic peptide with between six and ten amino-acid residues. According to the hypothesis proposed by one of us to explain the molecular storage of acquired information[14], peptides of this size would have adequate information content to secure the new neural connexions required for most types of learned behaviour.

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[1] Ungar, G., and Oceguera-Navarro, C., *Nature*, **207**, 301 (1965).
[2] Ungar, G., in *Protides of the Biological Fluids*, 1965 (edit. by Peeters, H.), 151 (Elsevier, Amsterdam, 1966).
[3] Ungar, G., and Cohen, M., *Intern. J. Neuropharmacol.*, **5**, 183 (1966).
[4] Ungar, G., and Irwin, L. N., *Nature*, **214**, 453 (1967).
[5] Ungar, G., *J. Biol. Psychol.*, **9**, 12 (1967).
[6] Fjerdingstad, E. J., Nissen, T., and Røigaard-Petersen, H. H., *Scand. J. Psychol.*, **6**, 1 (1965).
[7] Reinis, S., *Activitas Nervosa Superior*, **7**, 2 (1965).
[8] Babich, F. R., Jacobson, A. L., Bubash, S., and Jacobson, A., *Science*, **149**, 656 (1965).
[9] Rosenblatt, F., Farrow, J. T., and Herblin, W. F., *Nature*, **209**, 46 (1966).
[10] Byrne, W. L., and Hughes, A., *Fed. Proc.*, **26**, 676 (1967).
[11] Dyal, J. A., Golub, A. M., and Marrone, R. I., *Nature*, **214**, 720 (1967).
[12] Byrne, W. L., *et al.*, *Science*, **153**, 658 (1966).
[13] Gay, R., and Raphelson, A., *Psychon. Sci.*, **8**, 369 (1967).
[14] Ungar, G., *Persp. Biol. Med.* (in the press).

Simplified Procedure for Immunodiffusion Techniques on Cellulose Acetate

METHODS using cellulose acetate membrane (CAM)* for immunodiffusion were introduced 8 yr ago[1,2], but were slow in gaining recognition, possibly because the immunodiffusion had to be carried out under oil to prevent evaporation from the thin, microporous membrane. Diffusion in simple moist chambers is not entirely reliable except perhaps when gelatinized CAM ('Cellogel') is used[3]. A simple device is described here which ensures constant saturation of the CAM during diffusion by resting this on an electrolyte solution soaked filter paper support.

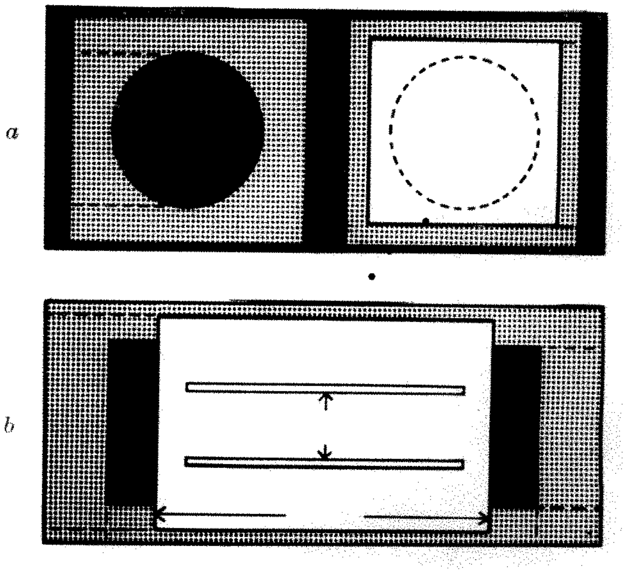

Fig. 1. *a*, Filter paper support cut out and prepared for bidimensional immunodiffusion. Dimensions: 5 mm thick, 5·5 × 5·5 cm with 3 cm diameter hole cut out. One CAM square with samples applied in position on pad. *b*, Filter paper support for immunoelectrophoresis with CAM strip placed in position. Dimensions: 5 mm thick, 5·5 × 12 cm with 3·5 × 10 cm rectangle cut out (for strips 7–10 × 5 cm). Antiserum strips applied to the CAM.

For bidimensional immunodiffusion, round holes (about 3 cm in diameter) are cut out from square filter paper pads, 4–6 mm thick (for example, a few layers of Whatman No. 3 MM), Fig. 1*a*. This forms a support which is placed on filter paper in a suitable flat container (about 2–3 cm high) with a well fitting lid and soaked with an appropriate electrolyte solution—the excess fluid being drained off; it should not be too wet. The CAM cut either in squares or disks, for example, 4 × 4 cm and of 4 cm in diameter, is marked with the required application pattern in indelible ink or pencil. It is then impregnated with electrolyte solution, blotted and placed on the pad in such a way that the diffusion area spans the gap and the periphery is in contact with the support, overlapping it by about 5–10 mm. The samples containing antigen and antibody are applied to the surface of the CAM as discrete drops by means of a micropipette or capillary, taking care to avoid "blow out" bubbles. The distance between the application sites will vary, usually it is about 5–10 mm; 0·5–2 μl. would be the usual volume for the peripheral applications and about twice as much for the central one. After the samples have been applied the lid can be closed. The diffusion time depends on the nature and concentration of the antigen and antibody, the distance of application sites and the temperature. With 1 cm distance, diffusion for 16–24 h at room temperature is usually adequate. After diffusion the CAM is removed and placed in an eluting fluid, such as 0·2 per cent 'Haemo-Sol' (Meinecke and Co., Inc., USA; Alfred Cox (Surgical),

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* All brands appear to be suitable for the purpose; Sartorius CAM was used in this study.

Ltd., UK) buffer or saline for about 10–20 min, preferably with agitation. After elution the CAM is stained, washed, dried and cleared as recommended for CAM electrophoresis. For weak precipitates 0·002 per cent Nigrosin in 2 per cent acetic acid is a most useful stain.

For immunoelectrophoresis, electrophoretic separation is carried out following the procedure recommended for CAM. For the most commonly used semi-micro technique, strips about 7–10 cm long, 3–5 cm wide and a bridge gap of 6–8 cm are used. One or two samples (about 0·5–2 μl.), applied as a drop or short streak, can be run, depending on the width. Bromophenol blue, added to the serum sample, will mark the size and shape and monitor the exact position of the albumin fraction, which should travel within about 15 mm from the bridge end. Good results have also been obtained with a separation pattern only 3 cm long. After electrophoretic separation the CAM strip is removed and placed on an impregnated rectangular filter paper support in a suitable container (Fig. 1*b*) in such a manner that only the sides of the strip are in contact with the support, overlapping it by about 5 mm. The size and shape of the support depend on the particular requirements; for example, for serum immunoelectrophoresis, 6 × 12 cm with a cut out central rectangle about 4 × 8 cm (Fig. 1*b*). Filter paper strips (about 1–2 mm wide) impregnated with antiserum are now applied to the electrophoretic strip in the desired pattern and distance, parallel to the separated fractions, about 3–4 mm as measured from the margin of the coloured albumin spot. The antiserum strips should be somewhat longer than the separation pattern and should not be applied closer than 5–6 mm from the edge of the support. Usually more antiserum will have to be added to the strip in position (carefully with a micropipette), for the ratio of serum to antiserum should be about 1 : 20–40 depending on the titre, the optimal ratio being established for each system. After the correct volume of antiserum has been applied, the container can be closed. Alternatively a ruling pen or a thin brush, guided along the edge of a ruler, proved to be quite a convenient and for some perhaps an easier way of applying the antiserum. Diffusion time for serum immunoelectrophoresis is usually 16–24 h; the optimal time is determined by experience. After diffusion, the strip is lifted from the support and the antiserum strips are washed off under the tap. The rest of the procedure is as described here for the bidimensional technique. Examples of patterns obtained are shown in Fig. 2*a* and *b*.

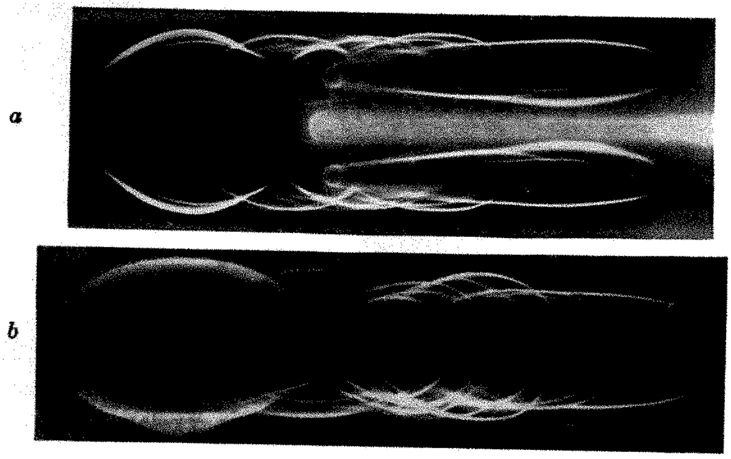

Fig. 2. *a*, Immunoelectrophoretic pattern. Stained with Ponceau S, uncleared strip, direct enlargement, negative print. Two samples of serum applied, lateral antiserum strips—anti human serum, central antiserum strip—anti immunoglobulin serum. Run for 1 h, 24 h diffusion period. *b*, Immunoelectrophoretic pattern. Ponceau S-stained uncleared strip, direct enlargement. Human serum against anti human serum, run for 1 h, 24 h diffusion period.

The most common errors of the techniques described here are: untidy application of samples; faulty electrophoretic technique; fingerprints; letting the CAM dry out at any stage before staining; imperfect adherence of CAM to support (if necessary a thin piece of impregnated filter paper, similar in shape and size to the support, placed on top of the CAM will ensure perfect contact); over saturated support pad; incorrect application and insufficient volume of antiserum; overcrowding, antigen and antiserum too close.

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[1] Kohn, J., and Consden, R., *Nature*, **183**, 1512 (1959).

[2] Kohn, J., in *Chromatographic and Electrophoretic Techniques* (edit. by Ivor Smith), **2** (Heinemann, second ed. in print).

[3] Muller-Beissenhirtz, W., and Keller, H., *Clin. Chim. Acta*, **13**, 95 (1966).

MICROBIOLOGY

Preservation of Coccidial Sporozoites by Freezing

Maintenance of cultures of coccidia for experimental purposes is laborious and expensive. To overcome these difficulties, we attempted to freeze and store sporozoites of *Eimeria meleagrimitis* Tyzzer, 1929, and *E. tenella* (Railliet and Lucet, 1891) Fantham, 1909, in liquid nitrogen vapour. Sporozoites, liberated in the usual manner[1], were suspended in media containing 7 per cent dimethyl sulphoxide and frozen to −80° C at the controlled rate of 1°/min; they were then stored in a cylindrical tank about 3 in. above liquid nitrogen.

After being stored for 1 month, sporozoites of each species were quickly thawed in water at 43° C and inoculated into cell cultures (bovine embryonic kidney; cell line, twentieth passage) and into the caeca or duodenum of chicks and turkey poults, respectively. Control cultures and birds were inoculated with unfrozen sporozoites. In cell culture, both species developed to mature schizonts in a manner previously described[2–4]. There seemed to be no significant difference between frozen and non-frozen sporozoites in their patterns of development. Frozen sporozoites inoculated into birds produced oocysts and lesions similar to those produced in birds inoculated with freshly excysted sporozoites.

Sporocysts containing sporozoites, released from oocysts by grinding[1], were also frozen. Oocysts were produced when the sporocysts were thawed and fed to birds. The intensity of the infection, however, was less than in birds fed freshly released sporocysts.

We have an abundant stock of frozen sporozoites and sporocysts of both species and intend to compare them with freshly released sporocysts or excysted sporozoites at regular intervals. Preservation of coccidia by freezing will make the maintenance of stock cultures less time consuming and make it possible to work with "parent strains" for an extended time.

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[1] Doran, D. J., and Farr, M. M., *J. Protozool.*, **9**, 154 (1962).

[2] Doran, D. J., and Vetterling, J. M., *Proc. Helm. Soc. Wash.*, **34**, 59 (1967).

[3] Doran, D. J., and Vetterling, J. M., *J. Protozool.*, **14**, 657 (1968).

[4] Patton, W. H., *Science*, **150**, 767 (1965).

Isolation of *Vibrio parahaemolyticus* from the Northwest Pacific

In Japan, *Vibrio parahaemolyticus* has been reported to be the major cause of gastroenteritis associated with the consumption of sea foods[1]. *V. parahaemolyticus* has been shown to occur in large numbers in coastal waters and marine sediments around Japan in summer months, but not in the winter[2]. This seasonal pattern is also apparent in food poisoning outbreaks due to *V. parahaemolyticus*[3].

The first reported isolation of *V. parahaemolyticus*-like organisms in the United States was from Puget Sound and Washington Coast sediments[4]. Approximately 10 per cent of the vibrios isolated from these sources showed characteristics similar to those described for *V. parahaemolyticus*. In this communication, work confirming the occurrence of *V. parahaemolyticus* in seawater, sediments and shellfish in the Puget Sound region and a procedure for the isolation and identification of this organism are reported.

Significant reductions in counts were obtained when Japanese strains of *V. parahaemolyticus* were quantitatively plated on media described by the Japanese workers[1,2,5,6] for the isolation of the organism from water, sediment and sea food materials. A satisfactory, semi-selective, solid medium was formulated on the basis of the invariant ability of *V. parahaemolyticus* to utilize starch. The components were: 0·3 per cent Bacto peptone; 0·1 per cent Bacto yeast extract; 0·5 per cent soluble potato starch; 1·5 per cent agar; 5 per cent sodium chloride; and distilled water to 100 per cent, adjusted to *p*H 7·5. Surface-inoculated plates of the medium were incubated in an anaerobic jar at 37° C for 36–48 h. Interfering organisms other than *Bacillus* types were suppressed and halophilic vibrios grew readily and could be recovered quantitatively. Where samples were known to contain large numbers of *Bacillus* types, such as marine sediments, the incorporation of 2 U/ml. of penicillin in the medium was found to be effective in greatly reducing contamination. Non-spreading, smooth, translucent colonies that showed starch hydrolysis were sub-cultured on human blood agar. Isolates that haemolysed blood at 37° C were considered presumptive positive. These isolates were then confirmed by testing in accordance with the method of Shewen[7] for Gram-negative bacteria and of Sakazaki[6] for halophilic vibrios. The testing procedure of Sakazaki is a scheme for the differentiation of *V. parahaemolyticus* from *V. alginolyticus* and *V. anguillarum* (Table 1). Sakazaki[6] reported that *V. alginolyticus* and *V. anguillarum* are the most commonly encountered vibrios from marine sources. The differentiation is based principally on the salt tolerance of these vibrios.

Table 1. DIFFERENTIATION OF *V. parahaemolyticus* FROM *V. alginolyticus* AND *V. anguillarum*

Characteristics	*Vibrio parahaemolyticus*	*Vibrio alginolyticus*	*Vibrio anguillarum*
Growth in 1% trypticase broth			
With 3% NaCl	+	+	+
With 7% NaCl	+	+	−
With 10% NaCl	−	+	−
Voges–Proskauer reaction	−	+	−
Sucrose fermentation	−	+	+ or −
Cellobiose fermentation within 24 h	−	−	+
Colony morphology on starch agar	Smooth	Swarming	Smooth

The differentiation scheme is based on Sakazaki data[6].

V. parahaemolyticus was found in relatively large numbers during the summer from all Puget Sound water, sediment and oyster samples. Numbers of this vibrio ranged from 30 to 350/ml. in the water samples, from 100 to 2,000/g in the sediment samples, and from 65 to 4,000/g in the oyster samples. In samples collected during the early spring and early fall, however, the counts of *V. parahaemolyticus* were very low; this situation paralleled that described by Japanese investigators[2]. Two hundred isolates from all the samples tested were studied extensively by standard taxonomic procedures. Simultaneously, 40 strains of *V. parahaemolyticus* obtained from Japan were tested. The more important characteristics of these organisms are shown in Table 2. Except for their ability to ferment sucrose, the Puget Sound isolates were physiologically identical. Sakazaki[1,6] reported that *V. parahaemolyticus* does not ferment sucrose; nevertheless, seven of the forty strains obtained from Japan fermented this sugar. The slow fermentation of cellobiose by *V. parahaemolyticus*, a differentiating property used by the Japanese investigators[6], was not found to be a useful taxonomic criterion. The forty Japanese strains tested showed various degrees of cellobiose fermentation, ranging from slow to very rapid. The same variability existed among the Puget Sound strains. On the basis of these data, it is concluded that the isolates from Puget Sound were physiologically indistinguishable from the Japanese strains. It is also apparent that the related biotypes *V. alginolyticus* and *V. anguillarum* (Table 1) were not encountered in the samples taken from Puget Sound.

Table 2. BIOCHEMICAL CHARACTERISTICS OF JAPANESE AND PUGET SOUND ISOLATES OF *V. parahaemolyticus*

	Organisms tested	
Reaction	Japanese strains *V. parahaemolyticus** (40)† Total positives	Puget Sound isolates (200) Total positives
(1) Growth in 1% trypticase broth		
With 0% NaCl	0	0
With 3% NaCl	40	200
With 7% NaCl	40	200
With 10% NaCl	0	3
(2) Glucose (acid)	40	200
(3) Lactose (acid)	0	0
(4) Sucrose (acid)	7	162
(5) Cellobiose (acid)	40	200
(6) Maltose (acid)	40	200
(7) Mannitol (acid)	40	200
(8) Starch (hydrolysis)	40	200
(9) Chitin digestion	33	194
(10) NO_3 reduction	40	200
(11) Gelatin liquefaction	40	200
(12) Hugh–Liefson (anaerobic) acid only	40	200
(13) Oxidase (positive)	40	200
(14) Haemolysis of blood	40	200
(15) Penicillin sensitivity (2·5 U)	0	0
(16) Single-polar flagella	40	200
(17) Gram stain (negative)	40	200

All media, unless designated, were made up with sea water or 3 per cent NaCl. All biochemical tests were run at 37° C.

* Cultures received from Japan.

† Number in parentheses indicates the number of cultures tested.

Serological identification of *V. parahaemolyticus* is not reliable[6], and so we tried to isolate bacteriophages specific for *V. parahaemolyticus* by enrichment of water, sediment and homogenized oyster preparations with the organism. It was hoped that such a procedure would provide definitive identification of the organism. Enrichment of thirty-five samples yielded two bacteriophages specific for *V. parahaemolyticus*, both from Pacific oyster homogenates. The two phages showed specificity to ten of forty Japanese strains and one Puget Sound strain of *V. parahaemolyticus*. The related biotypes, *V. alginolyticus* and *V. anguillarum*, were not lysed by these phages. It is expected that many of the Puget Sound strains are lysogenic, for there has been evidence of cross lysis between different strains. The isolation of specific bacteriophages supports the evidence that *V. parahaemolyticus* is not an exclusive inhabitant of Japanese waters and may be ubiquitous in coastal areas throughout the world. Furthermore, the association of specific vibrio phages with the Pacific oyster strongly implicates this animal as a potential carrier of *V. parahaemolyticus*.

The isolation of *V. parahaemolyticus* from inshore waters and its apparent association with oysters is of considerable significance, particularly in view of the outbreaks of food poisoning in Japan caused by this organism. The fact that many of the cases of food poisoning in the United States and other countries were due to agents of unknown aetiology[8] should justify the inclusion of specific tests for the detection of *V. parahaemolyticus* from suspected sources.

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Japanese strains of *V. parahaemolyticus* were given by R. R. Colwell, and *V. alginolyticus*, *V. anguillarum*, and additional strains of *V. parahaemolyticus* were given by R. Sakazaki.

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[1] Sakazaki, R., Iwanami, S., and Fukumi, H., *Jap. J. Med. Sci. and Biol.*, **16**, 161 (1963).

[2] Horie, S., Saheki, K., and Okuzumi, M., *Bull. Jap. Soc. Sci. Fish.*, **33**, 128 (1967).

[3] Aiso, K., Fujiwara, K., Simidu, U., Hatoh, H., Hasuo, K., Itsuka, T., Araki, T., and Uchida, A., *Ann. Rep. Inst. Food Microbiol.*, Chiba Univ., **18** (1965).

[4] Liston, J., Chan, J., and Baross, J., *Research in Fisheries*, Contr. No. 240, 51 (College of Fisheries, Univ. Washington, Seattle, 1967).

[5] Sakazaki, R., *Proc. Cholera Res. Symp.*, 30 (US Public Health Service, 1965).

[6] Sakazaki, R., undated publication of Nikeon Eiyo Kagaku Co., Ltd., Tokyo, Japan.

[7] Shewan, J. M., Hobbs, G., and Hodgkiss, W., *J. Appl. Bact.*, **23**, 379 (1960).

[8] Dack, G. M., *Food Tech.*, **20**, 39 (1966).

Identification of Rhinovirus Strain *D.C.*

In 1953, Andrewes et al.[1] reported the propagation of a virus in cultures of primary human embryonic lung from nasal washings of a patient (D. C.) with a common cold. Although no cytopathic effects were noted, culture fluids from the fourth to the tenth passage produced colds in volunteers whereas control fluids did not. At that time, attempts to repeat these experiments were unsuccessful. Using nasal washings from individuals inoculated with D. C. material, Tyrrell *et al.*[2] attempted to propagate the *D.C.* virus in primary human embryo kidney cultures in conditions favourable for recovery of rhinoviruses. Although these tests were also unsuccessful, these workers were able to propagate a virus, with production of cytopathic effects, in human diploid fibroblast cultures. This development led to the recognition of the agent as a rhinovirus. It is probable but not certain that this virus is the same as that which was in the original D. C. nasal washings.

Recently, a collaborative effort by several laboratories led to the internationally accepted numbering system for rhinoviruses. The initial report[3] by this group described a numbering system for fifty-five distinct (rhinovirus) serotypes and one subtype. Although *D.C.* virus was not included in this programme, it was of sufficient historical interest to warrant an attempt to identify it with antisera prepared against the fifty-six numbered rhinoviruses. Serum neutralization tests were carried out with *D.C.* virus and fifty-six rhinovirus antisera. Antiserum for rhinovirus type 9 (strain 211) neutralized *D.C.* virus. No reactions occurred with the remaining antisera. Subsequently, a complete cross neutralization test was performed with *D.C.* and type 9 viruses and antisera. Similar tests were made in the Salisbury laboratory using different antisera prepared against these viruses. The results of the tests are shown in Table 1. It is clear that *D.C.* virus is a strain of rhinovirus type 9 for which the prototype virus is strain 211, first described in 1961 (ref. 4).

At the time, the early experiments could only be interpreted as meaning that a virus which caused the common cold had been grown in cell culture. It is now evident that the virus was a typical rhinovirus, a member of the group of organisms found most commonly in this type of illness.

Table 1. CROSS NEUTRALIZATION TESTS WITH *D.C.* AND 211 STRAINS OF RHINOVIRUS TYPE 9

Virus strain	United States results Antiserum *D.C.* (Guinea-pig)	United States results Antiserum 211 (Goat)	United Kingdom results Antiserum *D.C.* (Rabbit)	United Kingdom results Antiserum 211 (Goat)
D.C.	5120/30*	2560/30	80/10	400/10
211	3840/30	5120/30	80/30	800/30

* Serum titre/TCD_{50}.

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[1] Andrewes, C. H., Chaproniere, D. M., Gompels, A. E. H., Pereira, H. G., and Roden, A. T., *Lancet*, i, 546 (1953).

[2] Tyrrell, D. A. J., Buckland, F. E., Bynoe, M. L., and Hayflick, L., *Lancet*, ii, 320 (1962).

[3] *Nature*, **213**, 761 (1967).

[4] Hamparian, V. V., Ketler, A., and Hilleman, M. R., *Proc. Soc. Exp. Biol. and Med.*, **108**, 444 (1961).

Primary Exo-erythrocytic Forms of *Plasmodium vinckei*

The first strain of *Plasmodium vinckei* Rodhain, 1952, was isolated by Vincke from *Anopheles dureni* caught by the River Kinga near Albertville (Katanga—Democratic Republic of the Congo). Unfortunately, at that time only the erythrocytic forms were described : cyclical transmission was not achieved in the laboratory.

P. chabaudi was found in 1965 by Landau[3] near Bangui (Central African Republic). The blood forms seemed to be very similar to *P. vinckei*.

In 1967, the isolation of a new strain of *P. vinckei* from *A. dureni* was reported[1]. The first observations on mosquito transmission[1] soon showed that the sporogony of this plasmodium is not as easy to perform as that of *P. berghei*. It not only requires a suitable temperature and humidity, an adapted invertebrate host, and a vertebrate host which would be not only susceptible to sporozoites but would also be a producer of gametocytes able to infect mosquitoes. In spite of the presence of gametocytes in laboratory white mice, only low infection rates were recorded in mosquitoes fed on these animals; only splenectomized mice were able to infect about 50 per cent of the mosquitoes. Hairless mice, however, produced a large number of viable gametocytes resulting in good infections in *A. stephensi* without splenectomy.

Out of five species of *Anopheles* (*A. stephensi*, *A. quadrimaculatus*, *A. gambiae*, *A. albimanus*, *A. atroparvus*) and *Aedes aegypti* only the first showed oocyst development and sporozoites in their salivary glands after an infective blood meal. In *A. quadrimaculatus* a high percentage of infections was obtained in the midgut. The oocysts grew normally to maturity and gave a great number of sporozoites, but not a single parasite could be found in the salivary glands.

Sporozoites were found in the salivary glands of *A. stephensi* after 14 days incubation at 20° C and after 11 days at 25° C. The size of mature oocysts varied between 40 and 70μ. The mean length of the sporozoites, measured *in vivo*, was 15μ (ranges 11–20μ). Incubation temperatures of 16° C and 27° C have been successfully used for the sporogony of *P. vinckei*.

Liver biopsies and autopsies were performed on seven mice and four rats (*Thamnomys surdaster*) inoculated with sporozoites of *P. vinckei* so as to find the exo-erythrocytic schizonts. The shortest maturation time of the tissue schizonts was determined by subinoculation of blood from these animals. The livers were fixed in Carnoy's fluid for 3 h, and the biopsies for 2 h. Sections (4μ) were stained by the Giemsa-colophonium method[2].

In one experiment, mosquitoes (*A. stephensi*) were fed on two hairless mice, on the fifth and sixth days after the mice had received an intraperitoneal inoculation of blood from a strain kept for 125 days in dry ice. The mosquitoes were incubated at 21° C. The infection rate of the salivary glands was 60 per cent. Two suspensions of sporozoites (from twenty-five and thirty-one mosquitoes) were prepared by a method used for standard inoculations of sporozoites of *P. berghei*[3] and were injected intravenously into two mice. The sporozoite count in a haemocytometer (Neubauer) showed fifty-six and seventy sporozoites/mm^3 (21,000 and 28,000 sporozoites were injected, respectively).

In another experiment, a young *Thamnomys surdaster*, probably the natural host of *P. vinckei* in Katanga, was inoculated with 45,000 sporozoites (0·9 ml. of a suspension of fifty sporozoites/mm^3). This suspension was made by crushing twenty-one mosquitoes infected after feeding on two hairless mice.

Sub-inoculation of blood from this *Thamnomys* after a single inoculation of sporozoites showed that the primary exo-erythrocytic schizonts of *P. vinckei* mature in 53 h. Schizonts were present in biopsies and autopsies of seven mice and four *Thamnomys* after single intravenous inoculation of sporozoites from various batches of mosquitoes.

At 48 h (mouse) the schizonts were immature, round, oval or irregular in shape and surrounded by a thin membrane. The nuclei are obscured by blue cytoplasmic aggregations. The mean measurement was about 24μ (19–27μ) (Fig. 1).

At 53 h (*Thamnomys*) mature and even ruptured schizonts were found. About twenty schizonts were found in each section. The mean measurements of the largest schizonts (twenty-one in number) were 33–42μ (range, 21–50μ) (Fig. 2).

At 54 h (mouse), 55 h (two mice and a tree rat), and 56 h (one mouse) most schizonts became mature. The nuclei are small, very numerous and regularly distributed and each is surrounded by a small blue cytoplasmic area. Some schizonts show rosette patterns or nuclei arranged in zones separated by areas of light blue cytoplasma. Other forms, although mature, have heavy blue cytoplasmic aggregations. Mean measurements were 23–30μ (range, 18–34μ). The schizonts found in the liver of tree rats are larger than those found in mice; mean measurements were 29–42μ (range, 23–51μ). Scanty mature forms were found in livers taken after 60–61 h from two mice and a rat.

It must be emphasized that many similarities can be found between *P. vinckei*, Rodhain, and *P. chabaudi* described by Landau and Killick-Kendrick[4] and studied by Wery[5]. For both these parasites healthy sporogony is possible at 21° C, 25° C and 27° C; sporozoites can be found in the salivary glands after 14 days at 21° C, and after 11 days at 25° C. Fully mature oocysts show a wide range of sizes from 40 to 70μ; the pigment of the oocysts is heavy and arranged rather in clumps than in lines.

The mean length of the sporozoites is 15μ. The earliest mature primary exo-erythrocytic schizonts are found at 52–53 h in *P. chabaudi* and at 53 h in *P. vinckei*. The nuclei are small and numerous, sometimes arranged in rosettes. Some schizonts have a sponge-like aspect, and blue cytoplasmic aggregations. The mean size at 53–54 h is about 30 × 40μ with a very wide range up to 51μ. It should also be remembered that both parasites are hardly distinguishable in the erythrocytic stages. Moreover, white rats and hamsters are refractory to both parasites. The course of infection in mice is more benign than *P. b. berghei*. Cross immunity between *P. chabaudi* and *P. vinckei* has been reported[6–8].

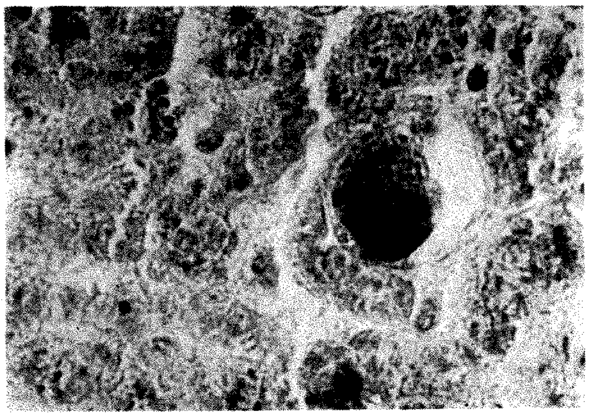

Fig. 1. Photomicrograph of section of liver (mouse) after 48 h showing immature exo-erythrocytic schizont of *P. vinckei*. Note basophilic aggregations of cytoplasma.

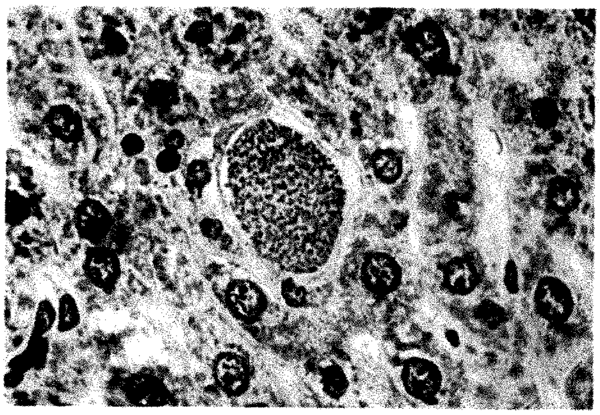

Fig. 2. Photomicrograph of section after 53 h of liver (*Thamnomys*) showing mature exo-erythrocytic schizont of *P. vinckei*.

It seems that *P. chabaudi* is morphologically and serologically very similar to *P. vinckei*, so *P. chabaudi* Landau, 1965, should be considered as a subspecies of *P. vinckei* Rhodhain, 1952. The taxonomic status of this species becomes *P. vinckei chabaudi* (Landau, 1965, nov. taxon).

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[1] Bafort, J., *Ann. Soc. Belge Méd. Trop.*, **47**, 3, 271 (1967).

[2] Bray, R. S., and Garnham, P. C. C., *Indian J. Malaria*, **16**, 153 (1962).

[3] Vincke, I. H., and Bafort. J., *Ann. Soc. Belge Méd. Trop.* (in the press, 1968).

[4] Landau, I., and Killick-Kendrick, R., *Trans. Roy. Soc. Trop. Med. Hyg.*, **60**, 5, 633 (1966).

[5] Wery, M., *Protistologica*, **3**, 275 (1967).

[6] Yoeli, M., Nussenzweig, R., Upmanis, R., and Most, H., *Nature*, **211**, 49 (1966).

[7] Cox, F. E. G., and Voller, A., *Ann. Trop. Med. Par.*, **60**, 3 (1966).

[8] Bafort, J., Vincke, I. H., and Timpermann, G., *Ann. Soc. Belge Med. Trop.* (in the press, 1968).

[9] Landau, I., *CR Acad. Sci.*, **260**, 3758 (1965).

Corrosion of Iron and Formation of Iron Phosphide by *Desulfovibrio desulfuricans*

THE classical theory of anaerobic bacterial corrosion, postulated by von Wolzogen Kühr and van der Vlugt[1], states that certain organisms, primarily those of the bacterial genus *Desulfovibrio*, remove hydrogen (electrons) that accumulate on the surface of iron (cathodic depolarization) by means of a hydrogenase, and reduce SO$_4^{--}$ to S^{--}. As a result of electron removal, iron dissolves as Fe^{++} ions at the anode.

It was recently demonstrated that a strain of *Desulfovibrio desulfuricans* would cathodically depolarize mild steel in a nitrogen atmosphere if the electron acceptor was the redox dye benzyl viologen (BV) (ref. 2). When a 1010 steel coupon was placed on a (2 per cent) agar (containing BV and *tris* buffer [*tris*(hydroxymethyl)aminomethane], *p*H 7·0 ± 0·1) surface so that half of the coupon (cathode) rested on a mass of cells (*D. desulfuricans*, mid-continent strain *A*), the BV was reduced by the cells. The iron dissolved as Fe^{++} ions at the half of the coupon (anode) that was not in contact with the cells. In summary, the reaction at the anode is: $Fe \rightarrow Fe^{++} + 2e$ and at the cathode

$$2e + 2H^+ \rightarrow 2H \rightarrow H_2 + 2BV \rightarrow 2BV_e + 2H^+$$

If a coupon was placed on 2 per cent yeast extract (YE) agar (*p*H 7·0 ± 0·1) plus BV (0·01 per cent) with cells of the same strain under one-half of the coupon, the results seemed to be the same. A depolarization current of 2 μA/cm² was obtained by the technique reported earlier[2]. When the experiment was repeated without BV in the YE agar, blackening occurred in the agar under the half of the coupon in contact with the cells. The black area became blue-green when developed with aqueous potassium ferricyanide solution (10 per cent w/v), indicating the presence of Fe^{++} ions. No Fe^{++} ions were found in the agar under the half of the coupon not in contact with the cells (Fig. 1). The black area, which extended several mm into the agar, seemed to consist of particles < 1μ in diameter. Black particles were also found in the cells. On treating the cells with the potassium ferricyanide solution, blue-green areas were found in the cells indicating local concentrations of Fe^{++}.

When $MgSO_4 \cdot 7H_2O$ (0·2 per cent) was substituted in place of BV as an electron acceptor in YE agar, the results seemed to be the same as with YE agar alone. The cells and agar under the coupon again became black. The substitution of $MgSO_4 \cdot 7H_2O$ (0·01 per cent) for BV in *tris* buffer agar produced no cathodic depolarization effect; that is, no increase in iron concentration at the anode as compared with a control system (no cells). In both of these systems, no sustained cathodic depolarization current was obtained, and the polarity of the system was unstable in that polarity continually reversed. These results seemed to indicate that the cells were reducing some material other than sulphate in the YE agar and a "short circuit" was occurring in the system (both anodic and cathodic reactions were occurring at the same area of the steel coupon that was in contact with the cells).

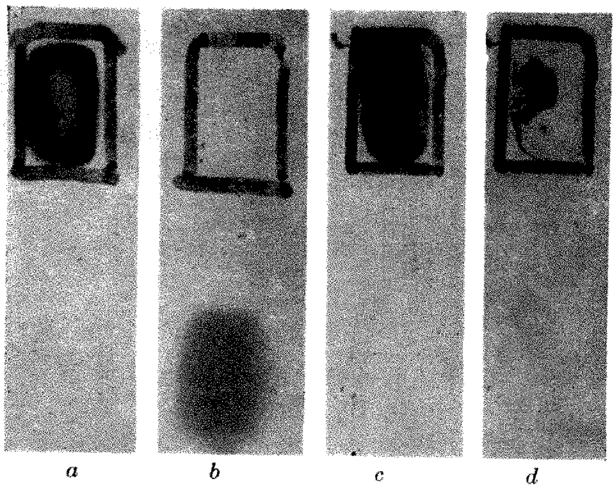

Fig. 1. Areas in agar under coupon indicating location of iron and reduced benzyl viologen (BV). *a*, Yeast extract (YE) agar plus BV surface immediately after removal of steel coupon. Dark area (cathode) caused by reduction of BV by *Desulfovibrio* cells. *b*, Same plate, 15 min after addition of aqueous potassium ferricyanide (10 per cent w/v) indicating a concentration of Fe^{++} ions at the anode (no cells). *c*, YE agar minus BV immediately after removal of steel coupon showing dark area under cells. *d*, Same plate 15 min after addition of aqueous potassium ferricyanide showing concentration of Fe^{++} ions.

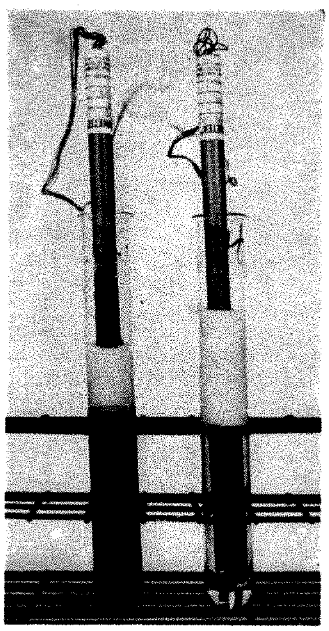

Fig. 2. Two corrosion probes (1010 steel) in yeast extract (2 per cent) (*p*H 7·0 ± 1) sealed with vaspar (equal portions of vaseline and paraffin). Tube at left, inoculated with culture of *Desulfovibrio desulfuricans* (mid-continent *A* strain). Tube at right, uninoculated control. After 2 days incubation at 26° ± 1° C.

Sterile YE broth (2 per cent) *p*H 7·0 ± 0·1, containing a steel coupon, when inoculated with a pure culture and incubated in a hydrogen atmosphere, often became black within 24h, depending on the effectiveness of oxygen removal. A clump of cells on the surface of the steel coupon often became black within 1 h or less. In a nitrogen atmosphere, the time for blackening was often delayed by a day or two. The same results were observed with three other cultures of *Desulfovibrio*, one of which was isolated from a tubercle in a water pipe, and a species of *Clostridium* isolated from tap water. The surface of the steel coupon usually remained just as bright as when it was placed in the YE broth; that is, except for where it came in direct contact with the black sediment at the bottom of the tube.

An attempt was made to measure this corrosion in tubes of inoculated YE (2 per cent) broth (10 ml./tube) using a corrosion probe (model *S*001, *S*4) of 1010 steel (Magna Corporation, Santa Fe Springs, California) with a Corrosometer (model *CK*, Crest Instrument Co., Santa Fe Springs)[3]. After several weeks' incubation at 26° ± 1° C, no detectable difference in resistance in the probe specimen could be seen between three probes in inoculated YE broth (0·14 mg SO_4^{--}, precipitated as the barium salt/ml.) and three probes in sterile YE broth. One of the inoculated tubes with probe and a sterile control are shown in Fig. 2. Because the corrosion rate was less than 2·6 mg/dm²/day (limit of sensitivity of metre for a 3-day interval), it may have been equal to or less than the corrosion rate established for the cathodic depolarization of 1010 steel using BV as the electron acceptor (2·5 mg/dm²/day[1]). Corrosion of probes could not be detected within 4 weeks in inoculated YE plus $MgSO_4 \cdot 7H_2O$ (0·2 per cent), and YE plus sodium lactate (4 ml./l.) and $MgSO_4 \cdot 7H_2O$ (0·2 per cent).

It was suspected from some observations made during the isolation of *Desulfovibrio* from tubercles in a water main that the blackening of the YE broth might be produced by a cell-free enzyme liberated in the medium. Seitz filtrates (cell-free) of 3-day-old YE broth cultures of the four strains of *Desulfovibrio* and the *Clostridium* sp.

containing a steel coupon were subsequently found, using the Thunberg technique, to reduce methyl viologen (Eo = −411 mV), and benzyl viologen (Eo = −315 mV) in a hydrogen atmosphere. No reduction was observed in a nitrogen atmosphere. A sterile 1010 steel coupon in the Seitz filtrate of these strains in a hydrogen atmosphere produced blackening of the filtrate, usually within 24 h, if oxygen removal was nearly completed. In the same system under a nitrogen atmosphere, a flocculent black precipitate was first formed on the surface of the metal. Complete blackening of the filtrate was delayed for about 24–48 h compared with the time of blackening in a hydrogen atmosphere. It seemed that the small amounts of hydrogen on the steel surface were evidently initially limiting the reaction.

A sterile Seitz filtrate of 3 day old blackened YE broth culture of the mid-continent strain in contact with 1010 steel coupons under H_2 was again allowed to react with sterile 1010 coupons. After 3 days under an H_2 atmosphere a black precipitate was obtained. X-ray diffraction analysis of this non-magnetic material (dried in vacuum) was negative for elemental iron or any compound of iron. When the black material was heated to 2,250° F in vacuum for 15 min and slowly allowed to cool to room temperature for about 20 h, it was magnetic. X-ray diffraction analysis of this material indicated that it was iron phosphide (Fe_2P). Mössbauer studies indicated that the material before heating was also iron phosphide, but colloidal in nature.

Further investigation of this reaction is in progress. It is suspected that phosphate in the yeast extract is reduced in the presence of hydrogen by the hydrogenase of *Desulfovibrio* to phosphine, which then reacts with the ferrous ions to produce iron phosphide[4,5]. If this assumption is validated, it would also indicate that the phosphate may be selectively reduced in preference to the sulphate, in the presence of iron, because both compounds are present in the yeast extract.

Because the black precipitate of iron phosphide seems to be similar to the black precipitate of iron sulphide, and because blackening of the media in which sulphate reducers are growing is usually assumed to be iron sulphide, further analysis of this material would seem warranted.

I thank C. J. Bechtoldt and Dr J. J. Spijkerman, US National Bureau of Standards, for the X-ray diffraction analysis and Mössbauer studies, respectively.

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[1] von Wolzogen Kühr, C. A. H., and van der Vlugt, L. S., *Water*, **18**, 147 (1934).
[2] Iverson, W. P., *Science*, **151**, 986 (1966).
[3] Roller, D., and Scott, W. R., *Corros. Tech.*, **8**, 71 (1961).
[4] Rudakov, K. J., *Zentr. Bakteriol, Parasitenk.*, Abt. II, **79**, 229 (1920).
[5] Tsubota, G., *Soil Plant Food*, **5**, 10 (1959).

IMMUNOLOGY

Synthesis of some Serum Proteins in Lymphocytes stimulated by Phytohaemagglutinin

SMALL lymphocytes are stimulated by phytohaemagglutinin (PHA) to increase the synthesis of RNA and DNA and to undergo transformation into blast-like cells. It has been shown by immunofluorescent and electrophoretic techniques, as well as by incorporation of amino-acids labelled with carbon-14, that stimulation with PHA results in the production of globulins[1-5], some 10 per cent or more of which can be precipitated by specific anti-γ-globulin[5]. In an investigation using radioimmunoelectrophoresis, Sell *et al.*[3] found no immunoglobulins, but did find α and β-globulins which were not clearly identified. The problem of *in vitro* synthesis of immunoglobulins in several lymphocytes is therefore still a matter of controversy.

We investigated the incorporation of lysine labelled with carbon-14 into the proteins of lymphocytes of healthy individuals stimulated with PHA and in patients with IgG and IgA plasmacytomas.

Lymphocyte suspensions of volume 2 ml. ($4–5 \times 10^6$ lymphocytes) were prepared in the usual way[6] using 8 ml. of medium 199 (Difco) with and without PHA. Twenty-four hours after the preparation of the cultures, lysine labelled with carbon-14 (Radiochemical Centre, Amersham; specific activity 41·0 μCi/mg) was added in a concentration of 0·5 μCi/10 ml. of culture. Lymphocytes of normal individuals were collected according to the method of Rabinowitz[7,8] and examined in the same conditions.

After 3 days, the cells were centrifuged and washed twice with Hanks solution. The cell sediments of several cultures were then combined, frozen and thawed four times. After centrifugation at 35,000g the precipitate—which consisted mostly of broken cells—was discarded and the supernatant diluted 1 : 1 with autologous serum as a carrier medium. Ouchterlony double diffusion tests and immunoelectrophoresis were performed in duplicate.

In the autoradiographic experiments, 'Currix' X-ray film (Gevaert, clear base) and *G*150 developer (Gevaert) were used. Autoradiography was carried out simultaneously on the immunoelectrophoreses of PHA-stimulated and non-stimulated lymphocyte preparations. The films were exposed for 1, 2, 4 and 6 months. The following antisera were used: two different polyvalent horse anti-human sera (Institute Pasteur, Paris), two different rabbit anti-human sera (Behring-Werke AG, Marburg), specific antisera against IgG, IgA, IgM, K-Bence-Jones, L-Bence-Jones, F(ab), F(c), β_{1a}, β_{1c}, α1-lipoprotein, α1-antitrypsin, α1-orosomucoid, and Gc-globulin (this antiserum was not strictly monospecific, but precipitated two other α-globulins besides Gc-globulin).

Proteins labelled with carbon-14 could be detected in all cultures to which PHA had been added. Their mobility toward the anode was similar to the α1, α2 and β-globulins of serum. The concentration of the proteins which moved towards the cathode was lower; usually two fairly well defined zones could be seen (Fig. 1). The findings in the lymphocyte lysates of both normal individuals and myeloma patients were identical. There was no increase in paraprotein concentration. In the non-stimulated lymphocytes no proteins directed towards the anode could be detected after exposure for 1–2 months, but traces of such proteins were, however, found after exposure for 6 months (Fig. 1*b*).

Using polyvalent anti-human antiserum two precipitates differing in concentration were recognized in the α1-globulin region: one precipitate in the α2-globulin region and a short weak precipitate in the γG-globulin region (Figs. 1*a*, 2). Using specific antisera the precipitate located in the α2-globulin region was identified as α2-macroglobulin.

The strong line of precipitate in the α1-globulin region corresponded with a protein precipitable by anti-Gc-globulin serum. Because this antiserum was not strictly monospecific against Gc-globulin, it remains to be seen whether this precipitate is indeed Gc-globulin (Hirschfeld's "group specific component"). After exposing the immunoelectrophoresis for 6 months this protein could also be shown in the non-stimulated cell lysates.

Furthermore, after stimulation with PHA and exposure of the films for 4–6 months an IgG-like precipitate could be detected. It was always very weak in concentration and therefore present only in a short zone within the IgG-precipitate of the carrier serum, located close to the well towards the cathode.

These findings suggest that three to five proteins are synthesized in lymphocytes stimulated by PHA. On the basis of immunoelectrophoretic criteria and Ouchterlony techniques it was shown that these proteins are identical with serum proteins. An adsorption of material labelled with carbon-14 by the precipitates of the carrier serum seems unlikely, because the ability to bind such material has been attributed to lipoproteins and albumin but not to the proteins described here. Although protein synthesis in non-stimulated lymphocytes is significantly lower, an α1-globulin (perhaps Gc-globulin) can also be demonstrated. In comparison with the results of Sell *et al.*[3] it was noted that their α-globulin-precipitate was probably identical with the protein we have identified as Gc-globulin (?). In their experiments, a relatively short exposure time was chosen which could explain why other serum proteins and IgG could not be identified. The concentration of IgG—which was not increased in myeloma—was extraordinarily low and was certainly not too high as was suggested by others[1,5]. On the basis of the sensitivity of the antisera and the extraordinarily long exposure time the concentration of IgG can be estimated to be about 1/100 to 1/1,000 of the total protein synthesis after PHA stimulation.

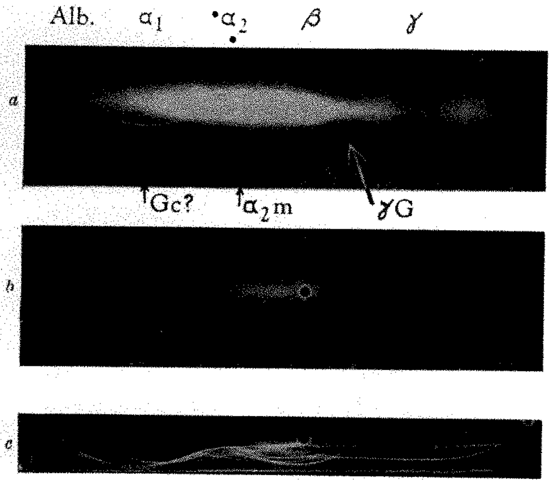

Fig. 1. Autoradioimmunoelectrophoretic analysis of carbon-14-labelled proteins in PHA-stimulated (*a*) and in normal lymphocytes (*b*); control immunoelectrophoresis of carrier serum (*c*). Antisera against normal human serum. Anode to the left; exposure, 6 months.

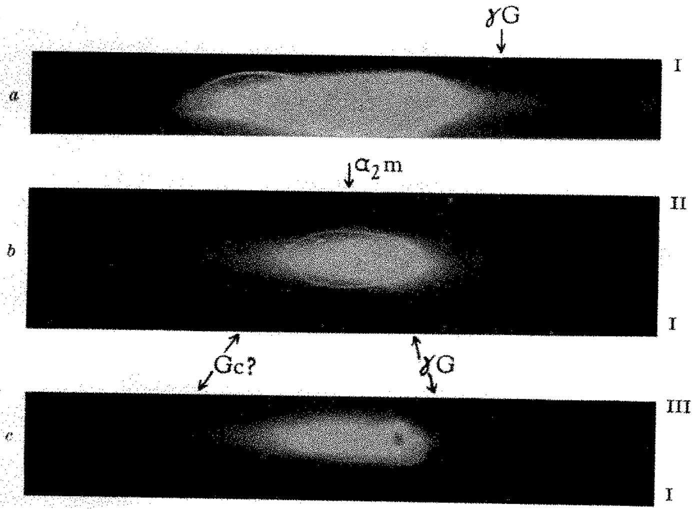

Fig. 2. Autoradioimmunoelectrophoresis of carbon-14-labelled proteins in PHA-stimulated lymphocytes. (*a*) γG-myeloma, (*b*) and (*c*) normal person. I, Horse antiserum against human serum. II, Rabbit antiserum against α_2 macroglobulins and, III, Gc-globulin. Anode to the left.

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[1] Bach, F., and Hirschhorn, K., *Exp. Cell Res.*, **32**, 592 (1963).
[2] Elves, M. W., Taylor, G., Roath, S., and Israels, M., *Lancet*, i, 1292 (1963).
[3] Sell, S., Rowe, D. S., and Gell, P. G. H., *J. Exp. Med.*, **122**, 823 (1965).
[4] Forbes, I. J., and Henderson, D. W., *Ann. Int. Med.*, **65**, 69 (1966).
[5] Huber, H., Winkler, H., Reiser, G., Huber, C., Gabl, F., and Braunsteiner, H., *Klin. Wschr.*, **45**, 204 (1967).
[6] Scheurlen, P. G., Pappas, A., and Ludwig, T., *Klin. Wschr.* (in the press).
[7] Rabinowitz, Y., *Blood*, **23**, 811 (1964).
[8] Rabinowitz, Y., *Blood*, **26**, 100 (1965).

Induction of Immunological Paralysis to Heterologous Tumours

TRANSPLANTATION studies have proved that the relative ease of tolerance induction in newborn animals is inversely proportional to the strength of the antigens to which tolerance is to be induced—that is to the strength of immunity they can induce in adults[1]. The rate and pattern of heterograft rejection, as well as the difficulty of inducing acquired tolerance to heterografts, has demonstrated the high strength of the interspecific histocompatibility barrier[2,3].

The failure of xenogeneic lymphoid cells to induce tolerance to heterografts when administered to the newborn may reveal some peculiarity of the host immunological apparatus, such as (*a*) a precocious maturation of the reactivity to heterologous antigens during ontogenesis[4,5], or (*b*) a genetically predetermined ability of a large part of the immunological system to react against some "obligatory" strong antigens[6,7].

A large part of the cell population reacting against xenogeneic antigens can be conceived not only on the grounds of an *a priori* immunological potency, but also as a consequence of a cumulative mechanism of action of the different non-self determinants present in the antigenic complex[1,8,9]. The more genetically dissimilar the antigen donor and recipient the larger are the doses of antigen needed[7]. Induction of heterograft tolerance would presumably depend on the administration of very large doses of donor antigens to inhibit the reactive population of immunocompetent cells.

It seems, however, that the most important condition for tolerance is the persistence of antigen[10]. If this is so, lack of the tolerogenic effect of the xenogeneic lymphoid cells might be the consequence, not of a violent host reaction but of an inability of the xenogeneic lymphoid cells to multiply[11] and thus cause persistence of antigens in the presence of an excess of the host's very strong antigens. Theoretically, fulfilment of this requirement for tolerance demands that antigen sould be repeatedly introduced. This principle is well illustrated by immunological paralysis to defined antigens, when, more often than not, unresponsiveness which is not actively maintained by continuous administration of the antigen, is eventually replaced by immunity[12].

To investigate which of these critical factors is essential to the induction of heterospecific tolerance, we have attempted to define the conditions of time and dosage required for the induction of tolerance to mouse tumours by administering xenogeneic spleen cells to rats. This communication is concerned with the onset and duration of unresponsiveness and with the kinetics of tolerance, as determined with tumour grafts. Tolerance was considered to be complete when actively growing tumours resulted after challenge. Animals which appeared tolerant at first but developed immunity later (rejecting the tumours) were considered to be partially tolerant.

For the paralysis treatment, spleen cells from *CBA* mice prepared by the method of Billingham[13], were inoculated intraperitoneally into newborn inbred Lewis rats, which were between 24 and 48 h old when the treatment began. The same amount of spleen cells (that is 250 to 450 × 10^6) per recipient was administered, either in single doses or in fractionated doses, given as five or ten injections at daily intervals.

The rats were challenged when 30 days old, by intramuscular inoculation of trypsinized cell suspensions containing 30 × 10^6 viable tumour cells. All except one group were challenged with tumour cells derived from a mammary carcinoma (*Ca/CBA*) that had appeared spontaneously in a *CBA* mouse. This tumour was in transplant generation 1–3 (performed by subcutaneous grafting of tumour fragments in *CBA* mice) when it was used in these experiments. In order to verify previous observations the non-specificity of heterologous tolerance to the isoantigens of the donors[14], we tested one group of rats with a sarcoma induced by methylcholanthrene in a *C57Bl/ScSn* mouse (*MC/Bl*$_1$ sarcoma).

Table 1. INDUCTION OF TOLERANCE IN NEWBORN RATS TREATED WITH MICE SPLEEN CELLS

Induction of tolerance		Challenge (30 day old rats)		No. of rats which developed tumours/No. of rats tested	No. of rats with developing tumours/No. of rats with regressing tumours
Injection period (days of life)	Total *CBA* spleen cell dose/rat	Days from treatment until challenge	Tumours (30 × 10^6 cells) used for challenge		
1	250	29	*Ca/CBA*	0/10	
1	350	29	*Ca/CBA*	0/10	
1	450	29	*Ca/CBA*	0/12	
1–5	250	25	*Ca/CBA*	0/8	
1–5	350	25	*Ca/CBA*	0/10	
1–5	450	25	*Ca/CBA*	0/12	
2–6	250	24	*Ca/CBA*	0/12	
2–6	350	24	*Ca/CBA*	0/8	
2–6	450	24	*Ca/CBA*	0/10	
1–10	250	20	*Ca/CBA*	7/12	2/5
1–10	350	20	*Ca/CBA*	10/12	4/6
1–10	450	20	*Ca/CBA*	16/16	16/0
2–11	250	19	*Ca/CBA*	5/10	0/5
2–11	350	19	*Ca/CBA*	8/10	2/6
2–11	450	19	*Ca/CBA*	10/12	8/2
1–10	450	20	*MC/Bl*$_1$	20/20	20/0

The results in Table 1 show that tolerance to the *Ca/CBA* tumour was not induced in rats by one or five injections of *CBA* spleen cells, irrespective of the dose used. In groups receiving ten injections of spleen cells, beginning in the first 24 or 48 h of life, tumours appeared after challenge, and their incidence seems to be directly related to the total number of spleen cells inoculated. The degree of tolerance also seems to depend on the dose of spleen cells inoculated. In groups treated with 450 × 10^6 cells, most animals developed lethal tumours when tested with *Ca/CBA* tumours and *MC/Bl*$_1$ sarcomas, and died at between 7 and 8 weeks of age; tumours also appeared in groups treated with smaller doses, but were rejected after 2 weeks in most rats.

Recovery from paralysis seems to be caused by the production of new immunocompetent cells by escape from suppression of previously tolerant cells, depleted of an internal store of antigen[15,16]. We conclude that in partially tolerant animals the conditions of tolerance and immunity may potentially coexist, the degree and duration of unresponsiveness being dependent on the ratio[14,17] of the number of blocked cells to the number of sensitized cells. On the assumption that one state can be converted into the other, depending on the concentration of antigen per cell and/or per population, we tried to reinduce tolerance by further supply of spleen cells in animals which had rejected the heterologous tumours.

Rats given, during the first 10 days of life, a total of 250–350 × 10^6 *CBA* spleen cells in ten injections, were challenged at 30 days with 30 × 10^6 *Ca/CBA* tumour cells. Animals which rejected the tumours were reinoculated between the fortieth and fiftieth days of life with 115–125 × 10^7 spleen cells given in ten injections. A control group of normal rats was treated at the same age with 150 × 10^7 *CBA* spleen cells. When 60 days old, they were inoculated intraperitoneally with 30 × 10^6 *Ca/CBA* tumour cells. Table 2 shows that although the total dose of spleen cells was the same for all groups (that is 150 × 10^7 cells), tolerance resulted only in rats treated during the neo-natal period. The incidence and degree of tolerance again seemed to be related to the dose administered within this period. This result suggests that the probability of success in tolerance reinduction depends on the degree of the host's unresponsiveness when the antigen is reintroduced.

Table 2. REINDUCTION OF HETEROGRAFT TOLERANCE IN POST-TOLERANT RATS

No. of *CBA* spleen cells (× 10^6) inoculated		Total spleen cell dose per rat (× 10^6)	No. of *Ca/CBA* tumour cells (× 10^6) inoculated: days of age:		Results obtained after challenge of 60 day old rats	
1–10 days of age	40–50 days of age		30	60	No. of rats which developed tumours/No. of rats tested	No. of rats with developing tumours/No. of rats with regressing tumours
250	1,250	1,500	30	30	6/18	3/3
350	1,150	1,500	30	30	10/17	7/3
—	1,500	1,500	—	30	0/20	—

We investigated mouse lymphoid cell chimaerism in tolerant rats. Chromosome patterns were studied in the thymus, femoral bone marrow and spleen of rats bearing tumours of lethal size. Neither rats challenged at 30 days nor those challenged at 60 days showed mouse metaphases in their lymphoid organs. These animals showed no symptoms of runt disease.

Histological examination (using haematoxylin and eosin) of tumours developed in heterologous hosts, revealed no modification of the original mouse tumour. *Ca/CBA* tumours invaded the rat thigh muscle and the peritoneal cavity (Figs. 1–2). If fragments of these tumours were back transplanted in *CBA* mice, typical *Ca/CBA* tumours developed. The mouse tumours growing in rats were not transplantable to untreated adult Lewis rats.

The fact that tolerance to heterologous tumours was inducible in 48 h old rats invalidates the assumption[4,5] that the host's immunological immaturity at the time of treatment with antigen is a necessary and short-lived condition for the induction of tolerance. On the other hand, the alternative hypothesis that tolerance is exclusively related to the establishment of an optimum relationship between the amount of antigen and the number of immunocompetent cells[15] does not fully account for our finding that whereas xenogeneic cells given in a single injection are inefficient, the same quantity of cells administered repeatedly is likely to induce tolerance. These results and earlier ones[14,17,18] show that the most important element in heterologous tolerance induction is the administration of antigen over a prolonged period. Heterologous spleen cells seem unable to achieve lymphoid chimaerism, and so it might seem that repeated inoculations are necessary to maintain the critical concentration of antigen. But because tolerance was reversed even after the onset of immunological rejection, it may be that heterospecific tolerance can be achieved gradually through the action of antigen in blocking the sensitized and hypersensitized cells if these are exposed continuously to large concentrations of antigen. The experiment now described was an attempt to determine whether paralysis can be induced in immunologically mature animals where a high

Table 3. INDUCTION OF HETEROGRAFT TOLERANCE IN IMMUNOLOGICALLY MATURE RATS

Tumour cells injected	Paralysis treatment Route of inoculation	Age (in days) of rats when injected	Results No. of rats which developed lethal tumours/No. of rats treated
385×10^6 *MC/Bl*$_1$ sarcoma cells/rat	Intraperitoneal + intramuscular alternately	17–87	10/10
385×10^6 *MC/Bl*$_1$ sarcoma cells/rat	Intraperitoneal + intramuscular alternately	27–97	10/10

degree of immunity is the rule[19], by injection of such strong antigens as heterologous cells.

The paralysis treatment consisted of a long course of tumour cell injections. The mouse sarcoma *MC/Bl*$_1$ in transplant generations 1–6, was used throughout this experiment. The tumour was maintained in the *C57Bl/ScSn* strain by intramuscular transfer of 5×10^6 cells at 2–3 week intervals. Two groups of Lewis rats were treated simultaneously (Table 3): the first received 385×10^6 viable *MC/Bl*$_1$ cells for 10 weeks, starting at 17 days of age; the second group received the same treatment, starting at 27 days of age. Injections were given daily, alternately intraperitoneal and intramuscular, during the first month; every other day during the second month, and once a week during the last 2 weeks. Tumours became palpable approximately 10 days after the last injection. In the next 18–20 days, all animals developed large tumours which had invaded the peritoneal cavity and lungs (Figs. 3–5). Transplantation of rat tumours back into *C57Bl/ScSn* mice confirmed their similarity to the original mouse tumours. These experiments seem to confirm our hypothesis that a process of gradual paralysis may be involved in the induction of heterospecific tolerance.

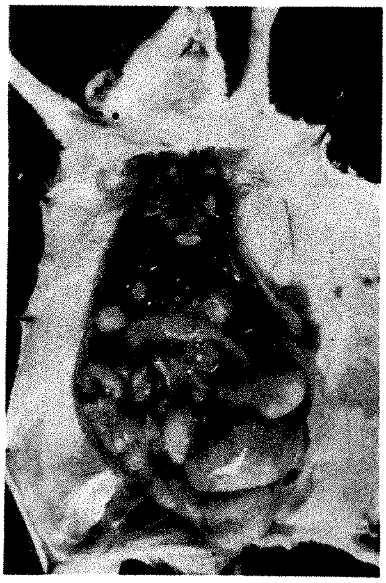

Fig. 1. An 85 day old rat, in which tolerance was reinduced by spleen cell treatment. The organs of its peritoneal cavity are invaded by a *Ca/CBA* tumour heterotransplant. Inoculation of tumour cells given on the 60th day of life.

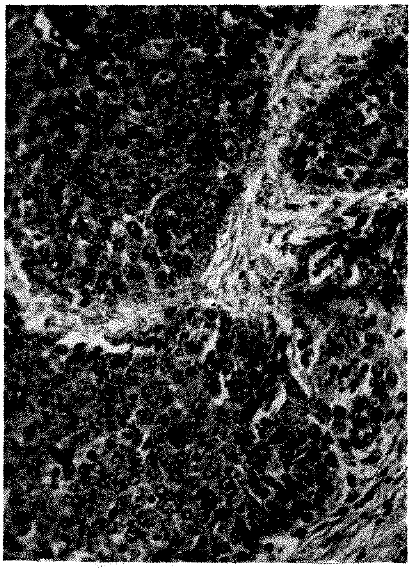

Fig. 2. *Ca/CBA* tumour growing in rat. (Haematoxylin and eosin. × c. 70.)

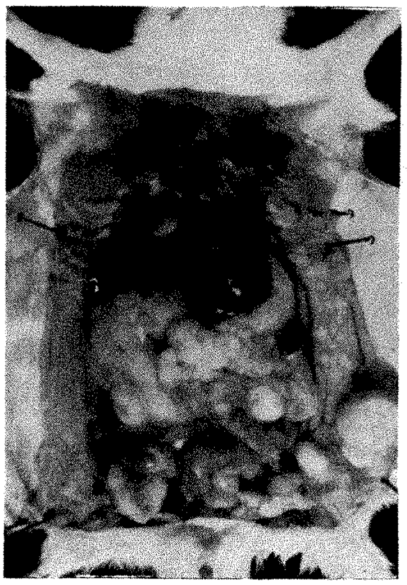

Fig. 3. Intraperitoneal invasion of the *MC/Bl*$_1$ mouse sarcoma in a 127 day old rat, treated with tumour cells between the twenty-seventh and ninety-seventh day of life.

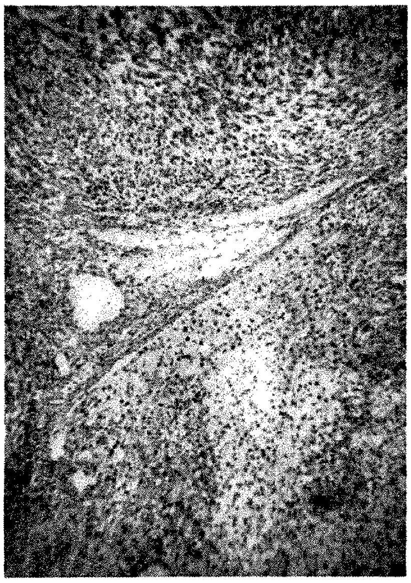

Fig. 4. *MC/Bl*$_1$ sarcoma growing in a 3 month old rat, showing tumour invasion of the liver. (Haematoxylin and eosin. × c. 70.)

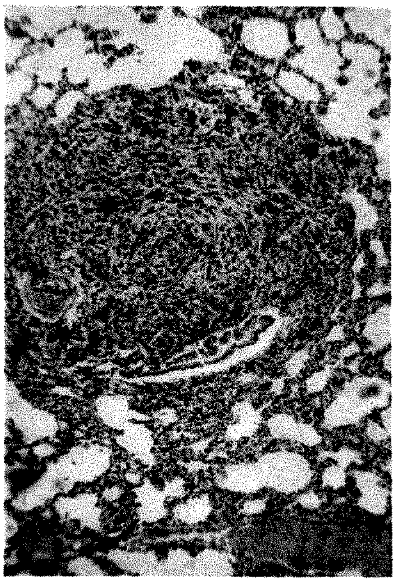

Fig. 5. The heterotransplanted MC/Bl_1 sarcoma invading the lung of a 3 month old rat.

Our findings show that: (*a*) immunological tolerance to heterografts can be induced through long-term paralysing treatment, not only in newborn animals but in adults as well; (*b*) tolerance can be maintained or reinduced by an adequate supply of antigen at the time when recovery from paralysis occurs; and (*c*) xenogeneic spleen cells have a tolerogenic effect without producing a graft versus host reaction.

Heterograft tolerance is specific to the donor's species but displays no specificity to the donor's isoantigen so it might be possible to supply antigens and transplants from heterogeneous donor populations.

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[1] Lengerová, A., and Matoušek, V., *Folia Biol.*, **12**, 319 (1966).

[2] Woodruff, M. F. A., *The Transplantation of Tissues and Organs* (Thomas, Springfield, 1960).

[3] Perper, R. J., and Najarian, J. S., *Transplantation*, **4**, 700 (1966).

[4] Billingham, R. E., Brent, L., and Medawar, P. B., *Nature*, **172**, 603 (1953).

[5] Billingham, R. E., Brent, L., and Medawar, P. B., *Phil. Trans. Roy. Soc.*, B, **239**, 357 (1956).

[6] McBride, R. A., and Simonsen, M., *Transplantation*, **3**, 140 (1965).

[7] McBride, R. A., Nisbet, N. W., and Skowron-Cendrzak, A., *Transplantation*, **5**, 569 (1967).

[8] Graff, R. J., Silvers, W. K., Billingham, R. E., Hildemann, W. H., and Snell, G. D., *Transplantation*, **4**, 605 (1966).

[9] Eichwald, E. J., Davidson, N., and Moore, M., *Transplantation*, **3**, 332 (1966).

[10] Mitchison, N. A., in *Mechanisms of Immunological Tolerance* (edit. by Hašek, M., and Lengerová, A.), 245 (Publ. House Czech. Acad. Sci., Prague, 1962).

[11] Simonsen, M., *Prog. Allergy*, **6**, 412 (1962).

[12] Coons, A. H., in *Tolérance Acquise et Tolérance Naturelle à l'Egard de Substances Antigéniques Définies*, 255 (C.N.R.S., Paris, 1963).

[13] Billingham, R. E., in *Transplantation of Tissues and Cells* (edit. by Billingham, R. E., and Silvers, W. K.), (87 Wistar Institute Press, Philadelphia, 1961).

[14] Suciu-Foca, N., and Nachtigal, M., *Cancer Research*, **27**, 1384 (1967).

[15] Nossal, G. J. V., in *Tolérance Acquise et Tolérance Naturelle à l'Egard de Substances Antigéniques Définies*, 429 (C.N.R.S., Paris, 1963).

[16] Mitchison, N. A., *Immunology*, **9**, 129 (1965).

[17] Suciu-Foca, N., and Nachtigal, M., *Oncology*, **21**, 143 (1967).

[18] Suciu-Foca, N., and Nachtigal, M., *Folia Biol.*, **13**, 350 (1967).

[19] Dresser, D. W., *Immunology*, **9**, 261 (1965).

PATHOLOGY

Mites in Bedroom Air

VOORHORST *et al.*[1] found that, among the many constituents of house dust causing allergic reactions of the respiratory tract (rhinitis and asthma) in people sensitive to house dust, a hitherto little known Pyroglyphid mite, *Dermatophagoides pteronyssinus*, contains allergens that are much more potent than those of other constituents. They also showed that excretory or secretory products of the mite contain the allergens. It therefore seemed probable that the mite could cause severe inhalation reactions should it become temporarily airborne and reach the upper or lower respiratory tract. Mechanical disturbance during bedmaking seemed a possible launching mechanism for making the mites airborne, especially because one of us (A. M. C.) found that *D. pteronyssinus* is very abundant in mattress dust.

To test this hypothesis a cyclone dust extractor, of diameter 15 cm, powered by centrifugal fans (giving an intake velocity of 300 cm/s in tests 1–4 (Table 1), and 1,900 cm/s in tests 5–7) was operated 2 ft. above the floor to collect dust from the air during bedmaking. In tests 1–4 the apparatus would extract particles efficiently down to the equivalent of 10μ spheres from 0·4 m^3 of air per min, and in tests 5–7 its efficiency should have been increased to retain 3μ spheres from 3·0 m^3 of air per min. The cyclone was operated during bedmaking (including turning the mattress) and after for 5 to 15 min each day. Samples were accumulated for several days in each test. The samples consisted of a loose pellet of fibrous material and a fine deposit on the wall of the receptacle consisting mainly of material from the stratum corneum.

Table 1. NUMBERS OF *Dermatophagoides pteronyssinus* CAUGHT IN CYCLONE DUST EXTRACTOR DURING BEDMAKING IN 1967

Sample	Collection date	Dust (mg/m^3 of air)	Mites/m^3 of air sampled	Mites/g of airborne dust
	Patient's bedroom, Harpenden			
1	March (7 days)	2·5	0·2	80
2	March (7 days)	—	0·2	62*
3	April (7 days)	—	0·34	136*
4	May–June (20 days)	2·1	0·043†	21†
	Patient's bedroom, Dulwich			
5	September (7 days)	1·7	0·04	44
	Men's ward, King's College Hospital			
6	October (6 days)	0·12	Nil	Nil
7	October (6 days)	0·25	Nil	Nil

* Estimated.

† After regular vacuum cleaning of mattress.

The samples were weighed, dispersed in 90 per cent lactic acid, held in this at 55° C for 24–48 h, and the suspension was examined for mites. These were removed, mounted for microscopic examination, identified and counted.

As well as *D. pteronyssinus*, a few representatives of other species were present. These included a second Pyroglyphid species, *Euroglyphus maynei*, the Acarid species *Acarus siro* and *Tyrophagus putrescentiae*, and the Glycyphagid mite *Glycyphagus domesticus*.

Clearly *D. pteronyssinus* can become airborne during bedmaking, and its numbers decreased with attempts to remove mattress dust (Table 1); it was not present in hospital air. Others have shown that mites can be inhaled[3-5]. Whole mites or considerable portions in the dust can be identified to species level microscopically, but this cannot yet be done with the finest debris of fragmented mites, which with their faecal pellets also occur in the airborne dust, and which may constitute a more serious source of allergens than the mites themselves.

We thank Professor A. C. Cunliffe for arranging the aerobiological sampling in hospital wards.

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[1] Voorhorst, R., Spieksma, M. I. A., and Spieksma, F. T. M., *Allergie Asthma*, **10**, 329 (1964).
[2] Cunnington, A. M., *Acta Allerg.*, **22**, 415 (1967).
[3] Carter, H. F., Wedd, G., and D'Abrera, V. St. E., *Indian Med. Gaz.*, **79**, 163 (1944).
[4] Soysa, E., and Jayawardena, M., *Brit. Med. J.*, No. 4383, 1 (1945).
[5] Taboada, M. de F., *Brit. Med. J.*, i, 437 (1954).

Absorption of *d*-Glucose by the Rabbit Jejunum during Cholera Toxin-induced Diarrhoea

CHOLERA exotoxin causes fluid production by the intestine when instilled into jejunal or ileal loops of anaesthetized rabbits[1-3]. Profuse quantities of isotonic fluid are produced by the small intestine without histological alteration of the mucosa[4]. The pathogenesis of the increased fluid production by the intestine in cholera is unsettled. It has not been determined whether the fluid production is the result of increased intestinal secretion, decreased intestinal absorption or a combination of these two factors. To assess the importance of altered intestinal absorption, absorption of *d*-glucose by the rabbit jejunum was measured both in the presence and absence of cholera diarrhoea.

Ten white male New Zealand rabbits weighing 2–3 kg were studied after an 18 h fast. The animals were anaesthetized with 10 mg/kg of sodium pentobarbital given intravenously. Hydration was maintained with intravenous Ringer lactate infused at a rate of 60 ml./h for the duration of the experiment (4 h). The abdomen was opened and a loop of jejunum approximately 15 cm long (7–10 vascular arcades) just distal to the ligament of Treitz was isolated. Both ends of this loop were transected between vascular arcades to preserve the blood supply[5]. The cut ends of the intestine were ligatured to prevent spillage.

The proximal end of the loop was cannulated with flanged polyethylene tubing (*PE* 200), while the distal end was cannulated with latex rubber tubing (internal diameter 3/16 in.). A similarly constructed loop was made in the adjacent jejunum thus creating two jejunal loops in each rabbit. The isolated loops were carefully washed with 40 ml. of Ringer lactate, emptied and then returned to the abdominal cavity. The abdomen was then closed, leaving only the catheters exposed. Rectal temperatures were monitored and the body temperature was maintained between 102° and 104° F with the aid of a heat lamp.

In one group of five rabbits (cholera group) 10 ml. of cholera toxin[6] prepared by Dr John Feeley (Lot No. *H*062867) was placed in both loops and allowed to remain there for 4 h. In five other control rabbits (control group) 10 ml. of Ringer lactate solution was instilled into both loops for 4 h, after which the loops were drained and emptied by flushing with air. Then, using an autoanalyser pump the loops of the control and the toxin treated animals were continuously perfused with a solution containing 26 mM *d*-glucose and 0·3 mM phenol red (PSP) in Ringer lactate. The PSP was employed as a non-absorbable marker. A sample of the test solution (50 ml.) was continuously perfused and the efflux from the loop was recirculated from a reservoir through the loop at a rate of 2 ml./min[7].

Before beginning the experiment, duplicate 1 ml. samples were obtained from the reservoir for determination of the initial concentration of glucose and PSP. The perfusion was begun and 1 ml. samples were collected from the reservoir at hourly intervals. The glucose concentration was determined on each sample using the method of Hoffman[8] modified for the 'Autoanalyser' (Technicon Instrument Corp., New York). PSP was determined colorimetrically by the method of Schedl *et al.*[9]. The final volume contained in the reservoir and the loop was obtained by dividing the amount of PSP placed in the system (corrected for PSP removed during the experimental period) by the final PSP concentration. The glucose absorbed was calculated by subtracting from the amount placed in the reservoir the amount in each sample and the amount remaining at the end.

After 4 h of perfusion the animal was killed and reopened. The loops were examined to ensure that the blood supply was intact, that drainage was free and that over-distension of the loops had not occurred. The loops were excised, opened along the antimesenteric border and the length was measured in centimetres. The fluid remaining in the reservoir beaker and drained from the loops was measured and compared with the fluid volume calculated from the PSP data. These two volumes were usually within 1 or 2 ml. of one another. Sections of the loops were taken from representative animals for histological examination. These showed no histological abnormalities.

The data as shown in the figure indicated that in the cholera group the mean *d*-glucose absorption was 0·038 ± 0·003 (*SEM*) mM/cm/4 h, as compared with 0·033 ± 0·003 (*SEM*) mM/cm/4 h in the control group. The mean net fluid production in the cholera group was 2·438 ± 0·122 (*SEM*) ml./cm/4 h as compared with the mean net fluid absorption of 0·312 ± 0·062 ml./cm/4 h in the control group.

In the control experiments, absorption of glucose was accompanied by net fluid absorption. On the other hand, in the loops treated with toxin equal glucose absorption occurred despite the production of copious quantities of isotonic fluid.

These studies indicate that the rate of jejunal glucose absorption is unaltered by profuse cholera toxin induced diarrhoea. It is suggested that cholera diarrhoea results from increased secretion by the intestine rather than by any alteration of intestinal absorption, supporting the hypothesis initially proposed by Cohnheim[10].

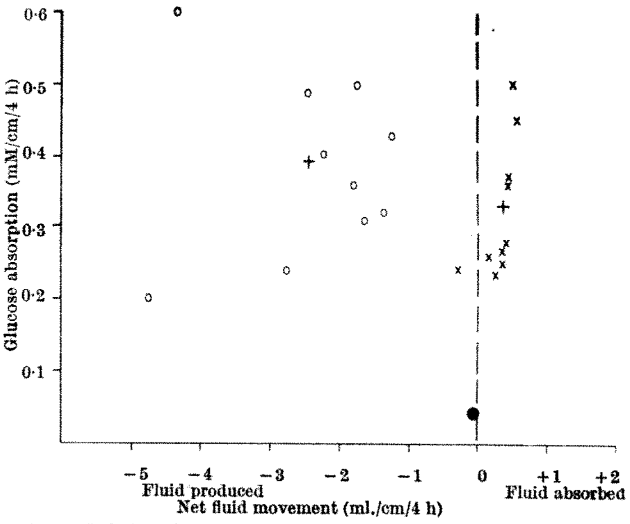

Fig. 1. Relationship between glucose absorption and net fluid movement in control and cholera toxin treated rabbit jejunal loops. Glucose absorption in mM/cm/4 h is plotted along the vertical axis and net fluid movement ml./cm/4 h along the horizontal axis. ○, Cholera loop; ×, control loop.

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[1] De, S. N., *Nature*, **183**, 1533 (1959).

[2] Norris, H. T., Schultz, S. G., and Curran, P. F., *J. Infect. Dis.*, **117**, 193 (1967).

[3] Leitch, G. J., Burrows, W., and Stolle, C., *J. Infect. Dis.*, **117**, 197 (1967).

[4] Elliott, H. L., and Yardley, J. H., *Symposium on Cholera* (US–Japan Co-operative Med. Science Program, California July 1967).

[5] Cocco, A. E., Salem, A. A., and Hendrix, T. R., *Bull. Johns Hopkins Hospital*, **117**, 1 (1965).

[6] Finkelstein, R. A., Atthasanpunna, P., Chalusmaya, N., and Charunmethee, P., *J. Immunol.*, **96**, 440 (1966).

[7] Scheff, M. F., and Smyth, D. H., *J. Physiol.*, **128**, 67P (1955).

[8] Hoffman, W. S., *J. Biol. Chem.*, **120**, 51 (1937).

[9] Schedl, H. P., and Clifton, J. A., *Gastroenterology*, **41**, 492 (1961).

[10] Cohnheim, J., translation by McKee, A. B., *New Sydenham Soc.*, London, **3**, 941 (1890).

PHYSIOLOGY

Inactivation of Isoniazid in Burmese Subjects

INACTIVATION by acetylation of the anti-tuberculosis drug isoniazid *in vivo* is subject to genetic polymorphism[1–3]. Studies of the gene frequency for rapid inactivation in different regions have revealed considerable racial variations[4–7]. Because of the association of isoniazid neuropathy with slow inactivation of the drug in therapeutic practice[8,9], the recording of gene frequencies among different races and in different countries is of practical importance. It is also of ethnological value in studies of population migrations. This communication reports such an investigation among Burmese subjects.

Isoniazid (10 mg/kg orally) was administered to 121 Burmese subjects, seventy-nine males and forty-two females, aged between 18 and 38. Venous blood samples were taken 6 h later, the serum was separated and extracted and the concentration of isoniazid was estimated by a vanillin condensation method[10]. Internal standards were added to serum taken from untreated subjects. The distribution of concentrations found (Fig. 1) was bimodal with an antimode at 3 μg/ml. Calculation on the basis of the seventy-six rapid and forty-five slow inactivators thus separated yields a gene frequency of 0·390 for rapid inactivation. A scatter diagram on a logarithmic scale (Fig. 2) suggested that eighteen of the rapid inactivators were homozygotes and the remaining fifty-eight heterozygotes. This distribution yields a gene frequency for rapid inactivation of 0·388 (Chi squared = 0·0094, $P > 0{\cdot}99$).

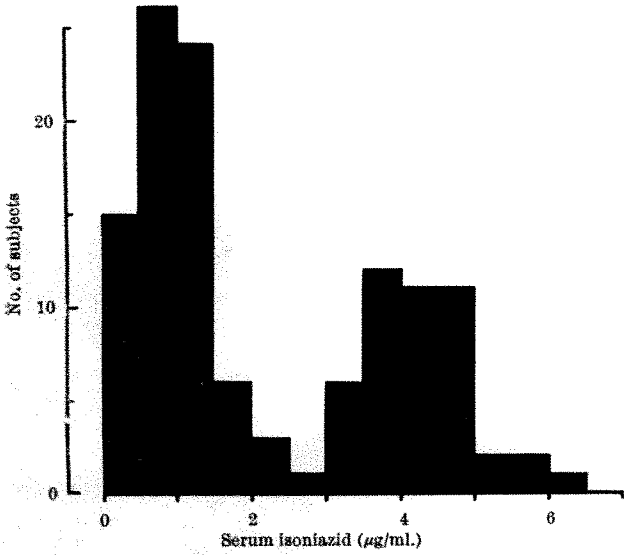

Fig. 1. Distribution of serum isoniazid concentrations in 121 Burmese subjects, 6 h after oral dose of 10 mg/kg.

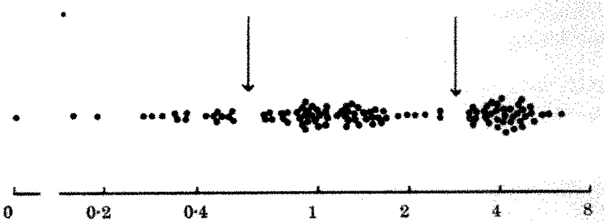

Fig. 2. Scatter diagram of serum isoniazid concentrations in 121 Burmese subjects, 6 h after oral dose of 10 mg/kg.

The gene frequency found indicates that approximately 37 per cent of Burmese subjects would carry the risk of neuropathy if clinically treated with isoniazid. This frequency is intermediate between those found among Thai[5] and South Indian[6] subjects, as might be expected both geographically and ethnologically (Table 1). The results of the present investigation are therefore consistent with current opinion of the Mongoloid origin of the gene responsible for the hepatic acetyltransferase involved.

Table 1. GENE FREQUENCIES FOR ISONIAZID INACTIVATION IN DIFFERENT POPULATIONS

Race	No. studied	Gene frequency		Reference
		Rapid	Slow	
Caucasian and Negro	485	0·28	0·72	2
Caucasian	105	0·24	0·76	7
Negro	116	0·27	0·73	
Japanese	209	0·71	0·29	
Japanese	1,808	0·66	0·34	5
Ainu	86	0·69	0·31	
Korean	65	0·67	0·33	
Ryukyan	124	0·60	0·40	
Thai	108	0·46	0·54	
Burmese	121	0·39	0·61	This work
S. Indian	321	0·22	0·78	6

Isoniazid tablets were donated by Dr A. M. C. Duffus, Roche Products, Ltd. One of us (S. E. S.) was an adviser in pharmacology to the Government of the Union of Burma under the Colombo Plan.

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[1] Knight, R. A., Selin, M. J., and Harris, H. W., *Trans. Eighteenth Cogn. Chemother. Tuberc.*, 52 (Veterans Administration, Washington, 1959).

[2] Evans, D. A. P., Manley, K. A., and McKusick, V. A., *Brit. Med. J.*, ii, 485 (1960).

[3] Evans, D. A. P., and White, T. A., *J. Lab. Clin. Med.*, **63**, 387 (1964).

[4] Harris, H. W., Knight, R. A., and Selin, M. J., *Amer. Rev. Tuberc. Pulm. Dis.*, **78**, 944 (1958).

[5] Sunahara, S., Urano, M., and Ogawa, M., *Science*, **134**, 1530 (1961).

[6] Gangadharam, P. R. J., Bhatia, A. L., Radhakrishna, S., and Selkon, J. B., *Bull. WHO*, **25**, 765 (1961).

[7] Dufour, A. P., Knight, R. A., and Harris, H. W., *Science*, **145**, 391 (1964).

[8] Devadatta, S., Gangadharam, P. R. J., Andrews, R. H., Fox, W., Ramakrishnan, C. V., Selkon, J. B., and Velu, S., *Bull. WHO*, **23**, 587 (1960).

[9] Mitchison, D. A., in *Drugs, Parasites and Hosts* (edit. by Goodwin, L. G. and Nimmo-Smith, R. H.), 267 (Churchill, London, 1962).

[10] Maher, J. R., Whitney, J. M., Chambers, J. S., and Stanonis, D. J., *Amer. Rev. Tuberc. Pulm. Dis.*, **76**, 852 (1957).

Neurotrophic Control of Contracture in Slow Muscle Fibres

SOME frog muscles, such as the iliofibularis, contain two types of muscle fibres: the "slow" fibres, with multiple innervations by small nerve fibres and capable of producing a maintained contracture when depolarized; and the "twitch" fibres which respond normally with a transient contracture and are innervated by larger motor nerve fibres[1,2]. Contracture characteristics of these muscle fibres seem to be determined by the type of innervation they receive, because the iliofibularis muscle loses its prolonged contracture after its original innervation has been replaced by the nerve to the sartorius, which is a purely twitch muscle[3].

To further investigate this problem we decided to see what would happen if the denervated iliofibularis muscle was re-innervated by a mixed nerve, containing large and small motor axons. It is known[4,5] that after nerve section the large motor fibres degenerate more rapidly than the small ones, and conceivably regeneration of the large fibres might also be faster. Furthermore, the proportion of large motor fibres far exceeds the small ones. It was therefore anticipated that by simply cutting the sciatic nerve and allowing it to regenerate, the slow muscle fibres might well become re-innervated by the more abundant, and presumably faster growing, large axons—a supposition which seems to be correct.

Iliofibularis muscles were examined at different times after section of the sciatic nerve. The muscle from the opposite leg was used as a control. The mechanical response of the normal iliofibularis to prolonged application of acetylcholine (about 10^{-5} g/ml.) or high K solution (50 or 100 mM) consists of two phases: a transient one resulting from activation of twitch fibres, and a more prolonged contracture caused by activation of the slow fibres[2]. If the nerve was prevented from growing back to the iliofibularis, the denervated muscles gave a well maintained contracture, in response to acetylcholine or K, during the first 3 months after denervation. If, however, denervation lasted more than 4 months (at 19°–20° C), the muscles atrophied and the maintained contracture was reduced, or even lost, in some cases. The explanation for this may be that an influence from the small nerve fibres usually conveys to slow fibres their ability to give a sustained contracture, and that this is lost when the neural influence is withdrawn for a sufficiently long time.

If nerve regeneration was allowed to occur the iliofibularis became re-innervated in 1–3 months (frogs kept at 10°–15° C), and when exposed to acetylcholine or high K gave only a transient contracture. That this loss of contracture is caused by the regenerating nerve fibres and is not simply a result of denervation alone is indicated by the fact that other muscles in the same frog, like the semitendinosus, had not yet been re-innervated and still responded with a maintained contracture. Furthermore, after crushing the sciatic nerve, regeneration is more rapid and the maintained contracture is lost in only 2 weeks; while the transformation caused by denervation alone occurs much later.

The loss of the sustained contracture in re-innervated muscles cannot be attributed either to degeneration, or to recognizable structural changes of the slow muscle fibres, because these could still be distinguished under the electron microscope. Furthermore, we have electron microscopic evidence that nerve fibres had reached the slow muscle fibres and formed new neuromuscular connexions. It is interesting that the muscle membrane at the new junctions had synaptic folds. This suggests that the nerve fibres forming the new contacts were large nerve fibres, originally connected to twitch muscle fibres; and that these nerve fibres induce in the muscle fibre the formation of synaptic folds which are usually absent in slow muscle fibres.

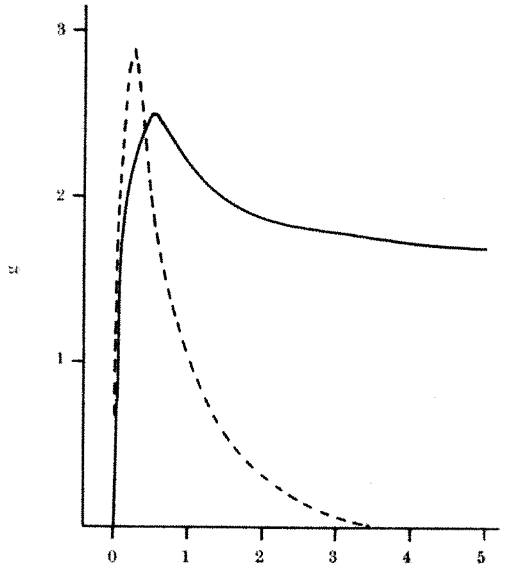

Fig. 1. Isometric contractures evoked by acetylcholine (5×10^{-6} g/ml.) in an iliofibularis 49 days after the sciatic nerve had been cut and allowed to regenerate (interrupted line); and in the control muscle in the opposite leg (solid line). The maintained contracture was already lost in the operated side even though neuromuscular transmission had not yet been restored. Frog kept at 10°–15° C after the operation.

In most respects the present results are similar to those obtained previously[3] with transplantation of the muscle, and re-innervation by the fast sartorius nerve; they confirm also that the slow muscle fibres retain their characteristic fine structural features, even though the contracture has been transformed.

An interesting new observation was the finding that muscle responses could be transformed even before neuromuscular transmission was restored as shown, for example, in Fig. 1. Similar results were obtained in several other muscles, 50–100 days after nerve section. Intracellular recording from end-plate regions showed no sign of re-innervation; and stimulation of the nerve did not elicit an electrical or mechanical response in the muscle fibres. It could be thought that the change in the duration of the contracture is accomplished even before the growing nerve fibres reach the muscle. To investigate this problem the iliofibularis nerve was dissected as it entered the muscle and examined under the electron microscope. Some Schwann cells could be seen among the still degenerating myelinated fibres. These cells contained filaments and a large number of microtubules, and were divided into several compartments all enclosed in a common basement membrane. During this, presumably early, stage of regeneration it was very difficult to ascertain whether any one of the profiles within the basement membrane was an axon or simply a process of the Schwann cell. Nevertheless, a few axons were in a more advanced stage of regeneration and could be recognized as such because they had the spiral beginnings of myelin formation. Thus it seemed that nerve fibres had definitely reached the muscle.

Fast or slow mammalian muscle can be partly converted to the opposite type when it is cross-innervated[6]. These muscles are subjected to different patterns of activity and it is not yet clear what significance, if any, the activity has in determining the type of contraction[6–9]. In our experiments activity can be ruled out as the determining factor because the contracture was changed before the transmission of impulses was restored.

These findings are of interest because they indicate, once again, that nerves are not limited simply to relaying electrical signals to the muscle, but also exert a direct influence which controls the characteristics of the contracture. They show further that the neural control is not dependent on nerve muscle impulses. The situation is somewhat analogous to the acetylcholine sensitivity of the muscle which is under a similar neural influence controlling the number and spread of acetylcholine receptors[10]. This analogy suggests also a possible way of explaining our results. It may be that there are Ca-receptors in the muscle fibre membranes, and that the number of receptors is controlled by a substance released by the nerve. To give a prolonged contracture a muscle fibre would require a high density of receptors in its membrane to supply the calcium necessary for contraction.

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[1] Tasaki, I., and Mizutani, K., *Jap. J. Med. Sci.*, **10**, 237 (1944).
[2] Kuffler, S. W., and Vaughan Williams, E. M., *J. Physiol.*, **121**, 318 (1953).
[3] Miledi, R., and Orkand, P., *Nature*, **209**. 717 (1966).
[4] Katz, B., and Miledi, R., *J. Physiol.*, **147**, 10P (1959).
[5] Birks, R., Katz, B., and Miledi, R., *J. Physiol.*, **150**, 145 (1960).
[6] Buller, A. J., Eccles, J. C., and Eccles, R. M., *J. Physiol.*, **150**, 417 (1960).
[7] Eccles, J. C., Eccles, R. M., and Kozak, W., *J. Physiol.*, **163**, 324 (1962).
[8] Buller, A. J., and Lewis, D. M., *J. Physiol.*, **178**, 343 (1965).
[9] Vrbová, G., *J. Physiol.*, **185**, 17P (1966).
[10] Miledi, R., in *The Effect of Use and Disuse on Neuromuscular Functions*, 35 (*Publ. House Czech. Acad. Sci.*, Prague, 1963).

Abnormalities in the Electrocardiograms of Young Sheep and Lambs grazing Natural Pastures low in Selenium

FRASER and I[1,2] have previously shown that abnormalities develop after a few weeks in the electrocardiograms of both rats and lambs fed dystrophogenic diets in experimental conditions. These abnormalities can be prevented by the administration of adequate selenium or tocopherol. Without such supplementation the rats die with hepatic necrosis, while the lambs develop lesions typical of white muscle disease (WMD) which in these experimental conditions always proves fatal

In an earlier communication[3], in which I reported abnormalities in the electrocardiograms of lambs in experimental conditions, I said: "It remains to be seen whether the same type of electrocardiogram change occurs in 'white muscle disease', a malady of sheep that are grazed in particular areas and which responds to selenium therapy". These experimental studies are open to the criticism that the lambs were being fed a somewhat unnatural diet, because the protein component was supplied as torula yeast.

I have now been able to examine electrocardiogram traces of young sheep and lambs grazing pastures which are demonstrably low in selenium, and in some cases of animals which have, at subsequent necropsy, shown lesions typical of WMD.

The findings are reported in summary in this communication for two reasons: first, to meet the criticism that electrocardiogram changes are found only in the unnatural conditions associated with the feeding of torula yeast-type diets, and second, to suggest that electrocardiogram records in field cases of muscular dystrophy might, at times, be a very useful method of early detection of the condition in a flock.

During this study, nine properties were visited in South Australia and one, where there was an outbreak of WMD, in Western Australia. The nine South Australian properties ranged from one which was selected as having no husbandry problems (to serve as "control") to one where WMD has been seen, including several cases during this work. The remaining properties provided examples, to a varying degree of severity, of muscular dystrophy encountered in the field.

The electrocardiogram equipment used and the technique adopted has been fully described elsewhere[2,4], the only additional feature of these studies being the use of a portable generator to power the electrocardiograph. It is possible with this mobile equipment to examine more than a hundred electrocardiogram patterns of sheep in a day, in field conditions.

Animals were randomly selected from the young ewes and lambs of the flock, and of those showing an abnormal electrocardiogram pattern, two or three were selected for slaughter. After post-mortem examination, pieces of skeletal muscle from the limbs, left and right ventricles and diaphragm were removed, fixed in buffered formalin and prepared for histological examination by paraffin embedding and staining sections with haematoxylin and eosin.

The property in Western Australia was visited during an outbreak of WMD, after several animals had died and the diagnosis made by the local veterinary officer. Thirty-eight records were obtained from this flock.

Pasture samples from the property in South Australia where WMD has been observed several times were analysed for selenium content by Watkinson's method[5]. Finally, whole blood samples were analysed for selenium from three groups of animals: (1) from an "unaffected" area in South Australia; (2) from the property in South Australia where WMD has occurred; and (3) from the property visited in Western Australia, which had a reported WMD.

Table 1 summarizes the chief findings from the properties in South Australia. The association of abnormal electrocardiogram records with histopathological lesions of myonecrosis was quite marked. Lesions were more severe in the right than the left ventricle and were always most pronounced in the skeletal muscle of the lambs. Clear cases of WMD were found in property "Gosse, K.I. II" and muscle damage was severe with advanced calcification. In such cases the diaphragm muscle is invaded by the lesion.

Table 1. ELECTROCARDIOGRAM EXAMINATION OF RANDOMLY SELECTED YOUNG SHEEP AND LAMBS ON NINE PROPERTIES IN SOUTH AUSTRALIA

Location of property	Number of animals examined	Number of abnormal electro-cardiograms	Number slaughtered	Number slaughtered with confirmed WMD
Keith I	25	5	3	3
Keith II	23	9	4	4
Keith III*	6	0	0	0
Gosse, K.I. I†	20	0	0	0
Gosse, K.I. II	21	10	5	5
Gosse, K.I. III	14	8	2	2
Karatta, K.I. I	13	1	2‡	2
Karatta, K.I. II	17	2	2	2
Karatta, K.I. III	10	1	2‡	2

K.I., Kangaroo Island.

* No husbandry problems.

† Poor flock, but there is an annual shortage of feed.

‡ The single electrocardiogram abnormality on each of the properties I and III were typical "WMD traces". The further two cases killed were showing severe arrhythmia and other mild irregularities. All cases at histological examination showed mild myonecrosis.

The electrocardiogram abnormalities were similar to those produced in the earlier experimental study, and one example with its corresponding muscle lesion is shown in Figs. 1 and 2.

Samples of pasture from property "Gosse, K.I. II" were shown to have the following range of selenium content; Yarloop clover, 0·005–0·007 p.p.m.; mixed native grasses, 0·007–0·01 p.p.m. These values are well below the levels quoted for "selenium responsive" situations in New Zealand where the threshold is considered to be 0·04 p.p.m. (ref. 6).

Whole blood selenium levels for the three groups of animals mentioned earlier gave the following range: (1) control animals from an "unaffected" area, 0·090–0·195 p.p.m. (twenty animals); (2) "Gosse K.I. II",

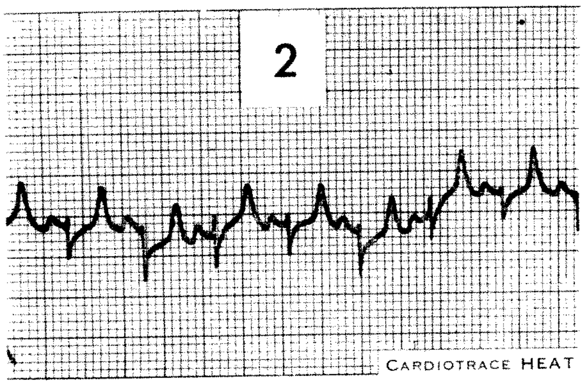

Fig. 1. Electrocardiogram record (lead II) of a lamb grazing pastures low in selenium. The animal was one of a random selection from a flock and was not clinically abnormal.

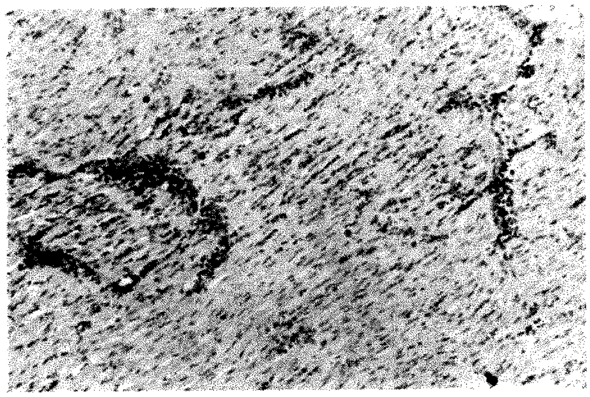

Fig. 2. Low power photomicrograph of an early lesion of myonecrosis found in the right ventricle of the lamb whose electrocardiogram is recorded in Fig. 1. Dystrophic lesions were quite widespread in the skeletal muscle of the same lamb. Stained haematoxylin and eosin. (×35.)

0·005–0·026 p.p.m. (sixteen animals); (3) "WMD flock" from Western Australia, 0·01–0·02 p.p.m. (four animals).

There is strong presumptive evidence from these findings that the electrocardiogram changes both in the field cases of muscular dystrophy and in those produced in experimental conditions have the same aetiology. In both situations the electrocardiogram patterns are similar and are associated with a low selenium intake in the lambs, which gives rise in both cases to lesions typical of WMD.

Further studies to determine whether administration of selenium can prevent the onset of these electrocardiogram changes in field cases of muscular dystrophy seem indicated. Furthermore, such studies might help to evaluate the use of the electrocardiogram as a guide in the decision to introduce prophylactic selenium treatment and in the early detection of white muscle disease.

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[1] Godwin, K. O., *Quart. J. Exp. Physiol.*, **50**, 282 (1965).

[2] Godwin, K. O., and Fraser, F. J., *Quart. J. Exp. Physiol.*, **51**, 94 (1966).

[3] Godwin, K. O., *Nature*, **209**, 1030 (1966).

[4] Godwin, K. O., and Fraser, F. J., *Biol.*, 277 (1965).

[5] Watkinson, J. H., *Anal. Chem.*, **38**, 92 (1966).

[6] Andrews, E. D., Grant, A. B., and Stephenson, B. J., *NZ J. Agric. Res.*, 7, 17 (1964).

Increased Receptor Utilization: Mechanism of Cocaine Potentiation

It has been known for more than 50 yr that cocaine causes potentiation of adrenergic responses. Several theories have been advanced to explain this phenomenon, but only two are ever given much attention. It has been suggested that cocaine blocks the site of noradrenaline (NA) uptake so that a higher concentration of NA is available at the receptors, resulting in potentiated responses[1]. On the other hand, some workers suggest that cocaine causes potentiation by acting on the effector cells and possibly at the α-receptors[2]. These two views are not necessarily exclusive of each other as might be inferred from some of the literature.

This work was carried out to find whether cocaine affects the receptor level or post-receptor level.

Phenoxybenzamine (POB) can irreversibly block α-receptors. It is possible, therefore, to block a sufficient number of receptors so that the maximal response to the agonist is reduced (that is, elimination of spare receptors) although the mechanical limits of the tissue have not been altered. In this case all unblocked α-receptors are assumed to be utilized in the presence of a supramaximal dose of NA. Thus the maximal response is dependent on the number of receptors remaining after POB and not on the mechanical limitations of the tissue. The maximal response of such a tissue to NA in the presence of cocaine can then only be modified by a receptor or post-receptor level mechanism.

Vasa deferentia from male albino rats were excised, trimmed and mounted in muscle baths containing Krebs-bicarbonate solution at 37° C and aerated with 95 per cent oxygen and 5 per cent carbon dioxide. Records of contraction were made isotonically (tension = 1/2 g) on a kymograph. Drug concentrations are given as the salts: noradrenaline bitartrate, cocaine hydrochloride, phenoxybenzamine hydrochloride.

After eliciting the maximum responses of the tissues by using 10^{-4} g/ml. NA, the tissues were washed and allowed to relax for 15 min at which time they were subjected to 5×10^{-9} g/ml. POB for 3 min then washed at 10 min intervals for 1·25 h. At this time the same concentration of

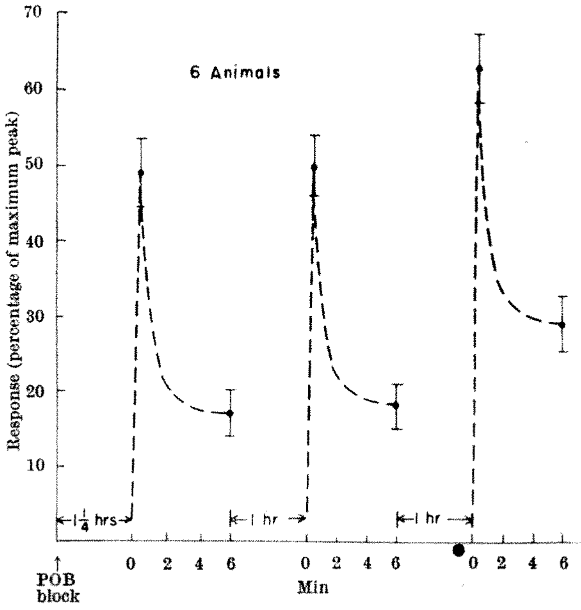

Fig. 1. Graph of mean responses of rat vas deferens in terms of the peak response to a maximal dose of noradrenaline (NA) 10^{-4} g/ml. Blockade of α-adrenergic receptors was effected 1·25 h before the graphed responses, by phenoxybenzamine, 5×10^{-9} g/ml. for 3 min. The last response was made in the presence of cocaine, 10^{-6} g/ml., added 1 min before the NA. Bars represent standard error of the mean (see Fig. 2 legend).

NA was given for 6 min, which allowed the tissue to produce a reduced rapid contraction (peak) and a steady state contraction (equilibrium) (Fig. 1). After an hour this concentration of NA was repeated and the previous response was closely reproduced (means of equilibria were within 1 per cent, Fig. 1). After another hour the tissue was treated with cocaine (10^{-6} g/ml.) 1 min before another dose of NA (10^{-4} g/ml.). The presence of cocaine increased both the maximal peaks and maximal equilibrium responses significantly ($P < 0{\cdot}01$, by paired data t test) (Fig. 1).

To ensure that the dose of NA which was supramaximal before treatment with POB was still supramaximal after, a different procedure was followed (Fig. 2). As before, the tissue maxima were obtained and POB blockade effected. After repeated washings for 1 h the tissues were subjected to 10^{-4} g/ml. NA for 6 min and then more NA was added, increasing the concentration to 2×10^{-4} g/ml., and left for another 6 min. This additional NA resulted in no change in equilibrium contraction. Subsequent addition of cocaine 6 min later, however, in the same concentration as used previously (10^{-6} g/ml.), resulted in a significant ($P < 0{\cdot}02$) increase in equilibrium within 3 or 4 min (Fig. 2).

After blockade with phenoxybenzamine it is seen that the presence of cocaine causes an increase in the maximum responses to NA. Cocaine introduced during responses to NA also causes an increased equilibrium. Because the portion of receptors remaining after POB blockade cannot produce the maximum response of which the tissue is capable, and because supramaximal doses of NA were used (no change occurred in the equilibrium with the additional NA), the increase in response in the presence of cocaine cannot be caused by increases in the local concentration of NA.

A series of six experiments similar to the first method outlined earlier was done, using methacholine instead of noradrenaline as the agonist. Only the peaks and not the peaks and equilibrium contraction (the latter are very small with methacholine) were compared. The dose of phenoxybenzamine used (5×10^{-8} g/ml. for 3 min) was sufficient to block noradrenaline by 90 per cent. Cocaine did not potentiate the peak responses to methacholine ($P > 0{\cdot}05$, paired-data t test).

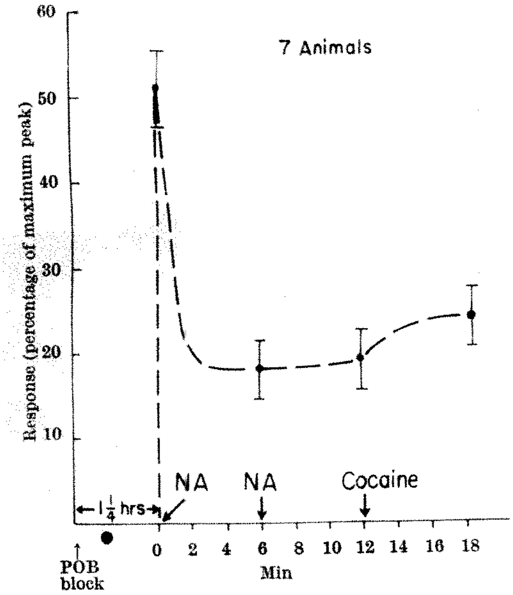

Fig. 2. Graph of mean response of rat vas deferens, in terms of the maximum peak response to noradrenaline (NA) 10^{-4} g/ml. before phenoxybenzamine blockade. POB (5×10^{-9} g/ml. for 3 min) was given 1·25 h before the responses graphed. Drugs were added in a cumulative manner. Note that, as in Fig. 1, the bars represent standard error of the means calculated on a non-paired basis, while statistical significance was assessed on a paired-data basis.

The increased responses of maximal equilibrium of these tissues can be attributed either to an increase in the effective utilization of receptors or to an increase of some post-receptor process such as excitation–contraction coupling. The process, however, by which cocaine potentiates is only involved with excitation by noradrenaline and not with excitation by methacholine, and it appears to be doubtful whether excitation–contraction coupling is involved. The increased utilization of receptors could be a result of increase of efficacy (intrinsic activity)[3]; a change in affinity would not produce any alteration of maximal response. As the response of the tissue follows qualitatively that predicted by the "rate theory of drug action"[4], we analysed our hypothesis of increased receptor utilization in terms of "rate-theory" kinetics. For the response of maximal equilibrium, Paton's equations reduce to

$$y_{\max} = \varphi \, k_2$$

where y is the equilibrium contraction, φ relates stimulation to response (and therefore includes excitation–contraction coupling) and k_2 is the dissociation or "offset" rate constant. Thus an increase in $y_{\max}$ could arise from increases in either φ or k_2. An increase in the latter appears to give a simpler solution since fewer assumptions about the process and the effects of cocaine would need to be made. It is reasonable that any interaction of cocaine near the adrenergic receptor might affect the equilibrium constant of the drug-receptor reaction. This postulated increase in k_2 would be sufficient to explain potentiation at any dose of noradrenaline. According to Paton's hypothesis, the equilibrium is limited by the availability of receptors for combination of the drug, and receptor availability depends on dissociation of existing complexes of drug receptor. An increase in k_2 would thus increase receptor availability, increase the combination rate of drug-receptor and potentiate the height of the equilibrium. The peak should also be potentiated since an increase in k_2 would slow development of fade.

It is seen that the maximal equilibrium attained in Fig. 2 is less than that of Fig. 1. This can be attributed either to protection of receptors by NA, which may interfere with the combination of cocaine–receptor, or inability of the tissue to respond further as a result of the prolonged exposure to high concentrations of NA.

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[1] MacMillan, W. H., *Brit. J. Pharmacol. Chemother.*, **14**, 385 (1959); Whitby, L. G., *et al.*, *Nature*, **187**, 604 (1960); Furchgott, R. F., *et al.*, *J. Pharmac. Exp. Ther.*, **142**, 39 (1963); de la Lande, J. S., and Waterson, J. G., *Nature*, **214**, 313 (1967).

[2] Maxwell, R. A., *J. Pharmac. Exp. Ther.*, **147**, 289 (1964); Maxwell, R. A., *et al.*, *J. Pharmac. Exp. Ther.*, **151**, 253 (1966); Bevan, J. A., and Verity, M. A., *J. Pharmac. Exp. Ther.*, **157**, 117 (1967).

[3] Stephenson, R. P., *Brit. J. Pharmacol. Chemother.*, **11**, 379 (1956); Ariens, E. J., *et al.*, in *Molecular Pharmacology* (edit. by Ariens, E. J.), 187 (New York, 1964).

[4] Paton, W. D. M., *Proc. Roy. Soc.*, B, **154**, 21 (1961).

Normocalcaemia in relation to Cortisone Secretion

THE hypercalcaemia which occurs in adrenalectomized animals or in patients with Cushing's disease treated by subtotal adrenalectomy has been thought to be the result of antagonism between secretions from the adrenal gland and parathyroid hormone[1]. Because cortisone produces hypocalcaemia, this hormone was thought to be the adrenal factor that influenced calcium homeostasis. A brief review of the history of these findings was given by Myers *et al.*[2], who investigated the subject further in adult adrenalectomized dogs. They reported the development of hypercalcaemia in these animals when cortisone therapy was withdrawn; however, subsequent thyroparathyroidectomy of the animals failed to prevent the hypercalcaemia of adrenal insufficiency. Our attention was caught by the fact that the dogs studied by Myers *et al.* lacked secretions from both the thyroids and the parathyroids. It has been shown that thyroxine and calcitonin, both coming from the thyroid gland, are important in mineral metabolism[3,4]. This report describes the results of a study designed to find out whether secretions from the thyroid, alone or in conjunction with parathyroid hormone, are important in the development of the hypercalcaemia of cortisone lack.

Adult mongrel dogs were divided into three groups of seven to thirteen animals each. In group 1, both thyroids and parathyroids were removed; the serum calcium and thyroxine levels decreased to values expected in thyroparathyroidectomized animals (serum calcium $< 6 \cdot 9$ mg/100 ml., serum thyroxine $< 1 \cdot 5$ μg/100 ml.). In group 2, the thyroids were removed but the superior parathyroids were left intact; the thyroxine levels decreased to thyroidectomy levels (serum thyroxine $< 1 \cdot 5$ μg/100 ml.) and the serum calcium values remained normal (serum calcium $> 9 \cdot 4$ mg/100 ml.). In group 3, the parathyroids were removed but the thyroids were left; the serum calcium levels decreased while thyroxine values remained normal (serum thyroxine $> 3 \cdot 5$ μg/100 ml.). Total adrenalectomy was done after neck surgery, generally 12 to 30 days later; however, in group 2, the adrenals were removed between 48 and 207 days after thyroidectomy. In all animals, 500 mg of sodium chloride per day and 5 mg of desoxycorticosterone ('Percorten') every 30 days were given throughout the experiment so that only corticosteroids were absent. Immediately before and for 3 days after adrenalectomy, prednisolone sodium phosphate was given until recovery from the operation was complete. The animals without parathyroids were maintained on a high-calcium diet until the adrenalectomy.

Fig. 1 shows the changes in serum calcium values after adrenalectomy, 100 per cent being the serum calcium value 3 days after cessation of corticosteroid replacement therapy. These values are corrected for specific gravity according to the method of Dent[5] and represent the mean values of the dogs in each group. The standard errors about each point in Fig. 1 are in all instances less than 20 per cent and the differences between curves are significant ($P < 0 \cdot 01$). Parathyroidectomy alone seemed to result in a precipitous increase in serum calcium, the animals dying as values of 17 mg/100 ml. or greater were reached unless the serum calcium was decreased by administration of corticosteroid. In the thyroidectomized animals there was little change in the serum calcium level, while the thyroparathyroidectomized animals showed a response midway between that of the two other groups.

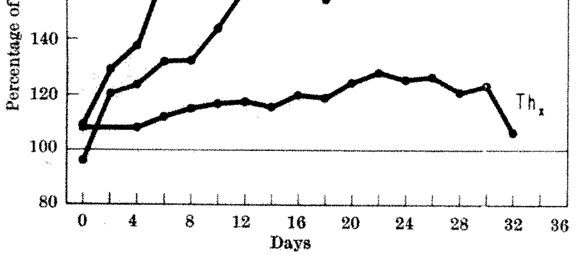

Fig. 1. Changes in serum calcium in adrenalectomized dogs which had been parathyroidectomized (Pth_x), thyroidectomized (Th_x), or thyroparathyroidectomized (Pth_xTh_x). Values are expressed in percentages of the postadrenalectomy serum calcium levels.

Feeding a diet deficient in calcium to these animals failed to prevent the hypercalcaemia, as shown by Walser *et al.*[6], although there appeared to be some delay in the initial development of hypercalcaemia. This suggested that absorption of calcium from the diet was not an important factor in the development of the hypercalcaemia. The alternative source of calcium is, of course, the skeleton. The animals showing hypercalcaemia demonstrated an increase, by a factor of three, in the amount of bone resorption taking place compared with normal cortical bone; most of the resorption occurred on the periosteal surface of bone.

The increase in serum calcium was accompanied by a decrease in the *p*H of the serum; at a serum calcium level of 10·0 mg/100 ml. the *p*H was approximately 7·30, and when the serum calcium was greater than 16·0 mg/100 ml. the *p*H was less than 7·28. In one dog, infusion of a solution of 1 per cent sodium hydroxide in 50 ml. of isotonic saline caused the *p*H to increase from 7·30 to 7·52 and the serum calcium to decrease from 15·4 to 13·5 mg/100 ml. These data suggested that changes in *p*H are important; in this context both Myers *et al.*[2] and Walser *et al.*[6] demonstrated an increase in plasma proteins and citric acid and a decrease in plasma bicarbonate. Both groups of investigators thought that only a small part of the hypercalcaemia could be related to the increase in serum proteins, while the latter group thought that an increase in the calcium-binding capacity of the protein might be an important factor. It is possible that the chief result of cortisone lack is to decrease blood *p*H and that bone acts as a buffer, releasing calcium into the blood. The buffering action seems to be more important physiologically than the control of serum calcium levels.

The failure of the thyroidectomized animals to show significant hypercalcaemia is related to the role of thyroxine in maintaining cell activity. This group of animals had been thyroidectomized for an average of 3 months before adrenalectomy, in contrast to the thyroparathyroidectomized animals which had undergone neck surgery only 12 to 30 days before adrenalectomy. The long-term absence of the thyroids ensured that the bone was in a hypothyroid state. Although the effect of cortisone lack appeared to be the same as far as *p*H changes were concerned, the serum calcium failed to increase. Administration of thyroxine to two thyroidectomized animals in this group produced a dramatic hypercalcaemia, the serum calcium increasing from 10·0 to 17·0 mg/100 ml. in 3 days. This observation both explains the difference between the groups and stresses the importance of thyroxine in maintaining normal cell metabolism. The difference between the thyroparathyroidectomized animals and the parathyroidectomized animals probably can be explained by a partial response of the bone to lack of thyroid hormones (partial because the period of hormonal lack was short). Calcitonin seems to be unimportant because thyroxine alone restored the response of the bone to hypocortisonism and produced hypercalcaemia.

These investigations will be incomplete until the mechanism explaining the hypercalcaemia of cortisone lack is clear. It is perhaps relevant, but more confusing, to remark that one of the main effects of cortisone in man, rats and rabbits seems to be the resorption of bone[6,7]. In fact, the osteoporosis of hypercortisonism is a rapidly developing bone disease, although it is not associated with hypercalcaemia[8]. Whatever the final explanation is, this report demonstrates the importance of the corticosteroids in maintaining normocalcaemia and also indicates that the hypercalcaemia of hypocortisonism seems to be independent of the parathyroids and to depend only on the presence of adequate amounts of thyroxine. The importance of thyroxine seems to be in maintaining the bone in a state in which it is able to respond by releasing calcium.

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[1] Eliel, L. P., Thomsen, C., and Chanes, R., *J. Clin. Endocrinol.*, **25**, 457 (1965).
[2] Myers, W. P. L., Rothschild, E. O., and Lawrence, jun., W., in *Bone and Tooth: Proceedings of the First European Symposium held at Somerville College, Oxford, April, 1963* (edit. by Blackwood, H. J. J.), 193 (Macmillan Company, New York, 1964).
[3] Adams, P., and Jowsey, Jenifer, *Endocrinology*, **81**, 735 (1967).
[4] Balogh, K., Roth, S. I., and Pechet, M. M., in *Program of the 49th Meeting of the Endocrine Society* (1967).
[5] Dent, C. E., *Brit. Med. J.*, ii, 1419 (1962).
[6] Walser, M., Robinson, B. H. B., and Duckett, jun., J. W., *J. Clin. Invest.*, **42**, 456 (1963).
[7] Storey, E., *Endocrinology*, **68**, 533 (1961).
[8] Follis, jun., R. H., *Bull. Johns Hopkins Hosp.*, **88**, 440 (1951).

Correlation of Loss of Hyaluronic Acid and Chondroitin Sulphate in Skin of Mice with Carcass Nitrogen Loss during Starvation

THE constituents of the ground substance of connective tissue respond dynamically to loss of systemic nitrogen. Marked changes have been reported to occur in the composition of the skin of mice during starvation[1]. This is accompanied by a significant reduction in hyaluronic acid (HA) and chondroitin sulphate (CS). Earlier studies[2] with rats suggested that hexosamine-containing materials in the skin decreased rapidly with small losses of carcass nitrogen (N), but when large losses occurred the additional decrement in the former was small. No attempt, however, was made to quantitate this relationship. In this work, decrement in HA and CS during starvation was correlated with loss of N in mice.

The procedures used were essentially those which have been described before[3,4]. In accordance with the general plan the quantities of HA and CS in the total pelt were determined and total N was measured in the carcass which remained after skinning. The length of the femur was measured to serve as a function of maximum growth. Regressions were derived correlating HA, CS and N with femur length in normal mice. In the starved mice, the quantity of HA, CS and N which were present before starvation could then be calculated from these regressions and the actual loss determined, using the observed values.

In order to establish the normal regressions twenty-seven male mice, weighing 24·0–40·3 g, were used. During starvation sixty-seven male mice, weighing 26–32 g, were individually housed in plastic containers (7 × 5 × 2·5 in.) on a layer of sawdust. Water was freely available. About ten mice were killed each day so that all had been killed after 7 days of starvation.

After cervical fracture the femurs were everted in a uniform manner and an X-ray picture was taken to be used for measurement of femur length. The pelt was removed in a uniform manner, dehaired, scraped free of subcutaneous tissue and analysed for HA hexosamine (HAH) and CS hexosamine (CSH) and the carcass was dissolved in formic acid ready for nitrogen analysis.

The regressions correlating HAH, CSH and N with femur length are as follows

$$\ln \mathrm{N} = 4{\cdot}97133 + 3{\cdot}75669 \ln F, r = 0{\cdot}871, P < 0{\cdot}001 \quad \text{(Fig. 1}a\text{)}$$

$$\ln \mathrm{HAH} = 3{\cdot}85427 + 5{\cdot}14034 \ln F, r = 0{\cdot}714, P < 0{\cdot}01 \quad \text{(Fig. 1}b\text{)}$$

$$\ln \mathrm{CSH} = 3{\cdot}71406 + 3{\cdot}77380 \ln F, r = 0{\cdot}598, P < 0{\cdot}01 \quad \text{(Fig. 1}c\text{)}$$

where N is mg of carcass nitrogen, HAH is µg of hyaluronic acid hexosamine, CSH is µg of chondroitin sulphate hexosamine, F is femur length in cm and r is the correlation coefficient. The high statistical significance is apparent.

From the femur length the pre-starvation values of HAH, CSH and N were calculated. Using the observed values, loss of HAH (ΔHAH), CSH (ΔCSH) and N (ΔN) were then obtained.

The data were correlated as $\ln \dfrac{\Delta\mathrm{HAH}}{\Delta\mathrm{N}}$ and $\ln \dfrac{\Delta\mathrm{CSH}}{\Delta\mathrm{N}}$ regressions on ln ΔN with high correlation coefficients and statistical significance as follows

$$\ln \frac{\Delta\mathrm{HAH}}{\Delta\mathrm{N}} = 3{\cdot}52280 - 0{\cdot}74516 \ln \Delta\mathrm{N}, n = 67, \quad r = 0{\cdot}799, \; P < 0{\cdot}001$$

$$\ln \frac{\Delta\mathrm{CSH}}{\Delta\mathrm{N}} = 2{\cdot}88043 - 0{\cdot}84072 \ln \Delta\mathrm{N}, \quad n = 43, r = 0{\cdot}704, \; P < 0{\cdot}01$$

Arithmetic or semi-logarithmic relationships were inadequately correlated. Written in exponential form

$$\frac{\Delta\mathrm{HAH}}{\Delta\mathrm{N}} = \mathrm{Constant}/\Delta\mathrm{N}^{0{\cdot}74516} \text{ and}$$

$$\frac{\Delta\mathrm{CSH}}{\Delta\mathrm{N}} = \mathrm{Constant}/\Delta\mathrm{N}^{0{\cdot}84072}$$

It is apparent that $\dfrac{\Delta\mathrm{HAH}}{\Delta\mathrm{N}}$ and $\dfrac{\Delta\mathrm{CSH}}{\Delta\mathrm{N}}$ decline rapidly with increasing ΔN. The reasons for this are not apparent, but several possibilities are suggested. These include (*a*) reduced rate of synthesis of fibroblast-produced substances during starvation in order to conserve materials and energy stores, and (*b*) release of protein present in the ground substance in order to conserve protein in the more vital organ. The ground substance contains plasma proteins as well as proteins, glycopeptides and mucoproteins synthesized by the fibroblasts and possibly other local cells. It was suggested earlier that in a restricted sense ground substance may serve as a protein storage site[2]. Although plasma proteins are "excluded" from hyaluronic acid gels according to the principles of gel filtration[5] the extracellular fluid-ground substance contains a considerable portion of plasma proteins, as much as half of the plasma albumin[6]. The protein in this phase suffers considerable reduction during starvation[1]. The nature of this feedback mechanism deserves intensive investigation.

The findings suggest a clinical application. Because collagen in the skin is relatively stable, the decline in hyaluronic acid/collagen in skin biopsies should follow loss of nitrogen during debilitating circumstances. This ratio has been measured in 8 mm punch biopsies taken during surgery and after realimentation and the difference has been correlated with loss of nitrogen during the period (unpublished work of Sobel and Cincotti).

This work was supported in part by a Navy Contract. One of us (R. C.) carried out this study while he was a scholar of the Committee for Advance Science Training. We thank Edward A. Warburton and the Western Research Support Center of the Veterans Administration for assistance with statistical evaluation of the data.

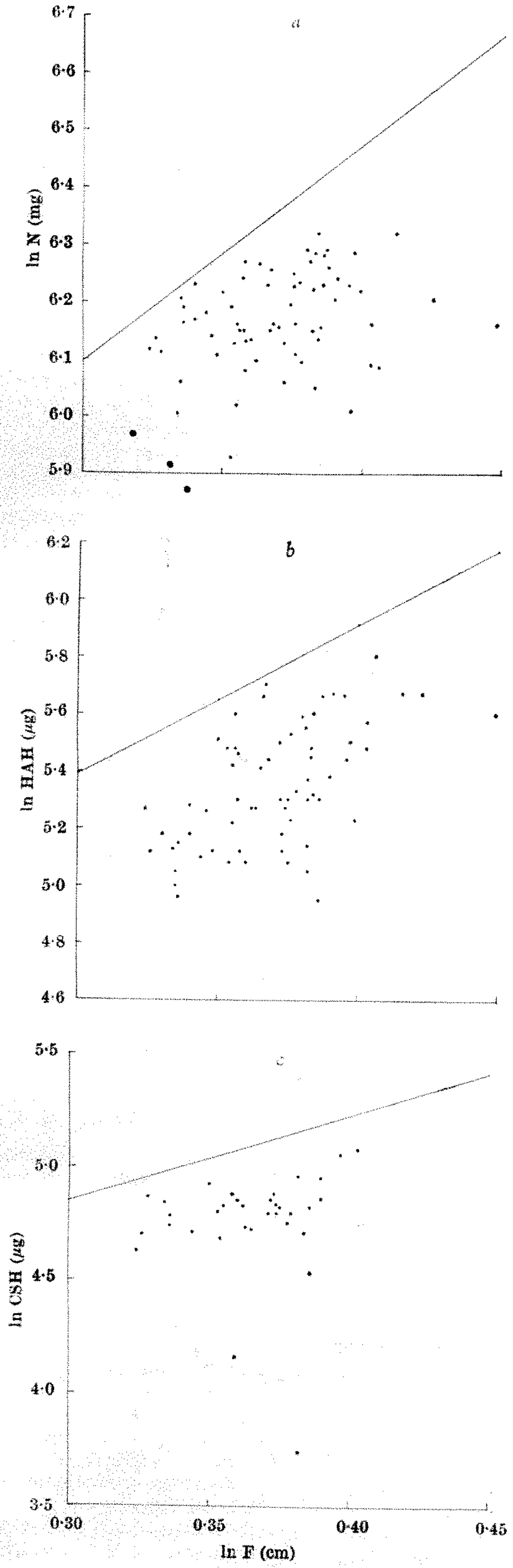

Fig. 1. Graphs of regressions correlating *a*, nitrogen; *b*, hyaluronic acid hexosamine; and *c*, chondroitin sulphate hexosamine with femur length. ●, Experimental values.

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[1] Sobel, H., Hewlett, M. J., Hoshek, S., and Sacker, I. M., *Amer. J. Physiol.*, **212**, 773 (1967).

[2] Sobel, H., Gabay, S., Johnson, C., and Hassan, B., *Metabolism*, **8**, 180 (1959).

[3] Sobel, H., Lee, K. D., and Hewlett, M. J., *Biochim. Biophys. Acta*, **101**, 225 (1965).

[4] Sobel, H., and Hewlett, M. J., *Proc. Soc. Exp. Biol. and Med.*, **126**, 149 (1967).

[5] Laurent, T. C., and Persson, H., *Biochim. Biophys. Acta*, **78**, 360 (1963).

[6] Wasserman, K., and Meyerson, H. S., *Amer. J. Physiol.*, **165**, 15 (1951).

Action of Insulin on Sodium Efflux from the Toad Oocyte

INSULIN applied *in vitro* reduces the internal sodium concentration of rat skeletal muscle[1-3] and stimulates the active transport of sodium in sodium-loaded frog muscle[3] and in toad bladder[4]. External application of insulin to single crab muscle fibres *in vitro* can cause a striking rise in the efflux of sodium[5]. Insulin stimulates protein synthesis in skeletal muscle in the presence of actinomycin D[6,7] and, because it has been shown that this inhibitor does not stop the hormone from stimulating the sodium pump[5], it is difficult to decide whether the primary action is on the efflux mechanism or induction of *de novo* protein synthesis. This communication describes the behaviour of another single symmetric cell, the toad oocyte, towards insulin.

Immature oocytes were obtained from the ovaries of healthy specimens of *Bufo bufo* by treatment with 0·5 per cent trypsin or by dissection. The methods employed for counting the sodium-24 activity of single cells and the effluent and the compositions of the Ringer and trypsin solutions used were those given by Dick and Lea[8] as modified by Bittar, Dick and Fry[9].

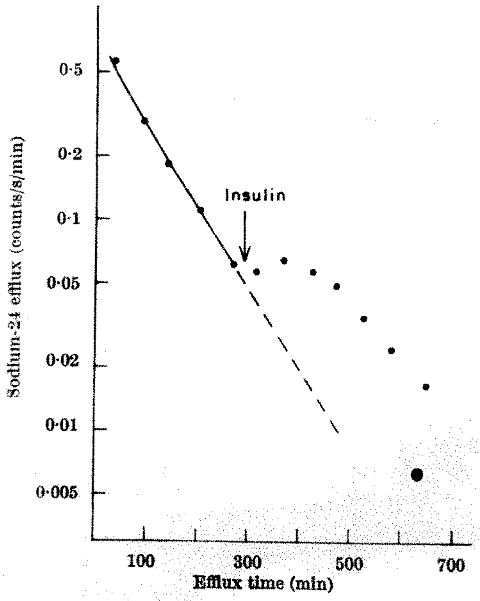

Fig. 1. The effect of 0·1 U/ml. of insulin on sodium efflux in a trypsinized oocyte (semi-log plot).

Normal dissected oocytes were insensitive to insulin: none of the twenty-four oocytes tested with 0·1 U/ml. of insulin showed a response, neither was any effect observed in four cells treated with 1 U/ml. of insulin, as compared with untreated controls. Occasional responses occurred in three different experimental conditions. First, three out of twenty oocytes pretreated with trypsin responded to 0·1 U/ml. of insulin by showing a prompt and significant rise in the efflux (Fig. 1). Second, six oocytes, from the same ovary, treated overnight with 20 μg/ml. of actinomycin *D*, showed a response like that in Fig. 2. (There was no evidence from control cells that actinomycin *D* influenced the behaviour of the efflux.) Third, two of a batch of four oocytes from the same ovary, sodium-loaded by cooling to approximately 4° C overnight, showed definite effects illustrated by Fig. 3. The sodium content of all these cells was between three and four times the normal of 10 mM/l. Twenty-six oocytes, sodium-loaded by overnight cooling, failed to respond to insulin, however, so factors besides a rise in the internal sodium must govern the response to insulin.

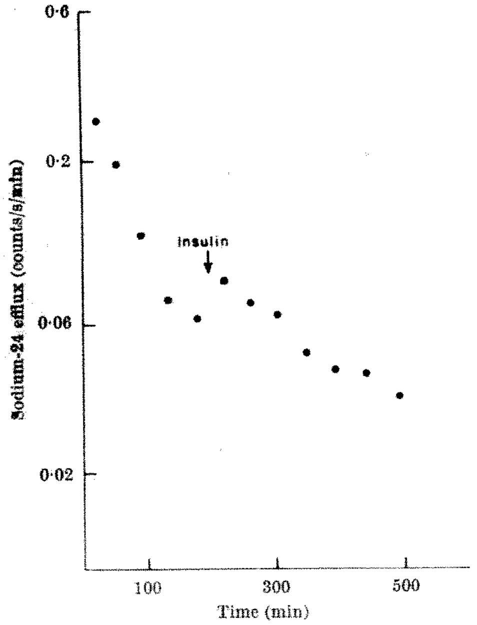

Fig. 2. Action of 0·1 U/ml. insulin on an oocyte treated with actinomycin. The oocyte had been exposed to 20 μg/ml. of actinomycin throughout the periods of sodium-24 loading and testing (that is, some 16 h).

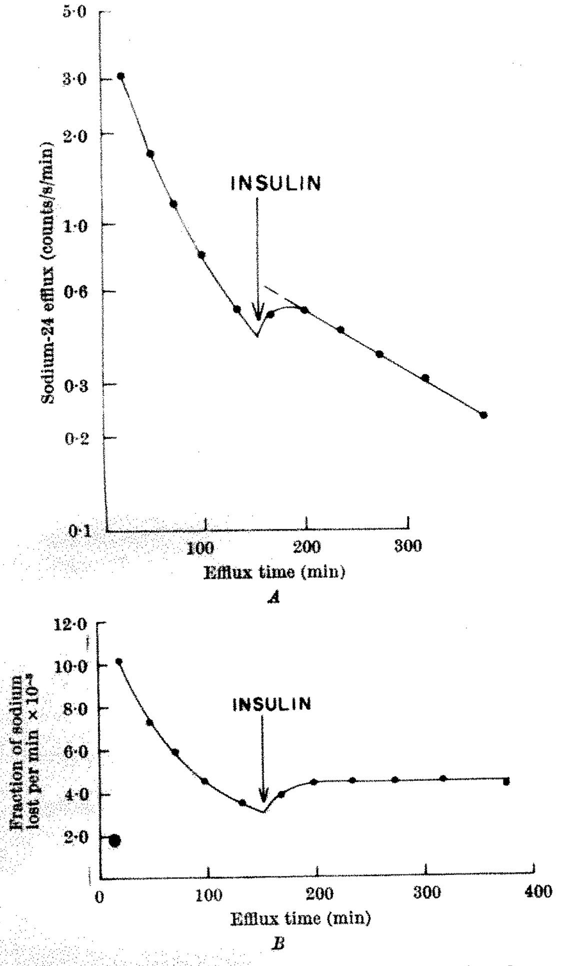

Fig. 3. The effect of 0·1 U/ml. of insulin on sodium-24 loss from an oocyte loaded with radio-sodium at an environmental temperature of about 4° C. *A*, The efflux curve; *B*, the rate constant curve.

The responses when they occurred were similar, despite differences in experimental conditions. The kinetics do not seem to fall into a simple pattern, however, because insulin increases the efflux of radio-sodium (Fig. 3*A*) and decreases its rate of decline. Also, in the presence of insulin, the fraction of sodium lost each minute becomes constant (Fig. 3*B*). The hormone probably acts by releasing sequestered sodium in some cells in which the rate of exchange between this and free intracellular sodium is slow. Response in actinomycin-treated oocytes implies that the action of the hormone does not depend on RNA synthesis. This is supported by the fact that actinomycin *D* is able to penetrate the oocyte[10] and abolish RNA formation in the resting cell[11].

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[1] Creese, R., and Northover, J., *J. Physiol.*, **155**, 343 (1961).
[2] Zierler, K. L., Rogus, E., and Hazlewood, C. F., *J. Gen. Physiol.*, **49**, 433 (1966).
[3] Kernan, R. P., *J. Physiol.*, **162**, 129 (1962).
[4] Herrera, F. C., *Amer. J. Physiol.*, **209**, 819 (1965).
[5] Bittar, E. E., *Nature*, **214**, 726 (1967).
[6] Eboué-Bonis, D., Chambaut, A. M., Volfin, P., and Clauser, H., *Nature*, **199**, 1183 (1963).
[7] Wool, I. G., and Cavicchi, P., *Proc. US Nat. Acad. Sci.*, **56**, 991 (1966).
[8] Dick, D. A. T., and Lea, E. J. A., *J. Physiol.*, **174**, 55 (1964).
[9] Bittar, E. E., Dick, D. A. T., and Fry, D. J., *J. Physiol.* (in the press).
[10] Brachet, J., and Ficq, A., *Exp. Cell Res.*, **38**, 153 (1965).
[11] Gross, P., Malkin, L., and Moyer, W., *Proc. US Nat. Acad. Sci.*, **51**, 407 (1964).

Effect of Heparin on Serum Calcium Concentrations in Mice

A HYPOCALCAEMIA induced by heparin has been the subject of several recent publications. In 1961 Heidland *et al.*[1] showed that a single intravenous injection of heparin elicited a significant lowering of serum calcium in dogs and they attributed this effect to the formation of a heparin–calcium complex in the serum. Goldsmith and Parry[2] also demonstrated heparin-induced hypocalcaemia in rabbits, and to a lesser extent in rats. The following mechanism for the phenomenon was suggested: heparin activates lipoprotein lipase with a resulting release of free

Table 1. EFFECT OF HEPARIN ON SERUM CALCIUM LEVELS IN MICE

Treatment*	Number of mice	mg Ca/100 ml. $\pm$ *S.E.M.*	% lowering†	t	Significance
1	48	6·65 ± 0·15	13·07	6·03	$P<0\cdot01$
2	43	7·26 ± 0·13	5·10	2·27	$P<0\cdot01$
3	56	6·66 ± 0·15	12·94	9·86	$P<0\cdot01$
4	131	7·65 ± 0·08			

* Groups 1, 2 and 3 received 0·25, 0·50, and 2·0 mg of heparin, respectively; group 4 received isotonic saline and served as control group.

$$\dagger\ \%\ \text{lowering} = \frac{(\text{mean control group} - \text{mean heparin group})}{\text{mean control group}} \times 100$$

fatty acids and subsequent formation of their insoluble calcium salts.

Because of the increasing use of heparinized blood from the laboratory mouse in studies of calcium homoeostasis, we have investigated the effect of a single intramuscular injection of heparin on serum calcium concentrations in mice.

Three hundred Swiss albino mice (60–90 days old) of both sexes weighing 25–30 g were randomly assigned to four experimental groups. Groups 1, 2 and 3 received 0·25, 0·50 and 2·0 mg of heparin, respectively; group 4, the control, received 0·5 ml. of isotonic saline. Two commercial heparin sources containing no detectable calcium (Wyeth, 100 USP units/mg, and Sigma, 160 and 170 USP units/mg) were used with no apparent difference in degree of hypocalcaemia. In all cases a single intramuscular injection with a total volume of 0·5 ml. was administered. Blood was collected by cardiac puncture and the serum was analysed for calcium 2 h after injection by the Mager–Farese[3] modification of the glyoxal *bis* (2-hydroxyanil) reaction[4]. All haemolysed sera were discarded. The results (Table 1) were analysed by a completely randomized design test. Treatment means were analysed by Student's t test when the calculated F ratio was shown to be significant. A 0·01 level of rejection was used. All the groups injected with heparin exhibited a statistically significant decrease in serum calcium when compared with controls, but no apparent dose response could be demonstrated.

In view of these findings, we recommend that caution be exercised in the utilization of heparin as an anticoagulant agent in studies concerning serum calcium.

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[1] Heidland, A., Klutsch, K., and Hochrein, H., *Klin. Wschr.*, **39**, 51 (1961).
[2] Goldsmith, M. W., and Parry, D. J., *Nature*, **210**, 1286 (1966).
[3] Mager, M., and Farese, G., *Clin. Chem.*, **12**, 234 (1966).
[4] Kerr, J. R. W., *Analyst*, **85**, 867 (1960).

BIOLOGY

Success of Eggs of the Shag on the Farne Islands, Northumberland, in relation to their Content of Dieldrin and *pp*'DDE

RECENT declines in the density and productivity of bird-eating predators, for example, the peregrine (*Falco peregrinus*) and sparrow hawk (*Accipiter nisus*), have been attributed to the presence of organochlorine pesticides in the tissues and eggs of these birds[1,2]. It has been suggested that the lethal dose in adults of these species resulted from the ingestion of contaminated grain-eating prey[3]. Granivorous birds, especially the wood pigeon (*Columba palumbus*), suffered considerable mortality in the period 1956–1961 when they ingested organochlorine seed dressings of the cyclodiene type, during spring sowing programmes[4,5].

Sub-lethal effects and effects on reproduction are more difficult to assess. Recent attempts[6-8] to compare the concentrations of organochlorines in eggs with their success have been thwarted by the scarcity of predators so that the sample sizes are inadequate for critical analysis. Comparable investigations in North America[9,10] are chiefly concerned with DDT which is only occasionally found in analytically significant amounts in the eggs of birds in the British Isles[11].

The concentrations of the organochlorine compounds in the eggs of the shag (*Phalacrocorax aristotelis*) from colonies on the Farne Islands, Northumberland, are higher than in other species of sea birds, except for the relatively non-toxic *pp*'DDE which is higher ($P<0\cdot01$) in the cormorant (*Phalacrocorax carbo*)[12]. The concentration of organochlorine insecticide in shag eggs, excluding *pp*'DDE, is similar to that recorded in the peregrine in which toxic effects have been claimed[2,13].

This report is based on analyses of shag eggs from the Farne Islands in each of the four seasons 1964–1967; the methods are described elsewhere[12]. The analyses were carried out by Shell Research Limited, Sittingbourne, Kent. Eggs were taken under licence, both at random and (most of them) by collecting addled (non-hatching) eggs. It is necessary to correct the concentration of insecticide in eggs to allow for water loss through evaporation, particularly because fertile and infertile eggs lose water and also carbon dioxide at different rates[14] and because more than 10 per cent of the addled eggs had extensive hair cracks through which water loss could occur. Where possible, egg or chick losses caused by inclement weather, predation and so on are excluded from this study. Success of the clutch or survival of the eggs and chicks was considered from the onset of incubation to the tenth day after hatching—a period of about 6 weeks. Earlier work has shown that the concentration of organochlorine insecticides in an individual shag declines from the time that it is first fed, so that at the tenth day the level is less than half that on hatching[12].

The within clutch variation in concentration of organochlorine is significantly less than the between clutch variation ($P<0\cdot001$; for example, twenty-one eggs of seven clutches, $F=10\cdot2$, for dieldrin). One egg of a clutch can therefore be taken as representative of the others. Embryo mortality was low, for only 17 per cent of addled eggs contained dead embryos. The "benefit of the doubt" was therefore given to live embryos in well incubated eggs collected at random. This method allows the consideration of addled eggs among the sample collected at random but means that the brood size is overestimated. A sample of 151 eggs were assayed from the 118 clutches which were selected for this study; the clutches contained 357 eggs.

There is no significant correlation between clutch size or brood size and the concentrations of either *pp*'DDE or dieldrin (Table 1). Total clutch or brood failure is not correlated with the concentrations of *pp*'DDE, which is usually considered much less toxic than dieldrin, but

Table 1. SUCCESS OF SHAG CLUTCHES FROM THE FARNE ISLANDS, NORTHUMBERLAND, ACCORDING TO THEIR ORGANOCHLORINE CONTENT

Concentration (p.p.m. wet weight) of whole egg, including shell assuming sp. gravity of 1·0	Clutches sampled	Total eggs in the clutches	Eggs per clutch	Chicks per brood, tenth day	Percentage clutches with no chicks surviving to tenth day (± *S.E.*)
(1) Dieldrin					
0·50–0·99	53	158	2·98	1·71	34·0 ± 6·5
1·00–1·49	39	122	3·13	1·81	33·3 ± 7·5
1·50–1·99	18	52	2·89	2·00	33·3 ± 11·2
2·00–3·00	8	25	3·12	2·00	75·0 ± 15·3
Total and mean	118	357	3·03	1·80	36·4 ± 4·4
(2) *pp*'DDE					
1·00–1·99	56	170	3·04	1·79	32·1 ± 6·2
2·00–2·99	42	129	3·07	1·88	40·1 ± 7·6
3·00–9·00	20	58	2·90	1·67	40·0 ± 10·9
Total and mean	118	357	3·03	1·80	36·4 ± 4·4

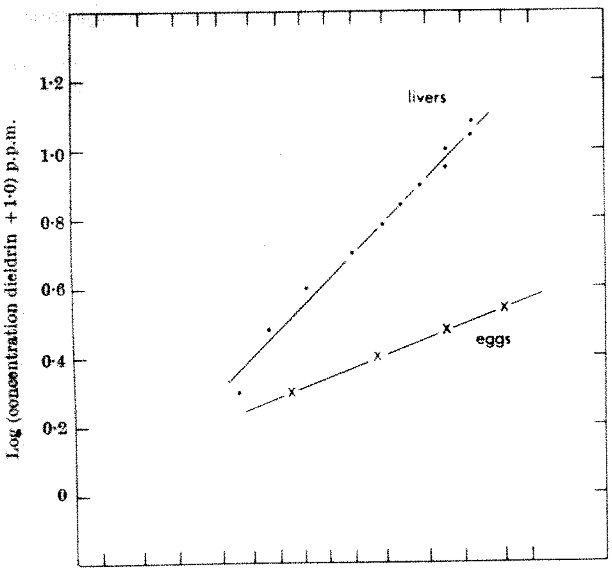

Fig. 1. Arithmetical probability plot of the accumulated log-normal frequency distribution curves of the concentrations of dieldrin in the liver and in the eggs of the shag.

there is a significant correlation ($2 \times 2\chi^2$, exact method; P (2 tail) $< 0{\cdot}04$), apparently of the threshold type, between the level of dieldrin and total clutch–brood failure. Such an association between the concentration of dieldrin and mortality is not related to parental age or experience and in any case the concentration of dieldrin in eggs is not related to the age of the female shag[12]. No investigation of the metabolism of dieldrin in birds has been carried out, but work on rabbits and rats suggests that a live embryo will break down dieldrin into its hydrophilic metabolites more quickly than substances in a "dead" egg[15,16]; however, this could not account for a correlation of a threshold type.

Laboratory studies using injection methods[17] have shown that the newly hatched chick is likely to be the stage during the development of birds which is most susceptible to dieldrin poisoning. For example, the survival of the embryo of the domestic fowl is not affected even at whole egg concentrations of 200 p.p.m. dieldrin[18], but concentrations of 17 p.p.m. in the egg can increase mortality if food shortage occurs immediately after hatching[19]. The toleration of different species of bird to dieldrin is claimed to vary three or four fold (refs. 20 and 3; compare ref. 21), but much of the work is non-critical, on a small scale and difficult to extrapolate to field conditions. It seems unlikely, however, that any poisoning occurred in the unsuccessful shag clutches which contained 2–3 p.p.m. of dieldrin because there is no evidence of an unusual proportion of deaths of chicks at hatching (Table 2).

Table 2. DETAILS OF NINE SHAG CLUTCHES CONTAINING EGGS WITH 2 P.P.M. DIELDRIN

	Whole egg conc. dieldrin (p.p.m.)	Year	Details of eggs		Total eggs
			Eggs assayed	Other eggs	
1	2·05	1967	ND, hair crack	Broken	2
2	2·08	1966	ND	ND	3
3	2·10	1965	ND	3 young > 10 days	4
4	2·10	1966	Live embryo (20 mm)	Female died, nest abandoned	4
5	2·22	19[illegible]6	Dead embryo (45 mm)	1 young died. 3 days 1 egg ND	3
6	2·31	1964	Out, ND	Broken	4
7	2·40	1965	Out, ND	?	(1)
8	2·65	1965	ND	Prob. ND	3
9	2·77	1965	ND	ND	2

ND, No embryonic development.
Out, Egg kicked out of nest by shags.
No. 7 is not included in Table 1; it was collected on the Isle of May, Fife, and was the only egg with > 2 p.p.m. dieldrin in a sample of twenty-eight (ten in 1965, eighteen in 1966).

It seems reasonable to consider the egg as a body "compartment"[22] and therefore to assume that the concentrations of dieldrin in other "compartments" of the laying female are correlated with that in the eggs. Analysis of the log-normal frequency distribution of concentrations of dieldrin in the livers of twenty-seven adult (of breeding age) shags and in the eggs (Fig. 1) suggest that concentrations of 2·5 p.p.m. in the egg reflect concentrations in the liver of about 9 p.p.m. A full investigation has revealed no evidence of dieldrin poisoning of adult shags from the Farne Islands[23].

The concentration of 9 p.p.m. in the liver is about one-fifth the mean concentration found in livers of experimentally poisoned quail (*Coturnix japonica*)[21], pigeons (*Columba livia var*)[5,21] and in duck poisoned with dieldrin in Colorado[24]. The sub-lethal effects of dieldrin are little known, but they have been described in the field[25,26] and in the laboratory, both *in vitro*[27] and *in vivo*[28]. Concentrations of DDT one-hundredth of the acute oral LD_{50} have been shown to increase the irritability of the central nervous system in rats dosed over a period of 30 days[28].

Recent increases of the rate of egg breaking in the sparrow hawk[29], peregrine[1,2,7] and golden eagle (*Aquila chrysaëtos*)[30] have been considered to be the result of sub-lethal effects of organochlorine insecticide contamination, and egg breaking also occurs in the shag (for example, clutches 1 and 6 in Table 2). Just over one-third of the egg breaking in the shag, and most cases of repeated breakages, were, however, the result of interference by extra females resulting from simultaneous polygyny at one nest. The survival curves of eggs of pairs and at nests with the polygyny are shown in Fig. 2. It is interesting to note that this kind of interference has been considered to be one of the causes of egg breaking in the peregrine[31]. Little is known of the causes of egg breaking in normal pairs of shags but the incidents were not related to the age or experience of the pair. Polygyny has been recorded in the shag before the use of organochlorine insecticides[32–34] and egg breaking has for long been recorded in several species[35,36].

It seems unlikely that organochlorine poisoning of embryo or chick shags has occurred on the Farne Islands and the adult mortality rate has not been increased with the use of dieldrin. On the other hand there is some evidence of a lowered breeding success among the few

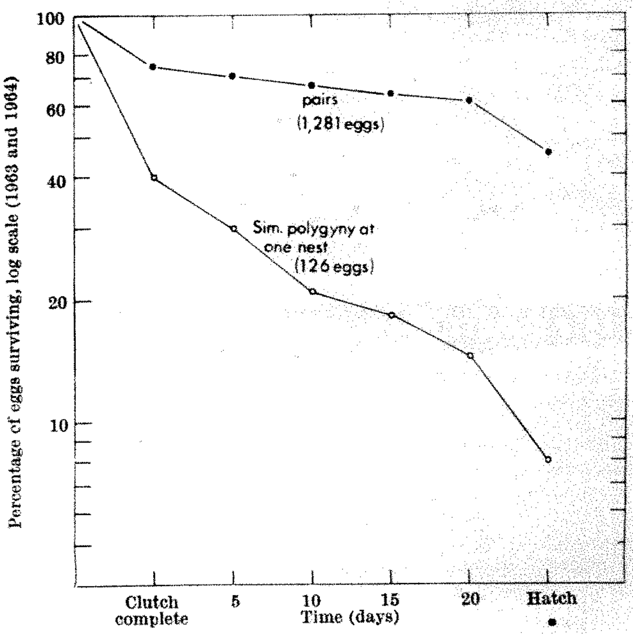

Fig. 2. Survival of eggs of the shag on the Farne Islands, Northumberland (1963 and 1964), comparing nests with normal pairs and those with simultaneous polygyny.

female shags (< 6 per cent) with liver concentrations of about 9 p.p.m. of dieldrin. It is possible that this is the result of sub-lethal effects, but much of the egg breaking is caused by polygyny. Much more detailed and critical attention than hitherto should be given to experimental studies of the sub-lethal effects of insecticides.

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[1] Ratcliffe, D. A., *Bird Study*, **10**, 56 (1963).
[2] Ratcliffe, D. A., *Nature*, **215**, 208 (1967).
[3] Jefferies, D. J., and Prestt, I., *Brit. Birds*, **59**, 49 (1966).
[4] Robinson, J., *Chem. Indust.*, **1967**, 1974 (1967).
[5] Turtle, E. E., Taylor, A., Wright, E. N., Thearle, R. J. P., Egan, J., Evans, W. H., and Soutar, N. M., *J. Sci. Fd. Agric.*, **14**, 567 (1963).
[6] Lockie, J. D., and Ratcliffe, D. A., *Brit. Birds*, **57**, 89 (1964).
[7] Ratcliffe, D. A., *Bird Study*, **14**, 238 (1967).
[8] Tubbs, C. R., *Brit. Birds*, **60**, 381 (1967).
[9] Keith, J. A., *J. Appl. Ecol.*, **3** (Suppl.), 71 (1966).
[10] Ames, P. L., *J. Appl. Ecol.*, **3** (Suppl.), 87 (1966).
[11] British Trust for Ornithology/Royal Society for the Protection of Birds, *Committee on Toxic Chemicals Reps.* 4, 5 and 6 (1964, 1965 and 1967).
[12] Robinson, J., Richardson, A., Crabtree, A. N., Coulson, J. C., and Potts G. R. *Nature*, **214**, 1307 (1967).
[13] Ratcliffe, D. A., *Brit. Birds*, **51**, 23 (1958).
[14] Romanoff, A. L., and Romanoff, A. J., *The Avian Egg* (Wiley, New York, 1949).
[15] Korte, F., and Arent, H., *Life Sciences*, **4**, 2017 (1965).
[16] Korte, F., *Botyu-Kagaku*, **32**, 46 (1967).
[17] McLaughlin, jun., J., Marliac, J. P., Verrett, M. J., Mutchler, M. K., and Fitzhugh, O. G., *Toxicol. Appl. Pharmacol.*, **5**, 760 (1963).
[18] Dunachie, J. F., and Fletcher, W. W., *Nature*, **212**, 1062 (1966).
[19] Koeman, J. H., Oudejans, R. C. H. M., and Huisman, E. A., *Nature*, **215**, 1094 (1967).
[20] *Ann. Rep. Patuxent Wildlife Research Center, Circ. Fish Wildl. Serv., Wash.*, 167, 199, 224 (1963, 1964 and 1965).
[21] Robinson, J., Brown, V. K. H., Richardson, A., and Roberts, M., *Life Sciences*, **6**, 1207 (1967).
[22] Robinson, J., *Nature*, **215**, 33 (1967).
[23] Potts, G. R., *J. Anim. Ecol.* (in the press).
[24] De Witt, J. B., Crabtree, D. G., Finley, R. B., and George, J. L., *Circ. Fish Wildl. Serv., Wash.*, **143**, 14 (1962).
[25] Rudd, R. L., *Pesticides and the Living Landscape* (Wisconsin, 1964).
[26] McEwen, L. C., and Brown, R. L., *J. Wildl. Mgmt.*, **30**, 604 (1966).
[27] Peakall, D. B., *Nature*, **216**, 505 (1967).
[28] Dési, I., Farkas, I., and Kemény, T., *Experientia*, **24**, 51 (1968).
[29] Ratcliffe, D. A., *Brit. Birds*, **53**, 128 (1960).
[30] Lockie, J. D., and Ratcliffe, D. A., *Brit. Birds*, **57**, 89 (1964).
[31] Treleaven, R. B., *Brit. Birds*, **54**, 136 (1961).
[32] Jourdain, F. C. R., *Brit. Birds*, **7**, 94 (1913).
[33] Robertson, D. J., *Brit. Birds*, **33**, 55 (1939).
[34] Williamson, K., *Brit. Birds*, **33**, 116 (1939).
[35] Marples, G., and Marples, A., *Sea Terns* (Country Life, London, 1934).
[36] Armstrong, E. A., *Bird Display and Behaviour* (Lindsay Drummond, London, 1947).

Discovery of *Rhabdopleura* (Hemichordata) at Plymouth

Rhabdopleura is one of three pterobranch genera which as hemichordates are important in our understanding of the evolution of the chordates[1]. Its biology and life cycle are very incompletely understood. So far, the most suitable marine laboratory for studying this genus has been Bergen, where *R. normani* has been well investigated[2]. This species, however, has to be kept at temperatures less than 12° C, and is dredged from a hard, rocky bottom in rather deep water.

The habitat of *R. normani*, the best known species, extends from within the Arctic Circle southward to the Azores, but, apart from a few records in shallow waters of some Norwegian fjords[2], it inhabits deep water, usually on the continental slope. In April 1967, specimens of *Rhabdopleura* were dredged for the first time from 1 to 2 miles south of Stoke Point in Devon. They have been identified as *R. compacta* Hincks[3], having the highly characteristic colonial form described by him. *R. compacta*, which was described inadequately from poorly preserved material, is not at present regarded as a valid species, but when a description of the new material has been prepared it is intended to re-instate it. *R. compacta* was first found off the coast of Northern Ireland in 1880. Since then it has been recorded once only, off Finistère. All the colonies from Stoke Point were found adhering to the concave sides of dead lamellibranch shells (mainly *Glycimeris glycimeris*) at a depth of 75–80 ft. They occurred on more than 60 per cent of the shells in one dredge haul and have also been obtained by diving.

The life cycle of *Rhabdopleura* is poorly understood. Recent authors agree with Schepotieff[4] that *Rhabdopleura* is dioecious. Observations on the material from Stoke Point have already revealed some points of importance. A number of female, but no male zooids have been found. They can be recognized by the presence of large ovoid eggs in the metacoel. It appears that these eggs may be released proximally, through a temporary opening at the posterior end of the metasome, to develop in the tube of the parent in much the same way as in *Cephalodiscus*. If this is so, the gonoduct seems to have no function except as a possible point of entry for spermatozoa. Fertilization in *Rhabdopleura* may therefore prove to be internal. After liberation into the tube the embryos develop and acquire a coating of cilia. They then probably crawl from the tube by squeezing past the zooid to begin their free life, presumably as larvae[5], although this stage has never been described.

Previous authors[4,6] have noted the rarity of sexual zooids and the disproportionate ratios of each sex. Vaney and Conte[6] believed *R. normani* to be a protogynous hermaphrodite, because they found a male with what they thought to be an ovary at the base of its stalk, but subsequently[4] this interpretation was considered to be erroneous, leaving their observations unexplained. Structures similar to those figured by them as an ovary (Pl. 8, Figs. 32, 36 and 42) have been found in *R. compacta*. It appears that the base of the contractile stalk may bud off gemmae which develop in the tube of the parent in the same way as the sexually produced embryos. A figure which Lankester[7] could not interpret (his Pl. 39, Fig. 2) provides corroborative evidence for this hypothesis. It shows part of the creeping tube of a zooid in which there are six embryos. One is attached to the contractile stalk, and appears to be budding from it. The colonies of *Rhabdopleura* may thus multiply asexually, as well as enlarging in this way.

It is hoped to develop these studies on *Rhabdopleura*. I wish to thank Drs E. and A. Southward and the Director of the Marine Biological Association's Laboratory for assistance, and also Professor E. W. Knight-Jones and Dr J. S. Ryland for helpful discussions. This work was supported by a Natural Environment Research Council research studentship.

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[1] Berrill, N. J., *The Origin of Vertebrates* (Oxford, 1955).
[2] Burdon-Jones, C., *Univ. Bergen Årb. Naturv. R.*, **11**, 1 (1954).
[3] Hincks, T., *History of British Marine Polyzoa* (London, 1880).
[4] Schepotieff, A., *Zool. Jb. (Anat.)*, **23**, 463 (1907).
[5] Burdon-Jones, C., in Parker and Haswell's *Textbook of Zoology*, **2**, Seventh edit. (revised by Marshall, A. J.) (London, 1964).
[6] Vaney, A., and Conte, A., *Rev. Suisse Zool.*, **14**, 143 (1906).
[7] Lankester, E. R., *Q. J. Microsc. Sci.*, **24**, 622 (1884).

β-Glycerophosphatase and Lateral Root Development

FOR many years plant anatomists[1] have believed that the emergence of lateral roots, which arise endogenously in the pericycle of the stele, occurs principally by mechanical rupture of the surrounding cortex. Torrey[2] expressed the view that emergence is probably assisted by hydrolytic enzymes, although to his knowledge there was no experimental evidence to support the postulate.

When investigating the distribution of β-glycerophosphatase in the roots of *Pisum sativum*, we found a large increase in activity of this enzyme in the region of lateral root development. Enzyme assays were carried out on 1 mm serial sections of 5 day old roots. The sections were ground into ice-cold buffer at *p*H 6·0 and an aliquot of the resultant homogenate was incubated with 10 mM Na β-glycerophosphate at 30° C. The rate of hydrolysis of the substrate was estimated by the rate of appearance of inorganic phosphate in the medium. Supplementary experiments had shown that no inhibitors or activators of the enzyme were present in the homogenates and that the rate of the reaction was proportional to the enzyme concentration.

The distribution of the enzyme activity along the axis of the root is shown in Fig. 1. There is a considerable increase in activity when expressed on a per section or per unit protein basis, in the region 2·5 cm to 4·0 cm from the root apex. This corresponds exactly with the region in which we observed lateral roots to be initiated. The results of Popham[3] show similarly that in 5 day old peas lateral roots were initiated from 2·3 cm behind the apex. This enzyme, in animal cells at least, seems to be associated with cell hydrolysis[4], so we thought the site of activity might be the cortex cells surrounding the lateral initials. To investigate this further, we carried out a histochemical examination of the localization of the enzyme. Cryostat sections were cut through the zone of lateral root development using the technique of Gahan *et al.*[5]. These sections were then incubated in the Gomori acid phosphatase reaction mixture[6] using 10 mM Na β-glycerophosphate buffered at *p*H 5·4. β-glycerophosphatase was localized as a brown–black precipitate of lead sulphide.

Examination of sections passing through the laterals showed activity clearly limited to the stelar cells and those cells of the cortex adjoining lateral roots (Fig. 2). Control sections to which either inhibitors or no substrate had been added showed no such activity. McGregor and Street[7] observed similar activity in the region of lateral root initials in tomato roots but they did not comment on its significance. Examination of sections under high power leaves no doubt that the activity surrounding the lateral occurs in the bordering cortex cells rather than in the outer cells of the lateral root itself (Fig. 3). The absence of activity around very young initials is a good indication that the β-glycerophosphatase activity is induced by wounding of the cortex cells, as a result of mechanical pressure exerted by the lengthening lateral initial. High glycerophosphatase activity has also been observed in the surface layers of roots where radial expansion is occurring, a situation where similar wounding is expected.

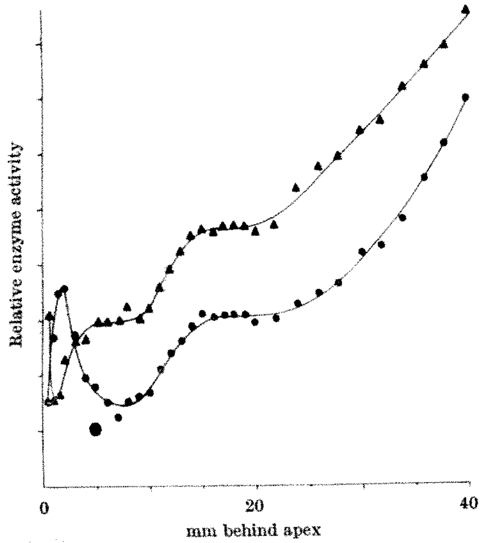

Fig. 1. β-Glycerophosphatase activity along the longitudinal axis of pea roots. Activity is expressed on a per section (●) and as a per unit protein (▲) basis.

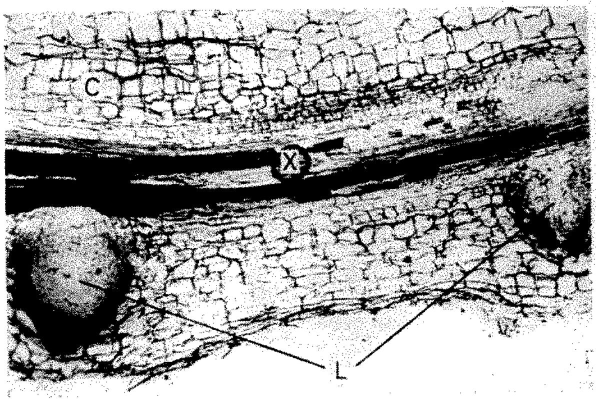

Fig. 2. Longitudinal section of a pea root through the region of lateral root formation. Incubated for 15 min, 30° C, in Gomori acid phosphatase medium. L, Lateral root initial; C, cortex; X, xylem cells. (× 40.)

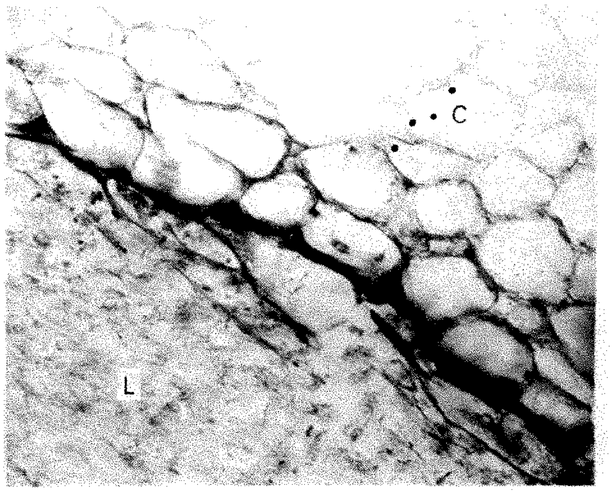

Fig. 3. Transverse section through the junction of a lateral initial and the cortex after incubating as in Fig. 2. L, Lateral root initial; C, cortex. (× 400.)

Our conclusion is that β-glycerophosphatase and perhaps other hydrolytic enzymes are induced by mechanical stimulation of cells surrounding the emerging lateral roots, and that this activity assists the lateral root in penetrating through the cortex.

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[1] Eames, A. J., and MacDaniels, L. M., *An Introduction to Plant Anatomy*, 286 (McGraw-Hill, 1951).

[2] Torrey, J. G., *Recent Advances in Botany Ninth Intern. Cong.*, **1**, 808 (1959).

[3] Popham, R. A., *Amer. J. Bot.*, **42**, 529 (1955).

[4] DeDuve, C., *Subcellular Particles* (edit. by Hayashi, T.), 128 (Ronald Press, 1959).

[5] Gahan, P. B., McLean, J., Kalina, M., and Sharma, W., *J. Exp. Bot.*, **18**, 151 (1967).

[6] Gomori, G., *Microscopic Histochemistry*, 189 (University of Chicago Press, 1952).

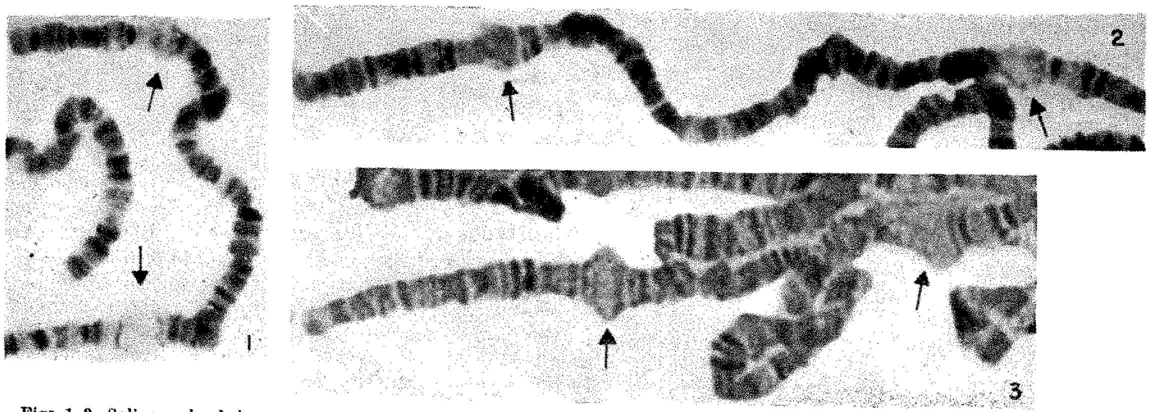
[7] McGregor, S., and Street, H. E., *Ann. Bot. N.S.*, **17**, 385 (1953).

CYTOLOGY

Inhibition of Development in *Drosophila* by Cortisone

GENE transcription, whereby enzyme reactions mediate the synthesis of RNA molecules from DNA templates, has been investigated mostly in microbial organisms, but studies with higher organisms have made fundamental contributions to our knowledge of this process. One of the more rewarding areas of study has centred on the role of hormones in the regulation of transcription. Investigations in vertebrates have revealed that RNA synthesis can be stimulated in some cases[1,2], and inhibited in others[3,4]. There have been analogous findings on the regulatory effects of hormones in invertebrates. The phenomenon of puff formation in the polytene chromosomes of certain Diptera[5] has been extensively investigated. Autoradiographic techniques have established unequivocally a direct relation between puff formation and RNA synthesis, and these studies have long been accepted as cytological evidence of gene transcription[6].

Recent observations in *Sciara*[7] have indicated that the steroid hormone, cortisone, can completely suppress the puffing process and the uptake of tritiated uridine by salivary gland chromosomes, while the normal developmental sequence, viability and fertility are unaffected. RNA production is undoubtedly necessary for normal development and normal development can apparently occur in the absence of puffing, implying that puffing and uptake of tritiated uridine are not accurate reflexions of RNA synthesis in the cell. Because of the importance of this process in studies of gene activity in *Drosophila*, it seemed necessary to confirm the findings in this organism.



Figs. 1–3. Salivary gland chromosome preparations from late third instar larvae showing large puffs at the distal end of chromosome 3L. 1, Cortisone-fed larva, 2 mg/ml. 2, Cortisone-fed larva, 2 mg/ml. 3, Control larva.

Three preliminary experiments are outlined. In the first experiment, designed to test the effects of cortisone on developmental time and viability, eggs were collected and placed on a medium of agar, sucrose, dried yeast and propionic acid in 10 per cent ethanol (to aid in cortisone solubilization). To this medium was added 0, 1·0, 2·0, 5·0 or 10·0 mg/ml. of cortisone (Sigma). Concentrations greater than 10 mg/ml. were lethal. The time of emergence and the relative viability were determined for each group. The distributions of control versus experimental groups were compared by means of the Kolmogorov–Smirnov test. Table 1 shows the mean time of emergence, the *P* value for each distribution and the percentage relative viability. In addition to reducing the viability of the experimental groups, it can be seen that cortisone, administered in doses of 2·0, 5·0 or 10·0 mg/ml., causes a delay in the developmental sequence.

Table 1. EMERGENCE AND VIABILITY OF LARVAE FROM EGGS PLACED ON MEDIUM CONTAINING CORTISONE

Concentration of cortisone (mg/ml.)	No. of eggs	Mean time of emergence (h)	Kolmogorov–Smirnov *P* value	Percentage viability
Control	60	279·6		83·3
1	60	274·8	⩾0·20	61·6
2	60	298·6	<0·01	63·3
5	60	292·7	<0·01	71·7
10	60	292·7	<0·01	68·3

In the second experiment, eggs were deposited on a medium containing cortisone (0·5, 1·0, 1·5, 2·0 and 2·5 mg/ml.) and allowed to develop to the prepupal stage. At this time their salivary gland chromosomes were examined, the presence of large puffs such as *74EF–75B* and *78D* being used as criteria of gene activity. At each concentration of cortisone employed, large puffs were in evidence (Figs. 1–3). Concentrations greater than 5 mg/ml. resulted in the death of the larvae or the production of prepupae with salivary glands unsuitable for cytological examination.

In the third experiment, eggs were placed on cortisone-free medium and allowed to develop to the late larval stage. Salivary glands were removed and one of each pair was incubated in a cortisone solution (0·0025, 0·025, 0·25, 0·5 and 1·0 mg/ml. of 10 per cent ethanol–Ringer's solution) while the other was incubated in a control of 10 per cent ethanol–Ringer's solution for periods of 15, 30, 45, 60, 90, 120 and 180 min. At each concentration for each time period, large puffs, similar to those shown in Figs. 1–3, were observed in both the treated and control glands. There were several where the large, easily identified puffs were not evident in either treated or control glands. No correlation existed between these and the concentration of cortisone or incubation time, so they can probably be explained by the unavoidable variability in the developmental status of the various larvae.

Although it may be argued that measurement of the uptake of tritiated uridine could provide a more accurate assessment of the effects of cortisone on RNA synthesis, present cytological observations clearly indicate that cortisone does not visibly suppress puff formation. The possibility still exists that cortisone may act to delay development by slightly inhibiting general RNA synthesis or by inhibiting the synthesis of specific RNA species.

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[1] Fujii, T., and Villee, C. A., *Proc. Second Inter. Cong. on Hormonal Steroids*, Milan, 29 (1966).

[2] Greenman, D. L., Wicks, W. D., and Kenney, F. T., *J. Biol. Chem.*, **240**, 4420 (1965).

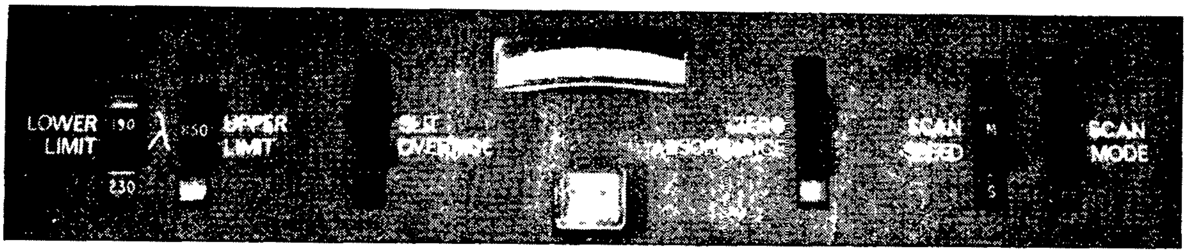
[3] White, A., and Makman, M. H., *Advances in Enzyme Regulation*, **5**, 317 (1967).

[4] Nakagawa, S., and White, A., *Proc. US Nat. Acad. Sci.*, **55**, 900 (1966).

[5] Clever, U., *Chromosoma*, **12**, 607 (1961).

[6] Pelling, C., *Nature*, **184**, 655 (1959).

[7] Goodman, R. M., Goidl, J., and Richart, R. M., *Proc. US Nat. Acad. Sci.*, **58**, 553 (1967).



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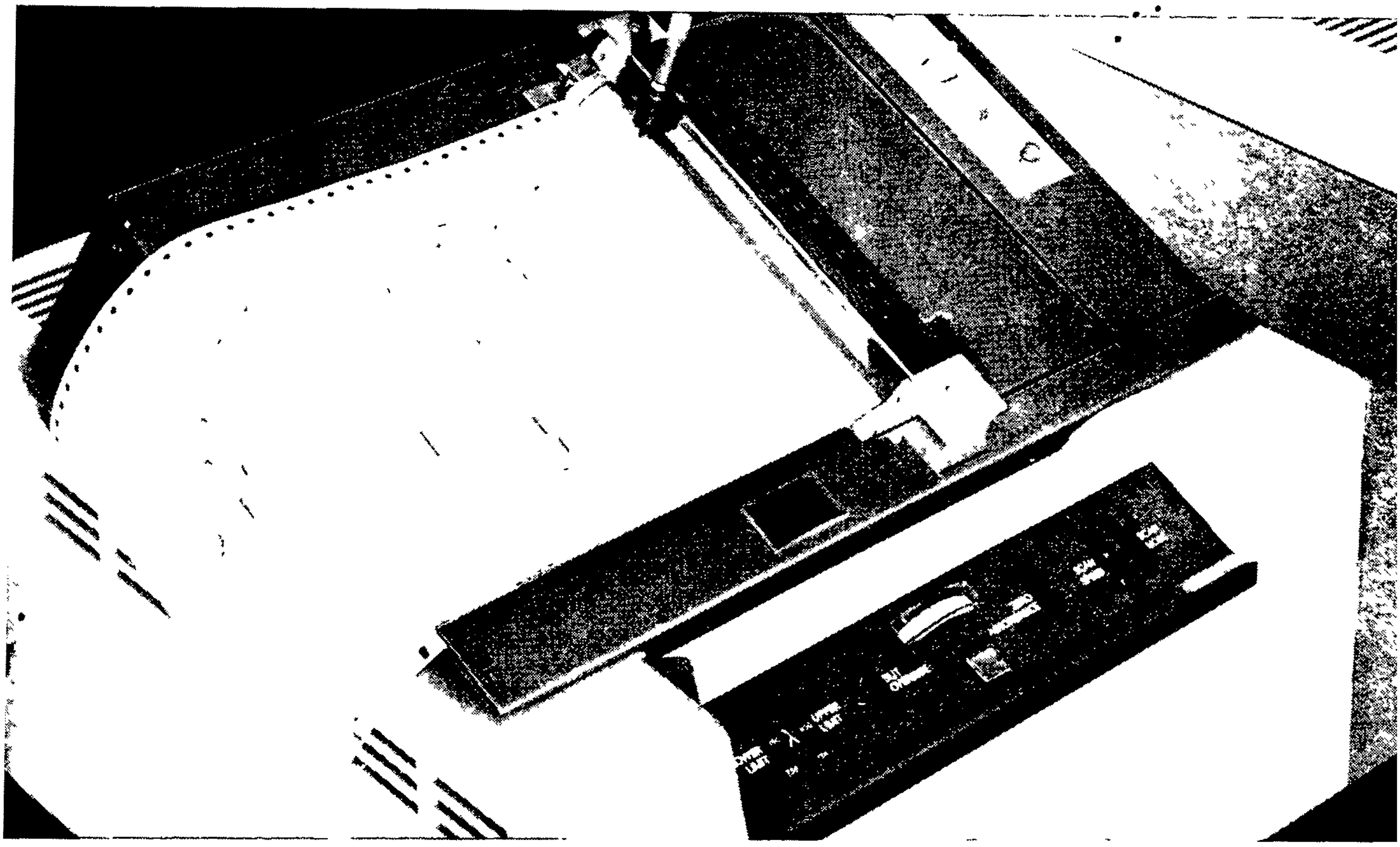

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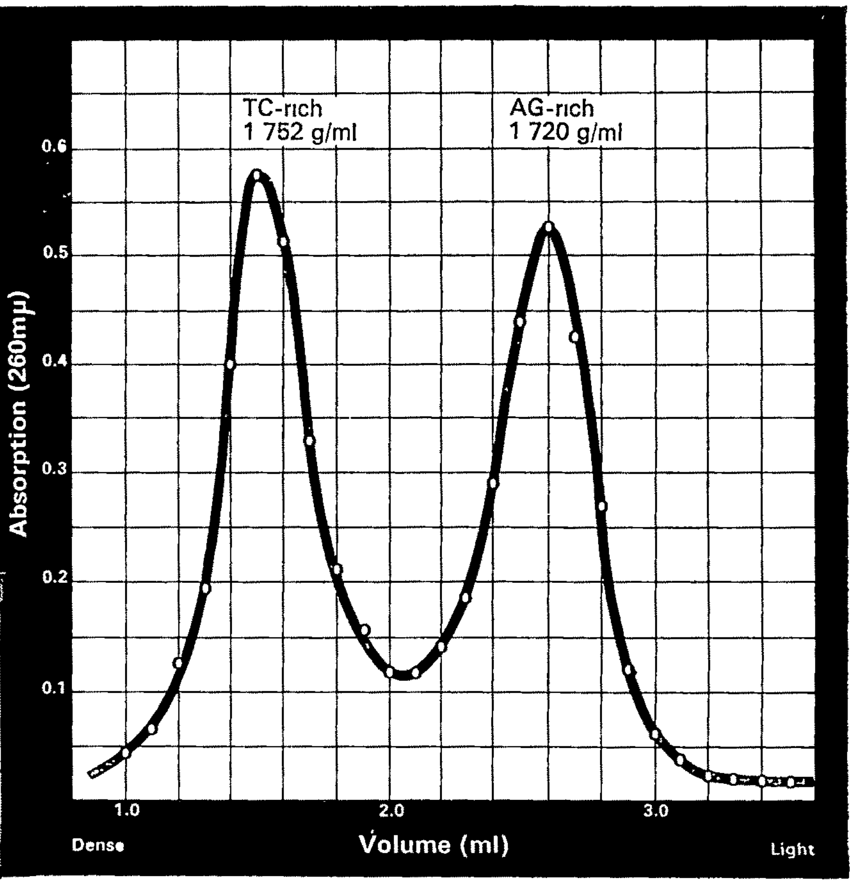

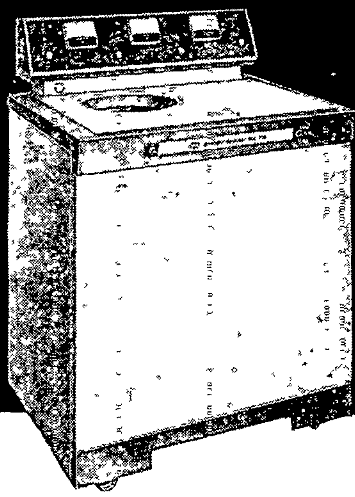

50 μg of mouse satellite DNA in Tris-HCl buffer was treated with sodium lauryl sulphate at pH 12.7 and centrifuged in a caesium chloride gradient (initial density 1.765 g/ml at 25°C) for 20hr at 42,000 r.p.m. at 25°C.

This separation was carried out on an MSE Super Speed 50 Preparative Ultracentrifuge using the 10 x 10ml angle rotor instead of the more usual swing-out rotor. Full details of the procedure are given in MSE Application Sheet No 2, available on request.

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APPLIED SCIENCE

Sintering in Gas Discharges

The application of gas-phase glow discharges to the activation of many chemical reactions is becoming increasingly important but the parallel application to various aspects of the processing of materials is not as yet so well known Dugdale[1] has described the mechanism and characteristics of the d c glow discharge and particular applications to both hollow cathode and hollow anode glow discharge heaters, which can be used for processes requiring an intense heat flux such as zone refining and evaporation machining

We have applied a microwave-stimulated electrodeless gas discharge to the sintering of various ceramics, metals, metalloids and mixtures and compounds thereof Particular consideration has been given to refractory oxides including alumina, beryllia, hafnia, magnesia and thoria and some results which are unusual in comparison with those achieved by conventional heating methods have been obtained This communication is principally concerned with the rate of sintering of alumina

Radio frequency power at 2,450 MHz is generated by a magnetron and fed to a rectangular wave guide by co-axial line The sintering chamber within which the discharge is established is a silica or alumina tube inserted transversely through the wave guide, which can be tuned so that the electric field intensity is a maximum in the zone in which the tube lies (Fig 1) At a fixed pressure in the sintering tube, the specimen temperature is controlled by varying the d c voltage applied to the magnetron

Thermocouples cannot be used to measure the temperature of the specimens because of direct heating by the glow discharge of the metallic elements of the couple Optical and radiation methods are also limited to some extent by interference from the radiation from the discharge below about 1,500° C true temperature, and it is then necessary to take measurements during short periods when the excitation is switched off Because of the low emissivity of the surfaces of oxide compacts and the "cold wall" conditions in the glow discharge, the apparent temperature is considerably below the true temperature Correction curves have been established by "push-out" experiments of pressed compacts of each oxide from a calibration furnace measured by reference thermocouple to a simulated "cold wall" environment where the apparent temperature is measured by successive approximations using optical and quick-reading radiation pyrometers

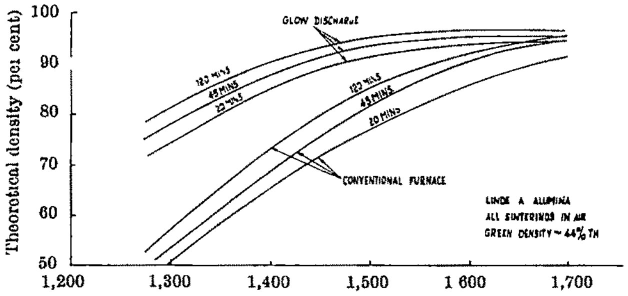

Fig 2 Isochronal densification curves for alumina

The pressure in the sintering tube is maintained at a chosen level in the range 1–50 torr by manipulation of a throttled backing pump and a controlled leak through which the required sintering atmosphere is admitted The nature of the atmosphere affects the maximum temperature which can be obtained Thus in hydrogen the peak temperatures are much lower (for example, 1,500° C) than in nitrogen, oxygen, air and carbon dioxide (about 2,000° C) for the same power input (1 kW) and experimental conditions A subsidiary consequence of change of operating pressure is the effect on the volume of the glow discharge For sintering small cylindrical pellets statically, or for the continuous sintering of long rods and wires, the discharge is usually constricted well inside the wave guide to give a short high temperature zone For the static sintering of long specimens, the discharge may be extended to envelop a length of more than 3 in The characteristics of the densification curves are such that very uniform sintering occurs over the full length of such a specimen

The densification achieved in pressed compacts of Linde *A* alumina by sintering for various time and temperature conditions in both glow discharge and conventional furnaces is plotted isochronally in Fig 2 as the percentage of theoretical density versus true sintering temperature The data illustrate the very marked increase in rate of densification in the discharge method It has been confirmed that the low pressure used in this method has not significantly affected the experimental results

The mechanical strengths of materials sintered in both ways are plotted in Fig 3 To obtain these results, small rods were isostatically pressed and sintered, and then machined to a square bar of a standard size used in these laboratories for four point bend tests While the average strength of the gas discharge sintered specimens does pass

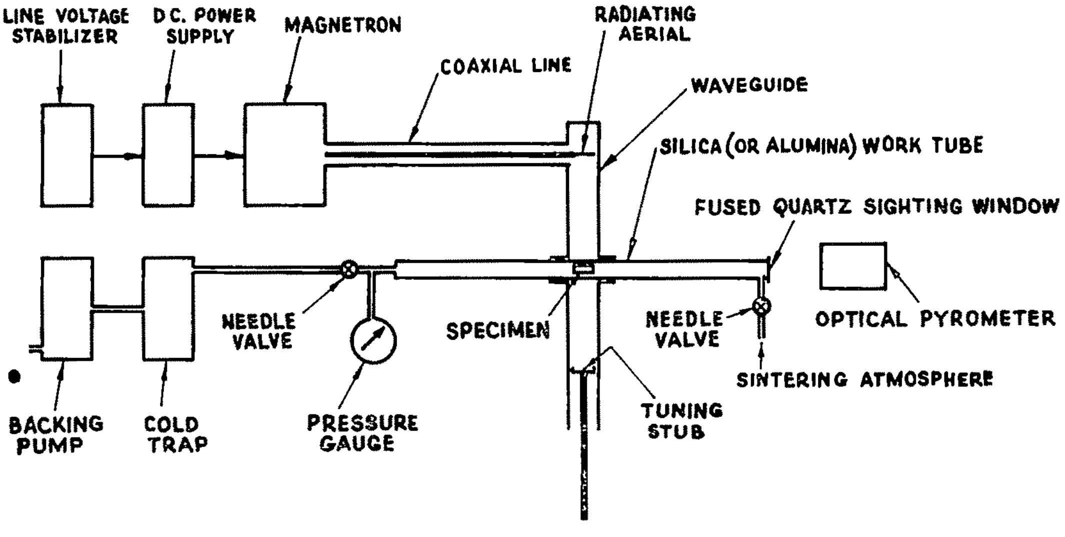

Fig 1 Gas discharge sintering apparatus

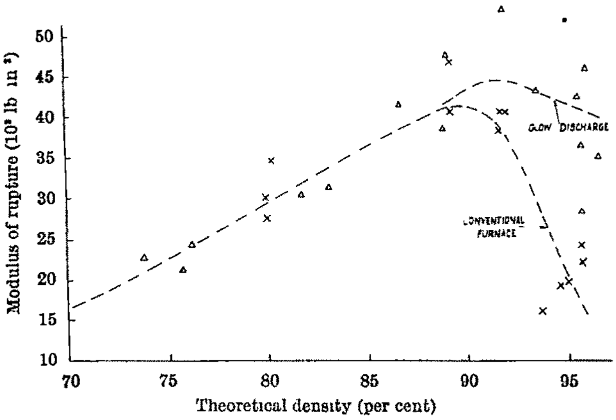

Fig 3 Strength-density relations for conventionally sintered and glow-discharge sintered alumina bars ×, Conventional furnace, Δ, glow discharge

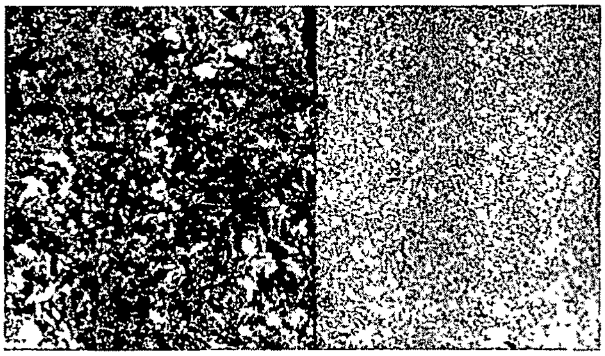

Fig 4 Fracture surfaces of coarse-grained conventionally sintered and fine-grained glow-discharge sintered bars (×16) (100 Å of aluminium vacuum deposited to increase reflectivity)

through a maximum and fall off slightly as high densities are reached, this effect is not nearly so marked as for conventionally sintered specimens of the same densities This difference in behaviour can be attributed at least in part to the much smaller grain size in discharge-sintered specimens (Fig 4) There is also the possibility that cleaner grain boundaries with fewer mechanical imperfections may contribute to the higher strength

The nature of the atmosphere is not a factor in obtaining the results given in Fig 2, for all specimens sintered at a chosen temperature in any atmosphere, the densities fall very close to the curve given, and the atmosphere influences only the efficiency of heating Chemical effects from this source can thus be discounted

While the experimental results given here are confined to Linde *A* alumina, the material which has been investigated most extensively, acceleration of densification has also been observed for aluminas from other sources and for other oxides The increase in rate of sintering is more marked with very fine highly sinterable powders like Linde *A* and Alcoa *XA*16 aluminas, and *NGK-CF* grade beryllia Even a moderately coarse oxide by these standards—Wah Chang Reactor Grade *S* hafnia, of nominal size 8μ as measured turbidimetrically—sintered to 90 3 per cent theoretical density in 25 min at 1,525°–1,550° C in an air glow discharge, however, whereas similar specimens treated in a normal furnace required a temperature of 1,700° C to reach 87 5 per cent density in the same time

The following hypotheses have been advanced to explain the observed rapid densification (1) an increased driving force through the increase in available surface energy associated with the cleaning of particle surfaces throughout the compact by release of adsorbed gases and precursor residues by ionic bombardment, (2) an increase in diffusion rate by the creation of vacancies and other mechanical phenomena caused by ionic bombardment, and (3) an increase in diffusion rate through local increases in temperature inside the pores of the compact The phenomenon of enhanced densification in gas discharge sintering is dependent on particle size and so it seems certain that the glow discharge is created within the pores; this is, of course, necessary for all three hypotheses. Kinetic and other relevant phenomenological studies are in progress

We thank Dr F K McTaggart, who developed the glow discharge device for use on the stimulation of chemical reactions, for many helpful consultations and Mr D P James for his contribution in the initial stages of the investigation

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[1] Dugdale, R A , *J Mat Sci* ,1, 160 (1966)

GENERAL

Improved Holographic Model of Temporal Recall

The static model of temporal recall which I recently proposed[1] as an alternative to Longuet-Higgins' model[2] is unsatisfactory in one respect From a fragment which is remembered it can recall earlier parts of the whole sequence This is unlike human memory which can readily recall later parts, but earlier parts only with great difficulty, if at all, especially if the sequence has been memorized by rote If we want to imitate human memory with models, we must take account of the weaknesses of the nervous system as well as of its powers Longuet-Higgins' model possesses this property, and so does the modified static system described here.

The original sequence is $A(t)$, $0<t<T$ We make a record of this in the form

$$\varphi(t_1) = \int_{t_1}^{T} A(\tau)A(\tau-t_1)\mathrm{d}\tau$$

This can be done only when the sequence has come to an end We can assume that first a straight "phonographic" record is made in the transient, short-time memory, and afterwards it is transcribed in a permanent memory in the form of the truncated autocorrelogram

We remember a fragment A' of A, which starts at t_0, so that

$$A'(t) = A(t+t_0)$$

up to some upper limit of t With this we form the recall function

$$R(t) = \int_{0}^{t} A'(t-t_1)\varphi(t_1)\mathrm{d}t_1$$

which is a truncated convolution of A' and φ. Substituting $\varphi(t_1)$, we obtain an integral of the triple product

$$A(\tau)A(\tau-t_1)A'(t-t_1)$$

over the trapezoidal area $t_1<\tau<T$, $0<t_1<t$, illustrated in Fig 1 For comparison the trapezoid used in the previous model is also shown

It is readily seen that if the sequence A is "noiselike" the correlation A', A will have a sharp maximum at $\tau=t+t_0$ and this will single out the value of $A(\tau)$ at this abscissa, so that apart from some noise

$$R(t) \propto A(t+t_0)$$

Thus we can recall the whole sequence A, but only from t_0 to T, and thereby the model imitates the weakness of a

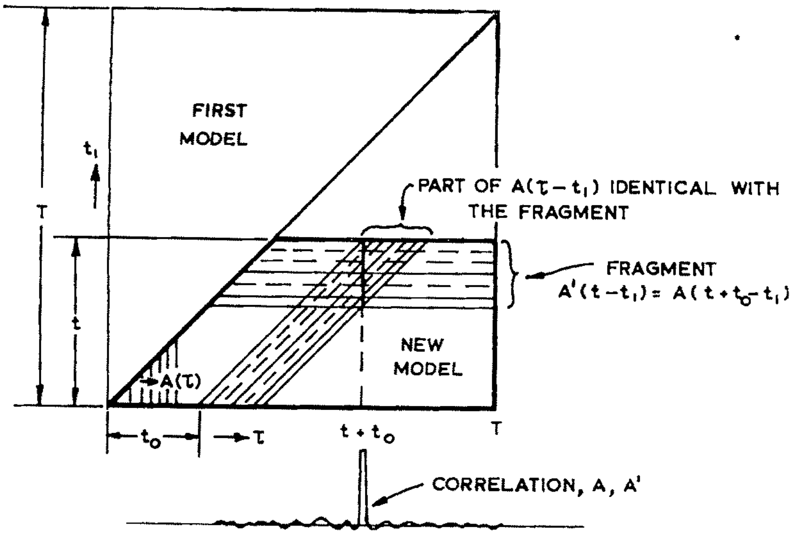
FIRST
MODEL
t_1
T
t
PART OF $A(\tau - t_1)$ IDENTICAL WITH THE FRAGMENT
FRAGMENT
$A'(t - t_1) = A(t + t_0 - t_1)$
NEW
MODEL
$A(\tau)$
t_0
τ
$t + t_0$
T
CORRELATION, A, A'

Fig 1

person who has memorized something by rote. My previous model, starting with a fragment which begins at t_0, could recall the sequence from $t=0$ to $T-t_0$ There was nothing to prevent it, after having obtained the beginning of the sequence, from starting again and recalling the whole This may be a better method if we want to construct a library for fragmentary recall, but it is rather "inhuman"

There is still a feature which distinguishes this model from Longuet-Higgins' bank of resonators It contains nothing which prevents reading the reconstructed sentence backwards, which is a difficult feat for the human memory It shares this feature with all static stores, such as a gramophone record or a tape recording, unless a "ratchet" is added which allows only one sense of reading Longuet-Higgins' bank of resonators contains such a ratchet, because it is a dynamic model

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[1] Gabor, D , *Nature*, 217, 584 (1968)
[2] Longuet-Higgins, H C , *Nature*, 217, 104 (1968)

Impotence Principle in Descriptive Morphology

It was recently proposed[1] that biological form should be specified by an expansion of the characteristic function of the form as a weighted sum of the elements of a complete set of orthonormal functions The method was applied to two-dimensional forms, using Walsh functions[2] In the discussion it was suggested that each weight might be maximized with respect to all possible transformations of the form caused by any combination of displacements, rotations and uniform contractions or expansions It was conjectured that this sequence of "maximal" weights constituted an unequivocal specification of the form, that is to say, two different forms would always be represented by different sequences In the case of the Walsh functions, these weights would be real numbers lying in the interval $-\frac{1}{2}$ to $+\frac{1}{2}$, except for the first one which would lie in the interval 0 to 1

If one accepts the notion of form in complete generality, this conjecture is false We have not been able to construct an actual non-trivial counter-example in the case of two-dimensional forms in a square frame that we had discussed[1]; but we have found one for a similar system using a fixed circular frame, in which the form is blown up or contracted so as to just fit into the frame and only rotational transformations need to be considered This counter-example led us to think that there may be some inherent limitation on the possibilities of mathematical description in morphology, and this is indeed the case We prove that it is impossible uniquely to specify all three-dimensional or two-dimensional forms by finite or denumerable sequences of real numbers.

We prove this for two-dimensional forms—it will then hold for three-dimensional forms, because all two-dimensional forms can obviously be put in one-to-one correspondence with a subset of all three-dimensional forms

For definiteness, consider a Cartesian co-ordinate system in the plane and let $f(x,y)$ be the characteristic function representing one specimen of a form, that is, it takes the value 1 at points covered by the specimen and 0 elsewhere.

Let $f\alpha(x,y)$ be the characteristic function representing any other specimen of this form, that is, the original form transformed by any combination of displacements, rotations and finite uniform contractions or expansions

Let $S_f = \{f\alpha(x,y)\}$ be the set of all such transformed functions derived from $f(x,y)$ The cardinality of S_f is clearly c, the cardinal number of the continuum

The cardinality of the set of all characteristic functions $f(x,y)$ whatever is obviously 2^c Because the S_fs constitute a partition of the set of all these characteristic functions into disjoint subsets, it follows that if k is the cardinal number of the set of S_fs, then

$$k \times c = 2^c \qquad (1)$$

k is clearly the cardinal number of all possible two-dimensional forms, each S_f corresponding uniquely to a single form

Suppose it were possible to assign to each S_f a finite or denumerable sequence of real numbers, expressed for example in binary digits To each such sequence we can make to correspond in one-to-one fashion a single real number, for example, if d_{ij} is the jth digit of the ith real number in the sequence one could form the real number $d_{11}\, d_{12}\, d_{21}\, d_{13}\, d_{22}\, d_{31}$ and so on, where in the obvious systematic way one writes down successively the digits, the sum of whose index numbers is respectively 2, 3, 4, . . , and so on. Hence one would have been able to set up a one-to-one matching correspondence between the S_fs and the real numbers or a subset of them One must therefore have

$$k \leq c \qquad (2)$$

From (1) and (2) it would follow that

$$2^c \leq c \times c$$

But

$$c \times c = c$$

Hence

$$2^c \leq c, \text{ which is impossible}$$

Thus we see that there just are not enough real numbers for the unique specification of all possible forms

This result does not mean that one should give up the numerical approach to morphological description. For, it depends on interpreting form in its fullest possible generality—for example, a leaf with an infinite number of smaller and smaller holes would under this rubric be a form Clearly, natural forms would constitute some subset of all forms, and it would be profitable to look for some general model for characterizing them. For such a restricted set of forms it may well turn out that real numbers or perhaps even rational numbers would be sufficient for unequivocal description One possibility, suggested by Professor J. A Robinson, is—in the two-dimensional case, for example—to consider only forms whose boundary can be approximated to any desired degree of accuracy by a finite number of polygons, other possibilities readily spring to mind

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[1] Meltzer, B , Searle, N H , and Brown, R , *Nature*, **216**, 32 (1967)

[2] Walsh, J L , *Amer J Math* , **45**, 5 (1923)

Incongruent Surfaces in the Human Hip Joint

Hammond and Charnley[1] have recently given support to the view that the femoral head is almost exactly spherical, that the surfaces of the acetabulum and femoral head are congruent so that "very perfect contact of all parts of the cartilaginous surfaces is to be expected in any position of the hip joint" In the past, however, several observers[2,3] were convinced that the surfaces of this joint are incongruent or, at best, fit one another in only one position If the latter view is correct, contact will not be made over the entire joint and the site and size of the actual contact areas in a given joint will depend on the relative position of the joint surfaces and on the magnitude and direction of the load transmitted through it

Goodfellow and Bullough[4] have shown that the surfaces of the human humero-ulnar joint are incongruent in youth but that, in old age, the surfaces become more closely fitting, they have further suggested[5] that the same effects exist in the hip joint Changes in joint profile with age were observed by Ogston in 1878 (ref 6) but he did not comment on the congruence of the surfaces

We have measured the curvature of both surfaces of the hip joint A three-legged gauge was used, the central leg was driven by a micrometer screw, and the outer legs had a span of 0.75 in Fifty-three hips, obtained at necropsy from subjects of different ages, were examined The radius of curvature was measured at four points, equidistant from each other along an equatorial circle, on each surface, taking four measurements at each point

It was found that there were substantial differences in curvature from point to point on both the acetabulum and the femoral head The difference between the maximum and the minimum radius, divided by the average of the four radii, was taken to be a measure of the departure from sphericity of each surface Fig 1 shows this ratio plotted against age for each of the 106 surfaces examined

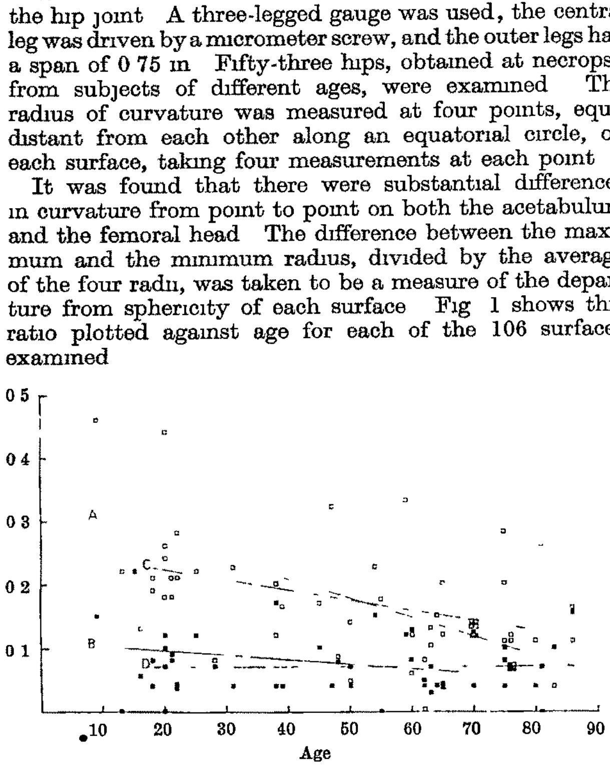

Fig 1 Departure from sphericity with age of the femoral head (■) and acetabulum (□) $y = R_{\max} - R_{\min}/R_{\mathrm{av}}$

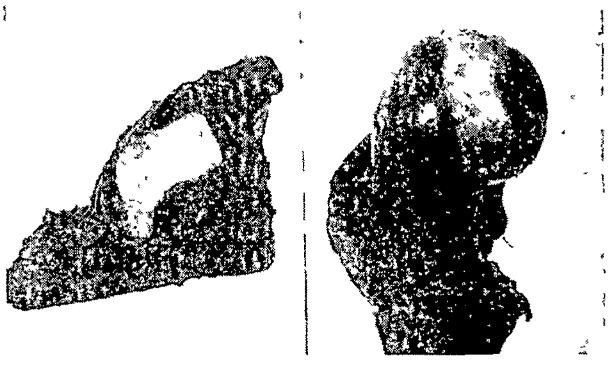

Fig 2 Disarticulated hip from a young male The non contact areas in 10 degrees of flexion are dyed

A statistical analysis was carried out on these data, fitting "least square" straight lines to the data for the acetabulum and for the femoral head These calculations were made including and excluding the data for hips aged eighteen years and younger The equations of these lines are (with 95 per cent confidence limits)

A, Acetabulum $y_0 = -0.003\,X_0 + 0.327 \pm 0.221$ (more than 9 yr)

B, Femoral head $y_0 = -0.0005\,X_0 + 0.105 \pm 0.103$ (more than 9 yr)

C, Acetabulum $y_0 = -0.0016 + 0.2526 \pm 0.135$ (more than 18 yr, that is excluding growth period)

D, Femoral head $y_0 =$ horizontal (more than 18 yr, that is excluding growth period)

Despite the scatter in these data, some conclusions can be drawn Neither the acetabulum nor the femoral head is spherical, the acetabulum is less spherical than the femoral head, and there is a "highly significant" decrease in the asphericity of the acetabulum with increasing age (correlation coefficients -0.399 for line *A*, -0.362 for line *C*) It is worth noting that nine of the ten specimens examined by Hammond and Charnley came from subjects aged 60 or more

The joint surfaces are not spherical, and so they cannot be congruent, although these data do not necessarily deny the proposition that the surfaces may be more congruent in some positions than in others

Because the hip joint is incongruent we would suggest that the site and size of the actual contact areas will depend on the position of the joint and the magnitude and direction of the applied load We have built a loading frame to apply static loads of known magnitude and direction to a hip joint in a variety of positions in simulated body conditions. The contact areas can be detected by adding dye to a bath of Ringer solution in which the loaded hip is immersed The results of a preliminary test, made on a young male hip in ten degrees of flexion, hre shown in Fig 2 The white areas on both the femoral aead and the acetabulum are taken to be the zones of contact which clearly do not cover the entire available surface

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[1] Hammond, B T , and Charnley, J , *Med Biol Eng* , **5**, 445 (1967)

[2] MacConaill, M A , *J Bone Joint Surg* , **32**, B, 244 (1950)

[3] Walmsley, T , *J Bone Joint Surg* ,**10**, 40 (1928)

[4] Goodfellow, J , and Bullough, P , *J Bone Joint Surg* , **49**, B, 175 (1967)

[5] Goodfellow, J , and Bullough, P , *J Bone Joint Surg* (*Brit*) (in the press)

[6] Ogston, A N , *J Anat Physiol* ,**12**, 503 (1878)

BOOK REVIEWS

CANCER CHEMOTHERAPY

Immunity, Cancer, and Chemotherapy

Basic Relationships on the Cellular Level. Edited by Enrico Mihich. Pp. xxiii + 390. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1967.) 144s.

WITH as yet very few exceptions, drugs used in the treatment of malignant disease are effective because they have the capacity to kill dividing cells in general and their action is not restricted to cancer cells. Cell division forms an essential part in the genesis of the immune response, and so it is not surprising that many anti-cancer agents interfere both with the formation of antibody and the rejection of foreign tissues. This dual action poses some extremely serious problems for cancer chemotherapy, for many tumours in experimental animals are now known to contain antigens in their membranes that are not present in the normal cells of the animals. These antigens introduce a slight degree of "foreignness" and in many experimental situations the growth of primary tumours is slowed down by an immunologically mediated host reaction. The possibility exists, therefore, that the immuno-suppressive action of anti-cancer drugs may impair the anti-tumour host response, and this has been observed with grafted tumours where the drugs reduced the magnitude of a primary immune reaction. No information appears to be available for primary tumours where an immuno-suppressive action would have to be directed against an already established immune response.

In man, the situation is still more complex, because the techniques used in animals to detect the presence of an anti-tumour host reaction cannot be applied clinically and the therapist does not know whether such a response exists in a particular patient. The theoretical danger of immuno-suppression is clearly not an argument against cytotoxic drugs when there is extensive disseminated disease but there are other circumstances such as prophylactic administration post-operatively when their use may be questioned.

A thorough discussion of the available knowledge of the influence of immuno-suppression on chemotherapy would clearly be most valuable but the book under review does not meet this requirement. It is the report of a symposium that took place in Buffalo, New York, but there is no indication as to when the meeting was actually held and none of the papers reproduced is, in fact, very topical. In some respects the most interesting part of this volume is the report of the discussion, which is both stimulating and informative, and the editor deserves great credit for what must have been a time-consuming and difficult task.

The title is misleading, for only one of the eighteen papers deals specifically with cancer and there are three papers by Sterzl, Schwartz and Berenbaum respectively devoted to the mode of action of immuno-suppressive agents without, however, going into their anti-tumour properties. Three-quarters of the book is concerned with different facets of immunology and includes such problems as the nature of the cellular response to antigens, the processing and localization of antigens, as well as the biochemical mechanism of protein synthesis. This vast field is covered too thinly for this book to make a worthwhile contribution to the scientific literature.

PETER ALEXANDER

IMMUNOLOGICAL VADE-MECUM

Handbook of Experimental Immunology

Edited by D. M. Weir. Pp. xiv + 1245. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967.) 160s. net.

IMMUNOLOGY was derived from bacteriology, but has long since developed into a discipline in its own right which impinges upon, and is useful to, other disciplines such as biochemistry, medicine, genetics or parasitology and has a peculiar fascination for molecular biologists. In doing so it has developed its own jargon and its own techniques. Although these may appear frightening to persons unfamiliar with them, some of the techniques are astonishingly simple, others are complicated in concept, though technically easy (provided—and this is a big proviso—that suitable reagents are available), and others are just plain complicated. There is no corpus of accepted experimental techniques, although good monographs exist relating to some of them, and Kabat and Mayer's *Experimental Immunochemistry* (especially the first edition, which made less demands on its readers) has probably done more than any other single work to advance immunology in those fields which it covered. It has been agreed for quite a number of years that a comprehensive book which explained immunological techniques in sufficient practical detail to make them usable by non-immunologists as well as immunologists was highly desirable. The questions were who could be persuaded to write it, and how much or how little theory would be required to make the techniques more than plain cookery. The publication of a *Handbook of Experimental Immunology* has therefore been awaited with considerable eagerness.

It contains thirty-six chapters and two appendices, written by twenty-nine authors from Britain, ten from the United States and three from the mainland of Europe. All the authors have contributed to the practical aspects of the methods which they discuss, and some are their originators. This has ensured that the presentation is authoritative. On the whole a fair balance has been kept between the need to supply a theoretical background and the provision of sufficient technical detail to allow the user to put the methods described into practice without further resort to other books or journals. Since each chapter contains a bibliography (which is well selected so far as I could judge) it is usually possible to find a reference to some specialized technique such as the isolation of a specific anti-hapten antibody.

The handbook contains more than 1,200 pages, and covers methods for isolating and characterizing immunoglobulins, for study of their synthesis *in vitro*, and for examining human immunoglobulin allotypes. There are useful chapters on preparation of viral, tissue and bacterial antigens (but surprisingly omitting the pneumococcal antigens, which are well characterized chemically, even if now of lesser medical importance than before the introduction of antibiotics). Several chapters deal with different aspects of primary and secondary antigen–antibody interaction, including immunofluorescence and immunoferritin techniques, the use of radio-iodinated proteins, and methods for gel diffusion, micro-complement fixation and passive haemagglutination; specific applications in virology, mycology and parasitology, methods for study of anti-tissue antibodies and of various aspects of cell mediated immunity, especially in relation to transplantation, demonstration of antibody forming cells by localized haemolysis in gels, methods for studying reaginic antibodies and pharmacological mediators, and there are appendices on statistical methods and on the use of emulsions for immunization.

The choice of what to include or exclude is inevitably somewhat arbitrary and to satisfy everyone's needs in one volume is probably impossible. Most laboratories concerned with immunology are likely to find that the bulk of their methodology has been catered for, even if indirectly (for example, the commonly used method for quantitative assay of immunoglobulin concentrations by radial diffusion against monospecific antisera is mentioned only in the barest outline). A future edition, however, might benefit by consideration of chromium-51 labelling of red cells and leucocytes; mixed cell agglutination and mixed antiglobulin tests; radio-immuno assay of pharmacologically active peptides; cytophilic antibodies other than reagins, methods involving inhibition on stimulation of cell migration, and perhaps by an extension of the chapter on methods for isolation of specific antibodies. It would certainly benefit by a better index, for a good index enables the reader, who is unlikely to read from cover to cover, to make the fullest use of the wealth of information incorporated in the handbook. Furthermore, quite a brief use of the index revealed to me an uncomfortably large number of errors. These are minor criticisms. The book fulfils a real need and will not disappoint the expectations of the many who have awaited its publication. At 160*s* its price is very reasonable. J. H. HUMPHREY

PLANT PATHOLOGY

Physiological Plant Pathology

(Botanical Monographs, Vol. 6.) By R. K. S. Wood. Pp. xi+570. (Oxford and Edinburgh: Blackwell Scientific Publications, 1967.) 63*s* net.

UNLIKE animals, plants have few specialized organs and, with this generalized organization, the symptoms of plant disease are less specific and informative than the symptoms of animal disease. Symptoms only provide the most general expression of the relation between the host and the parasite. A deeper understanding of this relationship must be built up by integrating into a coherent whole the results of a variety of observations and experiments, which are usually open to various interpretations which can be highly subjective. Professor Wood tries to give an unbiased picture by presenting as complete an account as he can of the available evidence with suitable comment and leaving the reader to make his own judgments. The result is about 500 pages of comment supported by a bibliography of about 1,300 references ranging in time from 1866 to 1965. This approach leads to some curious anomalies in treatment since well founded and generally accepted concepts tend to generate less literature than those that are more controversial. For example, the role of gibberellins in the development of bakanae disease of rice or the relationship between protocatechuic acid and resistance to onion smudge are dismissed in four and one pages respectively, while the very doubtful part played by fusaric acid and lycomarasmin in fusarium wilt of tomatoes, which is far from accepted, is discussed for sixteen pages. The current state of knowledge and opinion relating to the effect of toxins in tomato wilt is admirably summarized in the first two paragraphs of the tenth chapter: this would have sufficed for the casual reader while the addition of a short introduction to the bibliography would have introduced readers, more deeply concerned, to the full controversy. If this approach had been applied to other sections of the book the reader would have a clearer picture of the development of the subject and the benefit of Professor Wood's long experience in the field in assessing the present position.

The book is of unquestionable value to specialists, and students wishing to find their way into almost any branch of the subject will find appropriate references and comments to launch them, whether for teaching or research. However, a shorter and more critical text introducing the references would have filled this need equally well and would also have provided the student with a better balanced and more readable account of the subject: one which, by thinning the trees, could reveal more of the wood. However, it is fair to say that except in the case of balance, which is, after all, a matter of personal opinion, the book fulfils the aim of the series to produce a "carefully balanced account which will inform the advanced student, assist the lecturer and be a point of departure for the specialist".

There are a few typographical errors, but only two were noted which were likely to cause confusion: in Figs. 9.3 and 9.4 the "N" of pyridine is shown as if substituted on a benzene ring. It is also a pity that the plates come adrift so easily when the book is used. All in all, *Physiological Plant Pathology* is a very useful book and at present day prices represents a good money's worth.

S. H. CROWDY

WHAT GOES ON IN CELLS

Progress in Nucleic Acid Research and Molecular Biology

Vol. 7. Edited by J. N. Davidson and Waldo E. Cohn. Pp. xv+467. (New York: Academic Press, Inc. (London), Ltd., 1967.) 140*s*.

MUCH progress in molecular biology has been achieved by putting a searchlight on a few types of micro-organisms. Although the concepts that have been developed in this way are of inestimable value for the study of higher organisms, we still understand very little about the structure and replication of the cytologist's chromosomes, the regulation of chromosomal activity, the stability of messenger RNA, or the precise form in which it passes to the sites of protein synthesis.

Five of the nine essays in this welcome volume are concerned with crucial problems of molecular-cum-cell biology in higher organisms. Gavosto gives a clear and concise account of autoradiographic studies of DNA replication in human chromosomes. He describes how various chromosomes differ both temporally and spatially in their patterns of DNA replication and discusses the interesting differences that have been found between the replication patterns of chromosomes from normal and leukaemic cells. Hnilica's essay on the nuclear proteins is a comprehensive review which will prove to be valuable both for the specialist and for advanced students. Chantrenne and his colleagues, Burny and Marbaix, have written a clear and critical account of the search for the messenger RNA (*m*RNA) of haemoglobin. Although the concept of *m*RNA is now firmly held, many important questions about its nature and stability have not been satisfactorily answered even in bacterial systems. Reticulocytes ought to be an ideal source of a specific *m*RNA from higher organisms, for these cells make only one protein. Unfortunately, despite almost ten years of intensive effort in several laboratories, a clear demonstration of the haemoglobin messenger has not been achieved. In the next article, Hadjiolov presents a wider discussion of the nature of *m*RNA in animal cells and the theme is extended further by Nemer in the next article—an excellent and readable survey of information transfer by RNA during embryogenesis. Will embryos provide the principal experimental system to unlock the secrets of *m*RNA in animal cells?

Among the other articles in the book, there is a long and thoughtful review of the genetic code by Woese, who includes the mechanism of its operation and its possible origin and evolution. Larsson and Reichard give a first class account of the enzyme reduction of ribonucleotides to deoxyribonucleotides. This key process is controlled by various ribo and deoxyribonucleotides in a complex manner for which Larsson and Reichard have been able to suggest an elegant model. Phillips and Brown survey the mechanism of action of hydroxylamine—one of the most specific chemical mutagens known and the only one to have been used confidently in codon assignment. Nevertheless, it is still difficult to decide which particular chemical reactions cause the mutations

Phillips and Brown lead us along the firmer evidence that is available and argue a convincing case for the mechanisms that they propose The final article, "Mammalian Nucleolytic Enzymes and their Localization", is by Shugar and Sierakowska and covers both the techniques that are used and the possible functions of nucleases

The articles emanate from seven countries and are all of high standard, even when measured against the merits of the earlier volumes of the series The editors and publishers are to be congratulated not only for the quality of their product but also for their apparent success in publishing it quickly and so making its contents available when they are still young enough to have their greatest impact. K BURTON

COASTAL DEVELOPMENT

Processes of Coastal Development

By V. P Zenkovitch Edited by J A Steers Translated by D G Fry. Pp xv+738 (Edinburgh and London Oliver and Boyd, Ltd , 1967) 252*s*

PROFESSOR ZENKOVITCH has long been known personally to western scientists working on problems of coastal evolution As a member of the Commission on Coastal Geomorphology of the International Geographical Union, he has visited many universities and research establishments outside Russia His own contributions in this field are thus widely known, despite problems of language This English edition of a book first published in 1962, however, may well surprise even professional coastal geomorphologists at the depth and breadth of coastal research in the Soviet Union It will certainly make available for the first time to a wider audience the work of a large number of Soviet scientists whose names are not known outside communist countries Similarly, it will present work on coasts which, until now, could only be regarded by most of the world's students of coastal problems as *terrae incognitae* in the geomorphological sense

Inevitably, comparisons will be made between this book and the classic *Shore Processes and Shoreline Developments* by D. W. Johnson, published almost fifty years earlier It is a current criticism of the Davisian approach, which Johnson applied to coasts, that process is neglected and too much emphasis is placed on "stage" Perhaps the reverse criticism may come to be applied to Zenkovitch in the sense that contemporary processes are emphasized at the expense of those which, however significant in coastal development, have ceased to operate. There is growing realization in Britain, for example, that much beach sediment may be of glacial origin winnowed by the waves of the transgressing Flandrian sea from sea floors now below the depths from which modern waves can move material shoreward Zenkovitch does not ignore fluctuations of sea level during the Quaternary, but he does not devote as much attention to their contribution to the form of modern coasts as some might wish

Perhaps the greatest contrast between Johnson and Zenkovitch lies in the Russian's insistence that coastal geomorphology is an experimental science rather than an exercise in observation and description In his view, theory must be based on experiments in the field rather than in wave tanks, in which theories can be validated but not conceived As an example of this approach, the large-scale field trials of the kind carried out with fluorescent tracers, at Anapa on the Black Sea coast, using aerial ropeways to allow for the continuation of work in storm conditions, are significant They have led to notable advances Zenkovitch's division of accumulation forms into those developing on coasts with saturated and unsaturated sediment flow, is a major result of quantitatively based studies His theoretical treatment of the development of sectors of deposition and erosion, where coastal trends change, will be widely studied and applied

Zenkovitch incorporates much of the results of work outside the Soviet Union in his interpretation of coastal processes and form Thus, for example, he revives Cornaglia's neutral line concept[1], relating to the development of shore profiles of equilibrium, which has largely been overlooked by contemporary western workers His ideas on surf base, as distinct from wave base, echo Dietz's criticisms of some of Johnson's approach[2]

Although much of Zenkovitch's work best known in the West has been sited in the Black Sea, the book includes studies by himself and others of all the seas of the Soviet Union The examples taken from Arctic coasts are of particular interest as being outside the experience of most non-Soviet students He can claim to have worked in tidal as well as in non-tidal environments, and in conditions dominated by ocean swell as well as in areas dominated by storm waves However, some of the major problems associated with the differences between these environments have not been sufficiently emphasized The chapter on tidal conditions is unsatisfying and the significant role of tidal streams on open coasts in inhibiting shoreward transport of sediment is largely overlooked Similarly, the views of Australian writers on the importance of sediment supplied from the nearshore zone, under the influence of ocean swell, in the development of spits and bars, are given little or no attention

These are, however, only differences of emphasis They do not detract significantly from the value of a book which is destined to be a required text for many years to come It sets a standard against which future work will be measured and establishes the Shore Division of the Institute of Oceanography in Moscow as one of the world's leading Coastal Studies Institutes In presenting this major treatise to the English-speaking world, the translator and the editors have, themselves, made notable contributions C KIDSON

[1] Cornaglia, P , 1881, *Du flot de fond dans les liquides en etat d'ondulation, Annls Ponts Chauss*

[2] Dietz, R S , 1963, Wave Base, Marine Profile of Equilibrium and Wave built Terraces, a critical appraisal, *Geol Soc Am Bull* , 74, 971/990

UNSALEABLE KNOWLEDGE

Scienza e Tecnica 68

Annuario della Enciclopedia della Scienza e della Tecnica Pp viii+484 (Milano Arnoldo Mondadori Editore, 1967) Lire 14,000

THIS is a beautifully produced book for which it is hard to see any definitive use It is the first annual supplement to Mondadori's *Enciclopedia della Scienza e della Tecnica* and contains information of two kinds The first thirty pages are short articles written in newspaper form which describe events of major scientific interest, including the awarding of the Nobel Prizes, between October 1966 and October 1967 The remainder of the book is a series of forty-four essays "which has as its theme the great problems to-day being tackled by science and technology in order to satisfy our basic needs and to broaden the limits of our knowledge"

On the face of it, the volume has much to commend it The essays and articles, many by distinguished scientists, are well written and excellently illustrated Within the six sections of the book the selection of topics must inevitably be arbitrary and partial, but subject to this limitation the editors' choice is not, on the whole, to be quarrelled with In the first section, devoted to the transformation of the environment, Dr B R Sen, director general of the FAO, gives a useful synopsis of the world food problem concluding on the surprising note that "it is possible to hope for a definite improvement in the condition of the human race during the following decades". Professor B G Bogorov, of the University of Moscow gives an interesting account of the oceanic food chain, while Dr A Nyberg, director-general of the World Meteorological Service, expands on the possibilities of a universal weather service

The second section, on the adaptation of man to his environment, includes essays on contraception, hallucinogens, gerontology and reanimation, while the third section discusses nuclear reactors, real time computers in medicine and bionics The composition of the fourth section, which aspires to cover problems of research and development, may give some ground for surprise In reviewing the period in question many might include on their list of interesting advances topics from cosmology, oceanography, molecular biology, immunology and particle physics The editors have selected none of these topics, instead they have included in their limited space an interesting but not primarily important article on melanin, two articles on photochemistry and a discussion of the lattice energy of ionic crystals If the articles here selected are intended to represent the major themes of contemporary interest, the editors' judgment would seem to be seriously awry For which reason this is probably the least satisfactory section of the book, its best piece, which does not properly belong to it, is a discussion of the conservation of works of art and the restoration of those damaged in the Florence floods

The next section, devoted to the mathematics, logic and philosophy of science, is a praiseworthy attempt to cover a not particularly tractable subject But the value may well be questioned of including an essay on the theory of distributions which almost inevitably will lose the non-mathematical reader in its first few paragraphs The last section of the book, "The Politics of Scientific Research", discusses the science research policies of various countries, excluding the USSR, more by way of description than analysis, and without attempt at serious comparison The newspaper style articles which begin the book are perhaps an artificial but certainly successful means of conveying to the general reader some of the immediacy of science

In substance the book contains a wide variety of attractively presented information, is well planned except in its fourth section, and is excellently produced But although the publishers presumably know their business, it is hard to see for what class of reader the book is intended and by whom it will be bought Its price puts it beyond the shelves or coffee table of most individuals, and librarians may consider that it is not a work of reference, because it does not attempt to give an inclusive coverage of its chosen field The clarity of a publisher's intentions may always be questioned when it is left to the blurb writer, not to the editor in his preface, to state the purpose of a book, and such is the case with *Scienza e Tecnica 68* The volume will apparently be "useful both for the specialist who wants to find an authoritative account of current research in fields other than his own" as well as for the general reader In the English speaking world at least, this purpose is more topically and cheaply covered by the relevant scientific magazines, and even among readers of Italian the only buyers on impulse may be those who already possess the parent encyclopaedia, to which indeed the articles in the book are copiously referenced

N M L Wade

OBITUARIES

Professor E R Redfearn

Eric Reginald Redfearn, professor of biochemistry at the University of Leicester, died on March 6 in a road accident which also took the life of his elder son Redfearn's death, at the tragically early age of 43, ended a career in biochemical research which spanned only 16 years but sufficed to establish him as an internationally recognized authority on the mechanism of biological electron transport

Redfearn was educated at Wallasey Grammar School, which he left in 1943 to join the Royal Air Force Early work with airborne radar, and later service as a flight sergeant instructor in the education branch, confirmed him in his resolve to devote himself to science, and provided him with an understanding of electronics which was to stand him in good stead in his later work On demobilization in 1947 he spent a short period in industry before entering the University of Liverpool in 1948, he gained his B Sc with honours in biochemistry in 1952 Working under the supervision of Dr J Glover on the biogenesis of vitamin A and retinoic acid, Redfearn took his Ph D in 1954 and was appointed to a lectureship at The Queen's University, Belfast, where he continued and extended this work His return to a lectureship at Liverpool in 1957 coincided with the discovery, by Professor R A Morton and his colleagues, of coenzyme Q (ubiquinone) Redfearn's interest in this work was immediately aroused and he developed methods, which are still widely used, for the isolation and characterization of the oxidized and reduced forms of the various quinones His work was instrumental in establishing ubiquinone as a physiologically important electron carrier and in defining its site of action

In 1962, Redfearn was appointed to a senior lectureship in biochemistry at the University of Leicester Here his teaching experience and enthusiasm proved most valuable in developing the newly established department and in planning for the move and integration of that department into a separately housed school of biology In 1964, the University of Leicester conferred on Redfearn a personal professorship, in the same year the University of Liverpool awarded him the D Sc degree

In recent years, Redfearn's interests in ubiquinone and plastoquinone had widened into a general study of energy transductions in living systems This was stimulated partly by two extended periods of work with Dr Britton Chance at the Johnson Foundation of the University of Pennsylvania and one with Dr Tsoo E King at Oregon State University, and partly by his collaboration with a lively group of research students and postdoctoral fellows who were attracted to join him at Leicester Of particular note was the work which established rotenone as a specific inhibitor of mitochondrial NADH-oxidase, and the elegant elucidation of the two pathways by which electrons pass to separate terminal oxidases in *Azotobacter vinelandii* At the time of his death, Redfearn was turning his attention to photosynthetic bacteria, his most recent and uncompleted work provides evidence that the structure of chlorobium quinone requires to be modified

Eric Redfearn was a dedicated scientist, a loyal colleague and a delightful companion His untimely death will long remain a source of sorrow to all who were privileged to know him.

H L Kornberg

Dr H. M. Muir-Wood

Helen Marguerite Muir-Wood, for more than thirty years one of the leading authorities on the phylum Brachiopoda, died as the result of a stroke on January 16, little more than a month before her seventy-third birthday.

Born in Hampstead, north London, she took her first degree at Bedford College in 1918 and later studied under Professor E J Garwood at University College, London, where she developed an interest in Middle and Upper Palaeozoic rocks and their brachiopod faunas

In 1919 she was employed as a part-time curator in the department of geology (now the department of palaeontology), of the British Museum (Natural History), and the following year she accepted the position of assistant with full charge of the Brachiopod collections, a post she held, with appropriate promotions, until her retirement in 1965 at the age of seventy

During a full working life Helen Muir-Wood produced more than fifty memoirs and papers on all aspects of the Brachiopoda including a monograph on the Carboniferous Productoids and memoirs on the Jurassic and Palaeozoic faunas, not only of Great Britain, but of India, Sikkim, Malaya, Irak, Persia, Palestine and Somaliland She held office as a member of the Council of the Geological Society of London, the Palaeontographical Society, the Ray Society and the Systematics Association

Although her chief research interests included the morphology, systematics and evolution of the Lower Palaeozoic to Recent genera, she became particularly well known in the thirties for her pioneer work in establishing a classification of Mesozoic genera and species on their internal structures which were ascertained by transverse serial sections This technique, hitherto used only broadly by a few continental workers, provided the first major step forward in the understanding of the evolution and morphological variation of the group, replacing the somewhat arbitrary methods adopted by S S Buckman

Some of the early research work in connexion with her ideas on the classification was made possible by an award of the Lyell Fund which was presented to her by the Geological Society in 1930 On the completion of this work and in respect of her earlier publications, she received the degree of Doctor of Science from the University of London in 1934

During the Second World War, from 1942 to 1945, she served in the secretariat of the Admiralty at Bath and although this took her away for a time from her palaeontological researches, she managed to maintain an interest by collecting fossils from beds of the Great Oolite Series exposed in the Bath–Bristol district

After the war she returned to work at the British Museum (Natural History), continuing her researches on Middle and Upper Palaeozoic genera In 1950 she agreed to joint authorship, with Dr G A Cooper of the Smithsonian Institution, Washington, on a work exclusively on the Productoid genera and for this purpose visited the United States on two occasions The result of this collaboration appeared as "Morphology, classification and life habits of the Productoidea (Brachiopoda)" in 1960 and was quickly recognized by brachiopod workers as being one of the greatest contributions to the study of the phylum It was largely as a result of her researches in connexion with the publication that she was invited by the American editorial board to contribute, supervise and partly edit the Brachiopoda section for the *Treatise on Invertebrate Palaeontology* This tremendous undertaking required a concentration of both mental and physical resources second to none, but despite this she willingly accepted the additional post of deputy keeper of the department of palaeontology, British Museum (Natural History) in 1955, involving herself with a host of administrative problems which she discharged with consummate skill and her usual thoroughness This appointment, previously held only by a male member of the department, counted for more than a major triumph in her life, for her views were distinctly those of a feminist These views may have been strengthened by her early difficulties in establishing herself in what had always been a strictly masculine profession

In recognition of her services to geology, Helen Muir-Wood received the coveted distinction of the Lyell Medal which was presented to her by the Geological Society in 1958

Always sympathetic to the younger worker, Dr Muir-Wood will be remembered for her wise counsel by numerous enthusiastic research students, many now prominent names in this field of research themselves Aware of the many pitfalls associated with the systematics of the group, she was always keen to guide the unwary newcomer to safe ground with liberal understanding and kindness

Ellis Owen

University News

London

Dr J E Houldin, senior lecturer at Chelsea College of Science and Technology, has been appointed to the chair of electronics tenable at that college and Dr Richard Creese, reader at St Mary's Hospital Medical School, has been appointed to the chair of physiology tenable at that school The title of professor of bacteriology (infectious diseases) has been conferred on Mr D A Mitchison in respect of his post at the Royal Postgraduate Medical School

Nottingham

Dr J W. Barrett, research director of Monsanto Chemicals Ltd , has been appointed special professor of industrial chemistry, and Dr S Shone, senior administrative medical officer of the Sheffield Regional Hospital Board, has been appointed special professor of medical administration

Announcements

The Nuffield Foundation has awarded its first travelling fellowship in tropical marine biology to Dr E R Trueman, senior lecturer in zoology in the University of Hull He will work at the University of the West Indies, Jamaica, and intends to investigate aspects of the littoral and sublittoral fauna of the island

The Institute of Physics and the Physical Society have awarded the Maxwell Medal and Prize jointly to Professor K W H Stevens, professor of theoretical physics at the University of Nottingham, and Dr R J Elliott of the University of Oxford

The following medals were awarded at the spring meeting of the Optical Society of America the *David Richardson Medal* to Dr H E Edgerton of the Department of Electrical Engineering, Massachusetts Institute of Technology, the *1968 Adolph Lomb Medal* to Dr D C Sinclair of Spectra-Physics, Inc , Mountain View, California

Meetings

Electron Microprobe Analysis, July 31–August 2, Chicago (D R Beaman, The Dow Chemical Company, 241 Building, Midland, Michigan)

Progress in Overhead Lines and Cables for 220 kV and above, September 16–20, The Institution of Electrical Engineers (The Conference Department, The Institution of Electrical Engineers, Savoy Place, London, WC2)

Erratum In the announcement section of the March 23 issue of *Nature* (217, 1192, 1968) it was incorrectly stated that Professor W Morgan was director of the Lister Institute of Preventive Medicine The director is Sir Ashley Miles and Professor Morgan is deputy director

Erratum By an unfortunate misunderstanding in the *Nature* office, a number of references in the paper, "Late DNA Synthesis in Heterochromatin" by A Lima-de-Faria and Halina Jaworska (*Nature*, 217, 138, 1968), were incorrectly quoted The following changes should therefore be made Page 138, column 1, reference 1 should be 1–3, references 2, 3 should be 4, 5, column 2, references 4, 5 should be 6, reference 7 should be 7–11 Page 140, column 2, reference 43 should be 44, 45 In the first line of Table 2 on page 140, the references 4, 57 should in each case be replaced by 4, 5, 52 In Table 3 on the same page, reference 44 in the first line should be 45 and reference 69 should be 44 In the last line, the group of references 29–43 should be 29–31, 34–38, 40–43, in the same line, references 42, 43 and 70–72 should be 70, 71, 42, 43, 35 On page 141, column 1, the group of references given as 2, 52 should be 4, 5, 52 Lower down, reference 55 should be 55, 56 In column 2, reference 57 should be 57–59

CORRESPONDENCE

Cutting Cakes Blindfold

DEAR SIR,—I am surprised by your comments on the Science Research Council (*Nature*, 217, 991, 1968) Surely all those establishments outside the universities are overlooked, as indeed they always were, by boards and committees drawn both from the universities and from industry I do not see how their work would be improved, therefore, by "giving" them to the universities Neither am I prepared to suppose that because research is "university" research it is necessarily good research, this simply is not so Then again, a good deal of the cost to which you object will still be there whoever has these establishments, it is spent on buildings, electricity, administration, heating, wages, etc I would think that a good half of it must be spent in this way

And what do you propose for the staff, some of whom are first-rate men however much you may dislike what they are doing ? That they should join the brain-drain, or the unemployed ? Would it not be more intelligent to suggest that the distribution of these establishments is not all that it might be, and that they could be used by other departments, some of which are still expanding and needing staff ?

And what is "the common good", which the Radio and Space Research Station is apparently not concerned with ? In my understanding of the phrase, I do not include the spending of vast amounts of public money in discovering the "exciting new pulsations", an expenditure about to be greatly increased if I read the signs correctly The lot of 99 999 per cent of us would not change if all astronomy stopped to-morrow In any case, half the work of the station has to do, again as it always has, with propagation studies and the like a study which in most other countries has vastly more support than it has here, not less

Yours faithfully,
F V. BALE

The Coppice,
67 Switchback Road,
Maidenhead, Berks

Cows, Sheep, Pigs and Pounds

SIR,—You recently published a set of rules on the metrication of units (*Nature*, 217, 308, 1968) One was a reaffirmation of the convention that M means "multiply by a million" and m means "divide by a thousand" Although it was not explicitly stated, it also seemed that the convention that the number should precede the unit was being adhered to But your article on foot and mouth disease (*Nature*, 217, 1088, 1968) is in chaotic contravention The amount paid in compensation is given as £26 5 million Why not 26 5 M£ ? And the national herd is given as 12 342 m cattle, 28 885 m sheep and 7 107 m pigs Were they millipigs?

Until the end of the past century, a sum of money was usually specified by a number followed by an italic *l* Then the £ was introduced and its grandeur seems to have made people think that the frontal position was more suitable Why should we not, at any rate in scientific journals, use the same conventions when referring to pigs and pounds that we use when referring to grams and joules ?

Yours faithfully,
N W PIRIE

42 Leyton Road,
Harpenden

Orgueil Meteorite

SIR,—In relation to the letter "Occurrence of Olivine in a Type I Carbonaceous Meteorite" from J F Kerridge of this laboratory in your issue of February 24, 1968 (*Nature*, 217, 729, 1968), I would like to make some comments, particularly on his interpretation of his observations, with which I do not agree

I find it difficult to understand why the occurrence of olivine in a type I carbonaceous meteorite should have aroused scepticism This discovery is by no means a new one Olivine single crystals were reported and photographed in an optical microscope as long ago as 1962 by Professor G Mueller ("Interpretation of Microstructures in Carbonaceous Meteorites", *Nature*, 196, 929, 1962) The matter has also been referred to in another paper by Professor G Mueller and myself ("Significance of Inclusions in Carbonaceous Meteorites", *Nature*, 210, 151, 1966) and may be accepted as firm scientific knowledge

The Orgueil meteorite is extremely heterogeneous It still remains an unsolved problem how well crystallized olivine could co-exist with a low melting point carbonaceous phase The obvious solution is that the olivine crystals were formed first in the form of a high temperature dust, *nuéé ardente*, subsequently becoming incorporated in the stone, but the details of the process are not clear As to the composition of the olivine phase, the optical method is inadequate to determine it but the presence in almost every case of an iron-rich core supports Mr Kerridge's hypothesis that it is largely of the Fayalite type

In the 1966 paper referred to here, Professor Mueller and I pointed out that proof of a strict petrological character showed that these inclusions in various meteorites are fragments of meteorites of other types, particularly in the Sharps meteorite, indicating that all have the same parent body These views are quite compatible with the idea that the first stage of growth of the carbonaceous chondrites may have occurred from condensation of primary solar material

Yours sincerely,
J. D BERNAL

Department of Crystallography,
Birkbeck College,
Malet Street,
London, WC1

Electrophoresis for Free

SIR,—Your Molecular Biology correspondent (*Nature*, 217, 902, 1968) highlights the difficulties and errors in determining the sub-unit relationships of proteins, despite the use of sophisticated equipment and exemplary technique One little known technique with applications in this area is two dimensional electrophoresis (*Science*, 138, 152, 1962) Although not as precise as the ultracentrifuge, it can give useful results with heterogeneous preparations One may suppose that the reason this method is not more widely used is the unavailability of the equipment required Hence, the following offer is made to readers of *Nature*

For a limited period we will process protein samples and return a two dimensional pattern, with interpretation, gratis Samples should be accompanied by reference materials to bracket the postulated molecular weight, together with data on gross electrophoretic properties Protein concentration should be high enough to permit staining with amido black (ca 5 mg/ml)

We hope by this means to illustrate the value of this general technique, since it offers the possibility of obtaining valid data on the size–charge relationship of proteins with a minimal investment in both time and apparatus

Yours faithfully,
J BROOME

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Philadelphia,
Pa 19104

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, April 1

Institution of Electrical Engineers (at Savoy Place London WC2) Colloquium on "Injection Luminescence"

Institution of Mechanical Engineers, Medical Engineering Working Party (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m — Discussion Meeting on "Kidney Machines—Technical Medical and Social Problems"

Royal Society of Arts (at John Adam Street Adelphi, London, WC2) at 6 p m —"Industry and Commerce" (Last of three Cantor Lectures on "Modern China")

Society of Engineers (at the Geological Society Burlington House Piccadilly, London, W1), at 6 p m —Dr R Davies "High Speed Forming of Metals"

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, SW1), at 6 30 p m —Dr N W Pirie FRS "The Use of Novel Proteins in Human Food"

Monday, April 1—Wednesday April 3

British Biophysical Society (at the University of Nottingham)—Meeting on "Biological Semiconductors"

Tuesday, April 2

Society of Chemical Industry, Pesticides Group—Physicochemical and Biophysical Panel (at 14 Belgrave Square, London, SW1), at 10 a m —Symposium on "Physicochemical Aspects of Penetration through Plant Cuticle"

Institution of Civil Engineers (at Great George Street, Westminster, London, SW1) at 5 30 p m —Informal Discussion on "Sludge Disposal" introduced by Mr P Coackley

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Discussion Meeting on "Microwave Electrostatic Wattmeter" opened by Professor H E M Barlow

Institution of Electronic and Radio Engineers, Joint IERE/IEE Computer Groups (at the London School of Hygiene and Tropical Medicine Keppel Street, Gower Street, London, WC1), at 6 p m —Discussion Meeting on "Assessing Computer Performance"

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, SW1), at 6 p m —Discussion Meeting on "Mechanical Handling of Freight Containers in Port Marshalling Yards"

Tuesday, April 2—Wednesday, April 3

Institute of Physics and the Physical Society, Materials and Testing Group (in conjunction with the National Physical Laboratory, at Teddington, Middlesex)—Conference on "Solid–Solid Interfaces"

Tuesday, April 2—Thursday, April 4

Faraday Society (at the University of Newcastle upon Tyne)—General Discussion on "Electrode Reactions of Organic Compounds"

Wednesday April 3

Colour Group (Great Britain) (at the Institute of Ophthalmology, Judd Street, London, WC1), at 2 30 p m —Meeting on "Colourvision" Dr R A Weale "Further Experiments on Human Vision Pigments"

Geological Society of London (at Burlington House, Piccadilly London, W1), at 5 p m —Dr P G Llewellyn and Mr R Stabbins "Core Material from the Anhydrite Series Carboniferous Limestone, Hathern Borehole, Leicestershire" (Demonstration), Mr D R C Kempe "The Kilonwa Syenite, Tanzania" (Paper), Dr C B Phipps and Dr F A E Reeve "Structural Geology of the Malvern, Abberley and Ledbury Hills" (Paper)

Royal Meteorological Society (at 49 Cromwell Road London, SW7), at 5 p m —Professor G W Platzman "The Rossby Wave" (Symons Memorial Lecture)

Society of Chemical Industry, Road and Building Materials Group (joint meeting with the Institution of Highway Engineers, at the Institution of Structural Engineers, Upper Belgrave Street, London, SW1), at 5 p m —Meeting on "Engineering and Related Properties of Pulverized Fuel Ash"

Royal Society of Medicine, History of Medicine Section at 1 Wimpole Street, London, W1), at 5 15 p m —Sir John Charles "Twenty Years of the World Health Organization"

Institute of Petroleum (at 61 New Cavendish Street London, W1), at 5 30 p m —Mr R A Vere "New Aviation Turbo-Fuel Specifications Adequately Control Fuel Quality"

Institution of Civil Engineers (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "Experience in the Implementation of Mechanized Maintenance of Track" introduced by Mr F J J Prior and Mr K W Pearce

Institution of Electrical Engineers (at Savoy Place, London, WC2) at 5 30 p m —Mr J Reeve "Logical Behaviour of H V D C Convertors During Normal and Abnormal Conditions and Direct Digital Protection"

Thursday, April 4

Society for Analytical Chemistry, Automatic Methods Group, and the Association of Clinical Biochemists (Southern Region) (in the Department of Electrical Engineering, Imperial College, London, SW7), at 9 30 a m —Lecture and Demonstration Meeting on "Newer Systems in Automatic Analysis"

Royal Society of Arts, Commonwealth Section (at John Adam Street, Adelphi, London, WC2), at 2 30 p m —Mr Malcolm Ford "New Zealand's Field Work in the Antarctic"

Royal Society (at 6 Carlton House Terrace, London, SW1), at 4 30 p m —Dr M A Grace FRS "The Alinement of Nuclei" (Review Lecture)

Institute of Mathematics and Its Applications (at the Royal Institution Albemarle Street, London W1), at 5 p m —Dr P G Wakely "Industry and the Mathematician" (Presidential Address) *

Institution of Electrical Engineers (at Savoy Place London WC2), at 5 45 p m —Lord Jackson of Burnley, FRS Fourteenth Graham Clark Lecture

Institute of Refrigeration (at the National College for Heating, Ventilating Refrigeration and Fan Engineering, Southwark Bridge Road London SE1), at 6 p m —Mr A R Went and Mr A P Ivers "Design and Operation Considerations—Insulated and Refrigerated Transport"

Institution of Mechanical Engineers Process Engineering Group (at 1 Birdcage Walk, Westminster London SW1) at 6 p m —Discussion Meeting on "An Approach to the Design of Half-Coil Pressure Vessels"

Thursday, April 4—Saturday, April 6

British Society for the History of Science (at Loughborough University of Technology, Loughborough)—Symposium on "The Teaching of the History of Science (In Schools and Colleges)"

Friday, April 5

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Mr L C L Dale, Mr L B Knowles and Mr T H Morrison "Gas Turbine Plants for Peak Load Generation and Synchronous Compensation—a review of the Installations for the Central Electricity Generating Board"

Monday, April 8

Medical Research Council (at the National Institute for Medical Research, Mill Hill, London, NW7), at 4 30 p m —Professor A G Ogston, FRS (Australian National University, Canberra) "Some Aspects of Interactions Between Macromolecules"

Institution of Civil Engineers, Piling Committee (at Great George Street, Westminster, London, SW1), at 5 30 p m —Informal Discussion on "Use of *In situ* Diaphragms—Continuous Bored Pile—Slurry Trench—Thin Diaphragm"

Institution of Electrical Engineers (at Savoy Place, London, WC2), at 5 30 p m —Mr E A Lloyd "Eddy Currents and Automatic Inspection"

Monday, April 8—Tuesday, April 9

Biological Council (in the Edward Lewis Theatre, Middlesex Hospital Medical School, Cleveland Street, London, W1)—Symposium on "Scientific Basis of Drug Dependence".

Institution of Electronic and Radio Engineers, Institution of Electrical Engineers, Electronics Division, at Imperial College London, SW7) at 9 a m daily—Joint Conference on "Thick Film Technology"

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Lecturer (with a Ph D and able to give evidence of the ability to teach and conduct research in the field of rotating machines, with particular reference to their use in control systems) in Electrical Engineering—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (April 8)

Visiting Lecturer in Chemistry at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (April 8)

Lecturer (with interests in the following fields of specialization fluid mechanics, structural mechanical and computer systems, design synthesis, transportation engineering operation research and systems and geotechnical engineering) in Civil Engineering—The Registrar, University College of Swansea, Singleton Park Swansea, South Wales (April 11)

Professor (with a higher degree, preferably a doctorate, and research experience in the field of entomology) of Entomology at the University of Queensland, Australia—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (London and Brisbane, April 12)

Head of the Department of Biology—Clerk to the Governing Body, Paisley College of Technology, High Street, Paisley (April 15)

Lecturer or Assistant Lecturer (Probationary) (preferably with an interest in animal behaviour) in Zoology—The Secretary Bedford College (University of London), Regent's Park, London, NW1 (April 15)

Lecturer/Senior Demonstrator (with a higher degree in geology with particular interests in the fields of palaeontology and stratigraphy, and preferably some previous teaching experience) in Geology at the University College of Townsville, University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (Australia and London April 15)

Lecturer in Physics—The Secretary, Trinity College (University of Dublin), Dublin 2 Republic of Ireland (April 16)

Assistant Lecturer (preferably with a special interest in logic) in the Department of Philosophy—The Registrar, The University, Liverpool, quoting Ref RV/414 (April 17)

Professor, Senior Lecturers (2), and Lecturers (2) in the Department of Mathematics, University of Ife, Nigeria—The Inter-University Council, 33 Bedford Place, London, WC1 (April 23)

Chair of Archaeology of the Roman Provinces at the Institute of Archaeology—The Academic Registrar, University of London, Senate House, London, WC1 (April 25)

Lecturer in Zoology and Curator of the Zoological Collections (which do not include insects)—The Secretary of the Faculties, University Registry, Broad Street, Oxford (April 27)

Lecturer or Assistant Lecturer in Biology at the University of the West Indies, Barbados—The Inter-University Council, 33 Bedford Place, London, WC1 (April 29)

Lecturer in Organic Chemistry—The Secretary of the University Court, The University, Glasgow (April 30)

Lecturer/Senior Lecturer in Microbiology at Massey University, Palmerston North, New Zealand—The Secretary-General, Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, April 30)

SENIOR LECTURER or LECTURER IN BIOCHEMISTRY at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (New Zealand and London, April 30)

SENIOR LECTURER or LECTURER IN BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Association of Commonwealth Universities (Branch Office) Marlborough House, Pall Mall, London, SW1 (New Zealand and London, April 30)

UNIVERSITY STUDENTSHIPS AND FELLOWSHIPS for experimental and theoretical research in nuclear and elementary particle physics and solid state physics and also for theoretical research in plasma physics—the Secretary to the University Court, The University, Glasgow (May 4)

LECTURER (with a special interest in mineralogy/petrology and prepared to lecture in palaeontology up to second year level) in GEOLOGY at Makerere University College, Uganda (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, WC1 (May 7)

SENIOR LECTURER and LECTURERS (2) IN THE DEPARTMENT OF PHYSIOLOGY Makerere University College, Uganda (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, WC1 (May 8)

SENIOR LECTURERS or LECTURERS (with qualifications in either pure or applied mathematics and preferably a qualification in algebra) in MATHEMATICS at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, SW1 (May 15)

RESEARCH ASSISTANTS (with, or expecting to obtain, a good honours degree in agricultural botany or botany) in the DEPARTMENT OF AGRICULTURAL BOTANY, for research in the field of genetics, crop physiology or plant pathology—The Secretary, University College of Wales Aberystwyth

RESEARCH ASSOCIATE ELECTRON MICROSCOPIST to join a Cell Research Group engaged in investigating the nature of the adhesive mechanisms at the cell surface—Professor B M Jones, Department of Zoology, University College of Wales, Aberystwyth

SENIOR LECTURER (preferably with experience or interest in the fields of mineralogy, petrology or geochemistry) in GEOLOGY—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N7

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Transport Road Research Laboratory RRL Report LR89 The RRL Reflecting Kerb By J R Lake and J W Tyler Pp 4+5 plates (Crowthorne Road Research Laboratory, 1967) *Gratis* [151

Royal Observatory Bulletins No 131 *G–R* and *R–I* Colours of Late-Type Dwarfs By J B Alexander and B D Yallop Pp E371–E381 2*s* 6*d* net No 132 Photoheliographic Results 1960 Pp C99–C192 12*s* 6*d* net No 133 Time and Latitude Service 1966, January–March Pp B101–B116 3*s* net (London H M Stationery Office, 1967) [151

Bulletin of the British Museum (Natural History) Zoology Vol 16, No 2 A Revision of the Elephant-Shrews, Family Macroscelididae By G B Corbet and J Hanks Pp 45–111+1 plate (London British Museum (Natural History), 1968) 36*s* * [161

Sealing in U K and Canadian Waters (Papers submitted to a UFAW Symposium on 18th January, 1968) Pp 32 (London The Universities Federation for Animal Welfare, 1968) [171

General Register Office The Registrar General's Annual Estimates of the Population of England and Wales and of Local Authority Areas, 1967 Pp 16 (London H M Stationery Office, 1968) 2*s* 6*d* net [171

The Registrar General's Decennial Supplement, England and Wales, 1961, Area Mortality Tables Pp xii+149 (London H M Stationery Office, 1967) 35*s* net [181

Ministry of Health and General Register Office Report on Hospital In-Patient Enquiry for the Year 1964 Part 1 Tables Pp xi+288 (London H M Stationery Office, 1967) 31*s* net [181

Ambassade de France, Service de Presse et d'Information The City of Grenoble Pp 26 (London Ambassade de France, Service de Presse et d'Information, 1968) [181

Cabinet Office The Torrey Canyon Report of the Committee of Scientists on the Scientific and Technological Aspects of the Torrey Canyon Disaster Pp viii+48+8 plates (London H M Stationery Office, 1967) 5*s* 9*d* net [181

Northern Ireland Ministry of Agriculture Leaflet No 5 Dishorning Cattle Pp 3 Leaflet No 44 Cattle Diseases Pp 12 Leaflet No 60 Part time Agricultural Classes Pp 4 Leaflet No 61 Care and Management of Breeding Bulls Pp 12 Leaflet No 91 Parasitic Worm Diseases of Farm Livestock Pp 4 Leaflet No 102 Mastitis in Cattle Pp 7 Leaflet No 115 Safety on the Farm Pp 15 (Belfast Ministry of Agriculture, 1967 and 1968) [181

Other Countries

Directory of Meteorite Collections and Meteorite Research Pp 50 (Paris Unesco, 1968) [221

United States Department of the Interior Geological Survey Bulletin 1247 Low-Temperature Reaction Zones and Alpine Ultramafic Rocks of California Oregon and Washington By Robert G Coleman Pp iv+49 $0 25 Bulletin 1251–B Stratigraphy, Age, and Paleotectonic Significance of the Cottonwood Canyon Member of the Madison Limestone in Wyoming and Montana By Charles A Sandberg and Gilbert Klapper Pp iv+70 $0 30 Bulletin 1252–D Phosphate Deposits By V E McKelvey Pp iii+21 $0 15 Bulletin 2260–A, B Studies Related to Wilderness—Wildlife Refuges Bitter Lake and Bosque del Apache, New Mexico By George O Bachman Pp viii+19 $0 15 Bulletin 1260–C Studies Related to Wilderness—Wildlife Refuges Bear River Migratory Bird Refuge, Utah By Lowell S Hilpert Pp v+10 $0 15 Water-Supply Paper 1576–G Geology and Availability of Ground Water on the Ute Mountain Indian Reservation, Colorado and New Mexico By James H Irwin Pp v+109+plates 1–3 (Washington, D C Government Printing Office, 1966 and 1967) [221

Smithsonian Miscellaneous Collections, Vol 152, No 6 Solar Magnetism and World Weather By Dr C G Abbot Pp 7 Exploring Space and Sea By M Scott Carpenter, Commander, USN (Edwin A Link Lecture Series) Pp 28 $0 50 Masters of the Air Highlights of Aeronautical History fo the Young Reader By Glenn O Blough and Paul E Garber Pp 32 (Washington, D C The Smithsonian Institution Press, 1967) [231

Science in India—Some Aspects By Dr Atma Ran (Presidential Address, Fifty-fifth Indian Science Congress, Varanasi, 1968) Pp 15 (Calcutta Indian Science Congress Association, 1968) [231

Comptes Rendus des Travaux du Laboratoire Carlsberg Vol 36, No 7 Growth Rate Dependency of Protein and Nucleic Acid Composition of Tetrahymena Pyriformis and the Control of Synthesis of Ribosomal and Transfer RNA By Vagn Leick Pp 113–126 3 kr Vol 36, No 8 Synchronous DNA-Synthesis Induced by Synchronous Cell Division in Tetrahymena By K K Hjelm and E Zeuthen Pp 127–160 6 kr Vol 36, No 9–10 Studies on Endocytosis in Amoebae, the Distribution of Pinocytically Ingested Dyes in Relation to Food Vacuoles in *Chaos chaos* 1 Light Microscopic Observations By Cicily Chapman-Andresen 2 Electron Microscopic Observations Using Alcian Blue By Cicily Chapman-Andresen and Jytte Reichstein Nilsson Pp 161–208+6 plates 8 kr Vol 36, No 11 Malarial Periodicity and Body Temperature—an Experimental Study of *Plasmodium lophurae* in Chicken Embryos By Milan Kovic and Erik Zeuthen Pp 209–224 3 kr Vol 36, No 12 Geycogen Synthetase Activity in *Amoeba proteus* By Bernhard Larner Pp 225–234 2 kr Vol 36, No 13 Studies on the Carbamylation of Subtilisin Type Novo By I Svendsen Pp 235–246 3 kr Vol 36, No 14 Hydrogen–Deuterium Exchange in Ovalbumin By Lars Willumsen Pp 247–263 3 kr (Copenhague Danish Science Press, Ltd, 1967) [231

U S Department of Agriculture—Economic Research Service Foreign Agricultural Economic Report No 37 Agricultural Policies in the Far East and Oceania (Washington, D C Government Printing Office, 1967) [231

Annals of the South African Museum Vol 50, Part 6 Additions to the Cyrtosiinae (Bombyliidae) of South Africa By A J Hesse Pp 89–130 R 1 20 Vol 50, Part 7 The Cranial Morphology of the Permo-Triassic Anomodont *Pristerodon buffaloensis* with special reference to the Neural Endocranium and Visceral Arch Skeleton By T H Barry Pp 131–161+2 plates R 1 50 Vol 50, Part 8 Neue Spezies von *Notiophygus* Gory Nebst Erganzungen (Discolomidae Col) Von Hans John Pp 163–168+3 plates R 0 60 Vol 50, Part 9 Hydroids from the South-West Indian Ocean By N A H Millard Pp 169–194 R 1 (Cape Town South African Museum, 1967) [241

Académie Royale de Belgique Classe des Lettres et des Sciences Morales et Politiques Memoires Tome LIX, Fascicule 1 Les Caecilii Metelli de la République Par J Van Ooteghem, SJ Pp 349 (Bruxelles Académie Royale de Belgique, 1967) 320 francs [241

Republic of South Africa Department of Industries Division of Sea Fisheries Investigation Report, No 64 Reproduction of the South African Abalone *Haliotis midae* By G G Newman Pp 24 (Sea Point, Cape Town Director of Sea Fisheries, 1967) *Gratis* [241

United States Department of the Interior Geological Survey Water Supply Paper 1857 Ground Water in the Eola-Amity Hills Area, Northern Willamette Valley, Oregon By Don Price Pp iv+66+plate 1 Professional Paper 352-I Rates of Slope Degradation as Determined from Botanical Evidence, White Mountains, California By V C LaMarche, Jr Pp iv+377+plates 10 and 11 $0 75 (Washington, D C Government Printing Office, 1967 and 1968) [251

Journal of Radioanalytical Chemistry, Vol 1, No 1 (January 1968) Pp 1–94 Subscription price 165*s*, $22 50, 82 50 D Fl per volume, plus postage Published bimonthly (Amsterdam Elsevier Publishing Co, Budapest Akadémiai Kiadó, 1968) [261

New Zealand Department of Scientific and Industrial Research Geophysics Division Seismological Observatory Bulletin E-144 Seismological Report 1963 Pp 344 (Wellington Department of Scientific and Industrial Research, Geophysics Division, 1967) [132

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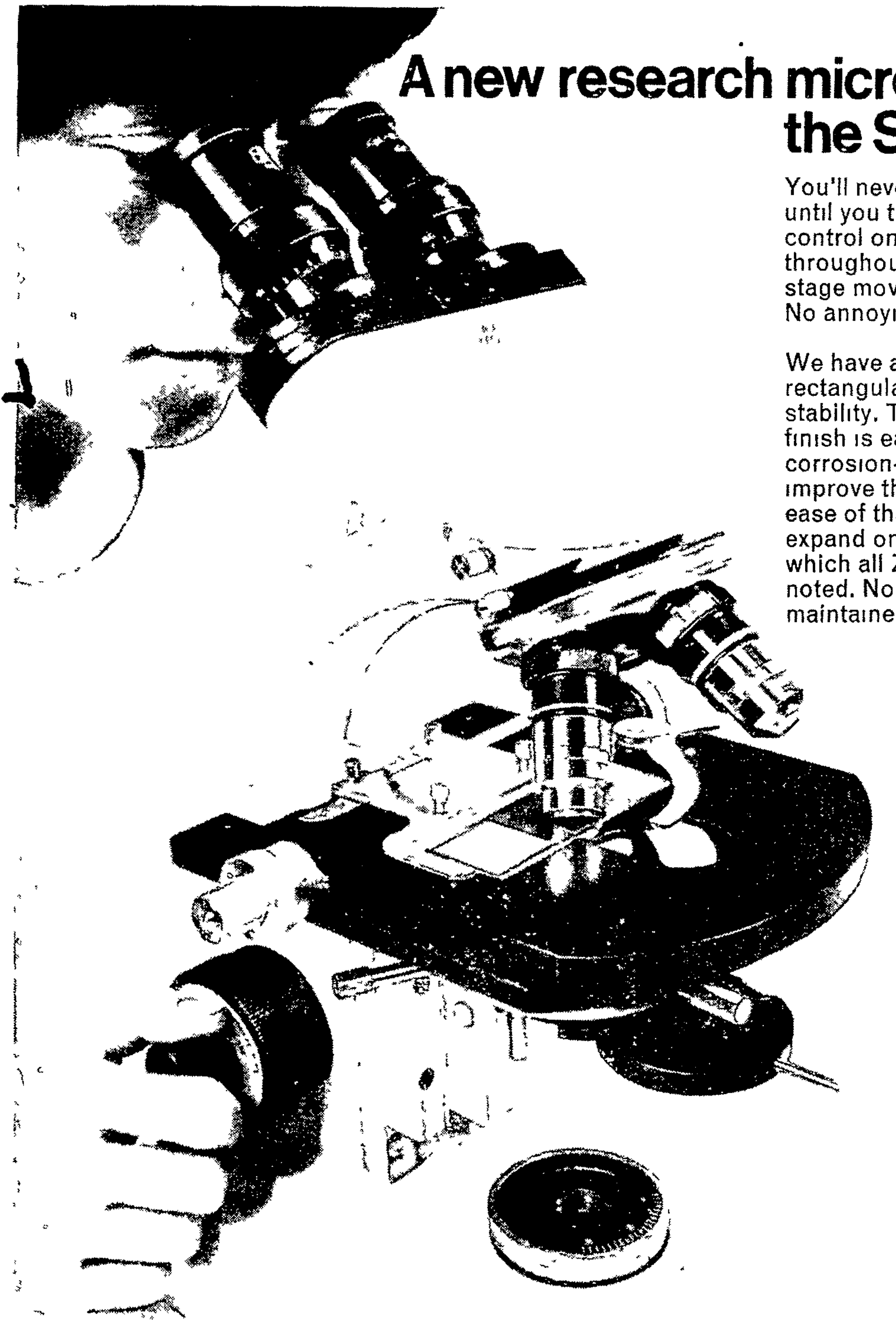

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APPOINTMENTS VACANT

UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF AGRICULTURAL BOTANY

Applications are invited for the post of LECTURER/ASSISTANT LECTURER in the above Department, with a special interest in Genetics and Biological Statistics Postgraduate experience desirable

Application forms are obtainable from the Registrar to whom all applications should be sent by April 30, 1968 (1194)

UNIVERSITY OF LIVERPOOL

Applications are invited for the post of Assistant Lecturer or Lecturer at the Tidal Institute and Observatory, Bidston, Birkenhead, Cheshire

The successful candidate will be expected to engage in research in numerical analysis, with particular reference to tidal and other geophysical time series

Salaries will be within the range £1,105 to £1,340 per annum for an Assistant Lecturer and £1,470 to £2,630 per annum for a Lecturer

Applications stating age, qualifications and experience, together with the names of three referees, should be received not later than April 15, 1968, by the Registrar from whom further particulars may be obtained Please quote Ref RV/415/N (1202)

MEDICAL RESEARCH COUNCIL UNIT FOR METABOLIC STUDIES IN PSYCHIATRY

UNIVERSITY OF SHEFFIELD

PHYSIOLOGIST, preferably post-doctoral required to work on abnormal biological rhythms, or the application of bioassays of vasopressin, gonadotrophins or oestrogens, or related problems Excellent facilities

RESEARCH BIOCHEMIST with clinical interest required to work on adrenal thyroid, and/or pituitary function in patients with regularly recurring psychoses Excellent facilities

All enquiries to Professor F A Jenner Whitley Wood Clinic, Sheffield, S10 3TL (1198)

UNIVERSITY OF EAST AFRICA

MAKERERE UNIVERSITY COLLEGE UGANDA

Applications are invited for Lectureship in Department of Biochemistry, Faculty of Medicine Preference given to candidates with experience or interest in (a) teaching medical students and (b) biochemical research in relation to medical problems Salary £EA1 950 to £EA2 280 p a (one EA shilling = 1s 2d sterling) Salary supplemented in appropriate cases under British Expatriates Supplementation Scheme in range £345 to £410 per annum (sterling) F S S U Family passages, biennial overseas leave

Detailed applications (six copies) naming three referees, by May 2, 1968, to Inter-University Council 33 Bedford Place London, W C 1, from whom particulars may be obtained (1139)

RADIATION BIOLOGY AND CELLULAR KINETIC RESEARCH GROUP

Staff positions open in renal physiology cancer biology immunology and biochemistry

SCIENTISTS with significant post-doctoral experience are invited to submit curricula vitae to Samuel W Lesher, Ph D, Director Division Radiation Biology Department of Radiology Allegheny General Hospital 320 E North Avenue Pittsburgh, Pennsylvania 15212 (1022)

INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS

COMMITTEE ON DATA FOR SCIENCE AND TECHNOLOGY

SCIENTIFIC WRITER/EDITOR

Applications are invited from graduates (male or female) for the position of Scientific Writer/Editor on the small staff of the Central Office of the Committee on Data for Science and Technology (CODATA) of the International Council of Scientific Unions (ICSU), located in Frankfurt (Main), Germany Starting date as soon as possible Knowledge of German would be helpful but is not essential

The main task will be to prepare new editions of the *International Compendium of Numerical Data Projects* which includes a detailed description of the organization and activities of world wide numerical data centres and a review and analysis of their publications In addition the editor will be responsible for the publication of the CODATA Newsletter

This appointment would interest a science graduate with a sound chemical and/or physical background, together with the ability to present scientific information in clear concise English

Salary in the range of £2,000 to £2,700 per annum according to experience (plus fringe benefits) and with addition for Good Honours Degree

Applications, stating full details of age, qualifications, experience, present work and the names of two referees, should be sent to The Executive Secretary, The Royal Society, 6 Carlton House Terrace London, S W 1 by April 19, 1968 (1204)

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOMATHEMATICS

Applications are invited for the post of Temporary Research Assistant to assist in a statistical research project supervised by the Professor of Biomathematics, to whom applications should be sent Degree in mathematics or similar professional qualification required Knowledge of statistics and/or computing an advantage Starting salary about £950 F S S U, subject to qualifications (1268)

UNIVERSITY OF OXFORD

INSTITUTE OF EXPERIMENTAL PSYCHOLOGY

An Electronic Specialist is required about June 1 1968, to direct design, development and maintenance of apparatus, and to take charge of a section of the Institute in a new building now under construction Candidates should have an appropriate University degree or equivalent professional qualification The salary will be on the Departmental Research Assistant scale £1 450 to £2 350 depending upon age and experience F S S U benefits

Please apply with details of career and names of two referees to the Administrator Institute of Experimental Psychology, 1 South Parks Road Oxford, as soon as possible (1148)

UNIVERSITY OF GLASGOW

WELLCOME LABORATORIES FOR EXPERIMENTAL PARASITOLOGY

Applications are invited for a Research Assistantship to work on the nutrition of tapeworms Appointment tenable for three years Initial salary up to £1,200 per annum with F S S U and annual increments of £100

Further particulars from Dr C A Hopkins University Veterinary Hospital, Bearsden Road Bearsden Glasgow to whom applications with the names of two referees, should be sent by April 30 1968 (1129)

CLINICAL CHEMIST, PH D FOR 800-BED teaching hospital of University of Toronto Responsibilities include sharing in supervision of modern routine and special procedures laboratories as well as method development Opportunity for post-doctoral training in clinical chemistry if necessary Write Dr A Rapoport Toronto Western Hospital 399 Bathurst Street, Toronto 2B Canada (1247)

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY

Research worker required with an interest in animal behaviour to work on the problems of communication within a primate group A background in zoology or physiological psychology or an interest in social organization would be an advantage Three-year appointment

Enquiries to Dr Richard P Michael, Primate Research Centre, Bethlem Royal Hospital Monks Orchard Road Beckenham, Kent Telephone 01-777 7887 (1206)

THE NORTH OF SCOTLAND COLLEGE OF AGRICULTURE AND DEPARTMENT OF AGRICULTURE, UNIVERSITY OF ABERDEEN

SCHOOL OF AGRICULTURE

Applications are invited for the post of HEAD OF THE CROP HUSBANDRY DIVISION with responsibilities for specialis[illegible] advisory work and College and University studies Salary scale Grade I, £2,651 to £3 345 per annum Superannuation scheme Medical examination

Further particulars, together with Forms of Application, and particulars of the appointment may be obtained from the Secretary, The North of Scotland College of Agriculture, 41½ Union Street, Aberdeen, AB9 1UD, with whom applications should be lodged not later than April 15 1968 (1245)

UNIVERSITY OF DURHAM

Applications are invited for the post of Research Assistant in Botany to work with Dr Margaret E Bradshaw on the population dynamics of selected species in Upper Teesdale The appointment will be for three years and will commence as soon after the closing date as possible The salary will be on the scale £850 to £950 with F S S U

Applications (3 copies) should be sent by Saturday April 13, 1968 to the Registrar and Secretary, Old Shire Hall, Durham from whom further particulars may be obtained (1236)

ROYAL UNIVERSITY OF MALTA

Applications are invited for a Lectureship in Biology The vacancy is for a Botanist Salar (including overseas allowance) £1,500 by £7 to £2 025 per annum F S S U Passages provided for appointee, wife and children under 1 years of age, up to a maximum of £75 per hea on appointment and normal termination Detailed applications (6 copies) naming thre referees by April 29, 1968, to Inter-Universit Council, 33 Bedford Place, London, W C 1, fro whom particulars are obtainable (1233)

UNIVERSITY OF THE WEST INDIES—TRINIDAD

Applications are invited for (a) Research Fellowship, or (b) Junior Research Fellowship i Faculty of Agriculture Candidates should hav a degree in agriculture agricultural science o equivalent qualification preferably with post graduate experience and experience of tropica soils and agriculture Appointee may be required to work in any of the islands served b the University Salary scales (a) £1 550 t £2 630 per annum, (b) £1,250 to £1,410 pe annum Child allowance F S S U Famil passages triennial study leave Detailed applications (6 copies) naming 3 referees should b sent by April 30, 1968, by persons living in th Americas and Caribbean area, to Secretary University of the West Indies, St Augustine Trinidad and by all others to Inter-Universit Council 33 Bedford Place, London, W C 1 Further particulars obtainable similarly (1232)

UNIVERSITY OF EAST AFRICA

MAKERERE UNIVERSITY COLLEGE—UGANDA

Applications are invited for Lectureship i Geology Candidates should have a speci interest in Mineralogy/Petrology and should als be prepared to lecture in Palaeontology up t second-year level An interest in Geochemistr would be of further advantage Salary scal £EA1,350 to £EA2,230 per annum (one E shilling equals 1/2d sterling) Salary supplemented in appropriate cases under British Expatr ates Supplementation Scheme in the range £34 to £410 per annum (sterling) F S S U Famil passages, biennial overseas leave Detailed appl cations (6 copies) naming 3 referees, by May 1968, to Inter-University Council, 33 Bedfor Place, London, W C 1, from whom particula are available (1231)

E.MERCK AG E Merck

Steroid Research

is of growing importance within our development programme We have made substantial progress and are seeking to extend our efforts in this field.

The Department of

Experimental Endocrinology

plays a vital part in this programme

We require a suitable person as

Head of Department

He should have experience in scientific teamwork, be conversant with the technique of screening and assessing steroid compounds also in methods used for developing new products

Whilst our Research Department is located in Germany, we would welcome a scientist with English as his mother tongue—the more so since E. Merck, Darmstadt is international Command of the German language would be useful (but is not essential)

Please write to us, even if your qualifications do not quite match this outline Scientific and human integrity apart, responsibility and initiative are our principal requirements

Salary and fringe benefits will be a matter for personal negotiation They will reflect the importance we place on this appointment

E. Merck Aktiengesellschaft, 61 Darmstadt/Germany
Personal-Abteilung I

(1297)

CSIRO RESEARCH
AUSTRALIA SCIENTISTS

BOVINE TUBERCULOSIS

DIVISION OF ANIMAL HEALTH

GENERAL The Division of Animal Health of the Commonwealth Scientific and Industrial Research Organization has three major laboratories—the Animal Health Research Laboratory, Melbourne, the McMaster Laboratory, Sydney, and the Veterinary Parasitology Laboratory, Brisbane

The major areas of research at the Animal Health Research Laboratory, Parkville, Victoria, include bovine pleuropneumonia, virus diseases, tuberculosis, infectious bovine infertility, ovine footrot, parasitic diseases of sheep and cattle in the southern winter rainfall zone and investigations into plant poisons

It is proposed to set up a section for tuberculosis research at the Animal Health Research Laboratory, and applications are invited for appointment as Research Scientist to each of two positions

MICROBIOLOGIST—IMMUNOLOGIST

DUTIES The Division is expanding its programme of fundamental immunological research, particularly in those fields relevant to mycobacterial infections Work is already in progress with special reference to ruminant immune globulins including immediate-type hypersensitivity It is hoped that future work will widen and supplement these studies into areas of cellular immunology and delayed-type hypersensitivity

The Laboratory is well equipped for these studies and possesses an analytical ultracentrifuge, electron microscope, fractionation equipment, cold room and extensive small and large animal accommodation

QUALIFICATIONS Applicants should have a Ph D degree in Science or in Applied Science with emphasis on Biology, or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Principal Research Scientist, $A8,242–$A9,490 p a, or Senior Principal Research Scientist, $A9,918–$A10,230 p a Salary rates for women are $A428 p a less than the corresponding rates for men

VETERINARIAN

DUTIES The appointee will be required to collaborate in the immunological research on bovine tuberculosis, particularly in the applied aspects of the programme He may be required to study the epidemiological aspects He would liaise with the meat inspection services, with the State veterinary services and with the Northern Territory Administration

QUALIFICATIONS Applicants should have a degree in Veterinary Science and a Ph D degree in a relevant discipline, or post-graduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability

SALARY Depending upon qualifications and experience, the appointment will be made within the salary ranges of Research Scientist, $A5,250–$A6,622 p a or Senior Research Scientist, $A6,892–$A7,974 p a Salary rates for women are $A428 p a less than the corresponding rates for men

CONDITIONS The appointments, which carry Commonwealth Superannuation Fund or Provident Account privileges, will be conditional upon passing a medical examination An initial probationary period of 12 months may be specified

Consideration would be given to appointment for an initial period of three years at the conclusion of which an extension of tenure or appointment for an indefinite period would be considered

Applications, quoting Appointment No 201/301 (Microbiologist-Immunologist) or 201/302 (Veterinarian), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant s academic and professional standing, should reach

Mr R F Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
Africa House, Kingsway,
London, W C 2, by the **24th May, 1968** (1259)

UNIVERSITY COLLEGE OF NORTH WALES

MATERIALS SCIENCE

SCHOOL OF ENGINEERING SCIENCE

Opportunities for research leading to the degree of Ph D are available in the Division of Materials Science which has a special interest in the electronic properties of materials Research in progress is linked whenever possible, with industrial laboratories and includes studies of

Atomic structure of metal surfaces and interfaces
Single crystal whisker growth and evaluation
Epitaxy and trace contamination in semiconductors
Local conduction in oxide and organic polymer films
Ultra-high vacuum preparation and assessment of semiconductor thin films
Conduction in liquid dielectrics and construction of liquid electrostatic devices
Emission mechanisms and motion of high current discharges

Excellent facilities are available, especially for surface and thin film studies, and include Scanning and Transmission Electron Microscopes, Field Emission and Field Ion-Microscopes, a Microprobe, X-ray Diffraction Equipment, an Infra-red Spectrometer and Ultra-high Vacuum facilities

A full programme of lectures forming part of an M Sc Course on Electronic Materials and Devices is available to all research workers

Further particulars and application forms may be obtained from the Registrar, U C N W, Bangor (1241)

THE UNIVERSITY OF MANCHESTER
MANCHESTER, 13

ASSISTANT LECTURER IN PHYSICS

Applications invited for above post associated with Low Temperature group under Professor H E Hall The research interests of the group are liquid helium and the transport properties of superconductors Candidates intending to pursue research in superconductivity preferred Salary range £1,105 to £1,340 per annum F S S U Particulars and application forms (returnable by May 10) from the Registrar Quote ref 41/68/Na (1240)

UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF AGRICULTURAL BOTANY

Applications are invited for RESEARCH ASSISTANTSHIPS in the field of Genetics Crop Physiology or Plant Pathology Applicants should have or expect to obtain a good honours degree in Agricultural Botany or Botany Permission to register for a higher degree will be granted in suitable cases (1239)

UNIVERSITY OF CAMBRIDGE

HISTOLOGIST (Technical Assistant £828 to £1 160) wanted for work on nervous and reproductive systems of birds and mammals Suitable applicant might occupy modern house adjoining laboratories in return for light caretaking duties

Apply, naming two referees, to Dr P Bateson Sub-Department of Animal Behaviour Madingley Cambridge (1267)

SCIENTIFICALLY QUALIFIED BACTERIOlogical assistant required in the Enteric Reference Laboratory to work with Dr E S Anderson, F R S Degree should be preferably in a biological subject The work is mainly con[illegible] with transferable drug resistance in the Enterobacteria —Applications with full details of age, qualifications and experience should be sent to Personnel Officer, Central Public Health Laboratory, Colindale Avenue N W 9 (1218)